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Friedhelm Beyersdorf

Ischemia-Reperfusion Injury in Cardiac Surgery

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FOREWORD

It has become increasingly clear that ischemia during cardiac surgery causes a variety of events that can cause, if unaltered by the control of reperfusion, myocyte endothelial necrosis. This volume describes the injury and operative procedures that can be employed to improve surgical results.

The metabolic changes associated with ischemia and reperfusion can be mitigated before, during, and after aortic clamping. This book propounds two fundamental principles; 1) protection against ischemic injury is related to how the heart is managed metabolically during ischemia, rather than merely aortic clamp time and 2) the reperfusion process, that can in part be controlled by alteration of temperature, metabolic substrates, pressure, and substrate supplementation, can be adjusted to avoid the deleterious and potentially lethal consequences of ischemia only when the normal blood supply is restored.

Three examples are selected to illustrate these principles. First, cardiac recovery after 45 minutes of global normothermic ischemia is marginal, with only 30% return of function and high morbidity and mortality after normal blood reperfusion without myocardial protection during ischemia. In contrast, 4 hours of aortic clamping with blood cardioplegia can completely protect:

1. the normal heart from damage, and
2. more importantly, hearts that have previously undergone 45 minutes of normothermic ischemia followed by 2 more hours of ischemia with warm and cold blood cardioplegia, with associated interventions.

This confirms that the way the heart is managed during aortic clamping is a more important variable than the rapidity of operation if no protection is provided, and reliance is placed only upon the speed of the surgeon. Clearly, surgical skill can reduce ischemic/reperfusion damage, but protective interventions can avoid it, despite a longer period of aortic clamping.

Second, 2 hours of regional ischemia causes extensive myocardial infarction, with only minimal salvage if regional tissue or normal blood supply is restored in a beating working heart (i.e., PTCA after acute myocardial infarction). Under these regional circumstances, there exists a condition where no control of the ischemic process is possible so that only the reperfusate modification will define subsequent improvement. Studies, both experimental and clinical, show that this ischemic/reperfusion lesion can be limited remarkably by controlling *only* the reperfusate conditions and composition.

Third, inadequate distribution of cardioprotective agents must be delivered homogeneously to limit ischemic damage and/or reverse reperfusion damage. This allows assessment of the adequacy of the benefits of surgical correction of lesions requiring ischemia to facilitate technical excellence during operative repair.

These observations have major surgical implications, since:

1. some problems cannot be offset under global circumstances, if temperature alone is used to reduce metabolic work;
2. responsible processes can be modified surgically to offset the lethal metabolic and functional changes that cause reperfusion damage when only normal blood supply is restored;

3. avoidance of reperfusion injury in the regional ischemic heart without any form of protection during acute myocardial infarction demonstrates the benefits of controlled reperfusion since this is the only method available to deal with the consequences of ischemia and reoxygenation. The absence of myocardial protection indicates clearly the occurrence of reperfusion injury and its modification by knowledge of its consequences.

In this book, alterations of myocytes and endothelium caused by ischemia and reperfusion are described in order to provide a comprehensive basis for consideration of the elements of effective cardioprotection. These elements include (a) characterization of myocardial injury; (b) reduction of endothelial injury by modifying the potential production of nitric oxide (NO) and augmenting NO production with metabolic precursors such as L-arginine. Restoration of NO allows more normal vasodilatation and decreases the adherence of neutrophils to the endothelium and platelet accumulation with ensuing thrombosis. Futher elements in the development of effective cardioprotection include: (c) manipulation of the intracellular buffering system by a selective sodium/hydrogen ion exchange inhibitor to limit postischemic sodium influx and subsequent calcium influx and accumulation; (d) providing substrates for metabolic support of the mitochondria by replacing amino acids depleted by ischemia and reperfusion; (e) defining cellular changes during ischemia and reoxygenation so that a reperfusate can be developed that decreases calcium entry and limits hypercontracture; (f) limiting reperfusion injury by reducing oxygen levels and adding antioxidants to limit reoxygenation injury; (g) further understanding preconditioning at the cellular level to limit post-ischemic stunning and thereby setting the stage for pharmacologic treatment to limit damage without enhancing ischemia; (h) supplementing the cardioplegic solution with magnesium to further limit calcium influx; and (i) changing the method of arrest by using repolarizing agents like pencidil to alter calcium influx and adding a beta adrenergic blocker (esmolol) simultaneously limit calcium influx and cardiac work after aortic unclamping. In any event, the ideal mode of cardioplegic delivery, whether antegrade, retrograde, simultaneous antegrade/retrograde or even pulsatile perfusion. must open vessels closed by ischemia to establish homogeneous postischemic distribution in steady state flow.

With prolonged ischemia without protection, as in acute myocardial infarction, myocardial damage occurs that cannot be altered by integrated modification of reperfusion. For surgical ischemia with protection, the metabolic environment can be changed by cardioplegic substrates delivered antegrade and/or retrograde. Yet normal reflow establishes a sequence of metabolic and functional events that may render the heart susceptible to extensive damage. This damage can be limited by modifying the conditions of ischemia so that the initial reperfusate restores a more normal cellular metabolism to allow subsequent ischemia to be better tolerated. Our goal in this book is to provide information on ischemia and reperfusion so as to promote efforts in cardioprotection.

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PREFACE

Cardiac surgery was always associated with ischemia and consequently reperfusion from its very beginning. After the recognition that the various techniques used to perform open heart surgery resulted in some form of ischemia, many different techniques evolved to protect the myocardium during that period of interruption of blood supply. In recent years, convincing data evolved showing that the reintroduction of normal blood to previously ischemic myocardium results in further injury or reperfusion damage. Great efforts have been undertaken to avoid the problems during more and more complex cardiac operations. These efforts included:

- Improved myocardial protection during ischemia
- Avoidance or at least reduction of the subsequent reperfusion injury
- Avoidance of ischemia at all (e.g., continuous delivery of oxygenated cardioplegic solutions, beating heart operations with or without extracorporeal circulation)
- Increased knowledge of the basic mechanisms underlying ischemia and reperfusion processes

This book will present the current knowledge of world-renowned experts in their field. They report their clinical and experimental data and give an overview of their entire subspecialty.

A successful cardiac operation requires both a perfect anatomical repair and conditions for myocardial protection that allow these repair techniques to occur. Even though great improvements have occurred in cardiac surgery over the years allowing more and more complex operations with better results for the patients to be performed, we as cardiac surgeons have to further improve both our anatomical repair techniques as well as the methods with which these techniques can be applied.

I thank all the contributors for the tremendous work they have done with their chapters, and I do hope that this will be of benefit for our patients and our surgical community.

Friedhelm Beyersdorf, M.D.
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Section I:
Basic Science
in Ischemia-Reperfusion Injury

CHAPTER 1

Harnessing the Cardioprotective Potential of Nitric Oxide in Nonsurgical and Surgical Ischemic-Reperfusion Injury

Jakob Vinten-Johansen and Russell S. Ronson

In cardiac surgery, there are numerous opportunities during the conduct of the operation for both unplanned and hence unprotected ischemia with subsequent reperfusion. These periods of potential injury include 1) antecedent ischemia (regional coronary occlusion, profound hypotension) occurring before institution of cardiopulmonary bypass or other means of hemodynamic support, 2) "protected" ischemia encountered between infusions of cardioplegia solution, but which may be complicated by maldistribution of cardioplegia solution by coronary obstructions or inadequate delivery pressures, and 3) inadvertent ischemia occurring after reperfusion has been initiated.[1,2] Each ischemic event not only carries the potential for producing damage in its own right, but may also interact with each other in a cumulative fashion. Each interval of ischemia also carries the potential for subsequent reperfusion injury, defined as injury extending beyond that present during ischemia. Whether this is an active process of dynamic injury development leading to new necrosis or dysfunction, or whether reperfusion injury is simply a passive phenotypic expression of morphologic changes that have occurred during ischemia, and that interventions to alter reperfusion injury are altering the course of that expression are issues that are fervently debated.[3,4] Therefore additional reperfusion injury may follow 1) resuscitation or hemodynamic restabilization before cardiopulmonary bypass, 2) infusion of cardioplegia solution through a newly revascularized segment, or initialization of blood flow through an internal mammary artery conduit, or 3) removal of the aortic cross clamp. The targets of ischemic-reperfusion injury are not restricted to myocytes alone in the form of necrosis or contractile dysfunction, but also include the vascular endothelium and its ability to elaborate important endogenous factors such as nitric oxide ($NO^{\bullet}$) and adenosine which are active in regulation of blood flow, blood pressure and cell-cell (neutrophil-endothelial cell) interactions.[5] Additionally, extracorporeal circuits used in many cardiac surgical procedures contribute to the pathophysiology of ischemia and reperfusion injuries by activating complement and cytokines which recruit neutrophils and other inflammatory cells and thereby amplify the inflammatory process.[6] This extracorporeal inflammatory component makes surgical ischemic-reperfusion injury uniquely different from its nonsurgical counterpart.

Nitric oxide is a naturally occurring autacoid that has a plethora of physiological actions as diverse as producing vasodilation to acting as a neuronal signaling molecule responsible for many of the biological actions of excitatory amines. However, an extremely important physiological effect is the inhibition of inflammatory processes, including endothelial cell activation and expression of adhesion molecules, and inhibition of neutrophil actions (Fig. 1.1). $NO^{\bullet}$ is a radical species that has an

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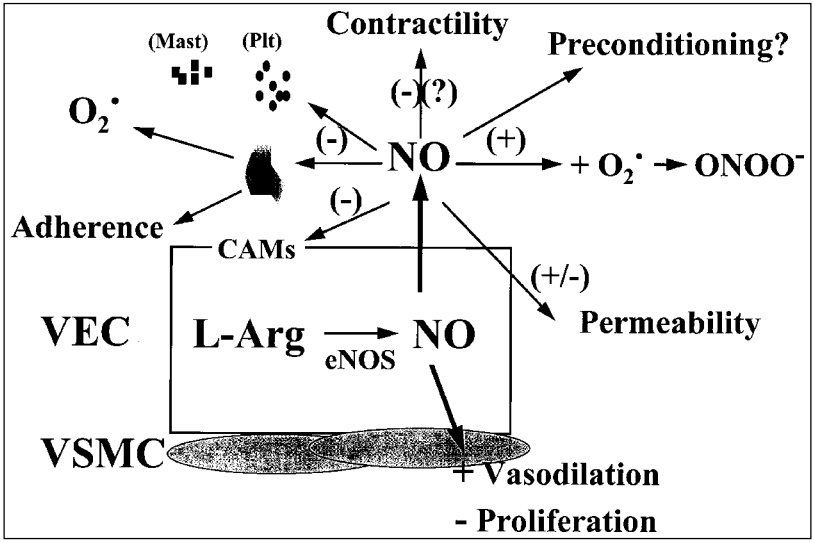

Fig. 1.1. The plethora of physiological effects of nitric oxide ($NO^{\bullet}$) are summarized. $NO^{\bullet}$ inhibits (-) neutrophil activation and adherence to endothelial cells (VEC), as well as inhibits platelet (plt) and mast cell (Mast) aggregation. $NO^{\bullet}$ also directly inhibits the expression or upregulation of cellular adhesion molecules (CAMs) on VEC. $NO^{\bullet}$ has been implicated in promoting (+) the second window of preconditioning and is a substrate for the extremely rapid biradical reaction with $^{\bullet}O^{-}_{2}$ to form peroxynitrite (ONOO-) which may have deleterious effects in crystalloid solutions because of a lack of detoxifying reactions that prevent ONOO- accumulation, but which may also have beneficial effects in blood media (i.e., blood cardioplegia) secondary to the presence of those detoxifying pathways. $NO^{\bullet}$ is most widely known for its effects on vasodilation thereby mediating in part autoregulation of blood flow and reactive hyperemia. Other abbreviations: L-Arg = L-arginine; eNOS = endothelial nitric oxide synthase; VSMC = vascular smooth muscle cells.

extremely short half-life, and hence can diffuse short distances before being inactivated, but this molecule nonetheless exerts potent physiological actions. $NO^{\bullet}$ formed by the vascular endothelium is released into the intravascular compartment and the perivascular and interstitial compartments. This close proximity to compartments which are important in the pathogenesis of ischemia-reperfusion injury places $NO^{\bullet}$ in a unique and powerful position in modulating cell-cell interactions characteristic of the inflammatory component of postischemic processes. In recent years, experimental research has shown that $NO^{\bullet}$ exerts potent cardioprotection from ischemic-reperfusion injury in both surgical and nonsurgical settings by a number of mechanisms, including inhibiting neutrophils and mast cells,[7] and attenuating adhesion molecule expression on vascular endothelium.[8-10] In contrast, $NO^{\bullet}$ has also been implicated in promulgating injury because of its actions as a radical species or the generation of potentially deleterious metabolites such as peroxynitrite ($ONOO^{-}$) and subsequently hydroxyl radical ($^{\bullet}OH$). Therefore, a duality of opposing physiological actions is associated with endogenous and exogenous $NO^{\bullet}$, the mechanisms of which are not fully known. This chapter summarizes the role of $NO^{\bullet}$ derived either endogenously from the vascular endothelium or provided exogenously from its physiological precursor L-arginine or molecules that donate $NO^{\bullet}$ spontaneously in modulating nonsurgical and surgical ischemic-reperfusion injury.

Chemistry of $NO^{\bullet}$ Generation

Nitric oxide is a free radical gas formed by the five electron oxidation of L-arginine to the products $NO^{\bullet}$ and L-citrulline by the

monoxygenase enzyme, nitric oxide synthase (NOS) (Fig. 1.2). The reaction requires molecular oxygen, NADPH as the electron donor, and tetrahydrobiopterin. There are three isoforms of NOS which are products of three separate genes and which share 50-60% homology between them: 1) neuronal NOS (nNOS or NOS1) is constitutively present in neuronal tissue; 2) inducible NOS (iNOS or NOS2) is stimulated to generate $NO^{\bullet}$ by selected cytokines and endotoxin over relatively long periods of time (hours); 3) endothelial NOS (eNOS or NOS3) which, like nNOS, is constitutively expressed and is rapidly responsive to selected physiological and pharmacological stimuli (agonists acetylcholine, bradykinin, Ca^{2+} ionophores). eNOS was initially described as originating from endothelial cells but is now known to be expressed by both vascular and endocardial endothelium as well as by myocytes. eNOS will be the focus of this chapter as the primary source of $NO^{\bullet}$ relevant to myocardial ischemic-reperfusion injury.

eNOS is a heme-containing enzyme that is Ca^{2+}-calmodulin sensitive. In contrast, iNOS is Ca^{2+} insensitive due to the very tight binding of calmodulin to the binding domain which is not regulated by physiological concentrations of Ca^{2+}. iNOS is therefore not regulated by physiological concentrations of Ca^{2+}, but rather by other slow-response mechanisms. eNOS is tethered to the sarcolemmal membrane in microdomains known as caveolea; these specialized areas of the cell plasmalemma are involved generally in transcytosis of large molecules and the uptake of smaller molecules. Interactions of agonists that stimulate eNOS, such as acetylcholine and bradykinin, dissociate eNOS from the membrane with subsequent translocation into the cytosol where it is phosphorylated, thereby activating the enzyme.

eNOS enzyme activity is regulated by physiological concentrations of Ca^{2+} through the binding of calmodulin to the binding domain of eNOS, thereby initiating electron flow involved in the production of the $NO^{\bullet}$ radical. The enzyme has a calmodulin-binding domain that undergoes a conformational change when Ca^{2+} and calmodulin bind to this site, which then facilitates the conversion of molecular O_2 and L-arginine to $NO^{\bullet}$ and L-citrulline. Tetrahydrobiopterin acts to facilitate the flow of electrons from NADPH to the heme moiety of eNOS. Therefore, the binding and dissociation of Ca^{2+}-calmodulin acts as a molecular toggle switch turning the enzyme on and off. Agonists stimulate an increase in intracellular Ca^{2+} through receptor-dependent mechanisms which transduce the signal by the phosphoinositide second messenger system (Fig. 1.2). This second messenger generates inositol 1,4,5-trisphosphate (IP_3), which, in turn, binds to receptors on the endoplasmic reticulum and subsequently, stimulates the release of Ca^{2+} from intracellular stores. This receptor-dependent Ca^{2+} signal then stimulates eNOS to increase $NO^{\bullet}$ production and release. Therefore, acetylcholine and bradykinin stimulate eNOS in a concentration-dependent manner. Ca^{2+} ionophores such as A23187 will also stimulate eNOS to release $NO^{\bullet}$ by receptor-independent mechanisms (Fig. 1.2) which helps to differentiate receptor-related from nonreceptor-related impaired $NO^{\bullet}$ release. Vasodilatory responses to receptor-dependent and receptor-independent stimulators of eNOS forms the basis of bioassay systems interrogating the functional capacity of vascular tissue to generate $NO^{\bullet}$. In addition to intracellular Ca^{2+}, eNOS is also regulated by vascular wall shear stress created by the pressure-flow interactions in the vessel[11,12] and by deformation of the vascular endothelium, both of which accompany pulsatile perfusion of the vasculature. The potent vasodilator effects of $NO^{\bullet}$, coupled with its physiological regulation by intracellular calcium put this autacoid in an effective position to contribute to the regulation of blood pressure and organ blood flow.[12] Accordingly, endothelium-derived $NO^{\bullet}$ has been implicated in the regulation of normal cardiovascular homeostatic processes such as autoregulation[13,14] and reactive hyperemia,[15,16] and in the pathophysiology of disease states including shock[17,18] atherosclerosis[19,20] hypertension,[21,22] and hypercholesterolemia.[20,23] Attenuated $NO^{\bullet}$ release after cardiopulmonary bypass has also been implicated in the etiology of pul-

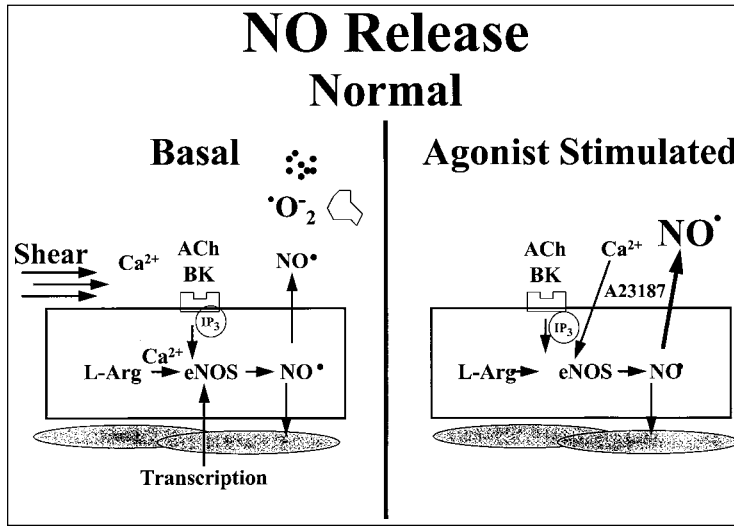

Fig. 1.2. Nitric oxide ($NO^{\bullet}$) is produced by the oxidation of the guanidino moiety of L-arginine by endothelial nitric oxide synthase (eNOS or NOS III). Basal release of nitric oxide is stimulated by vascular shear stress and physiological agonists such as acetylcholine (ACl) and bradykinin (BK) through receptor interactions. Nitric oxide rapidly diffuses ablumenally when it causes vascular smooth muscle relaxation and vasodilation. Diffusion intralumenally inhibits platelet aggregation, attenuates neutrophil activation, and interacts with superoxide anion $^{\bullet}O^{-}_{2}$ to form peroxynitrite ($ONOO^{-}$). Under experimental conditions such as in organ chambers, pharmacological agonists can stimulate eNOS by receptor-dependent (ACh, BK) or receptor-independent (Ca^{2+} ionophore A23187) to produce degrees of vasorelaxation which are dependent on concentration of agonists, efficiency of the receptor mechanism, or the function of eNOS.

monary vasoconstriction and pulmonary hypertension after cardiopulmonary bypass.[24]

Physiological Actions of $NO^{\bullet}$ Relevant to Ischemia-Reperfusion

Vasodilation

The role of $NO^{\bullet}$ (endothelium-derived relaxing factor, later identified as $NO^{\bullet}$ or a nitrosylated intermediate) as a physiological regulator of vascular tone was first identified by the classic studies of Furchgott and Zawadski[25] in which the endothelium was shown to play an obligatory role. $NO^{\bullet}$ has subsequently been identified as the endogenous nitrovasodilator, and the dilating component in vasoactive agents such as nitroglycerin, nitroprusside and other organic nitrates. $NO^{\bullet}$ released either through basal or agonist-mediated mechanisms diffuses both lumenally and ablumenally. A key target of ablumenally released $NO^{\bullet}$ is the iron center at the active site of guanylate cyclase. $NO^{\bullet}$ stimulates guanylate cyclase by binding to iron in the heme center, which stimulates the enzyme to generate cGMP and induce vasorelaxation through a number of mechanisms. The vasorelaxation response to acetylcholine of isolated coronary vessels with intact endothelium in organ chambers is consistent with vasodilator and hypotensive responses observed in vivo. The degree of vasorelaxation is related to the amount of $NO^{\bullet}$ released and the sensitivity of guanylate cyclase. The implication of this vasodilator effect on macrovessels (conduit or conductance vessels) and microvessels (resistance vessels) in normal as well as in postischemic myocardium is that the loss of regulation of local vascular resistance by $NO^{\bullet}$, secondary to endothelial dysfunction and impaired release of $NO^{\bullet}$, may lead to defects in reactive hyperemic responses and in global

and regional postischemic blood flow.[12,16,26] Impaired reactive hyperemia may also involve the neutralization of $NO^{\bullet}$ by superoxide radicals generated during even brief ischemia.[27]

Inhibition of Metabolism and Contractile Function

$NO^{\bullet}$ may decrease the rate of glycolysis by mechanisms independent of guanylate cyclase.[28] $NO^{\bullet}$ stimulates the ribosylation of ADP; stimulation of auto-ADP ribosylation of glyceraldehyce-3-phosphate dehydrogenase (GAPDH) inhibits the enzyme and in turn inhibits glycolysis. Ostensibly, the inhibition of metabolism and ATP generation may accordingly reduce contractile function during early reperfusion. The negative metabolic effect of $NO^{\bullet}$ may therefore have implications in the genesis of myocardial stunning[29] and hibernation in which glycolysis plays a large role. $NO^{\bullet}$ may also have a negative modulatory effect on oxidative metabolism by inhibiting mitochondrial oxidative phosphorylation and hence oxygen consumption.[30] However, this effect on oxygen consumption is not universally reported.[31]

The inhibition of glycolysis may have some implication in the purported negative inotropic effects of $NO^{\bullet}$ in normal myocardium.[32] However, there is controversy over the effects of $NO^{\bullet}$ on the inotropic state and contractile function of the heart. Finkel et al[32] first reported a negative inotropic effect of cytokines (TNF-α) in papillary muscle preparations which they attributed to the effects of $NO^{\bullet}$. This observation would have relevance to procedures using cardiopulmonary bypass since cytokines,[33,34] as well as complement,[6,35,36] are elevated during bypass by exposure of blood to foreign surfaces and the triggering of cytokine and complement fragment release by ischemia-reperfusion. In support of stimulated release of $NO^{\bullet}$ during bypass, transmyocardial $NO^{\bullet}$ levels as well as NOS activity have been observed to be elevated after release of the crossclamp during cardiac surgery,[29] a time when cardiac depression is often observed. However, other studies have reported no negative inotropic effects of *physiological* concentrations of $NO^{\bullet}$.[37,38] The disparate observations may be related to the amount of $NO^{\bullet}$ released, which may be higher (micromolar range) with cytokine stimulation (i.e., of iNOS) compared to either endogenously released $NO^{\bullet}$ (0.1-1 nMol in coronary circulation)[39] or therapeutic concentrations of $NO^{\bullet}$ donor agents (approximately 500 nMol). Higher concentrations of $NO^{\bullet}$ released by endotoxin stimulation may mediate cardiac depression during the later stages of septic shock. However, in surgically or nonsurgically reperfused postischemic hearts, any direct negative inotropic actions of $NO^{\bullet}$ may be overridden by the net cardioprotective effects of $NO^{\bullet}$ secondary to a reduction of neutrophil- and oxidant-mediated injury, resulting in better postischemic function.[40-44]

Decreased $NO^{\bullet}$ Release After Endothelial Damage from Ischemic-Reperfusion Injury

Under normal conditions, the endothelium tonically releases $NO^{\bullet}$[45-47] in the neighborhood of 0.1-1 nMol into the vascular and interstitial compartments. This basal $NO^{\bullet}$ release is regulated by vascular shear stress,[48-50] the tonic release of physiological agonists and humoral agents, and intracellular calcium. During cardiopulmonary bypass, a basal release of $NO^{\bullet}$ has been observed by Hattler et al.[29] Basal release of $NO^{\bullet}$ is inhibited by a number of pharmacological agents and pathological conditions as summarized in Figure 1.3. Guanidino-substituted L-arginine analogue inhibitors of eNOS such as N^{G}-nitro-L-arginine (L-NAME) and N^{G}-monomethyl-L-arginine (L-NMMA), as well as calmodulin antagonists,[45] reduce directly measured $NO^{\bullet}$. On the other hand, L-arginine but not D-arginine stimulates the basal release of $NO^{\bullet}$.[45,51] Because of the near diffusion-limited biradical reaction rate of $^{\bullet}O^{-}_{2}$ with $NO^{\bullet}$ to form $ONOO^{-}$,[52-55] the rapid degradation of $^{\bullet}O^{-}_{2}$ to H_2O_2 by superoxide dismutase also increases the amperometrically measured $NO^{\bullet}$ in vitro.[45] The neutralization of basally released $NO^{\bullet}$ and its vasodilator effects by $^{\bullet}O^{-}_{2}$ in conditions

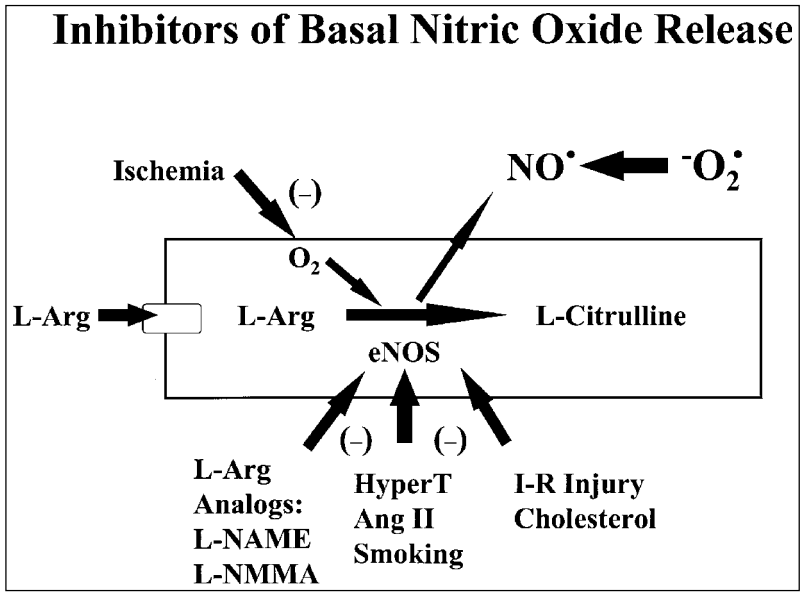

Fig. 1.3. Inhibitors of basal nitric oxide production and release by the vascular endothelium. L-arginine (L-Arg) is transported into the vascular endothelium and converted to NO• and L-citrulline by eNOS. This process requires the presence of molecular oxygen. The generation of NO• can be inhibited by lack of oxygen (ischemia, anoxia). Nonmetabolized analogues of L-arginine then inhibit enzyme activity (L-NAME, L-NMMA), and pathophysiological conditions such as angiotensin II (Ang-II)-dependent hypertension (HyperT), ischemia-reperfusion (I-R) and hypercholesterolemia.

such as angiotensin-related hypertension may be one of the pathophysiological mechanisms of high blood pressure. In addition, NO• itself may have a negative feedback regulation on eNOS activity. This was first reported by Rogers and Ignarro[56] for constitutive NOS in rat cerebellum, and later by Buga et al[57] for eNOS activity in cultured endothelial cells. Therefore, exogenous NO• may regulate its own synthase activity. As demonstrated by Ma et al,[31] exogenous NO• also regulates in vivo basal NO• release by a negative feedback mechanism on its own endothelial synthase activity. This inhibitory mechanism may involve binding of NO• to the heme moiety of eNOS, thereby inhibiting its catalytic activity. Clinically, this negative feedback inhibition may help explain impaired NO• -dependent autoregulation in patients on long-term nitrovasodilator therapy. Whether this autoinhibition has implications for less effective cardioprotection in those same patients is not clear.

Time Course of Endothelial Injury

Ischemia and reperfusion cause injury to the vascular endothelium, manifest as a reduction in basal and stimulated NO• release[58-61] and hence attenuated responses to agonist stimulators of eNOS.[59,62,63] In coronary occlusion models (regional ischemia), endothelial dysfunction is minimally expressed after the ischemic period (60-90 minutes), but is progressively expressed starting as early as 2.5 minutes after the start of reperfusion, and persists hours[58,64] to days[65] after reperfusion. Figure 1.4 shows coronary artery endothelial function (assayed as responsiveness to agonist stimulators of eNOS) 6, 24 and 48 hours after LAD occlusion. Notice that responses to acetylcholine were less in the LAD compared to the nonischemic left circumflex coronary artery

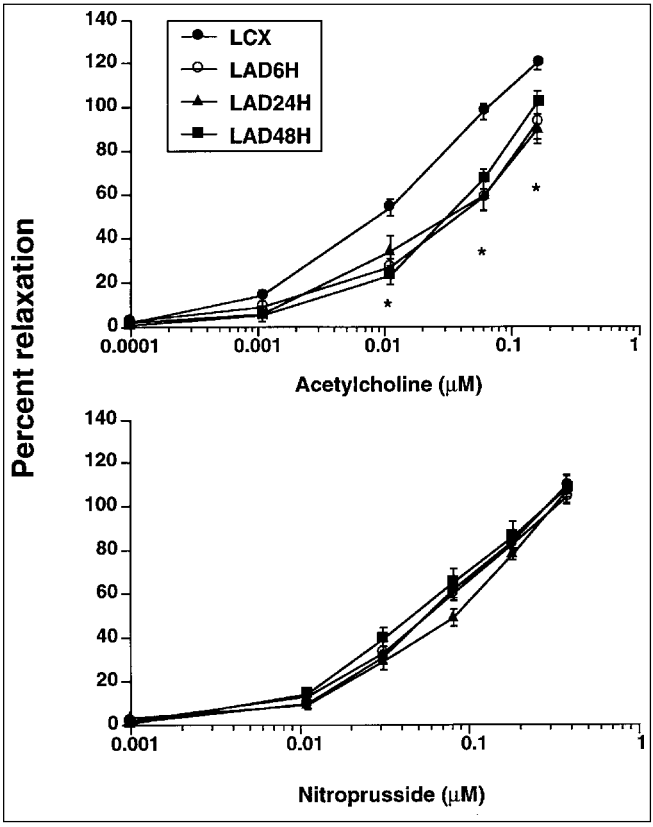

Fig. 1.4. Responses of preconstricted (U46619) postischemic coronary arteries to endothelium-dependent acetylcholine (receptor-dependent) and smooth muscle dilator sodium nitroprusside. LCX = normally perfused left circumflex coronary artery; LAD6H, LAD24H and LAD48H = ischemic (1 hour)-reperfused left anterior descending coronary artery reperfused for 6, 24, or 48 hours. Top Panel. Responses to acetylcholine. Bottom Panel. Responses to sodium nitroprusside. * $p < 0.05$ vs nonischemic LCX. Concentrations are final organ chamber concentrations. Note the persistent depression of endothelial responses over 48 hours of reperfusion. (From Zhao et al, unpublished results)

at any concentration. This impaired responsiveness persisted for 48 hours. In models of global ischemia, endothelial dysfunction has been shown to be present after reperfusion with little or no dysfunction expressed during ischemia[60,61] unless more prolonged periods of ischemia are imposed.[60] This endothelial dysfunction is expressed as a reduction in basal $NO^{\bullet}$ release as well as stimulated $NO^{\bullet}$ release, with obtunded relaxation responses to acetylcholine or other agonist stimulators of NOS. In the study by Nakanishi et al,[61] canine hearts were subjected to 45 minutes of global normothermic ischemia with or without unmodified blood reperfusion in cardiopulmonary bypass. The left anterior descending and left circumflex coronary arteries were excised, placed in organ chambers in a Krebs-Henseleit buffer medium, and agonist-stimulated endothelial function interrogated using acetylcholine for receptor-dependent vasorelaxation responses and the Ca^{2+} ionophore A23187 for receptor independent vasorelaxation responses. As shown in Fig. 1.5A, maximal vascular relaxation responses to acetylcholine in hearts subjected to ischemia

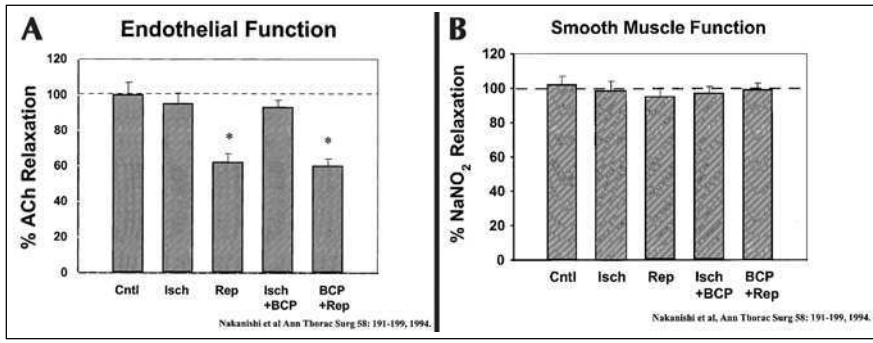

Fig. 1.5. Relaxation responses of preconstricted (U46619) postcardioplegia epicardial coronary arteries to agonists. PANEL A: Maximal relaxation responses to the endothelium-dependent, receptor-dependent agonist acetylcholine (ACl). PANEL B: Maximal relaxation responses to the direct (endothelium-independent) vascular smooth muscle dilator acidified $NaNO_2$. Cntl = normal control vessel; Isch = after 45 minutes global normothermic ischemia (no reperfusion); Rep = ischemia followed by one hour unmodified blood reperfusion; Isch + BCP = 45 minutes global normothermic ischemia followed by one hour intermittent (every 20 minutes) 4°C hyperkalemic blood cardioplegia (4 parts blood:1 part crystalloid); BCP + Rep = global ischemia plus one hour cardioplegia followed by one hour blood reperfusion. *$p < 0.05$ vs unstarred groups. Adapted from Nakanishi et al.[61]

without reperfusion were comparable to normal control hearts. In contrast, maximal vasorelaxation responses in coronary arteries subjected to both global ischemia and reperfusion were reduced by approximately 35-40% relative to control hearts and ischemia only hearts. A modest rightward shift in the entire concentration-relaxation response curve to acetylcholine occurred in ischemia—only coronary arteries (EC_{50} of $4.0 \pm 0.5 \times 10^{-8}$ Mol vs control $2.2 \pm 0.3 \times 10^{-8}$ Mol, $p < 0.05$), while a greater shift was seen in reperfused vessels ($6.0 \pm 0.2 \times 10^{-8}$ Mol) suggesting endothelial dysfunction. Similar impaired maximal and concentration-dependent responses to those in Figure 1.5A were also observed for the Ca^{2+} ionophore A23187. Smooth muscle relaxation responses to the $NO^{\bullet}$ donor acidified (pH 2.0) $NaNO_2$ were 100%, indicating no damage to the vascular smooth muscle (Fig. 1.5B). These data suggest that endothelial dysfunction involved not only damage to the receptor-transduction complex, but also to the more distal processes regulating eNOS activity or damage to the enzyme itself.[61] In this model of global ischemia[61] endothelial dysfunction was associated with morphological abnormalities in endothelial structure. In ischemic-only (no reperfusion) hearts, the coronary microvascular endothelium was largely normal, with normal and confluent attachment to the subendothelial matrix. Ischemic endothelial cells differed from normal tissue by the presence of vacuoles randomly distributed in the cytoplasm. In sharp contrast, tissue reperfused with unmodified blood demonstrated detached endothelium with exposed subendothelial matrix and endothelial cell fragmentation. In attached endothelial cells, the cytoplasm was dramatically hypervessiculated. These morphologic changes are in concordance with the severe dysfunction to the enzyme system described above. These data demonstrating impaired vasorelaxation responses to agonist stimulators of eNOS and morphologically-apparent damage are consistent with loss of basal $NO^{\bullet}$ release measured amperometrically, reported by Engelman et al.[47]

In regional ischemia, vasodilator responses to both receptor-dependent (acetylcholine) and receptor-independent (calcium ionophore A23187 which stimulates eNOS distal to the receptor-G-protein complex) stimulators of NOS are adversely effected, while in global models involving short (≤ 30 minutes) periods of normothermic ischemia before cardioplegia primarily the receptor-mediated endothelial vasodilator responses are

effected.[61,66] These data suggest that a different phenotype of injury may be expressed dependent on the type and duration of ischemia (regional vs global) imposed. Short-term global ischemia (i.e., ≤ 30 minutes) relevant to cardiac surgery may selectively target the receptor-G-protein complex,[66] while more severe regional ischemia-reperfusion (i.e., inducing myocardial necrosis and infarction) may target both the receptor-G-protein complex and the distal stimulus coupling mechanism, or the enzyme itself. In both cases, the acute injury to the endothelium may be mediated by oxygen radicals formed by 1) the endothelium (via xanthine oxidase activity or NAD(P)H oxidase activity), 2) by mitochondria or 3) by neutrophils adherent to the vascular endothelium. Superoxide dismutase reduces the injury to the endothelium following ischemia and reperfusion, while direct exposure of coronary artery endothelium to oxygen radicals produces endothelium-dependent injury to receptor-mediated dilation responses without effect to the receptor-independent dilator (smooth muscle) responses.[67] The oxygen radicals may not only damage the trigger mechanisms of $NO^{\bullet}$ but may also directly neutralize $NO^{\bullet}$ through the rapid biradical reaction with superoxide anion.

Endothelial Dysfunction and Neutrophils

Endothelial dysfunction plays a critical role in the pathogenesis of reperfusion injury in the myocardium.[64,68-70] This key role is thrust upon the endothelium because of its interaction with polymorphonuclear leukocytes or neutrophils and other inflammatory cell types at the vascular interface in the early and later phases of reperfusion. This interaction is mediated by a highly specific and temporally orchestrated sequence of interactions between adhesion molecules on both the endothelium and neutrophils. Thus, the initial interaction between endothelium and neutrophils is characterized by rolling of the neutrophils along the endothelial surface mediated by interaction between p-selectin on the endothelium and sialylated glycoprotein on the neutrophil, most likely the sialomucin P-selectin glycoprotein ligand-1 (PSGL-1).[71,72] This initial interaction is an obligatory step necessary for distal firm adherence interactions and transendothelial migration into the myocardial parenchyma and their physiological sequelae (no-reflow, necrosis).[73-76] After initial tethering of neutrophils by endothelial p-selectin, a well orchestrated sequence of neutrophil-endothelial cell interactions evolves with the endothelial expression of adhesion molecules, such as E-selectin and ICAM-1, and expression of adhesion counterligands on the neutrophils such as CD11/CD18, that allow firm adherence of the neutrophil to the endothelium. Nitric oxide also limits the capability of the endothelium to express both E-selectin and ICAM-1 after activation by cytokines released during ischemia and reperfusion.[77,78] The ability of nitric oxide to effect multiple points in the neutrophil-endothelial cell adhesion process not only makes it a powerful tool for minimizing reperfusion injury caused by neutrophils, but also leads to the possibility that $NO^{\bullet}$ is acting on a proximal common step in the formation of several types of adhesion molecules. DeCaterina et al have shown significant decreases in activation of NF-κB, a gene promoter for synthesis of adhesion molecules, following endothelial activation with TNF-α. This same decrease in NF-κB was not present in endothelium exposed to c-GMP, glutathione, or nitrite. [77] This important action of $NO^{\bullet}$ has the potential to decrease the accumulation of neutrophils in the myocardium following ischemia and reperfusion acutely as well as over longer term time frames (requiring protein synthesis).

After initial p-selectin-mediated engagement with the endothelium, the neutrophils accumulate in the ischemic-reperfused myocardium primarily after the onset of reperfusion.[68,79,80] Dreyer et al[79] have shown that the rate of neutrophil accumulation within the reperfused myocardial area at risk is rapid within the first hour; Lefer et al[64] have reported a similar time course using tissue myeloperoxidase activity as a marker of neutrophil accumulation. Furthermore, Lefer et al[68] have shown that this accumulation of neutrophils

in ischemic tissue is preceded by a more rapid rate of adherence of neutrophils to the coronary vascular endothelium. This adherence is associated closely with the time course of endothelial dysfunction related to the stimulated release of NO• and eventual accumulation of neutrophils in postischemic tissue in both surgical[40,41,81-83] and nonsurgical ischemia-reperfusion models. The reduction in basal release of NO• by postischemic coronary vascular endothelium resulting from the initial deleterious actions of neutrophils amplifies the obligatory interaction between neutrophils and endothelial cells, thereby triggering the adherence-dependent cascade of neutrophils and subsequent neutrophil-mediated inflammatory component of reperfusion injury.

NO•-Related Therapeutic Strategies

Enhancing Endogenous NO• Release

NO•-related therapeutic strategies for attenuating reperfusion injury are summarized in Table 1.1. Enhancing the release of *endogenous* NO• can be achieved by providing the precursor L-arginine. Although L-arginine appears in the blood in sufficient concentrations to saturate NOS, supplemental L-arginine has been shown to increase NO• release (directly or indirectly measured) by the coronary vascular endothelium.[45,84,85] In isolated rat aortic endothelial cells, 1 mM L-arginine increased the amperometrically measured release of NO• by approximately 40% above basal release.[45] D-arginine at the same concentration did not increase basally released NO•.

Supplementation with L-arginine up to 10 mM concentrations in vitro resulted in decreased neutrophil adherence to activated coronary artery endothelium, back to basal levels in unstimulated vessels, and significantly attenuated neutrophil-mediated injury although L-arginine did not directly attenuate neutrophil superoxide anion production, Figure 1.6.[84] D-arginine (10 mMol) did not inhibit adherence, and carboxy-PT10, 600 μM, a direct scavenger of NO•, reversed the effects of L-arginine.[51,84] Moreover, in a nonsurgical model of regional ischemia, intravenous[51] or intracoronary[155] L-arginine supplementation at the time of reperfusion significantly decreased postischemic coronary artery endothelial dysfunction and reduced infarct size; both were associated with decreased neutrophil adherence to endothelium and accumulation in the area at risk. In the study by Nakanishi et al, infarct size was reduced from 35 ± 2% of the area placed at risk to 18 ± 3% with 10mM intracoronary L-arginine. Infarct size reduction was not observed with D-arginine (10 mM, infarct size 49 ± 5% of area at risk).[63] Neither postischemic regional contractile function or myocardial blood flow were improved with L-arginine treatment.

Basally expressed NO• seems to provide endogenous cardioprotection which, when NOS is selectively attenuated with inhibitors such as L-NA or L-NAME, markers of injury are accentuated compared to noninhibited groups.[86,87] Consequently, in vivo infarct size was increased from 27 ± 2% area at risk in untreated rabbits, to 51 ± 2% in rabbits given intravenous L-NA at the time of reperfusion.[88] Similar observations have been made in surgically relevant models using cardiopulmonary bypass and cardioplegia. This reduction in the contribution of basally released NO• to endogenous cardioprotection, secondary to endothelial injury, may be a significant contributor to postischemic (postcardioplegia) infarction, systolic and diastolic dysfunction and vascular injury often observed in untreated groups.[2,40,44,61,89] In surgical models using antecedent ischemia (regional or global) with subsequent cardioplegia and reperfusion, supplementing with L-arginine to compensate for lost basal release of NO•, has shown significant benefit. Sato et al[83] used a canine model of regional (left anterior descending (LAD) coronary artery ligation for 90 minutes, followed by cardioplegic arrest using blood cardioplegia without (unsupplemented) or with 10 mM L-arginine with concomitant intravenous infusion (4 mg/kg/min) starting at release of the crossclamp. The LAD ligature

Table 1.1. Strategies in nitric oxide therapy

Endogenous Approach	Exogenous Approach
$NO^{\bullet}$ precursor L-arginine	Authentic $NO^{\bullet}$ Gas in solution Acidified $NaNO_2$
Agonist stimulators Acetylcholine Bradykinin	$NO^{\bullet}$ donors Bioconversions (NTG, SPM-5185) Direct donors
Transfection with eNOS	

was removed just before the second infusion of blood cardioplegia, thereby allowing delivery of cardioplegic solution to the previously ischemic (revascularized) myocardial region. Compared to the unsupplemented blood cardioplegia group, L-arginine supplementation resulted in 1) a 33% increase in postischemic systolic shortening of the area at risk and a significant decrease in segment stiffness, 2) a 30% reduction in infarct size (28 ± 4% vs 40 ± 4% of the area at risk, $p < 0.05$) and 3) near normalization of postischemic LAD endothelial responses to acetylcholine, suggesting improved endothelial function (Fig. 1.7). These beneficial effects were associated with lower myeloperoxidase activity levels in the area at risk used as a marker of neutrophil accumulation, suggesting a reduction of neutrophil-mediated injury with L-arginine supplemented blood cardioplegia.[83] These effects with L-arginine were reversed by inclusion of the NOS inhibitor L-nitro-arginine (L-NA) before administration of L-arginine-enhanced blood cardioplegia in a separate group. These results have been corroborated by other studies in which cardioplegia solutions were supplemented with L-arginine.[42] Interestingly, a preischemic infusion of L-arginine was found to be beneficial on postischemic variables by Engelman et al[90] in a nonsurgical model, while both pretreatment and supplementation of cardioplegia have been reported to be beneficial in a surgical model. Interestingly, 3 mMol L-arginine given *after* cardioplegia was detrimental, a subject addressed later in the discussion.[91] Along the same lines of pericardioplegia use of L-arginine, Hiramatsu et al[42,92] administered L-arginine during the early phase of reperfusion in blood perfused isolated hearts, ostensibly targeting reperfusion events, and reported better recovery of postcardioplegia systolic function compared to no adjunct treatment. This timing of administration of L-arginine is consistent with its antineutrophil effect in this and in vivo models. Similar beneficial effects of L-arginine administered at reperfusion were also reported by Armani et al[93] in a cell-free perfusate system, suggesting that $NO^{\bullet}$ derived from supplemental L-arginine may have beneficial effects based on other than its antineutrophil effects, i.e., quenching of superoxide anions. However, the benefits of L-arginine supplementation as a source of $NO^{\bullet}$ in cell-free systems is controversial.

Protection by Nitric Oxide Donors

In the study by Sato et al,[83] only a modest decrease in infarct size was achieved compared to other cardioprotective strategies, i.e., adenosine. This may represent a limitation of utilizing the endogenous cardioprotective mechanisms of the heart with L-arginine therapy in that the increased release of $NO^{\bullet}$ is dependent on the functional state of the endothelium. An injured endothelium in which $NO^{\bullet}$ generation mechanisms are impaired may show obtunded responses to

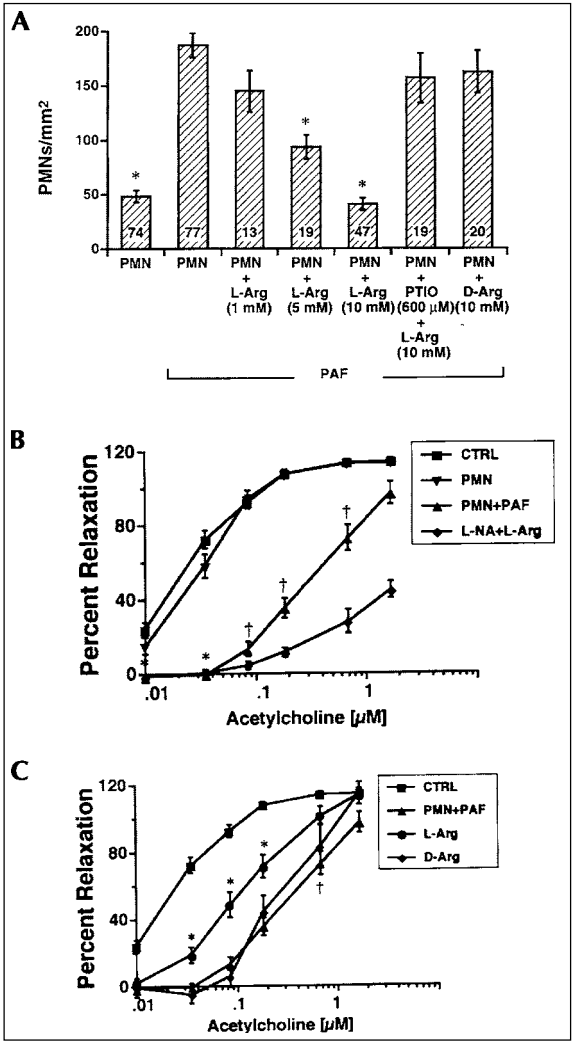

Fig. 1.6. PANEL A. Effects of different concentrations of L-arginine (L-Arg), the NO• scavenger carboxy-PTIO (PTIO) and D-arginine on PMN adherence to coronary endothelium. Coincubation with PAF is indicated by the bracket at the bottom. Results are presented as numbers of PMN per mm^2 of endothelium. Bar heights represent the mean ± s.e.m. *P<0.05 versus other groups without asterisks. P<0.05 versus all other groups. PAF concentration—100 nMol. Numbers in the bars represent numbers of coronary rings studied. PANEL B. Effects of unactivated and PAF-activated PMN, and L-NA on endothelium-dependent relaxation of coronary artery rings. There is no difference between the control (CTRL) rings not incubated with PMN and coincubation with unactivated PMN groups. PAF activation of PMN caused a rightward shift in the dose-response curve. L-NA + L-Arg showed decreased responses to acetylcholine. *P< 0.05 versus other groups. PANEL C. Effects of 10 mM L-arginine (L-Arg) and 10 mM D-Arginine (D-Arg) on PMN-mediated alterations in endothelium-dependent relaxation of coronary artery rings. Responses to acetylcholine were significantly greater in the L-Arg-treated rings compared with D-Arg and PMN + PAF rings. *P<0.05 versus other groups. P<0.05 PMN + PAF versus L-Arg and CTRL groups. D-Arg versus CTRL group. n = number of rings used. Adapted from Sato et al, Cardiovasc Res 31; 1996:63-72.

L-arginine. Therefore, this limitation in the endogenous capabilities of the heart may be overcome by parenteral administration of agents that donate NO• in vivo. This exogenous approach was first reported by Johnson et al using authentic nitric oxide gas (approximately 10-20 nM in vivo concentration)[94] and $NaNO_2$ as an NO• donor chemical[95] at subvasodilator concentrations, *administered only at the onset of reperfusion* in a feline model of regional (90 minutes LAD occlusion followed by 4.5 hours of reperfusion) ischemia-reperfusion. Both forms of NO• therapy decreased infarct size by about 75%, which was associated with decreased neutrophil accumulation in the area at risk (both non-necrotic and necrotic). These data suggest that NO• reduced infarct size by inhibiting neutrophil-mediated damage. Subsequent studies have been performed using a variety of organic NO• donor agents in nonsurgical models of coronary occlusion and reperfusion. These organic donors release NO• either spontaneously or after bioconversion reactions. Nitroglycerin is the prototype NO• donor agent, but is a poor NO• donor due to its prerequisite

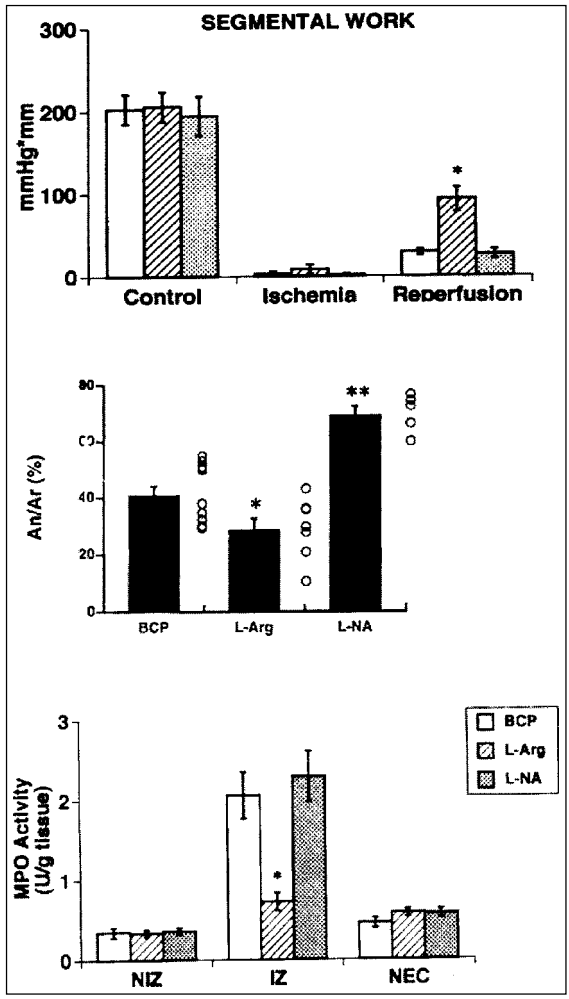

Fig. 1.7. Beneficial effects of blood cardioplegia supplemented with L-arginine. Top panel. Segmental work of the ischemic-reperfused myocardium derived by integrating the area of the pressure-segment length loop during control, after 40 minutes of LAD occlusion and during reperfusion after cardioplegia arrest. Open bar = unsupplemented blood cardioplegia; hatched bar = L-arginine supplemented blood cardioplegia; stippled bar = treatment with NOS inhibitor L-NA before cardioplegia. Middle panel. Infarct size as area of necrosis relative to the area at risk. Infarct size was reduced by approximately 30% in blood cardioplegia supplemented with L-arginine, and this infarct size reduction was reversed by preceding L-arginine with the NOS inhibitor L-nitro arginine (L-NA). Lower panel. Neutrophil accumulation in the nonischemic zone (NIZ), ischemic non-necrotic zone (IZ), and necrotic zone (NEZ) of the area at risk, assayed by the neutrophil-specific-enzyme myeloperoxidase in the myocardium. Group legend same for top panel. Adapted from Sato et al. J Thoracic Cardiovasc Surg 1995; 110: 302-314.[83]

bioconversion by a cysteine-containing enzyme that is partially depleted in the microvasculature after ischemia-reperfusion, and tolerance develops over time. One such cysteine-containing compound readily releases NO• after biotransformation, SPM-5185 (N-(3-hydroxy-pivaloyl)-S-(N'-acetylalanoyl)-L-cysteine ethyl ester (Schwarz Pharma AG, Monheim, Germany).[41,96] This NO•-donor agent, when administered during reperfusion after 1 hour LAD occlusion reduced infarct size from 41.7 ± 5.4% to 12.5 ± 3.2% of the area at risk, in association with a 58% reduction in transmural neutrophil accumulation (tissue myeloperoxidase activity) in the area at risk.[96] The nonactive form of the compound had no effects on any physiological variable.

In a study by Nakanishi et al[40] using a canine model of cardiopulmonary bypass and cardioplegia, hearts were subjected to 30 minutes of normothermic ischemia followed by one hour cardioplegia (4°C multidose blood cardioplegia, 4:1 blood: crystalloid, Table 1.2). Hearts received either standard blood cardioplegia (BCP) or blood cardioplegia with 10 μmol/L SPM-5185 (BCP + SPM). After one hour of cardioplegic arrest, the heart was reperfused for a total of 60 minutes, first in the beating empty state for 30 minutes and then after discontinuation of bypass for 30

minutes. Left ventricular function was assessed by the slope of the end-systolic pressure-volume (impedance catheter) relation. Postischemic end-systolic pressure-volume relation was depressed by 53.7% of preischemic values in the BCP group (from 8.2 ± 1.0 to 3.8 ± 0.3 mm Hg/ml). In contrast, there was nearly complete postischemic (Fig. 1.8) functional recovery in the BCP + SPM group (from 7.6 ± 1.1 to 7.2 ± 1.2 mm Hg/ml). In coronary arteries isolated from these hearts, endothelium-dependent maximal relaxation to acetylcholine was impaired by 27% in the BCP group, but recovered to ≥ 100% in the BCP+SPM group. Myeloperoxidase activity, an index of neutrophil accumulation in postischemic myocardium, was elevated in the BCP group (3.36 ± 0.58 U/100 mg tissue) but was significantly reduced in the BCP + SPM group to 1.27 ± 0.45 U/100 mg tissue. Therefore, this study demonstrated that addition of 10 μmol/L nitric oxide donor SPM-5185 in blood cardioplegia improves postischemic ventricular performance and postcardioplegia endothelial function in ischemically injured hearts, possibly via inhibition of neutrophil-mediated damage.

Peroxynitrite: The Potential Dark Side of Nitric Oxide Therapy

The biradical quenching reaction between $NO^{\bullet}$ and $^{\bullet}O^{-}_{2}$ may be a double edged sword. On the one hand, $NO^{\bullet}$ neutralizes a potentially deleterious species of oxygen radical. On the other hand, the reaction produces a potentially deleterious metabolite-peroxynitrite ($ONOO^{-}$) with subsequent degradation in the presence of transition metals to the highly biologically active hydroxyl radical. This reaction of $NO^{\bullet}$ and $^{\bullet}O^{-}_{2}$ in a 1:1 stoichiometry to form peroxynitrite progresses at essentially diffusion-limited rates ($6.7 \times 10^{9}\ M^{-1}\ sec^{-1}$), a rate which exceeds that between $^{\bullet}O^{-}_{2}$ and superoxide dismutase by a factor of three ($2 \times 10^{9}\ M^{-1}\ sec^{-1}$).[52] In addition, this reaction is exothermic with a release of 22 kcal/mol and is, therefore, essentially irreversible. As seen in equation 1, the amount of $ONOO^{-}$ produced through this reaction can be increased by increasing either $NO^{\bullet}$ or $^{\bullet}O^{-}_{2}$; the $^{\bullet}O^{-}_{2}$ specie is generated in larger quantities during the early moments of reperfusion, with lower but still significant quantities released on a persistent basis during the later phases of reperfusion. Under physiological concentrations of $NO^{\bullet}$ (~ 5-10 nM) and $^{\bullet}O^{-}_{2}$, endogenous and exogenous superoxide dismutase are effective scavengers. However, in micromolar concentrations as may be reached in vitro, $NO^{\bullet}$ effectively competes with superoxide dismutase to form peroxynitrite.

Equation 1:

$$NO^{\bullet} + {}^{\bullet}O^{-}_{2} \rightarrow ONOO^{-}$$
$$ONOO^{-} + H^{+} \rightarrow ONOOH$$
$$ONOOH \xrightarrow{(SOD,\ MPO)} {}^{\bullet}OH + NO_{2}^{+}$$

According to equation 1, the peroxynitrous acid (ONOOH) intermediate of $ONOO^{-}$ undergoes a heterolytic cleavage in the presence of transition metals (i.e., superoxide dismutase, myeloperoxidase) to form a hydroxyl anion ($^{\bullet}OH$) plus a nitronium ion (NO_{2}^{+}).[52] Therefore, in addition to reactive peroxynitrite, the potential for cellular or tissue damage exists by the potent oxidant actions of peroxynitrous acid (oxidation of iron and zinc containing molecular center) and the hydroxyl radical (lipid peroxidation, membrane damage).[52,53] Peroxynitrite and peroxynitrous acid may be major sources of cytotoxic hydroxyl radicals.[53] Accordingly, peroxynitrite has been directly or indirectly (through the hydroxyl radial) associated with attenuation of mitochondrial respiration,[97] cellular and tissue injury secondary to hemorrhagic shock, endotoxemia,[98] in normal[99] and postischemic tissue ischemia-reperfusion[100,101] and atherosclerosis. Peroxynitrite is bactericidal, nitrosylates sulfhydryl moieties on proteins, and causes protein cleavage and apoptosis. These latter events occur at micromolar concentrations.

On the other hand, numerous studies have reported physiological and cardioprotective effects of $ONOO^{-}$ similar to $NO^{\bullet}$. It has been reported that $ONOO^{-}$ attenuates platelet

Table 1.2. Composition of blood cardioplegia

Constituent	Value
Potassium ion (KCl) (μmol/L)	20-25 (induction, terminal infusions), 10 (intermittent infusions)
Calcium ion (CPD) (μmol/L)	0.3
Magnesium ion (derived from blood) (μmol/L)	0.5
pH (THAM buffer)	8.2 (at 4°C)
Osmolality (mOsm/L)	360-380
Hematocrit (%)	14-18 (or more)
Oxygen content (Ml O_2/100 ml)	8-10

CPD, Citrate-phosphate-dextrose (blood); THAM, tris(hydroxymethyl)-aminomethane

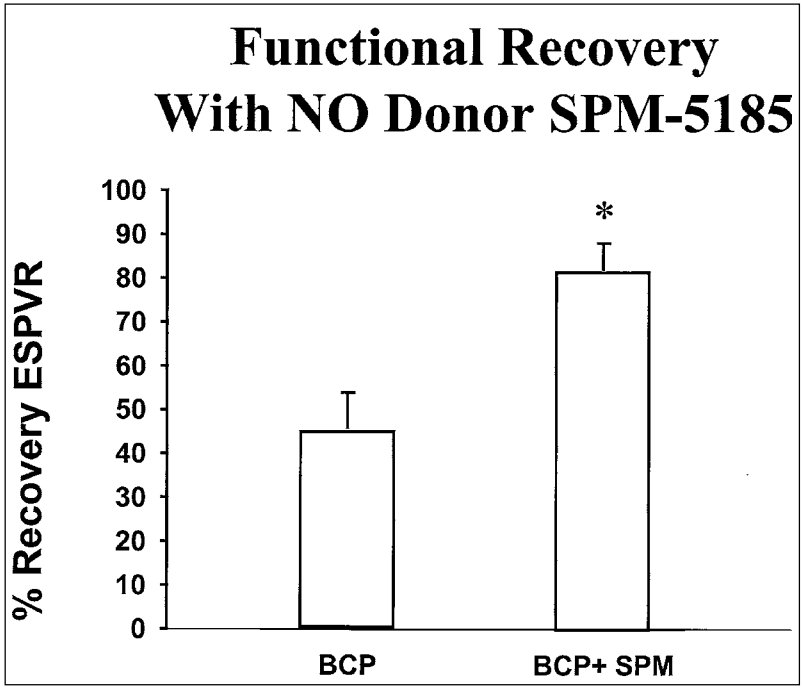

Fig. 1.8. Postcardioplegia recovery of end-systolic pressure-volume slope as a percent of preischemic values. There was a significant depression of left ventricular performance with unsupplemented blood cardioplegia (BCP) which was significantly improved with the addition of 10 μMol SPM-5185. *$p < 0.05$ vs BCP

aggregation, induces vasorelaxation, inhibits adherence of neutrophils to stimulated coronary artery[102] or mesenteric artery[103] endothelium, preserves postischemic vasorelaxation responses to acetylcholine (an agonist of endothelial NO• release), and reduces myocardial infarct size.[102] The reduction in neutrophil adherence by low micromolar concentrations of $ONOO^-$ is associated with an attenuation of p-selectin surface expression on endothelium[103] similar to the physiological effects of NO•. In the study by Nossuli et al[102] using a feline model of regional ischemia and reperfusion, an approximately 60% reduction in infarct size was associated with significantly less endothelial dysfunction (basal and stimulated release of NO• in bioassays) relative to a control group.

The resolution to this apparent schizophrenic effect of $ONOO^-$ where it is cast as a mediator of damage while in other conditions it exerts cardioprotective and vasculoprotective effects may be multifactoral, but may depend on 1) the concentrations of precursors NO• and $•O^-_2$ achieved under normal or pathophysiological conditions and, hence, the physiological concentrations of $ONOO^-$ achieved, 2) the environment in which $ONOO^-$ coexists with other cell types and tissues, and the presence or absence of endogenous detoxification mechanisms Figure 1.9. The balance between the formation and breakdown (degradation and detoxification) of $ONOO^-$ determines, in part, the physiological consequences of $ONOO^-$, particularly in pathological conditions.

Concentration of $ONOO^-$

The generation of $ONOO^-$ is favored when both NO• and $•O^-_2$ are formed at high concentrations; this condition may exist during the early moments of reperfusion with the reintroduction of molecular oxygen since the generation of both radical species requires molecular oxygen. Although there is substantial copper-zinc SOD present in the myocardium, potential oxygen radical scavengers are depleted during ischemia-reperfusion which increases the physiological concentrations of $•O^-_2$, and the rapid diffusion-limited reaction rate between NO• and $•O^-_2$ potentially outcompetes endogenous SOD for $•O^-_2$. Therefore, the presence of both NO• and $•O^-_2$ is favored under ischemia-reperfusion conditions. The tissue concentration of $ONOO^-$ may be in the nanomolar range in normal as well as pathological states, and that micromolar concentrations are not likely to be achieved. However, studies in which $ONOO^-$ has been shown to be deleterious have used micromolar to low millimolar concentrations of the anion (or its precursors), while studies in which nanomolar concentrations of $ONOO^-$ have been used report a protective effect.

Physiological Environment

In addition to the rate of formation of $ONOO^-$, degradation through breakdown reactions (Equation 1) and detoxification (Fig. 1.9) have a critical impact on the tissue levels of $ONOO^-$ and its biological actions. However, $ONOO^-$ is a relatively stable anion, particularly at alkaline pH, and this stability is sufficient to allow transcellular diffusion and to engage a biological target such as membrane lipids, DNA, and tyrosine moieties on proteins with which $ONOO^-$ can react in a destructive manner.

The cellular detoxification mechanisms that may drain $ONOO^-$ and prevent tissue accumulation of the anion include 1) plasma bicarbonate buffering system and 2) thiol containing molecules, including albumin and glutathione. NO• has been shown to S-nitrosylate plasma albumin[104] which, in addition to increasing its half-life, also serves as an NO• drain mechanism. Glutathione is present in cardiac tissue and is considered an endogenous antioxidant. $ONOO^-$ can nitrosylate reduced glutathione and other thiol compounds to form S-nitrosyl compounds which in turn can release NO•. The glutathione detoxification mechanism may be obtunded by oxidant-mediated or pathologically-induced endothelial injury[101] such as in hypercholesterolemia.[105] In addition, $ONOO^-$ may stimulate guanylate cyclase directly or through the formation of NO•. Therefore, $ONOO^-$ can serve as an alternative NO• donor and as a stimulator of

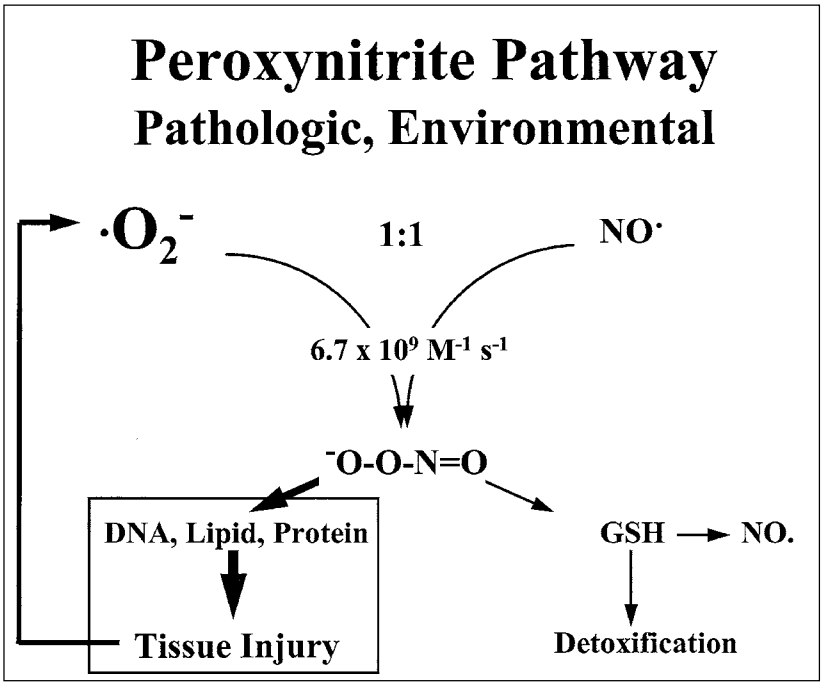

Fig. 1.9. The biradical reaction between nitric oxide NO• and superoxide radical •O^-_2 is nearly diffusion limited and mainly irreversible, and requires equimolar concentration of the two substrates. The end product, peroxynitrite ($ONOO^-$) can be detoxified in blood by nitrosylating thiol compounds such as glutathione (GSH) to form nitrosothiole, which may recycle to NO•. However, the lack of detoxifying reactions in crystalloid media allows build-up of $ONOO^-$ with protein nitrosylation, DNA damage and formation of the highly reactive hydroxyl radical. This pathway to injury may amplify the inflammatory response and •O^-_2 generation, thereby providing more substrate for $ONOO^-$ formation. (Adapted with permission from Xin-Liang Ma, M.D., Ph.D.)

guanylate cyclase activity and have the potential to be cardioprotective.

Crystalloid buffer systems (i.e., Langendorff isolated buffer perfused tissues, cell culture) do not contain endogenous compounds that may detoxify $ONOO^-$, while plasma and tissue contain these potentially detoxifying agents. In addition, buffer systems do not contain neutrophils as targets for NO• antiaggregatory and antiadhesion actions. Studies in which $ONOO^-$ has been shown to exert a deleterious effect were performed in cell-free buffer perfused models[99,101] while the studies which demonstrated a beneficial effect of authentic $ONOO^-$ were performed in in vivo models in which plasma and tissue borne detoxifying substances were present or buffer-perfused system contained neutrophils.[102,103] Therefore, $ONOO^-$ in *acellular* environments would not be detoxified and may therefore produce tissue injury, ostensibly either by direct or •OH related mechanisms, while in biological environments, $ONOO^-$ would not exert deleterious effects and may be protective through nitrosylation of thiols (such as glutathione) with subsequent NO• donation. This conclusion is supported by results presented by Ma et al[106] in which isolated hearts subjected to an ONOO- donor agent (SIN-1) in a blood environment were protected while those in a crystalloid environment were injured. This environmental effect may be important in cardiac surgery in which there are both crystalloid and blood formulations of cardioplegia solutions, with strong proponents for each. Currently there is great interest in the

use of NO• donors or precursors (L-arginine) as supplements to cardioplegia solutions. Whether a similar dichotomy in the physiological effects of NO• will be observed in this area of surgical myocardial protection has not yet been reported. Indeed, the deleterious effects of L-arginine administered during reperfusion reported by Engleman et al[91] for isolated crystalloid buffer perfused hearts may be related to this environment effect of NO• if indeed L-arginine supplementation of the crystalloid buffer released enough NO• to engage this NO•-ONOO-mechanism.

The question of whether $ONOO^-$ mediates differential effects depending on the blood or crystalloid environment was investigated by Ronson et al[107] in a canine model of myocardial protection with blood or crystalloid-cardioplegia solutions. Hearts were subjected to 30 minutes of normothermic global ischemia on cardiopulmonary bypass, followed by 60 minutes of hypothermic hyperkalemic intermittent cardioplegia using either crystalloid (Plegisol) or blood cardioplegia (8:1 blood:crystalloid), each with or without 5 μmol/L authentic ONOO-, using the Myocardial Protection Systems (MPS, Quest Medical, Inc.) delivery system to ensure accurate concentrations of $ONOO^-$. After 2 hours of reperfusion, systolic and diastolic function were determined using left ventricular pressure-volume (impedance catheter) relations analysis (Fig. 1.10A). In the crystalloid cardioplegia groups, the addition of $ONOO^-$ was associated with a 57% reduction (versus baseline) of systolic function compared to a 44 ± 2% reduction in the crystalloid cardioplegia group without $ONOO^-$ ($p < 0.05$). In contrast, postischemic left ventricular function was generally better than crystalloid cardioplegia with both formulations of blood cardioplegia, and there was significantly better recovery of systolic performance with BCP plus ONOO- compared to BCP without ONOO-. Myocardial edema (% tissue water) was significantly increased by $ONOO^-$ in crystalloid (81.1 ± 0.3 vs 79.6 ± 0.4%) and blood cardioplegia (78.9 ± 0.3% vs 76.4 ± 0.32%) in agreement with the chamber stiffness data. After the experiment, the left anterior descending and left circumflex epicardial coronary arteries were excised and placed in organ chambers to determine endothelial responses to agonist stimulators of nitric oxide synthase. Coronary arteries from postcardioplegic myocardium demonstrated marked differences with regard to both formulations and presence of $ONOO^-$. Generally, blood cardioplegia demonstrated better preservation of maximal endothelial responses to acetylcholine (receptor dependent) than crystalloid cardioplegia. The addition of $ONOO^-$ to blood cardioplegia demonstrated significantly greater responses (better function), while $ONOO^-$ in the crystalloid cardioplegia group was associated with significantly poorer function (Fig. 1.10B). Therefore, $ONOO^-$ in a crystalloid medium may indeed be deleterious, while it is beneficial in a blood medium with regards to postcardioplegia systolic function and coronary artery endothelial function.

Further investigation is necessary to determine the role of NO• and its metabolite $ONOO^-$ under different conditions. However, the majority of studies in *normal or ischemic-reperfused hearts* in which cardioplegia has been supplemented with NO• donors or the precursor L-arginine have reported net cardioprotective effects of NO•.[42,43,108,109]

Nitric Oxide in Hypoxia-Reoxygenation Injury

Cyanotic heart defects are often corrected surgically using cardiopulmonary bypass and elective chemical cardioplegia. Despite the well-known tolerance of the immature heart to ischemia and reperfusion[110-112] the immature myocardium is seemingly intolerant of hypoxia-reoxygenation using high blood oxygen tensions (≥ 400 mmHg).[113-115] Key characteristics in hypoxia-reoxygenation, with the latter being accomplished on cardiopulmonary bypass, are 1) increased production of NO• during the period of reoxygenation,[116,117] 2) a loss if the endogenous antioxidant reserve coupled with an increase in the production of oxygen-derived free radicals which is amenable to antioxidant therapy,[118] 3) increased production of oxygen-derived free radicals during reoxygenation, and 4) a reduction in the

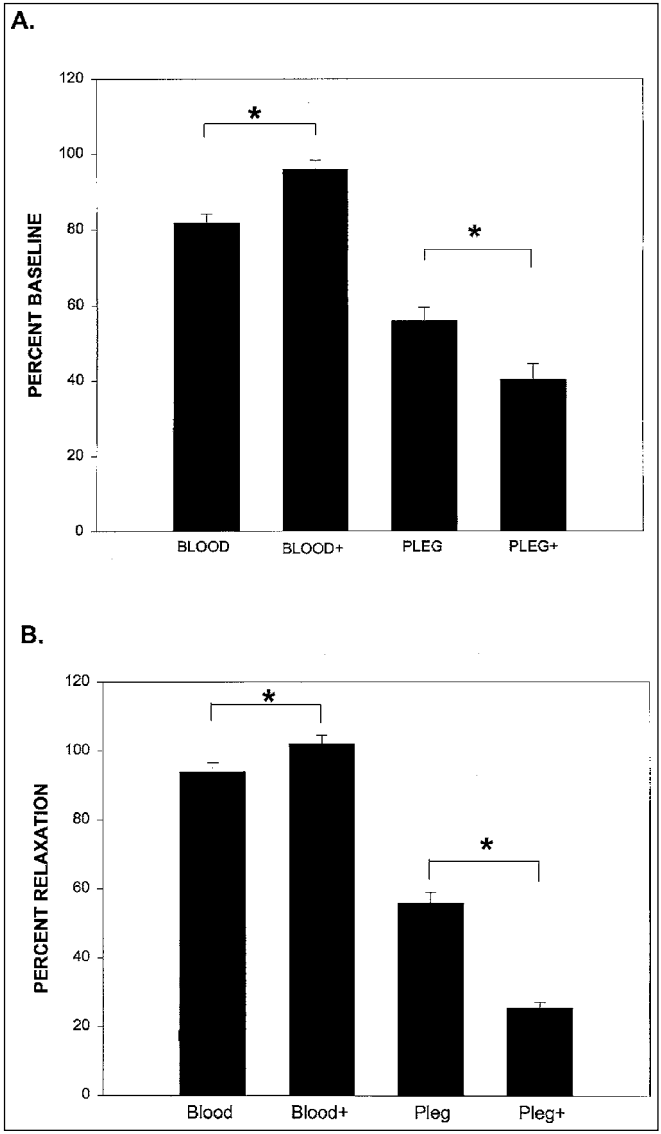

Fig. 1.10. Physiological effects of $ONOO^-$ in blood cardioplegia versus crystalloid cardioplegia (Plegisol) environments. Panel A. Recovery of end-systolic pressure-volume (impedance catheter) relations after 2 hours of reperfusion as a percent of baseline value of slope of the relationship. Panel B. Responses of post-cardioplegia epicardial coronary arteries to the endothelial-dependent, receptor- dependent stimulator of nitric oxide synthase, acetylcholine at 500 nM organ chamber concentration. Responses are maximal percent relaxation from preconstricted (U46619) values. In the BLOOD+ group, there was a significant left shift in the concentration-response curve compared to BLOOD group indicative of better function, while in the PLEG+ group there was a significant right shift relative to PLEG group indicative of receptor dysfunction. PLEG = Plegisol crystalloid cardioplegia without ONOO-; PLEG+ = Plegisol crystalloid cardioplegia with 5μM ONOO-; BLOOD = blood cardioplegia (8:1 blood: crystalloid) without $ONOO^-$; BLOOD+ = blood cardioplegia without 5 μM ONOO-. * = $p<0.05$ between indicated groups.

production of oxygen-derived free radicals and $NO^\bullet$ by low or hypoxic reoxygenation.[119] Seemingly paradoxical also is the effect of $NO^\bullet$ in the model of hypoxia-reoxygenation injury. In contrast to the cardioprotection reported for L-arginine or $NO^\bullet$-donor supplemented therapy in ischemia-reperfusion models as discussed above, the same therapy in hypoxia-reoxygenation models has deleterious effects.[116,120] and inhibitors of NOS had beneficial effects.[116,120] In this case of hypoxia-reoxygenation, ONOO—may be playing a major role in directing the effects of $NO^\bullet$-related therapy, even in the presence of blood-borne detoxification mechanisms. The combination of increased production of both $NO^\bullet$ and $^\bullet O^-_2$ during reoxygenation and the presence of reduced antioxidant reserve in the heart may have permitted the unbridled formation of ONOO- with subsequent production

of $^{\bullet}OH$, and hence, the deleterious effects. However, the hypothesis that overproduction of peroxynitrite in the hypoxic-reoxygenation injury model is responsible or contributory to the deleterious effects of $NO^{\bullet}$ has not been tested. In any event, the mechanisms of $NO^{\bullet}$ and related therapy are clearly different and independent of issues of different species, methods and laboratories since this same group showed that in an ischemic-reperfusion model (20 minutes antecedent normothermic ischemia before cardioplegia) using the same animal model, that L-arginine augmented $NO^{\bullet}$ production and was cardioprotective, and this effect was reversed by NOS inhibitors.[121]

Nitric Oxide and Myocardial Apoptosis

As summarized above, $NO^{\bullet}$ has important and potent modulatory effects on the inflammatory process related to postischemic injury and necrosis. Apoptosis, or genetically programmed cell death, as opposed to the explosive and inflammatory-initiating process of cellular necrosis,[122] is known to occur in the heart during failure,[123] infarction[124] and ischemia-reperfusion of the myocardium[125-127] due to a change in the expression of specific gene products and production of specific cytoplasmic proteins.[128-131] Some studies[127,132,133] have shown that ischemia with reperfusion, but not ischemia alone, induces apoptosis of the myocardium; hypoxia is also a stimulus for apoptosis.[134]

Apoptosis has been inhibited by a number of interventions including preconditioning.[135,136] In contrast, cardioprotective autacoids other than $NO^{\bullet}$ have been associated with induction of apoptosis.[137,138] However, the role nitric oxide plays in the pathophysiological development of apoptosis is not clear. Lopez-Farre et al[139] have demonstrated a link between nitric oxide and endothelial cell apoptosis. In cultured endothelial cells, inhibition of $NO^{\bullet}$ synthesis by L-NAME significantly increased endothelial cell apoptosis without altering any other environmental condition. Furthermore, the increased expression of apoptosis correlated with increased levels of c-myc and c-fos, gene products associated with induction of mitogenesis and apoptosis.[140] Other evidence of a protective role for $NO^{\bullet}$ against apoptotic cell death links the increase in $NO^{\bullet}$ production by shear stress to decreases in endothelial cell apoptosis. After exposure of the endothelium to TNF-α, a known stimulator of apoptosis,[141] shear stress almost completely abrogated the appearance of apoptosis compared to cells not subjected to shear forces. This effect on reducing apoptosis was mimicked by $NO^{\bullet}$ donors through an inhibition of ICE-like and cysteine proteases.[142] The potential increase in both endothelial and myocardial apoptosis in vivo secondary to decreases in endothelial nitric oxide production and release with endothelial cell dysfunction following ischemia and reperfusion is not known and needs to be further evaluated.

Summary

Nitric oxide, the molecule of the decade, is involved in a host of physiological effects, particularly in the modulation of neutrophils and endothelial cells—both individually and their interactions—during the complex processes instigated by ischemia and reperfusion. The endothelium is an effective target for augmenting endogenous $NO^{\bullet}$ because 1) it is the vascular interface between neutrophils, cytokines and other blood-borne proinflammatory mediators and the underlying tissue (myocytes, vascular smooth muscle, conductile tissue, architectural structures) that are themselves targets of injury, 2) the endothelium is the largest organ in the body, with an extensive surface area and distribution in organs, 3) it is responsive to a number of physiological stimuli such as shear stress. $NO^{\bullet}$ plays a key role in the attenuation of ischemic-reperfusion injury at numerous levels of the process (Fig. 1.11), including direct inhibition of the neutrophil (superoxide anion generation, adhesion molecule expression, adherence to endothelium), direct neutralization of superoxide radicals—a primary mediator of injury, inhibition of adhesion molecule expression on the surface of the

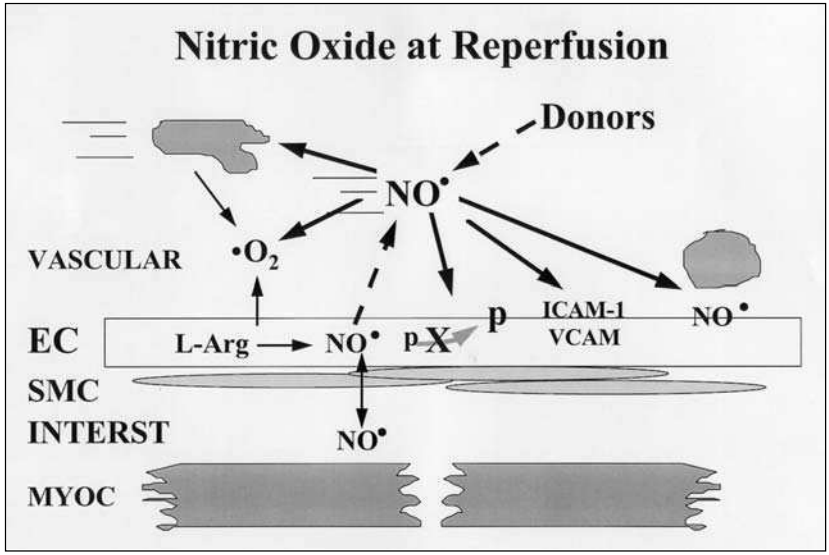

Fig. 1.11. The cardioprotective mechanisms of NO• related to inhibition of neutrophil and endothelial cell interactions during reperfusion. NO• inhibits surface expression of p-selectin (p) and intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule (VCAM). NO• also directly inhibits neutrophils and superoxide ($O^{\bullet}_2$) generation in addition to neutralizing $O^{\bullet}_2$ via direct quenching. NO• can be augmented by providing precursor L-arginine (L-arg) or administering direct NO• donor agents.

endothelium, and inhibition of synthesis of adhesion molecules at the level of nuclear transcription (NF-κB). Many studies support the observation that NO• exerts its cardioprotection primarily during the reperfusion phase, which would thereby guide the time course of its administration. Therapeutic strategies applicable to ischemic-reperfusion injury include provision of the precursor to NO•- L-arginine, which augments the endogenous generation of NO• for availability in the compartments (intravascular compartment, interstitial compartment) most involved in the acute and initiating steps in the inflammatory component of ischemic-reperfusion injury. However, a ceiling in the effectiveness of this approach may be encountered if the endothelium is dysfunctional and impaired in its ability to synthesize and release NO•. In this event, a wide variety of NO•-donor agents are available which may be administered. A word of caution must be given in that there may be a definite therapeutic window beyond which tissue damage may be caused by NO• itself or a metabolite such as peroxynitrite and hydroxyl radical, and hemodynamic (vasodilator) consequences may be precipitated that are unwanted. Although nature has evolved this pleuripotent mechanism of endogenous defense against inflammatory-like conditions, the unraveling of mechanisms and harnessing of this therapeutic potential will occupy scientific and drug development investigations for a long time to come.

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References

1. Vinten-Johansen J, Hammon JW, Jr. Cardiopulmonary Bypass: Principles and Practice. In: Utley, Gravlee, eds. New York: Williams & Wilkins 1993:155-206.
2. Vinten-Johansen J, Nakanishi K. Post-cardioplegia acute cardiac dysfunction and reperfusion injury. [Review]. J Cardiothorac Vasc Anesth 1993; 7:6-18.
3. Robicsek F, Schaper J. Reperfusion injury: Fact or myth? J Card Surg 1997; 12:133-7.
4. Vinten-Johansen J. Reperfusion injury: Idle curiosity or therapeutic vector? J Thrombosis Thrombolysis 1997; 4:59-61.
5. Vinten-Johansen J, Zhao Z-Q. Adenosine: Cardioprotection and Clinical Application. In: Mentzer, Kitakaze, Downey, Hori, eds. Kluwer Academic Publishers 1997:49-70.
6. Chenoweth DE. Pathophysiology and techniques of cardiopulmonary bypass. In: Utley, Betleski, eds. 2 ed. Baltimore: Williams & Wilkins 1983: 49-60.
7. Kubes P, Suzuki M, Granger N. Nitric oxide: An endogenous modulator of leukocyte adhesion. Proc Natl Acad Sci 1991; 88: 4651-5.
8. Hammon JW, Jr., Vinten-Johansen J. Augmentation of microvascular nitric oxide improves myocardial performance following global ischemia. J Card Surg 1995; 10:423-7.
9. Lefer AM, Lefer DJ. Therapeutic role of nitric oxide donors in the treatment of cardiovascular disease. Drugs of the Future 1994; 19:665-72.
10. Lefer DJ. Myocardial protective actions of nitric oxide donors after myocardial ischemia and reperfusion. [Review]. New Horiz 1995; 3:105-12.
11. Stewart JM, Wang J, Hintze TH. Role of EDRF in the regulation of shear rate in large coronary arteries in conscious dogs. J Mol Cell Cardiol 1994; 26(12):1625-33.
12. Smith TP, Jr., Canty JM, Jr. Modulation of coronary autoregulatory responses by nitric oxide. Evidence for flow-dependent resistance adjustments in conscious dogs. Circ Res 1993; 73:232-40.
13. Quyyumi AA, Dakak N, Andrews NP et al. Endothelium-derived factors, Vasodilation. Contribution of nitric oxide to metabolic coronary vasodilation in the human heart. Circulation 1995; 92:320-6.
14. Rubanyi GM. The role of endothelium in cardiovascular homeostasis and diseases. J Cardiovasc Pharmacol 1993; 23(Suppl 4): S1-S14.
15. Gryglewski RJ, Chlopicki S, Niezabitowski P. Coronary flow, Endothelium, Prostacyclin, Nitric oxide, Adenosine. Endothelial control of coronary flow in perfused guinea pig heart. Bas Res Cardiol 1995; 90:119-24.
16. Yamabe H, Okumura K, Ishizaka H et al. Role of endothelium-derived nitric oxide in myocardial reactive hyperemia. Am J Physiol 1992; 263(1 Pt 2):H8-14.
17. Lefer AM, Tsao PS, Ma X-L. Shock- and ischemia-induced mechanisms of impairment of endothelium-mediated vasodilation. [Review]. Chest 1991; 100:160S-3S.
18. Lefer AM, Lefer DJ. Pharmacology of the endothelium in ischemia-reperfusion and circulatory shock. Annual Review Pharmacology and Toxicology 1993; 33:71-90.
19. Harrison DG, Kurz MA, Quillen JE et al. Normal and pathophysiologic considerations of endothelial regulation of vascular tone and their relevance to nitrate therapy. [Review]. Am J Cardiol 1992; 70:11B-7B.
20. Vanhoutte PM. Hypercholesterolaemia, atherosclerosis and release of endothelium-derived relaxing factor by aggregating platelets. Eur Heart J 1991; 12:25-32.
21. Harrison DG. Endothelial dysfunction in atherosclerosis. [Review]. Bas Res Cardiol 1994; 89 Suppl 1:87-102.
22. Lüscher TF, Yang Z, Diederich D et al. Endothelium-derived vasoactive substances: Potential role in hypertension, atherosclerosis, and vascular occlusion. J Cardiovasc Pharmacol 1989; 13\4:S63-S69
23. Cooke JP, Andon NA, Girerd XJ et al. Arginine restores cholinergic relaxation of hypercholesterolemic rabbit thoracic aorta. Circulation 1991; 83:1057-62.
24. Morita K, Ihnken K, Buckberg GD et al. Pulmonary vasoconstriction due to impaired nitric oxide production after cardiopulmonary bypass. Ann Thorac Surg 1996; 61:1775-80.
25. Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 1980; 288.
26. Schrader J, Kostic MM, Borst M. Role of nitric oxide, adenosine and ATP during reactive hyperemia. [Abstract] Biology of Nitric Oxide 1991; 125.
27. Tsunoda R, Okumura K, Ishizaka H et al. Enhancement of myocardial reactive hyperemia with manganese- superoxide dismutase: Role of endothelium-derived nitric oxide. Cardiovasc Res 1996; 31(4):537-45.
28. Depre C, Vanoverschelde JL, Goudemant JF et al. Protection against ischemic injury by nonvasoactive concentrations of nitric oxide synthase inhibitors in the perfused rabbit heart. Circulation 1995; 92:1911-8.
29. Hattler BG, Gorcsan IJ, Shah N et al. A potential role for nitric oxide in myocardial stunning. J Card Surg 1994; 9:425-9.

30. Shen W, Xu X, Ochoa M et al. Role of nitric oxide in the regulation of oxygen consumption in conscious dogs. Circ Res 1994; 75:1086-95.
31. Ma X-L, Lopez BL, Christopher TA et al. Exogenous NO inhibits basal NO release from vascular endothelium in vitro and in vivo. Am J Physiol 1996; 271:H2045-H2051
32. Finkel MS, Oddis CV, Jacob TD et al. Negative inotropic effects of cytokines on the heart mediated by nitric oxide. Science 1992; 257:287-9.
33. Kawamura T, Wakusawa R, Okada K et al. Elevation of cytokines during open heart surgery with cardiopulmonary bypass: Participation of interleukin 8 and 6 in reperfusion injury [see comments]. Can J Anaesth 1993; 40:1016-21.
34. Steinberg JB, Kapelanski DP, Olson JD et al. Cytokine and complement levels in patients undergoing cardiopulmonary bypass. J Thorac Cardiovasc Surg 1993; 106:1008-16.
35. Davies SW, Bailey J, Wedzicha JA et al. Complement activation and lung permeability during cardiopulmonary bypass. Ann Thorac Surg 1991; 51:693-7.
36. Kirklin JK, Westaby S, Blackstone EH et al. Complement and the damaging effects of cardiopulmonary bypass. J Thorac Cardiovasc Surg 1983; 86:845-57.
37. Weyrich AS, Ma X-L, Buerke M et al. Physiological concentrations of nitric oxide do not elicit an acute negative inotropic effect in unstimulated cardiac muscle. Circ Res 1994; 75:692-700.
38. Hasebe N, Shen Y-T, Vatner SF. Inhibition of endothelium-derived relaxing factor enhances myocardial stunning in conscious dogs. Circulation 1993; 88:2862-71.
39. Kelm M, Schrader J. Control of coronary vascular tone by nitric oxide. Circ Res 1990; 66:1561-75.
40. Nakanishi K, Zhao Z-Q, Vinten-Johansen J et al. Blood cardioplegia enhanced with nitric oxide donor SPM-5185 counteracts postischemic endothelial and ventricular dysfunction. J Thorac Cardiovasc Surg 1995; 109: 1146-54.
41. Lefer DJ, Nakanishi K, Vinten-Johansen J. Endothelial and myocardial cell protection by a cysteine- containing nitric oxide donor after myocardial ischemia and reperfusion. J Cardiovasc Pharmacol 1993; 22 Suppl 7: S34-43.
42. Hiramatsu T, Forbess JM, Miura T et al. Effect of L-Arginine cardioplegia on recovery of neonatal lamb hearts after 2 hours of cold ischemia. Ann Thorac Surg 1995; 60: 1187-92.
43. Sato H, Zhao Z-Q, McGee DS et al. Supplementation of blood cardioplegia with L-arginine avoids regional postischemic injury by NO pathway. [Abstract] Ann Thorac Surg 1995.
44. Hiramatsu T, Forbess JM, Miura T et al. Effects of L-arginine and L-nitro-arginine methyl ester on recovery of neonatal lamb hearts after cold ischemia. Evidence for an important role of endothelial production of nitric oxide. J Thorac Cardiovasc Surg 1995; 109:81-7.
45. Guo J-P, Murohara T, Buerke M et al. Direct measurement of nitric oxide release from vascular endothelial cells. J Appl Physiol 1996; 81:774-9.
46. Ma X-L, Weyrich AS, Lefer DJ et al. Diminished basal nitric oxide release after myocardial ischemia and reperfusion promotes neutrophil adherence to coronary endothelium. Circ Res 1993; 72:403-12.
47. Engelman DT, Watanabe M, Engleman RM et al. Constitutive nitric oxide release is impaired after ischemia and reperfusion. J Thorac Cardiovasc Surg 1995; 110:1047-53.
48. Buga GM, Gold ME, Fukuto JM et al. Shear stress-induced release of nitric oxide from endothelial cells grown on beads. Hypertension 1991; 17:187-93.
49. Cooke JP, Stamler J, Andon N et al. Flow stimulates endothelial cells to release a nitrovasodilator that is potentiated by reduced thiol. Am J Physiol-Heart Circ Physiol 1990; 259:H804-H812.
50. Nerem RM, Harrison DG, Taylor WR et al. Hemodynamics and vascular endothelial biology. [Review]. J Cardiovasc Pharmacol 1993; 21 Suppl 1:S6-10.
51. Weyrich AS, Ma X-L, Lefer AM. The role of L-arginine in ameliorating reperfusion injury after myocardial ischemia in the cat. Circulation 1992; 86:279-88.
52. Beckman JS, Koppenol WH. Nitric oxide, superoxide, and peroxynitrite: The good, the bad, and the ugly. Am J Physiol 1996; 271:C1424-C1437
53. Beckman JS, Beckman TW, Chen J et al. Apparent hydroxyl radical production by peroxynitrite: Implications for endothelial injury from nitric oxide and superoxide. Proc Natl Acad Sci USA 1990; 87:1620-4.
54. Gryglewski RJ, Palmer RMJ, Moncada S. Superoxide anion is involved in the breakdown of endothelium-derived relaxing factor. Nature 1986; 320:454-60.
55. Rubanyi GM, Vanhoutte PM. Superoxide anions and hyperoxia inactivate endothelium-derived relaxing factor. Am J Physiol 1986; 250:H822-H827

56. Rogers NE, Ignarro LJ. Constitutive nitric oxide synthase from cerebellum is reversibly inhibited by nitric oxide formed from L-arginine. Biochem Biophys Res Commun 1992; 189:242-9.
57. Buga GM, Griscavage JM, Rogers NE et al. Negative feedback regulation of endothelial cell function by nitric oxide. Circ Res 1993; 73:808-12.
58. Tsao PS, Aoki N, Lefer DJ et al. Time course of endothelial dysfunction and myocardial injury during myocardial ischemia and reperfusion in the cat. Circulation 1990; 82:1402-12.
59. Tsao PS, Lefer AM. Time course and mechanism of endothelial dysfunction in isolated ischemic- and hypoxic-perfused rat hearts. Am J Physiol 1990; 259:H1660-H1666
60. Dignan RJ, Dyke CM, Abd-Elfattah AS et al. Coronary artery endothelial cell and smooth muscle dysfunction after global myocardial ischemia. Ann Thorac Surg 1992; 53:311-7.
61. Nakanishi K, Zhao Z-Q, Vinten-Johansen J et al. Coronary artery endothelial dysfunction after ischemia, blood cardioplegia, and reperfusion. Ann Thorac Surg 1994; 58: 191-9.
62. VanBenthuysen KM, McMurtry IF, Horwitz LD. Reperfusion after acute coronary occlusion in dogs impairs endothelium-dependent relaxation to acetylcholine and augments contractile reactivity in vitro. J Clin Invest 1987; 79:265-74.
63. Nakanishi K, Vinten-Johansen J, Lefer DJ et al. Intracoronary L-arginine during reperfusion improves endothelial function and reduces infarct size. Am J Physiol 1992; 263:H1650-H1658.
64. Lefer AM, Tsao PS, Lefer DJ et al. Role of endothelial dysfunction in the pathogenesis of reperfusion injury after myocardial ischemia. FASEB J 1991; 5:2029-34.
65. Kaeffer N, Richard V, Francois A et al. Preconditioning prevents chronic reperfusion-induced coronary endothelial dysfunction in rats. Am J Physiol 1996; 271:H842-H849
66. Seccombe JF, Schaff HV. Coronary artery endothelial function after myocardial ischemia and reperfusion. [Review]. Ann Thorac Surg 1995; 60:778-88.
67. Seccombe JF, Pearson PJ, Schaff HV. Oxygen radical-mediated vascular injury selectively inhibits receptor-dependent release of nitric oxide from canine coronary arteries. J Thorac Cardiovasc Surg 1994; 107:505-9.
68. Lefer AM, Ma X-L, Weyrich A et al. Endothelial dysfunction and neutrophil adherence as critical events in the development of reperfusion injury. Agents Actions Suppl. 1993; 41:127-35.
69. Lefer DJ, Nakanishi K, Vinten-Johansen J et al. Cardiac venous endothelial dysfunction after myocardial ischemia and reperfusion in dogs. Am J Physiol 1992; 263:H850-H856
70. Boyle EM, Pohlman TH, Cornejo CJ et al. Endothelial cell injury in cardiovascular surgery: Ischemia-reperfusion. Ann Thorac Surg 1996; 62:1868-75.
71. Moore KL, Patel KD, Bruehl RE et al. P-selectin glycoprotein ligand-1 mediates rolling of human neutrophils on p-selectin. J Cell Biol 1995; 128:661-71.
72. McEver RP, Cummings RD. Role of PSGL-1 Binding to selectins in Leukocyte Recruitment. J Clin Invest 1997; 100:485-92.
73. Davenpeck KL, Gauthier TW, Albertine KH et al. Role of P-selectin in microvascular leukocyte-endothelial interaction in splanchnic ischemia-reperfusion. Am J Physiol 1994; 267:H622-30.
74. Geng J-G, Bevilacqua MP, Moore KL et al. Rapid neutrophil adhesion to activated endothelium mediated by GMP-140. Nat 1990; 343:757-60.
75. Jerome SN, Dore M, Paulson JC et al. P-selectin and ICAM-1-dependent adherence reactions: Role in the genesis of postischemic no-reflow. Am J Physiol 1994; 266: H1316-21.
76. Kubes P, Jutila M, Payne D. Therapeutic potential of inhibiting leukocyte rolling in ischemia/reperfusion. J Clin Invest 1995; 95:2510-9.
77. DeCaterina R, Libby O, Peng H et al. Nitric oxide decreases cytokine-induced endothelial activation. J Clin Invest 1995; 96:60-8.
78. Ohashi Y, Kawashima S, Hirata K et al. Nitric oxide inhibits neutrophil adhesion to cytokine-activated cardiac myocytes. Am J Physiol 1997; 272:H2807-H2814
79. Dreyer WJ, Michael LH, West MW et al. Neutrophil accumulation in ischemic canine myocardium: Insights into time course, distribution, and mechanism of localization during early reperfusion. Circulation 1991; 84:400-11.
80. Entman ML, Michael L, Rossen RD et al. Inflammation in the course of early myocardial ischemia. FASEB J 1991; 5:2529-37.
81. Wilson I, Gillinov AM, Curtis WE et al. Inhibition of neutrophil adherence improves postischemic ventricular performance of the neonatal heart. Circulation 1993; 88:II372-9.
82. Byrne JG, Smith WJ, Murphy MP et al. Complete prevention of myocardial stunning, contracture, low- reflow, and edema after heart transplantation by blocking neutrophil adhesion molecules during reperfusion. J Thorac Cardiovasc Surg 1992; 104:1589-96.

83. Sato H, Zhao Z-Q, McGee DS et al. Supplemental L-arginine during cardioplegic arrest and reperfusion avoids regional postischemic injury. J Thorac Cardiovasc Surg 1995; 110:302-14.
84. Sato H, Zhao Z-Q, Vinten-Johansen J. L-arginine inhibits neutrophil adherence and coronary artery dysfunction. Cardiovasc Res 1996; 31:63-72.
85. Palmer RMJ, Rees DD, Ashton DS et al. L-arginine is the physiological precursor for the formation of nitric oxide in endothelium-dependent relaxation. Biochem Biophys Res Comm 1988; 153:1251-6.
86. Sato H, Zhao Z-Q, Jordan JE et al. Basal nitric oxide expresses endogenous cardioprotection during reperfusion by inhibition of neutrophil-mediated damage after surgical revascularization. [In Press] J Thorac Cardiovasc Surg 1996.
87. Sato H, Zhao Z-Q, Jordan JE et al. Basal nitric oxide expresses endogenous cardioprotection during reperfusion by inhibition of neutrophil-mediated damage after surgical revascularization. J Thorac Cardiovasc Surg 1997; 113:399-409.
88. Williams MW, Taft CS, Ramnauth S et al. Endogenous nitric oxide (NO) protects against ischaemia- reperfusion injury in the rabbit. Cardiovasc Res 1995; 30:79-86.
89. Hudspeth DA, Nakanishi K, Vinten-Johansen J et al. Adenosine in blood cardioplegia prevents postischemic dysfunction in ischemically injured hearts. Ann Thorac Surg 1994; 58:1637-44.
90. Engelman DT, Watanabe M, Maulik N et al. L-arginine reduces endothelial inflammation and myocardial stunning during ischemia reperfusion. Ann Thorac Surg 1995; 60: 1275-81.
91. Engelman DT, Watanabe M, Maulik N et al. Critical timing of nitric oxide supplementation in cardioplegic arrest and reperfusion. Circulation 1996; 94(suppl II):II-407-II-411
92. Hiramatsu T, Forbess JM, Miura T et al. Additive effects of L-arginine infusion and leukocyte depletion on recovery after hypothermic ischemia in neonatal lamb hearts. J Thorac Cardiovasc Surg 1995; 110:172-9.
93. Amrani M, Chester AH, Jayakumar J et al. L-arginine reverses low coronary reflow and enhances postischaemic recovery of cardiac mechanical function. Cardiovasc Res 1995; 30:200-4.
94. Johnson G, III, Tsao PS, Lefer AM. Cardioprotective effects of authentic nitric oxide in myocardial ischemia with reperfusion. Crit Care Med 1991; 19:244-52.
95. Johnson G, III, Tsao PS, Mulloy D et al. Cardioprotective effects of acidified sodium nitrite in myocardial ischemia with reperfusion. J Pharmacol Exp Thera 1990; 252: 35-41.
96. Lefer DJ, Nakanishi K, Johnston WE et al. Antineutrophil and myocardial protection actions of a novel nitric oxide donor after acute myocardial ischemia and reperfusion in dogs. Circulation 1993; 88:2337-50.
97. Xie YW, Wolin MS. Role of nitric oxide and its interaction with superoxide in the suppression of cardiac muscle mitochondrial respiration. Circulation 1996; 94:2580-6.
98. Szabo C, Farago M, Horvath I et al. Hemorrhagic hypotension impairs endothelium-dependent relaxations in the renal artery of the cat. Circulatory Shock 1992; 36(3): 238-41.
99. Lopez BL, Liu G-L, Christopher TA et al. Peroxynitrite, the product of nitric oxide and superoxide, causes myocardial injury in the isolated perfused rat heart. Cor Art Dis 1997; 8:149-53.
100. Schulz R, Wambolt R. Nitric oxide, Myocardial ischemia, Reperfusion, L-name, L-nmma, L-arginine, Stunning, Rabbit heart. Inhibition of nitric oxide synthesis protects the isolated working rabbit heart from ischaemia-reperfusion injury. Cardiovasc Res 1995; 30:432-9.
101. Ma X-L, Lopez BL, Liu G-L et al. Peroxynitrite aggravates myocardial reperfusion injury in the isolated perfused rat heart. Cardiovasc Res 1997.
102. Nossuli TO, Hayward R, Scalia R et al. Peroxynitrite reduces myocardial infarct size and preserves coronary endothelium after ischemia and reperfusion in cats. Circulation 1997; 96:2317-24.
103. Lefer DJ, Scalia R, Campbell B et al. Peroxynitrite inhibits leukocyte-endothelial cell interactions and protects against ischemia-reperfusion injury in rats. J Clin Invest 1997; 99:684-91.
104. Stamler JS, Jaraki O, Osborne J et al. Nitric oxide circulates in mammalian plasma primarily as an S-nitroso adduct of serum albumin. Proc Natl Acad Sci 1992; 89:7674-7.
105. Ma X-L, Lopez BL, Liu G-L et al. Hypercholesterolemia impairs a detoxification mechanism against peroxynitrite and renders the vascular tissue more susceptible to oxidative injury. Circ Res 1997; 80:894-901.
106. Lopez BL, Christopher TA, Ma X-L. The effects of peroxynitrite on the myocardium during ischemia-reperfusion are dependent upon the biological environment. [Abstract] Endothel 1995; 3:76

107. Ronson RS, Thourani VH, Ma X-L et al. The nitric oxide product peroxynitrite is beneficial in blood cardioplegia, but is deleterious in crystalloid cardioplegia in vivo. [In Press] Circulation (Suppl) 1998.
108. Nakanishi K, Zhao Z-Q, Vinten-Johansen J et al. Blood cardioplegia enhanced with an organic nitric oxide donor reverses postischemic ventricular dysfunction and coronary endothelial damage. Surg Forum 1993; 44:245-7.
109. Nakanishi K, Zhao Z-Q, Vinten-Johansen J et al. Blood cardioplegia enhanced with the nitric oxide donor SPM-5185 counteracts postischemic endothelial and ventricular dysfunction. J Thorac Cardiovasc Surg 1995; 109:1146-54.
110. Aoki M, Kawata H, Mayer JE, Jr. Coronary endothelial injury by cold crystalloid cardioplegic solution in neonatal lambs. Circulation 1992; 86:II346-51.
111. Kohman LJ, Veit LJ. Neonatal myocardium resists reperfusion injury. J Surg Res 1991; 51:133-7.
112. Hammon JW, Jr., Boucek RJ, Jr. Myocardial Protection in Cardiac Surgery. In: Roberts, ed. New York: Marcel Dekker, Inc.; 1987: 283-294.
113. Julia P, Kofsky ER, Buckberg GD et al. Studies of myocardial protection in the immature heart. III. Models of ischemic and hypoxic/ischemic injury in the immature puppy heart. J Thorac Cardiovasc Surg 1991; 101:14-22.
114. Ihnken K, Morita K, Buckberg GD et al. Studies of hypoxemic/reoxygenation injury: Without aortic clamping. III. Comparison of the magnitude of damage by hypoxemia/reoxygenation versus ischemia/reperfusion. J Thorac Cardiovasc Surg 1995; 110:1182-9.
115. Ihnken K, Morita K, Buckberg GD et al. Studies of hypoxemic/reoxygenation injury: Without aortic clamping. II. Evidence for reoxygenation damage. J Thorac Cardiovasc Surg 1995; 110:1171-81.
116. Morita K, Sherman MP, Buckberg GD et al. Studies of hypoxemic/reoxygenation injury: Without aortic clamping. V. Role of the L-arginine-nitric oxide pathway: The nitric oxide paradox. J Thorac Cardiovasc Surg 1995; 110:1200-11.

Chapter 2

The Cellular Basis of Immediate Lethal Reperfusion Injury

H. M. Piper and D. García-Dorado

In cardiac surgery, myocardium may suffer from ischemia either because it has been ischemic prior to the surgical intervention or because it is made intentionally or becomes inadvertently ischemic during the intervention. As a result of a successful revascularization or the end of the surgical manipulations, the ischemic myocardium may be reperfused. Ischemic or postischemic loss of viable myocardium is one of the major foes impairing an improvement of cardiac function after cardiac surgery. Many procedures have been designed for the purpose to protect myocardium against ischemic injury. The possibility—and need—to protect myocardium during the first minutes to hours of reperfusion against "reperfusion injury" has been largely neglected. This may seem surprising as the cardiac surgeon can control precisely the conditions of reperfusion. This chapter is focussed on the possibilities of protecting myocardial cells from cell death specifically by modifying the early conditions of reperfusion, i.e., to protect against the early causes of lethal reperfusion injury. It describes the novel strategies developed during the past decade in experimental research. The chapter does not deal with two other closely related topics which have been discussed extensively in several other recent reviews. These are: reversible postischemic dysfunction, so-called stunning, and delayed causes of lethal reperfusion injury, e.g., by activation of blood-borne factors.

Lethal Reperfusion Injury: Definition of Terms

Lethal reperfusion injury is defined as injury caused by restoration of blood flow after an ischemic episode leading to death of cells that were only reversibly injured during that preceding ischemic episode. For lethal reperfusion injury to occur, ischemia has to set the stage without producing irreversible injury already itself. The definition thus comes with the corollary that ischemic alterations of cellular conditions are necessary prerequisites for lethal reperfusion injury, but not in themselves sufficient causes for cell death. When after extended ischemia myocardium is reperfused by simple restoration of coronary blood flow, analysis of the developing necrosis does not permit to distinguish between cell death caused by the ischemia or by reperfusion. The only valid criterion for the existence of reperfusion injury is whether modification of the conditions of reperfusion can prevent cell death, otherwise occurring in ischemic-reperfused myocardium. This criterion appears simpler than it is to apply, since many modifications of reperfusion conditions are possible and failure to find one that reduces cell death in reperfused myocardium does not disprove the existence of reperfusion injury. As we argue below, causes of certain forms of lethal reperfusion injury have now been identified and thus some modifications of the conditions of reperfusion are indeed known to provide

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protection. If such a modification preventing cell death in reperfused myocardium has been identified, yet another question may be raised. In experimental models, cell death is normally assessed within a few hours of reperfusion. It therefore remains possible that the tested modifications of reperfusion conditions only delays the full expression of cell death beyond the time of assessment and thus falsely suggests prevention of lethal reperfusion injury.

The whole topic of lethal reperfusion injury can be differentiated conceptually as follows: Reversible injury must be delineated from lethal, irreversible injury. In this overview we will concentrate on the development of the acute form of cell death, i.e., necrosis occurring within the first minutes to first few hours of reperfusion. In the normal clinical setting, myocardial injury due to ischemia-reperfusion may appear as a single entity. For the understanding of reperfusion injury, ischemic injury must be clearly separated and the dependency of reperfusion injury on preceding ischemic changes must be clarified. Injury of ischemic-reperfused myocardium is complex, involving injury of vascular cells as well as cardiomyocytes. We will here primarily discuss whether lethal reperfusion injury of cardiomyocytes (as opposed to other cells in myocardium) can occur during the first minutes after reflow in the setting of ischemia-reperfusion, i.e., we will concentrate on *immediate* lethal (necrotic) reperfusion injury. This can be distinguished from *delayed* lethal (necrotic) reperfusion injury which, e. g., may be delivered to the cardiomyocytes by activated polymorphic neutrophils, and also from induction of apoptosis of cardiomyocytes in reperfused myocardium.

A Short Note on the Role of Oxygen Radicals

There is clear evidence that in reperfused myocardium oxygen radicals are generated at a higher rate than in normal myocardium. Several sources seem to contribute to that increased production of oxygen radicals:blood borne cells such as activated neutrophiles as well as constitutive cells of the myocardium such as cardiomyocytes and endothelial cells. The impact of oxygen radicals on the myocardial cells after ischemia may be increased since their antioxidative defense is reduced. Oxygen radicals are highly reactive and therefore potentially very toxic molecules. It is therefore understandable that they seemed to be a prime candidate for cause of lethal reperfusion injury. A large number of experimental studies has been performed in which ischemic-reperfused myocardium has been treated by means directed against oxygen radicals. Superoxide dismutase either alone or in combination with catalase upon reperfusion has been used in experimental studies aiming at reduction of infarct size by reperfusion protection. In a smaller number of studies chemical antioxidants were used for the same purpose.

Several reviews from the past ten years have summarized these studies and came to the sobering conclusion that the experimental studies have failed to prove the hypothesis that oxygen radicals are crucially involved in the genesis of lethal reperfusion injury.[1-5] This conclusion was drawn because there is about an equal number of studies demonstrating improvement or no improvement of infarct size when antioxidant strategies are applied during reperfusion. Part of the discrepancies among results may be explained by experimental flaws but a large part seems due to differences in the experimental models used. The general feeling is that a phenomenon depending so much on the specific features of an experimental model is not of general relevance. This is a plausible opinion. It must be emphasized, however, that the lack of unequivocal evidence from experimental animal studies does not exclude the possibility that the human case represents one of the positive cases where oxygen radicals contribute to myocardial reperfusion injury.

Many researchers who had tried hard to prove the importance of oxygen radicals have thereafter lost hope altogether that myocardium could be protected at the time of reperfusion or, in other words, they lost confidence in the existence of lethal reperfusion injury. This frustration does usually not apply to reversible reperfusion injury. In fact, even those researchers who feel that there is no such

thing as lethal reperfusion injury would normally admit that there are transient aggravations of myocardial metabolic or functional disorder during reperfusion which are caused by the reperfusion situation. Mechanical stunning and reperfusion arrhythmias are such phenomena.[6] In the pathogenesis of these reversible dysfunctions oxygen radicals seem in fact to play an important part. A critical discussion of these forms of reversible reperfusion injury is also interesting but not the objective of this chapter.

Three Initial Causes of Immediate Lethal Reperfusion Injury

We address the question whether causes for immediate lethal reperfusion injury of cardiomyocytes exist in mammalian hearts and how one can interfere with their mechanisms. Since specific strategies against immediate reperfusion injury have never been applied to human myocardium in vivo except for antioxidative treatment, it must remain open now if reperfusion protection represents a useful strategy in human therapy. Three potential initial causes of immediate reperfusion injury, apart from oxygen radicals, have been experimentally investigated in considerable detail, and will be briefly discussed:

cause 1: Re-energization,
cause 2: Rapid normalization of tissue pH,
cause 3: Rapid normalization of tissue osmolality.

These potential initial causes are not entirely independent. As outlined below mechanical disruption of the sarcolemma appears to be the endpoint of immediate lethal reperfusion injury. Hypercontracture of the myofibrils is probably one of the major final causes. Hypercontracture is made possible by re-energization of the ischemic cell (cause 1) in which destructive contractile forces are generated due to Ca^{2+} overload and increased cytoskeletal fragility. Ischemic acidosis can attenuate this contractile activation. Rapid normalization of tissue pH (cause 2) can act as a permissive factor for hypercontracture elicited by re-energization and can also contribute to further Ca^{2+} overload. Cell swelling is the other major final cause for immediate lethal reperfusion injury. It originates in the reperfusion situation from a too rapid normalization of extracellular osmolality (cause 3), leaving the intercellular fluid hyperosmolar.

Role of Re-Energization

About two decades ago Hearse and coworkers[7] demonstrated that in the oxygen depleted and reoxygenated myocardium severe myocardial injury, characterized by myofibrillar hypercontracture and sarcolemmal disruption, may develop with the onset of reoxygenation. It has been demonstrated by Ganote and co-workers[8,9] that this injury is due to the resumption of energy production upon reoxygenation. This phenomenon of severe cell injury immediately provoked by re-energization has been termed "oxygen paradox". It has remained an open question for a long time whether the oxygen paradox represents genuine "reoxygenation injury" or just a dramatic manifestation of injury that has already developed during the oxygen depletion period. The presence of contraction bands in infarcted myocardium is a histological indicator of oxygen paradox injury in ischemic-reperfused myocardium. Histologic analysis clearly demonstrates that when reperfusion is performed early enough to produce some myocardial salvage, infarcts are composed almost exclusively of contraction band necrosis reflecting hypercontracture of myocytes, and there is evidence indicating that this hypercontracture occurs during the first minutes of reflow. Although contraction bands can be observed in the absence of necrosis in specific circumstances (as an artifact in biopsies), in the setting of reperfusion hypercontractured myocytes invariably present signs of necrosis, indicating that, as opposed to what happens in isolated cardiomyocytes, reperfusion-induced hypercontracture is associated to sarcolemmal disruption and cell death.

Details of the causal mechanism of the oxygen paradox have now been identified in

experimental studies using isolated cardiomyocytes (see below). These studies have shown that the oxygen paradox is indeed injury

1. brought about by the process of reoxygenation and
2. based on a mechanism within the myocardial cell.

Re-energization causes lethal cell injury by provocation of hypercontracture. The mechanism is the following: After prolonged energy depletion, cytosolic Ca^{2+} concentration is dramatically increased. Upon re-energization of the myocardial cell, made possible by resupply of oxygen to mitochondria, two processes are simultaneously activated:

1. The energy supply to cation pumps initiates recovery of the cellular cation balance;
2. resupply of energy to the myofibrillar elements initiates contractile activation.

Under conditions of energy depletion, i.e., in ischemic or hypoxic myocardium, the cytosol of the myocardial cells becomes loaded with Na^+ and Ca^{2+}. Recovery of energy production upon the resupply with oxygen and metabolic substrates rapidly reactivates two major cation pumps (Fig. 2.1): namely the Ca^{2+} pump (Ca^{2+}-ATPase) of the sarcoplasmic reticulum (SR) and the Na^+ pump (Na^+-K^+-ATPase) of the sarcolemma, unless these pumps are themselves injured by the preceding ischemic conditions. Activation of the Ca^{2+} pump of the SR leads to a temporary sequestration of excess Ca^{2+} within this intracellular storage organelle.[10,11] If the capacity of this organelle is too small for the amount of Ca^{2+} accumulated in the cytosol, a cycle of continuous release and reuptake of Ca^{2+} from and into the SR is initiated. These spontaneous oscillations come to an end only if the major mechanism for Ca^{2+} extrusion from the cytosol is sufficiently activated, i.e., the Na^+/Ca^{2+} exchanger of the sarcolemma.[11] The ability of this exchanger to remove Ca^{2+} from the cytosol depends on the magnitude of the transsarcolemmal Na^+ gradient. Restoration of a sufficiently large Na^+ gradient across the sarcolemma is therefore the prerequisite for extrusion of Ca^{2+} from reoxygenated myocardial cells. It is essential that the Na^+ pump of the sarcolemma is rapidly activated to remove access Na^+ from the interior of the cell. It was shown on the cellular level that the re-energized cardiomyocyte can retain sufficient metabolic competence to rapidly reactivate the SR Ca^{2+} pump and the sarcolemmal Na^+ pump during the early phase of reoxygenation, even if the cell had been extensively depleted of its energy stores and suffered from severe Ca^{2+} and Na^+ overload before re-energization.[10-12] It seems that cells in which these pumps have been crucially damaged during the ischemic period are in principle unable to recover and cannot be, therefore, subject to reperfusion injury in the sense of the definition given above (Fig. 2.2).

It is the resupply of energy to the myofibrilar elements in the presence of an increase of cytosolic Ca^{2+} concentration which may become deleterious for the reoxygenated cell (Fig. 2.1, lower part). This is because during the initial phase of reoxygenation the cytosolic Ca^{2+} is still largely elevated and myofibrillar activation therefore leads to uncontrolled, excessive force generation. This sustained force generation causes hypercontraction. A hypercontracting cardiac muscle cell becomes severely injured in its cytoskeletal structures as the deformation of cytoskeletal elements beyond the degree found under normal contractile shortening is no longer readily reversible. The resulting state of irreversible cell shortening is called "hypercontracture." In tissue, hypercontraction of adjacent cells may lead to mutual cellular disruptions and necrosis. This pathomechanism of reoxygenation-induced mechanical injury can be prevented if the contractile machinery is inhibited during the first stage of energy recovery, for the time needed to reestablish a normal cellular cation control. It has been demonstrated in several studies that a direct blocker of the myofibrils, 2,3-butanedionemonoxime (BDM), can be used experimentally to inhibit the myofibrillar machinery during the early "vulnerable phase" of reoxygenation.[13-17] These studies from different groups have involved different models (isolated myocytes,[13] isolated rat heart,[14] isolated guinea pig heart,[17] in situ pig[15] and dog heart[16]), different conditions

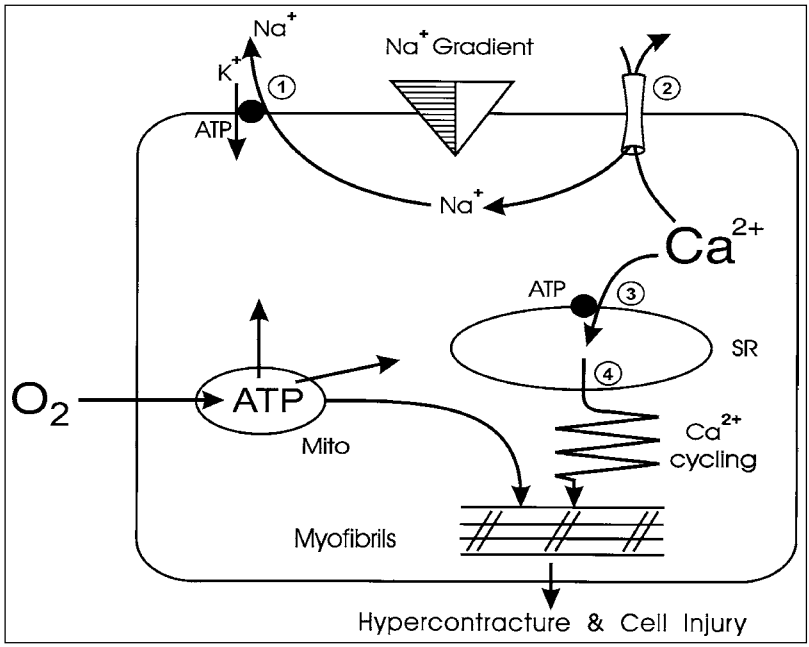

Fig. 2.1. Scheme of cation control and initiation of hypercontracture in the reoxygenated cardiomyocyte. On reactivation of oxidative phosphorylation in mitochondria (MITO), the Na^+ pump (1) is reactivated generating a transsarcolemmal Na^+ gradient which provides a driving force for the Na^+/Ca^{2+} exchanger (2) in its "forward mode". Re-energization of the Ca^{2+} pump (3) of the sarcoplasmic reticulum (SR) causes sequestration of excessive cytosolic Ca^{2+} into this organelle. Overload of the SR leads to Ca^{2+} release. Repetitive Ca^{2+} uptake and release by the SR results in Ca^{2+} cycling between SR and cytosol. Reactivation of oxidative phosphorylation in mitochondria provides energy also to the contractile machinery. Energy supply in the presence of high cytosolic Ca^{2+} concentration causes uncontrolled contractile activation and consecutively mechanical injury of cell structures.

(anoxia reoxygenation,[13,14] ischemia-reperfusion[17] transient coronary occlusion[15,16]) and different end-points including ventricular function,[17] hypercontracture,[13] histochemically determined infarct size[15,16] or extension of myocardial necrosis as assessed by quantitative histology after 24 hours of reperfusion.[15] In the study by García-Dorado et al,[15] for example, the left descending coronary artery was occluded for 45 min in an in vivo model (pig) of regional ischemia. Upon reperfusion, BDM was added to the coronary flow and remained there for the first 60 min of normoxic reperfusion. Infarct size determinations after 24 hours of reperfusion then demonstrated a reduction by half in BDM treated hearts.

It has recently been demonstrated that hypercontraction may also be elicited by a closely related mechanism.[19] In cells capable to reestablish a normal cation control, the initial phase of Ca^{2+} recovery in the reoxygenated cell may be divided into two stages:

1. An early stage during which the cytosolic Ca^{2+} level falls due to uptake of Ca^{2+} into the SR and
2. a second stage during which Ca^{2+} is shifted in an oscillatory manner between cytosol and SR until a sufficient proportion of the Ca^{2+} level is extruded across the sarcolemma.[10] The Ca^{2+} oscillations of this stage can also cause hypercontraction. This is not solely explained, however, by the magnitude of the Ca^{2+} peak concentrations occurring during oscillations. It has been shown that the susceptibility of reoxygenated cardiomyocytes to develop hypercontracture at a given elevation of

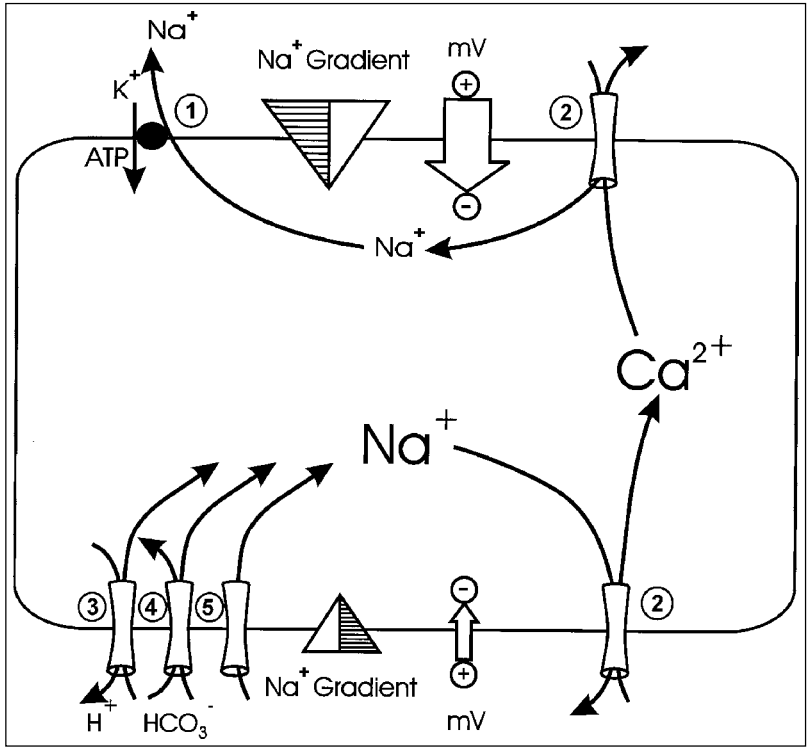

Fig. 2.2. Scheme of Na^+ and Ca^{2+} control in the reoxygenated cardiomyocyte. Upper half: When reactivation of the Na^+ pump (1) creates a large transsarcolemmal Na^+ gradient and the membrane potential (mV) is large, the Na^+/Ca^{2+} exchanger (2) is driven in its "forward mode". The cytosolic Ca^{2+} overload may thus be reduced. Lower half: When Na^+ influx through Na^+/H^+ exchanger (3), Na^+/HCO_3^- symporter (4) or Na^+ channels (5) diminishes the transsarcolemmal Na^+ gradient and the membrane potential is small, the Na^+/Ca^{2+} exchanger is driven in its "reverse mode". The cytosolic Ca^{2+} overload may thus be increased.

cytosolic Ca^{2+} is increased after a prolonged period of hypoxic energy depletion.[18] This means that hypercontraction in energy depleted and repleted myocardial cells may be elicited by Ca^{2+} concentrations in the cytosol which would not cause harm to a normal cell. The cause for this increased susceptibility seems to reside in an increased fragility of cytoskeletal elements which can no longer resist large contraction forces. An alternative explanation would be that the myofibrillar sensitivity to Ca^{2+} is increased in reoxygenated cardiomyocytes. This has not yet been studied directly. It seems unlikely, however, since studies on reperfused myocardium after short-lived ischemia, exhibiting stunning, have shown rather a reduction of myofibrillar Ca^{2+} sensitivity.[19] In vitro it is possible to protect the reoxygenated myocardial cell from hypercontracture by damping the oscillatory movements between Ca^{2+} and cytosol, thus reducing the high peak concentrations of cytosolic Ca^{2+}.[18] This can be achieved by specific blockade of Ca^{2+} uptake into or release from the SR, as with cyclopiazonic acid or ryanodine, respectively.[19] Interestingly, the volatile anesthetic halothane can also be used to inhibit SR function and thereby provide protection.[20] Halothane applied upon reoxygenation has been shown to

protect isolated cardiomyocytes, hypoxia-reoxygenated hearts[21] and ischemic-reperfused in vivo[22] myocardium against hypercontracture and lethal reperfusion injury.

Role of Rapid Normalization of Tissue pH

The cytosolic pH in cardiomyocytes in reperfused myocardium has a pronounced influence on the development of hypercontracture. After prolonged ischemia, the cytosolic pH is markedly lowered because anaerobic metabolism and the breakdown of ATP produce an excess of H^+. This leads to an acidification of both the intracellular and the interstitial spaces. Upon reperfusion the pH in the interstitial space is rapidly renormalized and a gradient is thus generated between the cytosol, containing still high H^+ concentrations, and the interstitium, where the H^+ concentrations are already re-normalized. This causes an activation of the H^+ extruding mechanisms of the cardiomyocytes, i. e., the Na^+/H^+ exchanger and the Na^+/HCO_3^- symporter.[23,24] This process has two consequences (Fig. 2.3):

1. Intracellular acidosis is rapidly reduced. Intracellular acidosis inhibits, however, the myofibrillar machinery, i.e., it exerts an effect similar to the presence of BDM during the early phase of reperfusion.[25] Rapid extrusion of excess H^+ from the reoxygenated cell thus removes a potentially protective agent.
2. Activation of the Na^+/H^+ exchanger causes a net influx of Na^+ into the cytosol. Depending on the ability of the Na^+ pump to remove this excess load of Na^+, it may come to a secondary activation of the Na^+/Ca^{2+} exchange mechanism, transporting Na^+ in the outward direction and Ca^{2+} in the inward direction. This coupled mechanism may enhance the preexisting Ca^{2+} overload of the cells.

Rapid H^+ removal and secondary Ca^{2+} uptake thus both favor the development of hypercontracture if ischemic-reperfused myocardial cells are allowed to restore a normal intracellular acid-base balance. It has been demonstrated in vitro that continuation of extracellular acidosis, and thereby intracellular acidosis, during the early phase of reoxygenation protects myocardial cells against the development of hypercontracture during this phase. For reperfusion of myocardium in vivo the situation is less clear. Inhibitors of the Na^+/H^+ exchanger were found to protect against the development of hypercontracture and necrosis during reperfusion only when present during the previous ischemic period.[26-28] The most likely explanation for the discrepancy between the in vitro and the in vivo studies is at present that in blood perfused hearts intracellular acidosis cannot be maintained for a sufficiently long time after initiation of reperfusion if only the Na^+/H^+ exchanger is inhibited. This is because the myocardial cell possesses also another route for the transsarcolemmal extrusion of acid equivalents, i.e., the Na^+/HCO_3^- symporter which works in parallel to the Na^+/H^+ exchanger and is active in normal bicarbonate containing fluids. Unfortunately, specific inhibitors for this mechanism are not yet available for research or therapy.

Role of Rapid Normalization of Tissue Osmolality

One of the major causes for water influx into the ischemic-reperfused myocardial cell seems to be cytosolic Na^+ overload. The Na^+/H^+ exchanger plays a major role in cell volume regulation.[29,30] In ischemic myocardium the end products of anaerobic metabolism also accumulate, thus increasing the osmotic load in the intracellular and the interstitial space.[30] If during reperfusion the extracellular excess of osmotically active molecules is rapidly washed out, an osmotic gradient between the intracellular and the extracellular space is generated.[31] Cellular uptake of water and, through the consecutive increase in intracellular pressure, mechanical stretch of the sarcolemma meets a myocardial cell whose mechanical fragility was increased during the preceding energy depletion.[32-34] As is the case for hypercontracture, swelling per se is

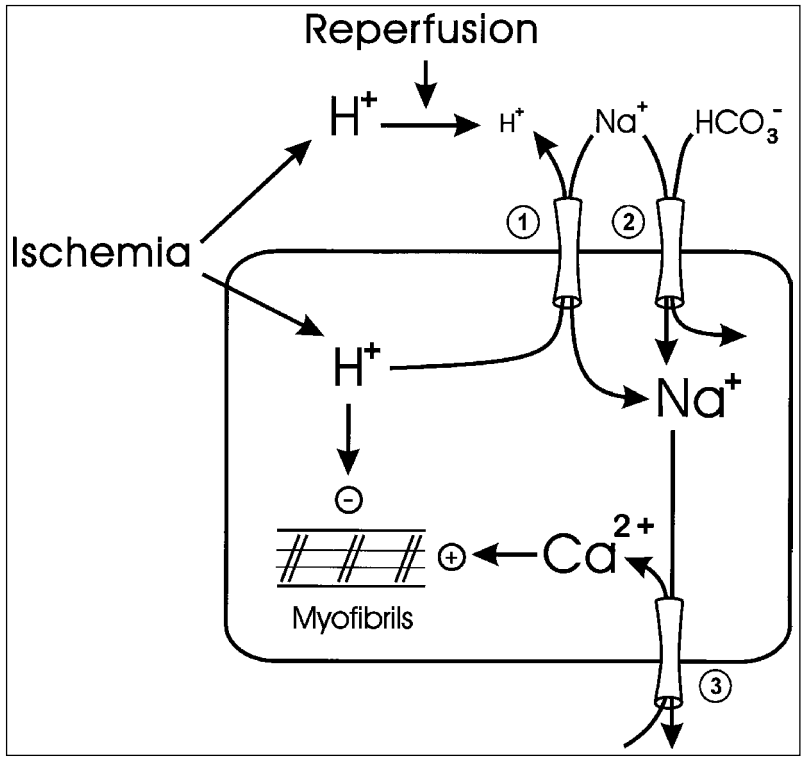

Fig. 2.3. Scheme of H^+ control and its consequences in the cardiomyocyte upon reperfusion. Ischemia causes intra- and extracellular acidification. Intracellular acidosis inhibits contractile activation. Upon reperfusion the extracellular H^+ concentration is rapidly reduced and thus a transsarcolemmal H^+ gradient created. Consecutively, intracellular excess H^+ is removed via the Na^+/H^+ exchanger (1) and the Na^+/HCO_3^- symporter (2). As long as high intracellular H^+ concentration is present, the contractile machinery is impaired and hypercontracture prevented. Na^+ influx through (1) and (2) can cause net Ca^{2+} influx through the Na^+/Ca^{2+} exchanger (3) in its "reverse mode". This increases cytosolic Ca^{2+} overload, favoring uncontrolled contractile activation.

normally not able to disrupt the sarcolemma, as demonstrated by the maintenance of sarcolemmal integrity in isolated cardiomyocytes subjected to osmotic stress in normoxic conditions. However, the mechanical stress caused by swelling may add up with other sources of stress and then result in cell deterioration (Fig. 2.4). In isolated cardiomyocytes, osmotic stress results in sarcolemmal disruption only if the cell develops hypercontracture and has previously been submitted to prolonged energy deprivation.[35] The combination of these factors seems to increase sarcolemmal fragility and render the cell thus more susceptible to damage by osmotic stress. The results of studies with highly hyperosmotic reperfusion indicate that attenuation of the additional mechanical stress imposed by swelling can limit myocardial necrosis during reperfusion.[36-38]

The mechanism of sarcolemmal fragility secondary to energy deprivation is not understood in detail. Alterations in the lipidic composition of the cell membrane, as suggested by the protective effect of treatments preserving the turnover of phospholipids during energy deprivation,[34] changes in sarcolemmal proteins[39,40] or changes in the sarcolemma-cytoskeleton anchorage[41,42] could play a role. There is also evidence that sarcolemmal fragility induced by ischemia can be aggravated during the first moments of reperfusion.[33,40] It has recently been demonstrated that

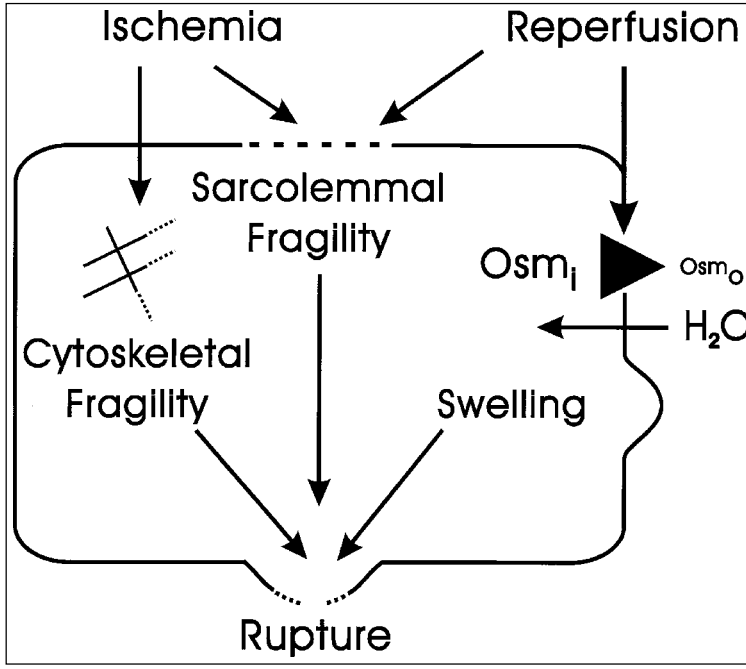

Fig. 2.4. Scheme of osmotic injury of the reoxygenated cardiomyocyte. Upon reperfusion a transsarcolemmal osmotic gradient is created. This leads to water influx and cell swelling. Cytoskeletal fragility increases during ischemia. Circumstances of ischemia and reperfusion augment independently sarcolemmal fragility. Increased cytoskeletal and sarcolemmal fragility and cell swelling together favor rupture of the sarcolemma.

enhanced susceptibility of reoxygenated myocardial cells to osmotic injury can be reduced by specific interventions during the early phase of reoxygenation.[33] Effective measures were additions of NO-donors in high concentration and of an antilipid peroxidant or means increasing the cellular glutathione pool. The results suggest that mechanical fragility of the sarcolemma is increased by radical mechanisms during the early period of reoxygenation. It must be said clearly that, in this role of enhancing sarcolemmal fragility, oxygen radicals are a factor of secondary importance for reperfusion injury, when seen in relation to the whole scenario.

Following Initial Causes: Spreading of Necrosis

Several lines of evidence indicate that reoxygenation-induced hypercontracture and sarcolemmal disruption are markedly influenced by cell-to-cell interactions.[43,44] Histologic observations have shown that the areas of contraction-band necrosis induced by transient coronary occlusion followed by reperfusion are composed of hypercontracted myocytes connected to each other to form a continuum, of which the often complex geometry cannot be explained by gradients of flow or microvascular distribution.[45] Computer simulation studies indicate that some kind of cell-to-cell interaction must be taken into account to explain these histological features, and that in the absence of such interaction, hypercontracted myocytes should be scattered across the area of risk instead of forming continuous zones of necrosis. It has been suggested that this cell-to-cell interaction could be mechanical, the exchange of forces imposed by tight intercellular junctions tearing apart the sarcolemma of myocytes hypercontracting during in situ reperfusion, and damaging the

sarcolemma of adjacent cells.[43,44] But the interaction between adjacent myocytes leading to cell-to-cell progression of hypercontracture also could be chemical. Ca^{2+} and other second messengers may diffuse freely through gap junctions and thereby transmit the trigger for hypercontracture. Recent studies in pairs of isolated cardiomyocytes have demonstrated that hypercontracture of one cell induced by sarcolemmal disruption or microinjection of Ca^{2+} can induce hypercontracture of adjacent cells, that this transmission is associated to passage of gap junction permanent dye to the adjacent cell, and that the gap junction uncoupler heptanol prevents both dye passage and transmission of hypercontracture. The intracoronary administration of heptanol during the first minutes of reperfusion significantly reduces infarct size in the in situ pig heart submitted to transient coronary occlusion.[46] These results are consistent with the hypothesis that cell-to-cell transmission of hypercontracture may cause spreading of necrosis, and thus contributes to reperfusion injury.

The Problem of Delay of Necrosis and Initiation of Apoptosis

Some interventions applied at the time of reperfusion can apparently reduce the extent of irreversible tissue injury when this is investigated early but in fact may only delay the manifestation of irreversible injury. If this is the case such an intervention does not provide true reperfusion protection. There are indeed some cases known where an apparent protective effect was later found to be only imitated by a time delay in development of the markers of necrotic tissue injury. This must be distinguished from injury of myocardium by causes appearing not during the early but during the late phase of reperfusion, e.g., tissue injury by invasion of activated neutrophils.

It is another question whether reperfused myocardium may also become subject to apoptosis, i.e., programmed cell death, even if effectively protected against immediate necrotic injury. Apoptosis is a transcriptionally controlled cellular response to moderate cell injury or to the influence of various cytokines. In contrast, necrotic cell death is the consequence of severe structural cell damage and is not transcriptionally regulated. Cells which have entered the apoptotic process retain physical integrity of the plasmalemma initially even though its chemical structure may change. The point where the process becomes irreversible seems to be the activation of endonucleases severing genomic DNA at internucleosomal sites, what is then taken as a characteristic feature of cell death by apoptosis. In a number of recent papers evidence for apoptotic cell injury in ischemic-reperfused myocardium and in border zones of ischemic myocardium has been demonstrated.[47-53] This gives rise to the question if reperfusion of severely ischemic myocardium can be followed by delayed apoptotic cell death which could abolish all short-lived protective effects against the acute onset of necrosis during reperfusion. The real contribution of apoptosis to cardiomyocyte death has not yet been established. To date, the factors initiating apoptosis in ischemic-reperfused myocardium are still unclear. It is also an open question whether the apoptotic process observed in reperfused myocardium is due to triggers during the time of ischemia or during reperfusion. If the latter is the case, it seems reasonable to expect that possibilities are found to inhibit the full development of apoptotic cell death as the whole process of apoptosis is a multi-stage metabolic mechanism with many possible sites of inhibition.

Conclusions

After prolonged periods of energy depletion the ischemic myocardial cell can be jeopardized by specific causes within the reperfusion period (Fig. 2.5). These causes can be viewed as unwanted aspects of the recovery process itself limiting its efficiency. Understanding of the basic causes has opened novel perspectives for specific interference with these serious pathomechanisms. The experimental results encourage the development of therapeutic approaches to reduce infarct size by specific measures applied during the early

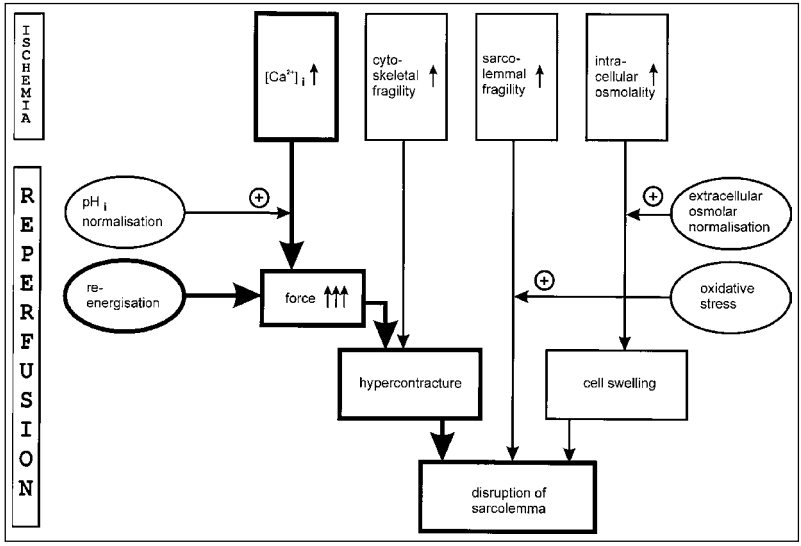

Fig. 2.5. Scheme of factors contributing to immediate lethal (necrotic) reperfusion injury of the cardiomyocyte.

phase of reperfusion. The principles of the protective interventions during the early stage of reperfusion are:

1. Inhibition of contractile activation;
2. prevention of intracellular Ca^{2+} oscillations;
3. preservation of intracellular acidosis;
4. suppression of causes favoring sarcolemmal fragility;
5. reduction of cell swelling;
6. prevention of spreading of necrosis.

This does not exclude that measures aimed to limit necrosis or apoptosis occurring later during reperfusion are of additional value.

To date, all these strategies for reperfusion protection have only been studied experimentally and the number of studies is relatively small. The results of these studies conclusively demonstrated that it is possible to markedly reduce myocardial necrosis by treatments applied at the time of reperfusion, and provide precise mechanistic explanation for this beneficial effect within the frame of our current understanding of myocyte death during ischemia-reperfusion. This is in contrast to the wealth of investigations testing the strategy of preventing injury due to oxygen free radicals in reperfusion injury. The failure of the latter strategy has hindered research on this important pathophysiological problem during recent years. Knowledge on the basic causal mechanisms of reperfusion injury, reviewed in this chapter, does no longer justify abstention from intensive research on new principles of reperfusion protection. Cardiac surgery may profit immediately from this research since in most cardiac surgery procedures the reperfusion conditions can be modified at will.

Acknowledgement

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References

1. Yellon DM, Jennings RB, eds. Myocardial Protection. The Pathophysiology of Reperfusion and Reperfusion injury. New York: Raven, 1992.
2. Przyklenk K, ed. Special Issue: Lethal Myocardial Reperfusion injury. J Thromb Thrombolysis 1997; 4:1-154.
3. Ferrari R, Hearse DJ. Reperfusion injury: Does it exist and does it have clinical relevance? J Thromb Thrombolysis 1997; 4: 25-34.

4. Kloner RA. Does reperfusion injury exist in humans? J Am Coll Cardiol 1993; 21: 537-545.
5. Downey JM, Yellon DM. Do free radicals contribute to myocardial cell death during ischemia-reperfusion? In: Yellon CM, Jennings RB, eds. Myocardial Protection: The Pathophysiology of Reperfusion and Reperfusion injury. New York: Raven, 1992:35-57.
6. Bolli R. Myocardial stunning in man. Circulation 1992; 86:1671-1691.
7. Hearse DJ, Humphrey SM, Chain EB. Abrupt reoxygenation of the anoxic potassium-arrested perfused rat heart: A study of myocardial enzyme release. J Mol Cell Cardiol 1973; 5:395-407.
8. Ganote CE. Contraction band necrosis and irreversible myocardial injury. J Mol Cell Cardiol 1983; 15:67-73.
9. Ganote CE, Vander Heide RS. Importance of mechanical factors in ischemic and reperfusion injury. In: Piper HM, ed. Pathophysiology of severe ischemic myocardial injury. Dordrecht: Kluwer, 1990:337-355.
10. Siegmund B, Zude R, Piper HM. Recovery of anoxic-reoxygenated cardiomyocytes from severe Ca^{2+} overload. Am J Physiol 1992; 263:H1262-H1269.
11. Siegmund B, Ladilov YV, Piper HM. Importance of Na^+ for the recovery of Ca^{2+} control in reoxygenated cardiomyocytes. Am J Physiol 1994; 267:H506-H513.
12. Siegmund B, Koop A, Klietz T et al. Sarcolemmal integrity and metabolic competence of cardiomyocytes under anoxia-reoxygenation. Am J Physiol 1990; 258: H285-H291.
13. Siegmund B, Klietz T, Schwartz P et al. Temporary contractile blockade prevents hypercontracture in anoxic-reoxygenated cardiomyocytes. Am J Physiol 1991; 260: H426-H435.
14. Schlüter KD, Schwartz P, Siegmund B et al. Prevention of the oxygen paradox in hypoxic-reoxygenated hearts. Am J Physiol 1991; 261:H416-H432.
15. García-Dorado D, Théroux P, Duran JM et al. Selective inhibition of the contractile apparatus: A new approach to modification of infarct size, infarct composition, and infarct geometry during coronary artery occlusion and reperfusion. Circulation 1992; 85: 1160-1174.
16. Schlack W, Uebing A, Schäfer M et al. Regional contractile blockade at the onset of reperfusion reduces infarct size in the dog heart. Pflügers Arch 1994; 428:134-141.
17. Habazettl H, Palmisano BW, Bosnjak ZJ et al. Initial reperfusion with 2,3 butanedione monoxime is better than hyperkalemic reperfusion after cardioplegic arrest in isolated guinea pig hearts. Eur J Cardiothorac Surg 1996; 10:897-904.
18. Ladilov YV, Siegmund B, Piper HM. Simulated ischemia increases the susceptibility of rat cardiomyocytes to hypercontracture. Circ Res 1997; 80:69-75.
19. Gao WD, Liu Y, Mellgren R et al. Intrinsic myofilament alterations underlying the decreased contractility of stunned myocardium. A consequence of Ca^{2+}-dependent proteolysis? Circ Res 1996; 78:455-465.
20. Siegmund B, Schlack W, Piper HM. Halothane protects cardiomyocytes against reoxygenation-induced hypercontracture. Circulation, in press.
21. Schlack W, Hollmann M, Stunneck J et al. Effect of halothane on myocardial reoxygenation injury in the isolated rat heart. Br J Anaesth 1996; 76:860-867.
22. Preckel B, Schlack W, Comfère T et al. Effects of inhalational anaesthetics on myocardial reperfusion injury in vivo. Pflügers Arch 1997, suppl to 433:R15.
23. Lagadic-Gossmann D, Vaughan-Jones RD. Coupling of dual acid extrusion in the guinea-pig isolated ventricular myocytes to a1 and b-adrenoceptor. J Physiol 1993; 464:49-73.
24. Piper HM, Balser C, Ladilov YV et al. The role of Na^+/H^+ exchange in ischemia-reperfusion. Basic Res Cardiol 1996; 91:191-202.
25. Ladilov YV, Siegmund B, Piper HM. Protection of the reoxygenated cardiomyocyte against hypercontracture by inhibition of Na^+/H^+ exchange. Am J Physiol 1995; 268: H1531-H1539.
26. Bugge E, Ytrehus K. Inhibition of sodium-hydrogen exchange reduces infarct size in the isolated rat heart—a protective additive to ischemic preconditioning. Cardiovasc Res 1995; 29:269-274.
27. Klein HH, Pich S, Bohle RM et al. Myocardial protection by Na^+/H^+ exchange inhibition in ischemic reperfused porcine hearts. Circulation 1995; 92:912-917.
28. García-Dorado D, González MA, Barrabés JA et al. Prevention of ischemic rigor contracture during coronary occlusion by inhibition of Na^+-H^+ exchange. Cardiovasc Res 1997; 96:3579-3586.
29. Inserte J, García-Dorado D, Ruiz-Meana M et al. The Na^+-H^+ exchange occurring during hypoxia in the genesis of reoxygenation-induced myocardial oedema. J Mol Cell Cardiol 1997; 29:1167-1175.

30. Grinstein S, Woodside M, Sardet C et al. Activation of the Na^+/H^+ antiporter during cell volume regulation. Evidence for a phosphorylation-independent mechanism. J Biol Chem 1992; 267:23828-23828.
31. García-Dorado D, Oliveras J. Myocardial edema: A preventable cause of reperfusion injury. Cardiovasc Res 1993; 27:1555-1563.
32. Armstrong SC, Ganote CE. Effects of the protein phosphatase inhibitors okadaic acid and calyculin A on metabolically inhibited and ischemic isolated myocytes. J Mol Cell Cardiol 1992; 24:869-884.
33. Schlüter KD, Jakob G, Ruiz-Meana M et al. Protection of reoxygenated cardiomyocytes against osmotic fragility by NO donors. Am J Physiol 1996; 271:H428-H434.
34. Ruiz-Meana M, García-Dorado D, Julia M. González MA et al. Pretreatment with trimetazidine increases sarcolemmal mechanical resistance in reoxygenated myocytes. Cardiovasc Res 1996; 32:587-592.
35. Ruiz-Meana M, García-Dorado D, González MA et al. Effect of osmotic stress on sarcolemmal integrity of isolated cardiomyocytes following transient metabolic inhibition. Cardiovasc Res 1995; 30:64-69.
36. Kloner RA, Reimer KA, Willerson JT et al. Reduction of experimental myocardial infarct size with hyperosmotic mannitol. Proc Soc Exp Biol Med 1976; 151:677-683.
37. Tranum-Jensen J, Janse MJ, Fiolet WT et al. Tissue osmolality, cell swelling, and reperfusion in acute regional myocardial ischemia in the isolated porcine heart. Circ Res 1981; 49:364-381.
38. García-Dorado D, Théroux P, Muñoz R et al. Favorable effects of hyperosmotic reperfusion on myocardial edema and infarct size. Am J Physiol 1992; 262:H17-H22.
39. Iizuka K, Kawaguchi H, Kitabatake A. Effects of thiol protease inhibitors on fodrin degradation during hypoxia in cultured myocytes. J Mol Cell Cardiol 1993; 25: 101-1109.
40. Yoshida K, Inui M, Saido TC et al. Reperfusion of rat heart after brief ischemia induced proteolysis of calspectin (nonerythroid spectrin of fodrin) by calpain. Circ Res 1995; 77:603-610.
41. Steenbergen C, Hill ML, Jennings RB. Cytoskeletal damage during myocardial ischemia: Changes in vinculin immunofluorescence staining during total in vitro ischemia in canine heart. Circ Res 1987; 60:478-486.
42. Ganote CE, Vander Heide RS. Cytoskeletal lesions in anoxic myocardial injury: A conventional and high voltage electron microscopic and immunofluorescence study. Am J Pathol 1987; 129:327-344.
43. Ganote CE. Cell-to-cell interactions contributing to the oxygen paradox. Bas Res Cardiol 1985; 80:141-146.
44. Frank JS, Brandy AJ, Fransworth BS et al. Ultrastructure and function of isolated myocytes after calcium depletion and repletion. Am J Physiol 1986; 250:H265-H275.
45. Solares J, García-Dorado D, Oliveras J et al. Contraction band necrosis at the lateral borders of the area at risk in reperfused infarcts. Observations in a pig model of in situ coronary occlusion. Virchows Arch 1995; 426: 393-399.
46. García-Dorado D, Inserte J, Ruiz-Meana M et al. Gap junction uncoupler heptanol prevents cell-to-cell progression of hypercontracture and limits necrosis during myocardial reperfusion. Circulation 1997; 35: 80-89.
47. Bardales RH, Hailey LS, Xie SS et al. In situ apoptosis assay for the detection of early acute myocardial infarction. Am J Pathol 1996; 149:821-829.
48. Fliss H, Gattinger D. Apoptosis in ischemic and reperfused rat myocardium. Circ Res 1996; 79:949-956.
49. Suzuki H, Wildhirt SM, Dudek RR et al. Induction of apoptosis in myocardial infarction and its possible relationship to nitric oxide synthase in macrophages. Tissue Cell 1996; 28:89-97.
50. Veinot JP, Gattinger DA, Fliss H. Early apoptosis in human myocardial infarcts. Hum Pathol 1997; 28:485-492.
51. Saraste A, Pulkki K, Kallajoki M et al. Apoptosis in human acute myocardial infarction. Circulation 1997; 95:320-323.
52. Kajstura J, Cheng W, Reiss K et al. Apoptotic and necrotic myocytes cell deaths are independent contributing variables of infarct size in rats. Lab Invest 1996; 74:86-107.
53. Gottlieb RA, Burleson KO, Kloner RA et al. Reperfusion injury induces apoptosis in rabbit cardiomyocytes. J Clin Invest 1994; 94:1621-1628.

CHAPTER 3

Apoptosis in Ischemia—Reperfusion Injury

Harald Darius, Waltraud Ibe and Michael Buerke

Apoptosis or "programmed cell death" has recently been recognized to be involved in several cardiovascular diseases. There are some histologic criteria as well as histochemical, biochemical and gel electrophoretic methods developed for the detection of apoptosis in isolated cells, tissues or organs. In myocarditis and heart failure evidence is accumulating that apoptosis may play a role in their pathophysiology. In isolated cardiomyocytes hypoxia and reoxygenation cause apoptosis, cytokines at physiologic concentrations induce apoptosis and intracellular promotors and inhibitors of apoptosis have been identified. In animal models of myocardial ischemia and reperfusion apoptotic cells have been detected in the area at risk not undergoing necrosis. Reperfusion itself either seems to induce apoptosis or at least significantly augments this process, depending on the experimental conditions and animal species studied. In autopsy studies of human myocardial infarction, victims apoptotic cells were identified in the border zone between successfully reperfused myocardium and the core ischemic area undergoing necrosis. Intracellular regulatory proteins (e.g., Bcl-2, Bax, Fas, p53) were identified to be involved in apoptosis induction or reduced expression. The pathophysiologic role of apoptosis in human myocardial infarction and reperfusion as well as in surgical ischemia and reperfusion still has to be determined. If the reduction or induction of apoptosis may be an useful therapeutic goal in the future to aim at is still unresolved.

Characteristics of Apoptosis

Apoptosis and necrosis are two independent phenomena determining the survival of cells within an organ structure or isolated cells in response to environmental factors. Since necrosis is more like an accident of nature due to malnutrition of a cell or tissue damage caused by ischemia or toxic concentrations of environmental toxins or biological mediators e.g., oxygen derived radicals, apoptosis represents a genetically determined process. During necrosis the membrane permeability is increased, swelling of cells and organelles results from altered ion pump activity leading to increased sodium retention. DNA degradation occurs nonspecifically resulting in fragments of nondefined length showing in gel electrophoresis as a smear. Cell swelling and nuclear destruction result in cytolysis, release of cytoplasmic constituents and induction of an inflammatory reaction.

In contrast to necrosis, apoptosis or programmed cell death (PCD) represents a normal feature of cell and tissue development (Table 3.1). This is of specific relevance in the fetus and in some instances of cell replacement in adult tissues (e.g., thymus). PCD is a tightly regulated and energy dependent process induced by a genetic program and requiring gene transcription. Cells undergoing necrosis

Ischemia-Reperfusion Injury in Cardiac Surgery, edited by Friedhelm Beyersdorf.

Table 3.1. Specific features of apoptosis in contrast to necrosis

Necrosis	Apoptosis
- Death of tissue segments	- Death limited to single cells
- Membrane destruction	- Membrane remains intact
- Cell swelling	- Cell shrinkage
- Nonspecific DNA degradation	- Chromatin condensation
- Inflammatory response	- Phagocytosis without inflammation
- No energy required	- Energy required
- Nonregulated event	- Highly regulated event
- No gene requirement	- Gene transcription (de novo)

can be easily differentiated from those undergoing PCD since PCD results in cell shrinkage, blebbing of the cellular membrane and condensation of the nuclear chromatin. Since apoptotic bodies are formed no or very little cytoplasmic constituents are released into the surrounding environment and, unlike in necrosis, there is no evidence of a localized inflammatory reaction. The apoptotic bodies are phagocytosed instead. Nuclear condensation is followed by nuclear disintegration and DNA fragmentation into segments of 180-200 bp length or multiples of this. These fragments can become visualized easily in gel electrophoresis, where a laddering phenomenon is seen with characteristic bands of the respective segment length.

There is a wide variety of environmental factors and biological mediators that have been demonstrated to cause PCD in vitro in cell culture or isolated tissues (Table 3.2). In vivo however, a number of noncardiovascular and cardiovascular disorders have been identified to result in an accelerated rate of apoptosis (Table 3.3).

Detection of Apoptosis

There are a number of methods for the detection of apoptosis in isolated cells or tissues, however none of which is regarded as being entirely specific (Table 3.4). Thus, very often two or more independent methods are utilized for the confirmation of apoptotic cells. The methods are either based on histologic or cytologic examination or on detection of DNA fragments (Fig. 3.1A). Light microscopy, fluorescence microscopy or flow cytometry (Fig. 3.1B) may be used after labeling the DNA fragments using a TUNEL assay (**T**erminal deoxynucleotidyl transferase-mediated d**U**TP **N**ick **E**nd-**L**abeling) or Annexin V antibodies to detect the terminal DNA fragments. The most reliable but laborious method is the detection of the characteristic cellular features of apoptosis described above by electron microscopy. An alternative method for the detection of apoptosis in a tissue segment is the visualization of DNA strand breaks of a rather specific segment length between 180 and 200 base pairs in agarose gel electrophoresis by the characteristic laddering phenomenon.

Apoptosis in Cardiac Disease

Programmed cell death or apoptosis recently has been demonstrated in several cardiac disease states. Mallat et al[1] demonstrated convincing evidence of apoptosis being heavily involved in the degenerative process of the arrhythmogenic right ventricular dysplasia, which often is not differentiated from Uhl's disease. In end stage heart failure all samples derived from hearts of patients with dilated cardiomyopathy showed histologic evidence of apoptosis and a positive laddering phenomenon in gel electrophoresis. In contrast, only one out of three hearts from patients with end stage coronary artery disease had detectable DNA fragmentation as a sign of apoptosis.[2] When hearts from congestive cardiac failure patients were analyzed and compared to hearts

Table 3.2. Environmental factors and biologic mediators for which an increased rate of apoptosis has been demonstrated

Physiologic stimuli	Nonphysiologic stimuli
- steroids	- heat,
- calcium	- UV-irradiation
- tumor necrosis factor	- infections
- transforming growth factor	- cytotoxic T-cells
- depletion of essential growth factors	- oncogenes, tumor-suppressor gene
- loss of cell-cell contact	- chemotherapeutics

Table 3.3. Altered rates of apoptosis observed in noncardiovascular and in cardiovascular disorders

Noncardiovascular disorders	
Increased rate	**Diminished rate**
- AIDS	- malignancies
- IDDM I*	- autoimmune disease
- myelodysplastic syndrome	- viral infection
- ischemic lesions	
- neurodegenerate disorders	

*: IDDM-I: insulin dependent diabetes mellitus Type I

Cardiovascular disorders
Increased rate of apoptosis
- myocardial infarction
- reperfusion damage
- arterial hypertension
- arrhythmogenic right ventricular dysplasia
- dilated cardiomyopathy
- atherosclerotic plaque
- restenosis after ballooning

from patients who died due to motor accidents the morphologic features of apoptosis were increased 232-fold. In confocal microscopy DNA-strand breaks were associated with chromatin condensation and fragmentation.[3] Other groups presented evidence of apoptosis in myocarditis, left ventricular hypertrophy and coronary artery dilatation. Thus, apoptosis may play a major role in cardiomyocyte cell death and may help to explain some of the uniform reactions of the heart towards a wide variety of damaging agents or environmental factors.

Experimental and Clinical Evidence of Apoptosis in Acute Myocardial Infarction and Reperfusion

During the last few years increasing evidence became available from animal experiments and from human autopsy studies showing a role of apoptosis in myocardial ischemia with or without reperfusion. In isolated cardiac myocytes the hypoxia-induced decrease in intracellular glutathione seems to be responsible for the reoxygenation-dependent c-jun kinase

Table 3.4. Methods for the detection of apoptosis in isolated cells or tissues

Morphologic methods:
- Electron microscopy - TUNEL-Assay using peroxidase, alkaline phosphatase, or fluorescein labeling - Detection of DNA fragments in flow cytometry
Chemical methods detecting nuclear strand breaks:
- ELISA for histone detection (cell death detection assay) - Laddering phenomenon in gel electrophoresis - Binding of labeled Annexin V to phosphatidylserine in flow cytometry
Detection of regulatory proteins (e.g., Bcl-2, Bax, Fas) by antibody-based detection in cells (light microscopy) or tissue homogenates (ELISA) or Western blot

activation, which is selectively inhibited by tyrosine kinase inhibitors or N-acetyl cysteine.[4] A number of extracellular mediators and intracellular signaling proteins have been identified as contributing factors to cardiomyocyte apoptosis. Physiologic concentrations of TNF-alpha induced apoptosis in isolated rat cardiomyocytes probably causing apoptosis by activating the endogenous mediator sphingosine.[5] Other authors suggested the involvement of intracellular signaling proteins like p53,[6] Fas[7] and others.[8] However, these data were obtained in vitro using isolated rat cardiomyocytes in most studies. Thus, the physiologic relevance of these findings for the pathophysiologic sequealae of events in human myocardial ischemia and reperfusion is still unclear. In addition to the in vitro experiments there are a number of reports from animal experiments in vivo concerned with the detection of apoptosis during myocardial ischemia and/or reperfusion. It is not entirely clear yet if ischemia alone is a sufficiently strong stimulus to induce apoptosis. Gottlieb et al[9] were not able to detect signs of apoptosis in rabbit myocardial tissue undergoing permanent ischemia for 4.5 hours. In contrast, if the myocardium was reperfused for 4 hours following a period of 30 min of ischemia a substantial number of cardiomyocytes underwent apoptosis, detected either by gel electrophoresis, by in situ nick end labeling or electron microscopy. In transmission electron microscopy the pattern of nuclear chromatin condensation was markedly different between reperfused and permanently ischemic myocardium. Interestingly, induction of granulocytopenia in the rabbits did not alter the development of apoptosis, an effect very different from necrosis, which is highly dependent on neutrophil infiltration.[9] In contrast to the experiments performed in rabbit myocardial ischemia, rat myocardium seems to be more susceptible to ischemia for the induction of apoptosis. In a model of permanent coronary artery occlusion, Kajstura et al[10] analyzed infarct size and rate of apoptotic cells after 20 min-7 days. They report that apoptosis is the most prominent mechanism of cell death in the majority of cardiomyocytes within the first 2 days of infarction. This corresponds to the immunocytochemically detected increase in Bcl-2 and Fas expression, which are increased 18- and 131-fold.[10] The results reported by Fliss and Garringer[11] correspond quite well with the data referred to above, since Fliss and Garringer were able to show that development of apoptosis is present during the first hours of permanent coronary artery ligation in the rat; however reperfusion markedly accelerates and augments this process. Although the conditions in autopsy studies are far from being as standardized as compared to animal or in vitro experiments, there is growing evidence that apoptosis is a phenomenon also present in acute human myocardial infarction. Itoh et al[12] were able to identify apoptotic cells by nick end labeling and DNA

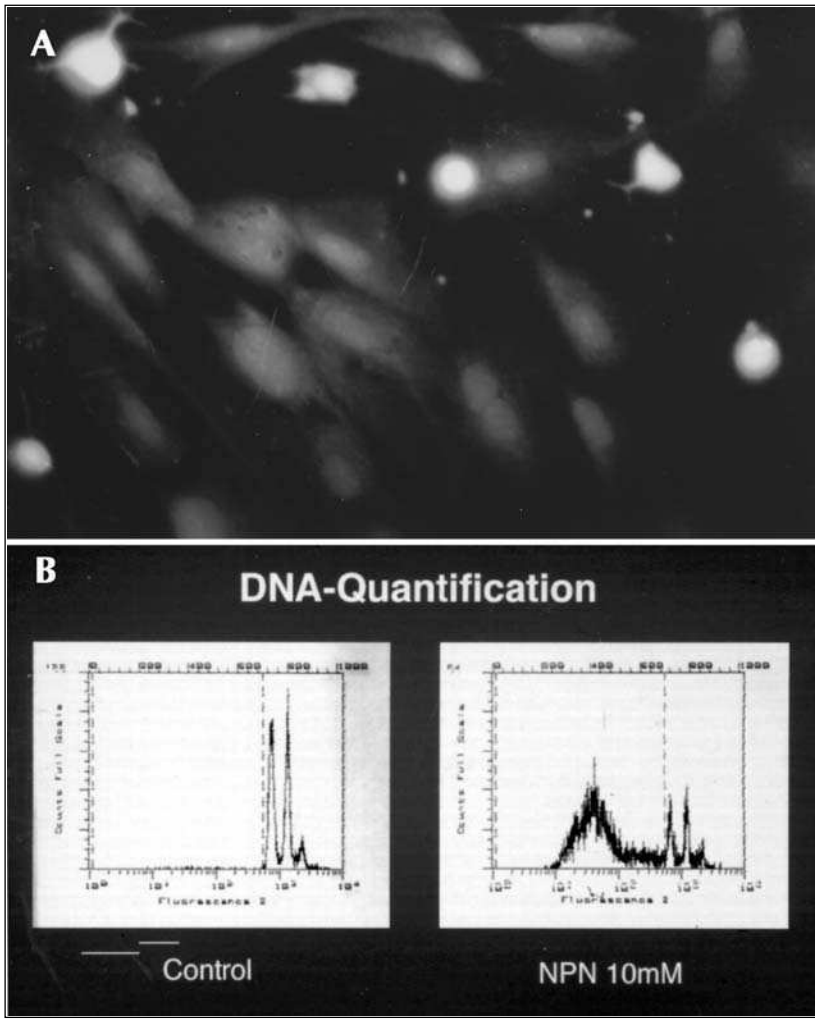

Fig. 3.1A.) Nuclear staining in rabbit vascular smooth muscle cells following incubation of the cells with actinomycin D. Nuclei show chromatin condensation and the formation of apoptotic bodies. B.) Flow cytometric DNA analysis of rabbit vascular smooth muscle cells. On the left panels cells under control conditions exert their DNA in haploid or diploid manner, with very few cells being in the S-phase (peak to the right). Following incubation with very high concentrations of nitroprusside sodium (NPN, 10 mM) cells undergo apoptosis and the number of haploid and diploid cells decreases markedly, with many cell fragments exerting DNA strands of varying length (peak to the left).

agarose gel electrophoresis in hearts of patients who died during acute myocardial infarctions. In their histologic examinations they observed most apoptotic cells in areas staining strongly with hematoxylin-eosin, commonly accepted as an indicator of necrosis. Other groups[13] suggested, that the early signs of apoptosis in human myocardium are already visible after about two hours of myocardial ischemia, thus possibly representing an early histologic sign of acute myocardial infarction. Saraste et al[14] analyzed human autopsy specimens and found that apoptotic cells were present primarily in the border zone of histologically infarcted

myocardium. Only very few apoptotic cells were detectable in areas remote to the infarct zone. The authors speculate that apoptosis may provide a possible target for therapeutic nterventions during evolving myocardial infarction in humans. Some of the intracellular signaling proteins possibly involved in human cardiomyocyte cell death during acute myocardial infarction were identified by Misao et al.[15] They found a very high incidence of Bcl-2 positive cells, an inhibitor of apoptosis, in areas salvaged by thrombolysis immediately adjacent to infarcted tissue. In contrast, in old myocardial infarctions they found almost no Bcl-2 positive cells. However, immunoreactivity for Bax, an accelerator of apoptosis, showed a very high incidence in old infarctions, whereas only 2 of 15 hearts with acute MI showed Bax positive specimens.

Prevention of Apoptosis During Ischemia and Reperfusion

In a rat model of acute myocardial ischemia and reperfusion, M. Buerke was able to demonstrate in two different sets of experiments performed in Allan M. Lefers group and in our group the prevention of apoptosis by pharmacologic interventions given immediately prior to initiation of reperfusion.[16,17] In ether anesthetized rats the heart was exteriorized briefly and a silk slip knot was placed around the left coronary artery. The heart was returned immediately to the chest cavity and the lungs excavated in order to allow the animals to breathe spontaneously. Following 20 min of ischemia the slip knot was removed to initiate reperfusion for 24 hours. Infarct size was quantitated by measuring the creatine kinase (CK) loss within the left ventricular free wall (ischemic region) if compared to the CK content in the septum i.e., nonischemic region. Leukocyte infiltration was measured by myeloperoxide content or visual analysis of histologic slides stained with hematoxylin-eosin. Histologic analysis was carried out in additional rats whose hearts were perfusion fixed after 24 and 48 hrs of reperfusion. Immunohistochemical staining for apoptosis was carried out by direct immunoperoxidase detection of digoxigenin labeled genomic DNA by the TUNEL reaction using 3'-OH end labeling with terminal deoxynucleotidyl transferase.

In nontreated rats ischemia and reperfusion resulted in a significant loss of creatine kinase activity from the ischemic area in the range of 570 IU/100 mg protein. This CK loss was almost completely prevented if the rats were pretreated with an intraperitoneal injection of insulin-like growth factor (10 μg) one hour prior to coronary artery ligation. A comparable but somewhat less pronounced cardioprotective effect was seen dose-dependently with injection of up to 100 μg/kg bw of C1-esterase inhibitor 2 min prior to initiation of reperfusion, preventing the activation of the classical complement pathway (Fig. 3.2). Similar cardioprotective effects of C1-esterase inhibitor have been described previously in a cat and a porcine model of myocardial ischemia and reperfusion.[17-19] Interestingly, both cardioprotective agents studied very effectively prevented neutrophil infiltration into the ischemic area as evidenced by a significantly reduced myeloperoxidase content, which was confirmed by histologic analysis. In both studies the prevention of neutrophil infiltration was thought to be intimately involved in the reduction of reperfusion damage or were even thought to represent the mechanisms of action (Fig. 3.2).

In both sets of experiments histologic evidence of apoptosis was obtained in the ischemic areas of vehicle treated rats and a high number of TUNEL positive i.e., cells undergoing apoptosis was identified. In the IGF-1 experiments sham operated rats had only 4% of apoptotic nuclei identified, whereas rats undergoing myocardial ischemia and reperfusion exerted up to 62% positive cells. The number of TUNEL positive nuclei was significantly reduced by treating the rats prior to reperfusion with 1 μg of IGF-1, which reduced the apoptotic cells to about 28%.[19] In a series of experiments with inhibition of complement activation, the number of TUNEL positive cells increased from 3% in sham operated animals to about 33% in rats undergoing ischemia and reperfusion. This substantial increase in

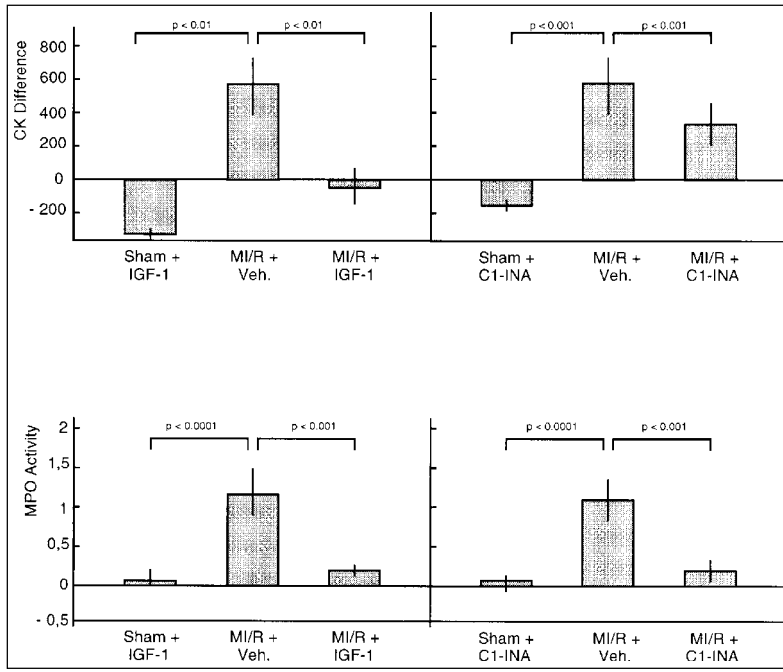

Fig. 3.2. Upper panel: Infarct size in rats with myocardial ischemia for 20 min and reperfusion for 24 hours measured as creatine kinase (CK) difference between the left ventricular free wall (ischemic area) and the interventricular septum (nonischemic area). Rats were treated by intraperitoneal injection of insulin like growth factor (IGF-1), 10 μg given 1 hr prior to coronary artery ligation or C1-esterase inhibitor (C1-INH) 100 μg/kg 2 min prior to reperfusion (data taken from references 16 and 19). Lower panel: Neutrophil infiltration in the infarcted myocardium quantified by measuring the activity of the neutrophil specific enzyme myeloperoxidase. Treatment with IGF-1 or C1-INH significantly reduced the neutrophil infiltration into the area at risk, a process thought to be the mechanism of action for the diminished reperfusion damage demonstrated by the reduced CK loss. (Data taken from references 16 and 19)

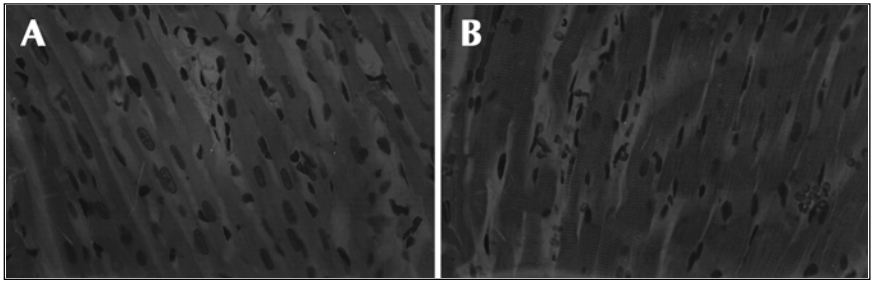

Fig. 3.3. TUNEL reaction positive cardiomyocytes in the border zone of infarcted rat myocardium. Rats underwent 20 min of ischemia and 24 hours of reperfusion. A: Apoptosisim vehicle treated animals; B: reduced apoptosis in rats treated with C1-INH.

apoptotic cells was significantly reduced in C1-esterase inhibitor treated animals to 13% (Fig. 3.3).

References

1. Mallat Z, Tedgui A, Fontalioran F et al. Evidence of apoptosis in arrhythmogenic right ventricular dysplasia. N Engl J Med 1996; 335:1190-11962.
2. Narula J, Haider N, Virmani R et al. Apoptosis in myocytes in end-stage heart failure. N Engl J Med 1996; 335:1182-9.
3. Olivetti G, Abbi R, Quaini F et al. Apoptosis in the failing human heart. N Engl J Med 1997; 336:1131-41
4. Laderoute KR, Webster KA. Hypoxia/reoxygenation stimulates Jun kinase activity through redox signaling in cardiac myocytes. Circ Res 1997; 80:336-3445.
5. Krown KA, Page MT, Nguyen C et al. Tumor necrosis factor alpha-induced apoptosis in cardiac myocytes. Involvement of the sphingolipid signaling cascade in cardiac cell death. J Clin Invest 1996; 98:2854-65.
6. Long X, Boluyt MO, Hipolito ML et al. p53 and the hypoxia-induced apoptosis of cultured neonatal rat cardiac myocytes. J Clin Invest 1997; 99:2635-43.
7. Tanaka M, Ito H, Adachi S et al. Hypoxia induces apoptosis with enhanced expression of Fas antigen messenger RNA in cultured neonatal rat cardiomyocytes. Circ Res 1994; 75:426-33.
8. Gottlieb RA, Gruol DL, Zhu JY et al. Preconditioning in rabbit cardiomyocytes. Role of pH, vacuolar proton ATPase, and apoptosis. J Clin Invest 1996; 97:2391-2398.
9. Gottlieb RA, Burleson KO, Kloner RA et al. Reperfusion injury induces apoptosis in rabbit cardiomyocytes. J Clin Invest 1994; 94:1621-810.
10. Kajstura J, Cheng W, Reiss K et al. Apoptotic and necrotic myocyte cell deaths are independent contributing variables of infarct size in rats. Lab Invest 1996; 74:86-107.
11. Fliss H, Garringer D. Apoptosis in ischemic and reperfused rat myocardium. Circ Res 1996; 79:949-56.
12. Itoh G, Tamura J, Suzuki M et al. DNA fragmentation of human infarcted myocardial cells demonstrated by the nick end labeling method and DNA agarose gel electrophoresis. Am J Pathol 1995; 146:1325-31.
13. Bardales RH, Hailey LS, Xie SS et al. In situ apoptosis assay for the detection of early acute myocardial infarction. Am J Pathol 1996; 149:821-9.
14. Saraste A, Pulkki K, Kallajoki M et al. Apoptosis in human acute myocardial infarction. Circulation. 1997; 95:320-3.
15. Misao J, Hayakawa Y, Ohne M et al. Expression of bcl-2 protein, an inhibitor of apoptosis, and Bax, an accelerator of apoptosis, in ventricular myocytes of human hearts with myocardial infarction. Circulation 1996, 94:1506-151216.
16. Buerke M, Murohara T, Skurk C et al. Cardioprotective effect of insulin-like growth factor I in myocardial ischemia followed by reperfusion. Proc Natl Acad Sci USA 1995; 92:8031-5.
17. Buerke M, Murohara T, Lefer AM. Cardioprotective effects of a C1 esterase inhibitor in myocardial ischemia and reperfusion. Circulation 1995; 91:393-402.
18. Horstick G, Heimann A, Gotze O et al. Intracoronary application of C1 esterase inhibitor improves cardiac function and reduces myocardial necrosis in an experimental model of ischemia and reperfusion. Circulation 1997; 95:701-8.
19. Buerke M, Prüfer D, Dahm M et al. Inhibition of the classical complement pathway reduces reperfusion injury and induction of apoptosis. Eur Heart J 1996; 17:597 (Abstr.).

Chapter 4

Changes in Myocardial Gene Expression Following Ischemia and Reperfusion

John W. C. Entwistle III and Andrew S. Wechsler

The heart has the remarkable ability to adapt to a wide variety of physiological and pathological conditions. Some examples of change in the structure and function of the heart include normal growth and development, response to pressure overload, volume overload, hypothyroidism and ischemic injury. While the external manifestations of these changes are very different, they share several features at the subcellular level. The single common denominator is an alteration in expression of the myocellular proteins. However, the initiating signals and subsequent subcellular changes are diverse. This creates the situation in which the morphological changes seen in the myocardium and the resultant changes in function fall into one of several defined categories, such as hypertrophy or cardiomyopathy. It is likely that most if not all of the myocardial responses to injury have a significant component based on changes in the expression of genes encoding structural or functional proteins.

Ischemia and reperfusion (IR) have profound effects on the function and structure of the myocardium. At one extreme, prolonged ischemia results in cellular necrosis. Since the adult myocyte is incapable of division, the surviving cells must hypertrophy to restore contractile function. However, when the ischemic insult is less severe, the result is myocardial stunning, in which the injured myocytes exhibit temporary contractile dysfunction despite adequate substrate delivery. By definition, stunned myocardium will regain full mechanical function given time. The mechanism through which the stunned heart responds to such injury is largely unknown, but it appears that the basis for the cellular response to IR injury is at the molecular level. While the initiating event in stunning may be transient, it may also be involved in the initiation of a complex and coordinated cascade of molecular changes.

Several factors have been implicated in the onset of myocardial stunning, including the loss of high-energy phosphates, oxidative stress through the generation of oxygen free radicals, and altered myocardial cytosolic calcium levels and myofibrillar calcium sensitivity (see reviews by Bolli[1] and Kusuoka[2]). In addition, sympathetic overstimulation occurs during ischemia through neuronal release of catecholamines, which may cause direct myocellular damage, compounding the injurious effects (see review by Schömig[3]). These factors may play a significant role in both the initiation of stunning and in the initiation of the molecular changes responsible for the altered myocardial phenotype seen during recovery.

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Basics of Myocardial Gene Expression

Adaptive and maladaptive changes in the heart are both geometric and the consequence of altered protein content and/or configuration. The protein composition of the myocyte can be regulated at one or more sites. Pretranslational control mechanisms include those that affect the rate at which specific mRNAs are produced (transcriptional), or those that affect their processing, nucleocytoplasmic transport and stability (posttranscriptional). The selection of mRNAs to be synthesized into protein and the rate at which this occurs are types of translational control. Posttranslational regulation involves the processing, transportation and stability of the protein products. Control may be exerted at one or more levels for each mRNA and protein product. Some well-described examples of the regulation of myocardial gene expression include those seen during growth and development,[4-6] or associated with overload-induced myocardial hypertrophy.[7-12] In the fetal heart, myosin and α-actin are present as "fetal" isoforms. Atrial natriuretic factor is produced by both the atria and ventricles in the fetus, and the proteins of the fetal sarcoplasmic reticulum (SR) are downregulated.[6,13] After birth and during development, there is a gradual shift in the expressed MHC and actin isoforms to those typical of the adult heart. In addition, atrial natriuretic factor (ANF) production in the ventricles disappears, and the expression of SR gene products increases. Both hormonal and mechanical stimuli govern these changes. Hypertrophy in adult tissue in response to pressure overload, but not volume overload,[14] mimics gene expression in the fetal heart. This includes a switch in the dominant isoforms for myosin and α-actin, re-expression of ANF by the ventricles, and decreased expression of the sarcoplasmic and endoplasmic reticulum calcium ATPase-2 (SERCA2) and phospholamban (plb). Changes in myocardial gene expression occur in response to a variety of other stimuli, and each elicits a characteristic pattern of gene expression. This phenotypic change permits remodeling of the myocardium as an adaptive response to external signals.

The phenotypic changes involved in myocardial recovery from IR injury may take hours to days to be detectable when monitoring protein levels because of the slow rate of change in steady-state protein concentration. Many models of IR injury, such as the isolated heart, are not suited for long-term studies. Thus, analysis of steady-state mRNA levels has become a popular method for detecting alterations in gene expression early after physiologic events. The major assumption, however, is that protein levels will eventually mirror those of their respective mRNAs. When studied in models of acute injury, mRNA levels generally predict the direction of change in the corresponding protein, indicating that transcriptional control mechanisms are important in regulating the phenotype of the heart after injury.

Studies of altered gene expression after myocardial injury have utilized a variety of models, including cultured myocytes, in vitro beating hearts, and in vivo preparations. While changes in gene expression seen in isolated myocyte preparations are clearly the result of IR injury to the myocytes, this may not represent changes in the intact heart. Fibroblasts and endothelial cells are just a few of the other cellular components of the heart capable of responding to IR injury. Indeed, both of these cell types are extremely active in regulating their local environment and produce substances that act in a paracrine manner to influence the myocytes. Thus, while it may be scientifically interesting to isolate myocytes in an attempt to localize changes in gene expression, failure to account for interactions with other cell types and alterations of the mechanical properties of the myocyte represent an important shortcoming of such studies.

In intact heart models of IR injury, both regional and global models of IR have been employed. Regional models of ischemia and reperfusion generally treat the nonischemic border zones as normal control tissue. However, there is a distinct possibility that these areas are also affected by the IR injury as the mechanical stresses seen in the "control" areas

are altered during ischemia and reperfusion, until the function of the ischemic area recovers fully. In addition, paracrine or nervous stimulation may also occur in these "control" areas as a result of injury, further altering the milieu of the cells in the border zone.

Mechanisms of Gene Changes in Stunning

Stunning, when severe, may persist for several days before recovery of contractile function occurs, and extends beyond the termination of the initial ischemic stimulus. While the cellular and molecular mechanisms that underlie this delay remain unknown, this adaptive process in response to IR may involve specific qualitative changes in cell phenotype that are the consequence of changes in myocardial gene expression. As the phenotype of the heart is altered, these changes could alter the function and/or cellular morphology of the stunned heart, and hence prolong recovery. Two putative causes of stunning are increased cytosolic calcium levels and alpha-adrenergic receptor stimulation. Both are potent signals that govern transcriptional activity of genes important for contractility and hypertrophy.[15-17] Thus, the events that initiate contractile dysfunction seen in stunning may also be stimuli for alterations in gene expression that may govern the rate and manner of myocardial recovery. Therefore, delayed recovery from contractile dysfunction may reflect changes in myocardial gene expression that are either a maladaptive response to noxious stimuli or an adaptive response to the hypertrophy signals from mechanical stress induced by stunning.

Calcium as a Regulator of Gene Expression

Calcium is an important second messenger in the cell, with a variety of effects ranging from a role in contraction to transcriptional control. Many genes are responsive to changes in the free calcium concentration in the cell. Calcium may bind to calmodulin, a calcium-binding regulatory protein. The calcium-calmodulin complex alters the activity of target proteins, including several kinases. Ca^{2+}/calmodulin-dependent protein kinase (CAM kinase) phosphorylates the Ca^{2+}/cAMP response element binding protein (CREB). The phosphorylated CREB then can bind to the Ca^{2+}/cAMP response element (CRE) in the promoter region of target genes, and influence the rate of transcription of those genes. Addition of the calcium ionophore A23187 to cultured cardiac myocytes induces an increase in the steady state mRNA level for the immediate early gene (IEG) c-fos.[18] c-fos and the other IEGs are transcription factors. Once expressed, these IEGs affect the rate of transcription of numerous other genes through binding to the TPA (12-O-tetradeconoylphorbol-13-acetate) response element (TRE) in the promoter region of its target genes. The CRE and TRE are found in the promoter region of many genes. Thus, the effects of calcium on gene expression, both directly through the CRE, and indirectly through specific IEGs and TRE, may be widespread.

Alpha-Adrenergic Receptor Stimulation as a Regulator of Gene Expression

The α_1-adrenergic receptor has been linked directly to changes in the expression of several genes involved in cardiac growth and development.[19;20] It may serve as one of the primary instigators in altering gene expression following pressure-overload or other forms of cardiac injury. In cultured neonatal rat ventricular cardiomyocytes, α_1-adrenergic stimulation with phenylephrine (PE) induced the production and release of atrial natriuretic factor (ANF) within 12 hours, with increases in ANF mRNA evident by 6 hours.[19] In addition to regulating ANF expression, PE induced expression of the myosin light chain-2 transcript, but failed to affect the mRNA level for the cardiac-specific sodium channel.[21] This indicates that induction of transcription by α_1-adrenergic receptor stimulation has specificity and is not a generalized process. α_1-adrenergic stimulation has a comprehensive effect on cardiac gene expression. PE exerts its effect on gene expression through a consensus

sequence in the promoter region of the responsive genes. In the ANF promoter, the sequence conferring PE-responsiveness has been identified and termed the phenylephrine response element (PERE). α skeletal actin (αSkA) and β-MHC contain homologous regions corresponding to the PERE of ANF.[22] The α_1-adrenergic system is thought to be at least partly responsible for the development of cardiac hypertrophy,[23,24] and it is characterized by increases in αSkA, β-MHC and ANF, which all respond to PE stimulation.

Myocellular Stretch as a Regulator of Gene Expression

In addition to changes in gene expression induced through chemical mediators such as calcium and α-adrenergic receptor agonists, mechanical factors such as myocyte stretch may play a role in myocardial remodeling and associated changes in myocardial gene expression. Myocardium in globally stunned hearts may be subject to abnormal stresses that elicit molecular markers typical of stretch-induced hypertrophy. For example, decreased contractility may lead to chamber dilatation. Stretch imposed upon cardiac myocytes alters the expression of several myocardial genes, including those encoding heat shock protein 68 (HSP68),[25] c-fos,[25,26] c-myc,[25] αSkA,[27] and β-MHC.[25] Ventricular loading, a determinant of stretch, is an independent regulator of gene expression in the absence of stunning. Both ventricular overloading, as in pressure overload hypertrophy, and underloading[28-30] have been linked to changes in myocardial gene expression. Such changes can be modulated by pacing the heterotopically transplanted heart.[31] These findings collectively suggest that the load upon the ventricle during reperfusion may affect the molecular phenotype of the stunned myocardium.

Changes in Gene Expression in Ischemia and Stunning

Regardless of the initiating signal(s) involved in stunning, the signals that alter the myocellular phenotype at a cellular and molecular level are important. Whether myocellular stretch, intracellular calcium concentrations, and/or catecholamine stimulation of the myocyte act together or independently in regulation of gene expression in stunned hearts is not well understood. These factors, however, initiate a complex series of events that eventually result in altered functional and structural characteristics. Among the first changes seen are induction of stress proteins and immediate-early genes (IEGs). Stress proteins serve to help the heart survive the initial insult, while the IEGs are transcription factors that direct the expression of a multitude of other genes under their control. Some of the genes with altered expression are structural and functional proteins which result in a new cellular phenotype. These changes in gene expression alter the functional characteristics of the myocyte and thus influence the way in which the heart recovers from injury.

Changes in Stress Proteins and IEGs

There is a large, highly conserved, family of proteins whose expression is altered under times of cellular stress. These stress-response proteins act to improve cellular survival under adverse conditions, and include members of the heat shock protein (HSP) and glucose-responsive protein (GRP) families. Hsp70 gene products are thought to assist in the import of nascent polypeptides into mitochondria[32] and to act as chaperones in the folding of denatured proteins.[33] Changes in levels of mRNA encoding heat shock proteins have been well established in hearts subjected to ischemia and reperfusion.[34-37]

The glucose response protein grp78 is another stress-response protein. Grp78 is a constitutively expressed protein of the endoplasmic reticulum (ER) that is induced during glucose starvation or increased calcium levels, and it affects protein translocation and folding (see review by Haas[38]). Thus, within the ER, grp78 appears to have a role similar to that of hsp70.[39,40] Despite this similarity, changes in expression of grp78 have not been seen in global IR injury.[37,41] However, we have

recently demonstrated an increase in the steady-state mRNA levels encoding grp78 only under conditions of ventricular unloading during reperfusion. Thus, while the hsp70 and grp78 share similar functional characteristics, the signals responsible for their expression in IR injury differ. This is consistent with previous data that some signals may coordinately or discordantly regulate hsp and grp expression.[42] The result, however, is that IR injury induces a pattern of expression amongst the stress-responsive proteins that appears to be protective in nature.

The immediate-early genes (IEGs) are nuclear transcription factors that are involved in the regulation of gene expression in normal growth and development, as well as in alterations of gene expression seen after injury. The net result of IEG expression is determined by the relative levels of the individual IEGs (see review by Herschman[43]). Members of the fos and jun families exert their actions as dimers. c-fos and jun-c are able to form heterodimers that bind to the AP-1 sequence(s) in the promoter region of certain genes. The presence of these binding sites provides the mechanism through which alterations in IEG expression change expression of structural cellular genes and thus alter the phenotype of the injured cell. The c-fos/jun-c dimer may regulate cardiac gene expression in a positive[44] or negative[45] manner, depending on the specific gene of interest. When the IEG jun-B binds to jun-c, c-fos binding activity of the dimer is inhibited.[46,47] Altered expression of the IEGs has been demonstrated in IR injury, with increases in the steady-state levels of mRNA encoding jun-c,[41,48-50] jun-B[48,50] c-fos,[37,41,48,50] egr-1[50] and c-myc.[41] However, the IEG induction is clearly not universal in IR injury as no significant induction was detected in the levels of mRNAs encoding the IEGs egr-1[41,48] or c-myc.[37,50-52]

Induction of IEGs in response to injury provides a mechanism through which an initial extracellular signal is transduced to upregulate other transcription factors which can then orchestrate further changes in gene expression, culminating in altered mechanical properties of the heart. Rapid induction of IEGs has been demonstrated after acute pressure overload of the ventricle,[53-55] regional stunning,[50] and in global myocardial ischemia and reperfusion.[37,51] The timing and pattern of IEG induction varies with the nature of the injury. The complicated interactions between the different transcription factors may in part dictate the response of the cell to ischemia/reperfusion injury, and the pattern of the IEG response may be responsible for the different phenotypic changes seen in different models of cardiac injury. Due to the complex interactions between the different IEGs, the temporal pattern of IEG expression has important implications in the resulting effects on the regulation of structural genes.

In global IR injury, we have demonstrated a temporal pattern of IEG expression, with different IEG mRNA profiles at 1 and 2 hours of reperfusion.[41] This pattern of rapid changes in levels of mRNA encoding individual IEGs has also been demonstrated in regional ischemia and reperfusion.[50]

In regional ischemia and reperfusion, expression of stress-related genes is dependent upon the region of the heart examined. Increased expression of the IEGs c-fos and jun-c, and the heat shock proteins hsc70 and hsp70 are limited to the ischemic area after IR injury, as demonstrated by in situ hybridization. However, mRNA encoding the IEG jun-B is seen in both the ischemic and nonischemic border zones.[48] Temporal and regional differences in IEG expression are important since the net result of IEG expression depends upon the ratio between jun-c, jun-B and c-fos, due to the interactions between these proteins.[46,47] However, altered IEG expression is only meaningful in that they directly impact upon the expression of other proteins within the cell that have functional importance.

Changes in Growth Factors

While the most important function of the heart is supplying blood and nutrients to the body, it also is the source of a variety of factors that influence the activity of other cells. Interleukin-8 (IL-8) affects adhesion of neutrophils to cardiomyocytes and results in cytotoxicity of myocytes in vitro. IL-8 mRNA

is increased after in vivo regional IR injury, suggesting that locally produced factors play a deleterious role in IR injury.[56] Similar increases in the mRNAs encoding IL-6, tumor necrosis factor alpha (TNF-alpha), and IL-1beta have been shown in the ischemic zone of hearts following 1-3 hours of reperfusion.[57] The mRNA encoding monocyte chemoattractant protein-1 (MCP-1), which may play a role in monocyte trafficking into IR-injured myocardium, is increased in the endothelium of small veins within the heart following IR injury.[58] While MCP-1 induction is not demonstrated in the myocytes themselves, the action of MCP-1 affects the environment of the myocytes, thus influencing recovery and function. A further link between IR injury and the initiation of the inflammatory response following acute myocardial ischemia is seen in the increase in the mRNA encoding endothelial leukocyte adhesion molecule 1 (E-selectin). E-selectin mRNA levels are not influenced by ischemia alone, but increase only after reperfusion.[59] In addition, intracellular adhesion molecule-1 (ICAM-1) has been implicated in neutrophil-mediated myocardial injury following IR. In situ hybridization techniques have been used to demonstrate that ICAM-1 mRNA levels are increased in ischemic myocardium within 3 hours of reperfusion, and are increased in nonischemic areas within the same heart by 24 hours.[60] The induction of ICAM-1 in the border zones may increase the area of injury due to additional damage to viable cells from the inflammatory process.

Endothelin-1 (ET-1), a potent vasoconstrictive peptide, is increased systemically after myocardial infarction. Some of the circulating ET-1 may be cardiac in origin, as the mRNA encoding ET-1 is increased in injured myocardium after 90 minutes of regional ischemia and 150 minutes of reperfusion.[61] This increase in mRNA levels is correlated with an increase in immunoreactivity for ET-1 in the area. These data demonstrate that changes in cardiac gene expression may play a substantial role in the overall response of the organism to myocardial stunning.

Cells within the heart are capable of producing a variety of growth factors under physiologic conditions. In addition, the heart appears to respond to IR injury through increased production of some of these factors. Insulin-like growth factor (IGF) has been implicated in DNA synthesis and cellular proliferation. Increases in IGF-1 and its receptor, IGF-1R, are seen in surviving myocytes after regional infarction.[62] In contrast, mRNA encoding IGF-2 is increased by operative, but not IR, stress following repetitive brief coronary artery occlusions, while the mRNA encoding one receptor subtype, IGF binding protein-5 (IGFBP-5), is increased following IR injury.[63] Since IGF exerts its action through IGFBP, the induction of the receptor may play an important role in the adaptive processes that occur in the heart en route to recovery. Similarly, the heart undergoing transient ischemia and reperfusion responds with an increase in mRNA encoding vascular endothelial growth factor (VEGF).[64,65] Local inducible VEGF production following IR injury may be the mechanism for coronary collateral development in ischemia. In addition, mRNAs encoding hepatocyte growth factor (HGF) and its receptor c-Met, which have been implicated in tissue regeneration and angiogensis, are increased after IR injury.[66] While HGF mRNA levels are increased in endothelial cells and interstitial cells, mRNA for c-Met was increased only in capillary endothelial cells. Following regional myocardial infarction, mRNA encoding the growth factor transforming growth factor β1 (TGF-β1) is increased after 24-48 hours.[67] The increase is localized to myocytes, whereas increase in mRNA for heparin-binding growth factor 1 (HBGF-1) is increased in the walls of blood vessels.[52] While the cells of origin of many of these growth factors are not myocytes, myocytes are influenced by the altered chemical environment that results.

Ischemia and reperfusion is associated with increased production of several factors that regulate the way in which the myocardium responds to its local environment. While these changes may be important in the injury response of the heart, the changes within the structure and function of the myocytes may

be even more important to the survival of the organism.

Changes in Structural or Functional Proteins

Alterations in either the amount or isoform prevalence of a structural or functional myocardial protein can have a significant impact on the contractile properties of the heart. This has been extensively illustrated in the contractile changes shown in association with normal development, acute injury, and end-stage heart disease. The mechanisms of myocardial recovery after IR injury may involve similar changes in the expression of structural or functional myocardial proteins.

We have studied changes in the expression of several proteins of the sarcoplasmic reticulum (SR). These include the SR calcium ATPase (SERCA2) responsible for calcium uptake into the SR, its regulatory protein, phospholamban, and the SR calcium release channel, the ryanodine receptor (RyR). Changes in the ratio of SERCA2 to phospholamban are associated with altered contractile properties of the myocardium during normal myocardial development or end-stage failure. After global IR injury, there is discordant regulation of the mRNA species encoding these proteins, such that altered SERCA2 to phospholamban ratio results.[41] Over time, these changes in steady state mRNA levels would be reflected in alterations at the protein level, which would produce altered contractile properties in the recovering heart. In contrast to the changes seen after a single period of global IR injury, a different pattern of change is seen after brief periods of repetitive ischemia in both a porcine model of regional IR[68] and rabbit model of global IR injury.[69] Thus, the manner in which IR injury occurs may influence the direction of change for individual mRNA species. Finally, we have found that the manner of reperfusion significantly impacts the expression of SR proteins. Ventricular unloading during reperfusion ameliorates changes in SR gene expression seen in global stunning.[41] Because alterations in the relative ratios of SR calcium regulatory proteins can have a dramatic impact upon intracellular calcium flux and therefore myocardial contraction, these data provide a possible mechanism to explain prolonged contractile dysfunction after global stunning.

Energy production and consumption are markedly altered in ischemic and postischemic myocardium. The expression of several genes involved in these processes is also altered in IR injury and may be related to some of the alterations observed. In regional ischemia, the mRNA encoding the cardiac glucose transporter GLUT1 is increased in both ischemic and nonischemic myocardium, whereas that for GLUT4 is unaltered.[70,71] The cardiac ATP-sensitive potassium channel (KATP) serves to protect the heart during ischemia. Its mRNA is increased in myocardium subjected to prolonged periods (60 minutes) of regional-ischemia followed by 24 hours of reperfusion.[72] While such changes cannot influence the immediate recovery of stunned myocardium, they may regulate the rate and degree of recovery.

The changes in gene expression seen in IR injury are selective. Most genes investigated show no significant change in mRNA levels. In addition, the mRNA levels of some genes are depressed by ischemia and reperfusion. However, this may be more difficult to detect in the early period following injury. Since the half-life of most mRNA species is measured in hours, an early decline in mRNA levels may signify an active degradation of the message as well as a decrease in active production of that message. The mRNA for creatine kinase M is decreased 40% in ischemic myocardium, whereas that encoding myosin heavy chain is unaltered.[36]

Implications of Changes in Gene Expression

The cellular stress response is a complex orchestration of molecular signals that allow the cell to adapt to a rapidly changing environment. These signals are ubiquitous, and their expression varies at different times during growth and development and at times of cellular stress or injury. It includes members

of the heat shock family of proteins and nuclear transcription factors. Ischemia and reperfusion injury has been associated with altered expression of several genes of this class.

Changes in myocardial gene expression have been demonstrated in a wide variety of injury models, suggesting that this phenomenon is a vital part of the adaptive mechanisms of the stressed heart. In particular, changes in contractile function have been correlated with altered expression of functional proteins, such as the genes encoding SR proteins. Indeed, changes in the expression of mRNAs for SR proteins have been noted in hypothyroidism,[7,73] hyperthyroidism,[7,73,74] ventricular hypertrophy following overload[7,8,75] and in congestive heart failure. In failed human hearts, decreased mRNA encoding SERCA2 was correlated with a decrease in the cardiac index prior to explantation.[76] Thus, changes in expression of SR gene products in several models of myocardial remodeling are accompanied by altered contractile properties, consistent with the importance of the SR phenotype in myocardial function.

Conclusions

Myocardial stunning has at least two major components: functional and molecular. The functional component is the reduced contractility of the myocardium and depressed organellar (mitochondrial and SR) function seen after a significant but nonlethal injury. There is also a myocardial molecular response to stunning. This coordinated molecular response reflects the degree of myocardial injury, since interventions known to improve myocardial function after global ischemia result in alterations in the pattern of this response. Given that amelioration of stunning is associated with specific changes in this molecular response, we propose that the injured myocardium has initiated a reparative program to restore proper myocardial function.

References

1. Bolli R. Mechanism of myocardial "stunning". Circ. 1990; 82:723-738.
2. Kusuoka H, Marban E. Cellular mechanisms of myocardial stunning. Ann Rev Physiol 1992; 54:243-256.
3. Schomig A. Catecholamines in myocardial ischemia: Systemic and cardiac release. Circ 1990; 82:II-13-II-22
4. Lompre AM, Anger M, Levitsky D. Sarco(endo)plasmic reticulum calcium pumps in the cardiovascular system: Function and gene expression. J Mol Cell Cardiol 1994; 26:1109-1121.
5. Moorman AFM, Vermeulen JLM, Koban MU et al. Patterns of expression of sarcoplasmic reticulum Ca^{2+}-ATPase and phospholamban mRNAs during rat heart development. Circ Res 1995;76:616-625
6. Brillantes AMB, Bezprozvannaya S, Marks AR. Developmental and tissue-specific regulation of rabbit skeletal and cardiac muscle calcium channels involved in excitation-contraction coupling. Circ Res 1994; 75: 503-510.
7. Nagai R, Zarain-Herzberg A, Brandl CJ et al. Regulation of myocardial Ca^{2+}-ATPase and phospholamban mRNA expression in response to pressure overload and thyroid hormone. Proc Natl Acad Sci USA 1989; 86: 2966-2970.
8. de la Bastie D, Levitsky D, Rappaport L et al.Function of the sarcoplasmic reticulum and expression of its Ca^{2+}-ATPase gene in pressure overload-induced cardiac hypertrophy in the rat. Circ Res 1990; 66:554-564.
9. Lattion AL, Michel JB, Arnauld E et al. Myocardial recruitment during ANF mRNA increase with volume overload in the rat. Am J Physiol 1986; 251:H890-H896.
10. Mercadier JJ, Samuel JL, Michel JB et al. Atrial natriuretic factor gene expression in rat ventricle during experimental hypertension. Am J Physiol 1989; 257:H979-H987.
11. Black FM, Packer SE, Parker TG et al. The vascular smooth muscle α-actin gene is reactivated during cardiac hypertrophy provoked by load. J Clin Invest 1991; 88:1581-1588.
12. Schiaffino S, Samuel JL, Sassoon D et al. Nonsynchronous accumulation of α-skeletal actin and β-myosin heavy chain mRNAs during early stages of pressure-overload-induced hypertrophy demonstrated by in situ hybridization. Circ Res 1989;64:937-948.
13. Knowlton KU. Atrial natriuretic factor: A molecular marker for cardiac specific, embryonic, and inducible gene expression. Heart Failure 1992; June/July:121-128.

14. Schaub MC, Hefti MA, Harder BA et al. Various hypertrophic stimuli induce distinct phenotypes in cardiomyocytes. J Mol Med 1997; 75:901-920.
15. Bishopric NH, Kedes L. Adrenergic regulation of the skeletal α-actin gene promoter during myocardial cell hypertrophy. Proc Natl Acad Sci USA 1991; 88:2132-2136.
16. Bishopric NH, Simpson PC, Ordahl CP. Induction of the skeletal α-actin gene in α_1-adrenoceptor-mediated hypertrophy of rat cardiac myocytes. J Clin Invest 1987;80:1194-1199.
17. Waspe LE, Ordahl CP, Simpson P. The cardiac β-myosin heavy chain isogene is induced selectively in α_1-adrenergic receptor-stimulated hypertrophy of cultured rat heart myocytes. J Clin Invest 1990;85:1206-1214.
18. Sadoshima J, Izumo S. Mechanical stretch rapidly activates multiple signal transduction pathways in cardiac myocytes: Potential involvement of an autocrine/paracrine mechanism. EMBO J 1993;12:1681-1692.
19. Knowlton KU, Baracchini E, Ross RS et al. Coregulation of the atrial natriuretic factor and cardiac myosin light chain-2 genes during α-adrenergic stimulation of neonatal rat ventricular cells: Identification of *cis* sequences within an embryonic and a constitutive contractile protein gene which mediate inducible expression. J Biol Chem 1991; 266: 7759-7768.
20. Springhorn JP, Ellingsen O, Berger H-J et al. Transcriptional regulation in cardiac muscle: Coordinate expression of Id with a neonatal phenotype during development and following a hypertrophic stimulus in adult rat ventricular myocytes in vitro. J Biol Chem 1992; 267:14360-14365.
21. Shubeita HE, McDonough PM, Harris AN et al. Endothelin induction of inositol phospholipid hydrolysis, sarcomere assembly, and cardiac gene expression in ventricular myocytes: A paracrine mechanism for myocardial cell hypertrophy. J Biol Chem 1990; 265: 20555-20562.
22. Ardati A, Nemer M. A nuclear pathway for α_1-adrenergic receptor signaling in cardiac cells. EMBO J 1993;12:5131-5139.
23. Simpson P. Norepinephrine-stimulated hypertrophy of cultured rat myocardial cells is an α_1-adrenergic response. J Clin Invest 1983; 72:732-738.
24. Simpson P, Bishopric N, Coughlin S et al. Dual trophic effects of the $alpha_1$-adrenergic receptor in cultured neonatal rat heart muscle cells. J Mol Cell Cardiol 1986; 18:45-58.
25. Delcayre C, Klug D, Van Thiem N et al. Aortic perfusion pressure as early determinant of β-isomyosin expression in perfused hearts. Am J Physiol 1992;263:H1537-H1545
26. Komuro I, Kaida T, Shibazaki Y et al. Stretching cardiac myocytes stimulates protooncogene expression. J Biol Chem 1990; 265:3595-3598.
27. Komuro I, Katoh Y, Kaida T et al. Mechanical loading stimulates cell hypertrophy and specific gene expression in cultured rat cardiac myocytes: Possible role of protein kinase C activation. J Biol Chem 1991; 266:1265-1268.
28. Samarel AM, Engelmann GL. Contractile activity modulates myosin heavy chain-β expression in neonatal rat heart cells. Am J Physiol 1991; 261:H1067-H1077.
29. Klein I, Ojamaa K, Samarel AM et al. Hemodynamic regulation of myosin heavy chain gene expression: Studies in the transplanted rat heart. J Clin Invest 1992; 89:68-73.
30. Buchwald A, Klein HH, Lindert S et al. Effect of intracoronary superoxide dismutase on regional function in stunned myocardium. J Cardiovasc Pharm 1989; 13:258-264.
31. Geenen DL, Malhotra A, Buttrick PM et al. Increased heart rate prevents the isomyosin shift after cardiac transplantation in the rat. Circ Res 1992; 70:554-558.
32. Gambill BD, Voos W, Kang PJ et al. A dual role for mitochondrial Heat Shock Protein 70 in membrane translocation of preproteins. J Cell Biol 1993; 123:109-117.
33. Lindquist S, Craig EA. The heat shock proteins. Ann Rev Genet 1988; 22:631-677.
34. Knowlton AA, Brecher P, Apstein CS. Rapid expression of heat shock protein after brief cardiac ischemia. J Clin Invest 1991; 87: 139-147.
35. Dillman WH, Mehta HB, Barrieux A et al. Ischemia of the dog heart induces the appearance of a cardiac mRNA coding for a protein with migration characteristics similar to heat-shock/stress protein 71. Circ Res 1986; 59:110-114.
36. Mehta HB, Popovich BK, Dillman WH. Ischemia induces changes in the level of mRNAs coding for stress protein 71 and creatine kinase M. Circ Res 1988; 63:512-517.
37. Wechsler AS, Entwistle JCI, Yeh T Jr et al. Early gene changes in myocardial ischemia. Ann Thorac Surg 1994; 58:1282-1284.
38. Haas IG. BiP (GRP78), an essential hsp70 resident protein in the endoplasmic reticulum. Experientia 1994; 50:1012-1020.
39. Kozutsumi Y, Segal M, Normington K. The presence of malfolded proteins in the endoplasmic reticulum signals the induction of glucose-regulated proteins. Nature 1988; 332:462-464.
40. Pelham HRB. Speculations on the functions of the major heat shock and glucose-related proteins. Cell 1986; 46:959-961.

41. Entwistle JWC III. Changes in the expression of sarcoplasmic reticulum protein and stress-related genes in stunned myocardium: Effects of unloading during early reperfusion. Virginia Commonwealth University 1998; 1-83.
42. Watowich SS, Morimoto RI. Complex regulation of heat shock- and glucose-responsive genes in human cells. Mol Cell Biol 1988; 8:393-405.
43. Herschman HR. Primary response genes induced by growth factors and tumor promoters. Annual Review of Biochemistry 1991; 60:281-319.
44. Bishopric NH, Jayasena V, Webster KA. Positive regulation of the skeletal α-actin gene by Fos and Jun in cardiac myocytes. J Biol Chem 1992; 267:25535-25540.
45. McBride K, Robitaille L, Tremblay S et al. fos/jun repression of cardiac-specific transcription in quiescent and growth-stimulated myocytes is targeted at a tissue-specific cis element. Mol Cell Biol 1993; 13:600-612.
46. Chiu R, Angel P, Karin M. Jun-b differs in its biological properties from, and is a negative regulator of, c-jun. Cell 1989; 59: 979-986.
47. Schutte J, Viallet J, Nau M et al. jun-b inhibits and c-fos stimulates the transforming and ***trans***-activating activities of c-jun. Cell 1989; 59:987-997.
48. Plumier JC, Robertson HA, Currie RW. Differential accumulation of mRNA for immediate early genes and heat shock genes in heart after ischemic injury. J Mol Cell Cardiol 1996; 28:1251-1260.
49. Mizukami Y, Yoshida K, Morimoto S. A novel mechanism of JNK1 activation. Nuclear translocation and activation of JNK1 during ischemia and reperfusion. J Biol Chem 1997; 272:16657-16662.
50. Brand T, Sharma HS, Fleischmann KE et al. Protooncogene expression in porcine myocardium subjected to ischemia and reperfusion. Circ Res 1992; 71:1351-1360.
51. Wechsler AS, Entwistle JWCI, Ding M et al. Myocardial stunning: Association with altered gene expression. J Card Surg 1994; 9: 537-542.
52. Sharma HS, Wünsch M, Brand T et al. Molecular biology of the coronary vascular and myocardial responses to ischemia. J Cardiovasc Pharm 1992; 20(Suppl 1): S23-S31.
53. Komuro I, Kurabayashi M, Takaku F et al. Expression of cellular oncogenes in the myocardium during the developmental stage and pressure-overloaded hypertrophy of the rat heart. Circ Res 1988; 62:1075-1079.
54. Pollack PS, Houser SR, Budjak R et al. c-myc gene expression is localized to the myocyte following hemodynamic overload in vivo. J Cell Biochem 1994; 54:78-84.
55. Rozich JD, Barnes MA, Schmid PG et al. Load effects on gene expression during cardiac hypertrophy. J Mol Cell Cardiol 1995; 27:485-499.
56. Kukielka GL, Smith CW, LaRosa GL et al. Interleukin-8 gene induction in the myocardium after ischemia and reperfusion in vivo. J Clin Invest 1995; 95:89-103.
57. Chandrasekar B, Colston JT, Freeman GL. Induction of proinflammatory cytokine and antioxidant enzyme gene expression following brief myocardial ischemia. Clin Exp Immunol 1997; 108:346-351.
58. Kumar AG, Ballantyne CM, Michael LH et al. Induction of monocyte chemoattractant protein-1 in the small veins of the ischemic and reperfused canine myocardium. Circ. 1997; 95:693-700.
59. Billups KL, Palladino MA, Hinton BT et al. Expression of E-selectin mRNA during ischemia/reperfusion injury. J Lab Clin Med 1995; 125:626-633.
60. Youker KA, Hawkins HK, Kukielka GL et al. Molecular evidence for induction of intracellular adhesion molecule- in the viable border zone associated with ischemia-reperfusion injury of the dog heart. Circ. 1994; 89:2736-2746.
61. Tonnessen T, Giaid A, Saleh D et al. Increased in vivo expression and production of endothelin-1 by porcine cardiomyocytes subjected to ischemia. Circ Res 1995; 76: 767-772.
62. Reiss K, Kajstura J, Zhang X et al. Acute myocardial infarction leads to upregulation of the IGF-1 autocrine system, DNA replication, and nuclear mitotic division in remaining viable cardiac myocytes. Exp Cell Res 1994; 213:463-472.
63. Kluge A, Zimmermann R, Münkel B et al. Insulin-like growth factor II is an experimental stress inducible gene in a porcine model of brief coronary occlusions. Cardiovasc Res 1995; 29:708-716.
64. Banai S, Shweiki D, Pinson A et al. Upregulation of vascular endothelial growth factor expression induced by myocardial ischaemia: Implications for coronary angiogenesis. Cardiovasc Res 1994; 28:1176-1179.
65. Hashimoto E, Ogita T, Nakaoka T et al. Rapid induction of vascular endothelial growth factor expression bt transient ischemia in rat heart. Am J Physiol 1994; 267:H1948-H1954.

66. Ono K, Matsumori A, Shioi T et al. Enhanced expression of hepatocyte growth factor/c-Met by myocardial ischemia and reperfusion in a rat model. Circ 1997; 95: 2552-2558.
67. Thompson NL, Bazoberry F, Speir EH et al. Transforming growth factor beta-1 in acute myocardial infarction in rats. Growth Factors 1988; 1:91-99.
68. Frass O, Sharma HS, Knöll R et al. Enhanced gene expression in stunned porcine myocardium. Cardiovasc Res 1993; 27:2037-2043.
69. Crandall JD. Modulation of sarcoplasmic reticulum gene expression following global myocardial ischemia and reperfusion. Virginia Commonwealth University 1996; 21-36.
70. Brosius FCI, Liu Y, Nguyen N et al. Persistent myocardial ischemia increases GLUT1 glucose transporter expression in both ischemic and nonischemic heart regions. J Mol Cell Cardiol 1997; 29:1675-1685.
71. Brosius FCI, Nguyen N, Egert S et al. Increased sarcolemml glucose transporter abundance in myocardial ischemia. Amer J Cardiol 1997; 80:77A-84A.
72. Akao M, Otani H, Horie M et al. Myocardial ischemia induces different regulation of KATP channel gene expression in rat hearts. J Clin Invest 1997; 100:3053-3059.
73. Arai M, Otsu K, MacLennan DH et al. Effect of thyroid hormone on the expression of mRNA encoding sarcoplasmic reticulum proteins. Circ Res 1991; 69:266-276.
74. Kimura Y, Otsu K, Nishida K et al. Thyroid hormone enhances Ca^{2+} pumping activity of the cardiac sarcoplasmic reticulum by increasing Ca^{2+}ATPase and decreasing phospholamban expression. J Mol Cell Cardiol 1994; 26:1145-1154.
75. Komuro I, Kurabayashi M, Shibazaki Y et al. Molecular cloning and characterization of a Ca^{2+} + Mg^{2+}-dependent adenosine triphosphatase from rat cardiac sarcoplasmic reticulum: Regulation of its expression by pressure overload and developmental stage. J Clin Invest 1989; 83:1102-1108.
76. Mercadier JJ, Lompre AM, Duc P et al. Altered sarcoplasmic reticulum Ca^{2+}-ATPase gene expression in the human ventricle during end-stage heart failure. J Clin Invest 1990; 85:305-309.

Section II:
Methods to Prevent Ischemia-Reperfusion Injury

Chapter 5

The NO-Donor L-Arginine Reduces the Reperfusion Injury after Heart Transplantation

Gábór Szabó

Ischemia-reperfusion injury is a common condition during cardiac surgery. Myocardial performance within the first hours after the surgical procedure determines the patient's state not only during the postoperative period but also for the long time outcome, especially after heart transplantation when an extended time of ischemia is followed by reperfusion. Most studies about the effects of myocardial ischemia and reperfusion focus on myocardial injury and the recovery of contractile function. It is now appreciated that the survival of the heart as a whole is in part dependent on the ability of the microcirculation to deliver and distribute blood flow adequately during reperfusion. Recent studies show the importance of protecting the microvasculature to attenuate reperfusion injury.[1-3] Preservation of microvascular reactivity may be especially important in those hearts undergoing cold ischemic storage for transplantation. Potential impairment of the right ventricular endothelium-dependent microvascular response after harvesting and implantation is also a likely contributing factor in right ventricular failure after cardiac transplantation.[2]

Effects of Nitric Oxide

During the past few years, several studies reported that the L-arginine-nitric oxide (NO) pathway plays an important role in ischemia-reperfusion injury. In the heart, NO is synthesized from oxygen and L-arginine by the coronary endothelium,[4] cardiac myocytes[5] and endocardial cells.[6] It has a host of physiological effects, including autoregulatory modulation of coronary blood flow[7,8] and inhibition of neutrophil endothelial interaction[9,10] as well as platelet aggregation. In contrast to the well understood physiological roles of NO in heart function, its significance under pathophysiological states remains unclear. On one hand NO is shown to have a beneficial effect by increasing postischemic blood flow and decreasing the 'no-reflow' phenomenon,[11] decreasing leukocyte adhesion and of cell adhesions molecules[12] and quenching free radicals, particularly superoxide radicals.[13] Contrarily, some authors report deleterious effects of NO during cardiac ischemia and reperfusion probably by a direct negative inotropic action of NO, or inhibition of mitochondrial respiration or formation of peroxynitrite, a precursor of highly oxidant species.[14-16]

In a recent study, we investigated the short and long time effects of altered NO-synthesis on reperfusion injury after reversible deep hypothermic ischemia in a modified heterotopic rat heart transplantation model. Enhancement of NO-synthesis was achieved by L-arginine and blocking of NO synthesis by L-NAME. Left ventricular pressure-volume

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relationships were measured by an intraventricular balloon which was connected to a pressure transducer and myocardial blood flow was assessed by the hydrogen-clearance technique.[17,18] This study demonstrated that the L-arginine-NO pathway has an important beneficial effect on reperfusion injury after reversible deep hypothermic ischemia in the heterotopically transplanted rat heart. The rapid infusion of the NO-precursor L-arginine (40 mg/kg) during the first five minutes of reperfusion led to an increased functional recovery after 60 minutes of reperfusion (Figs. 5.1, 5.2). Simultaneous administration of L-arginine and L-NAME (10 mg/kg), an inhibitor of endogenous NO-synthesis, antagonized these effects suggesting that functional changes can be attributed to NOS enhancement or inhibition, respectively. Furthermore, L-NAME alone reduced recovery of left ventricular function. While administration of L-arginine led to a leftward shift of the left ventricular systolic pressure-volume curves suggesting higher contractility in comparison to control, the left ventricular systolic pressure-volume curves showed a significant rightward shift in the L-NAME group indicating decreased systolic function after reperfusion. The slope of this relationship Emax was significantly higher in the L-arginine and significantly lower in the L-NAME group. Active diastolic function, myocardial relaxation, was significantly improved after L-arginine as indicated by the lower isovolumic relaxation constant (TE) and severely impaired by application of L-NAME (Fig. 5.2). Left ventricular compliance did not differ between the groups except the L-NAME treated animals after 60 minutes of reperfusion. The fact that passive diastolic function was also impaired (Fig. 5.2) suggests that the relative integrity of endogenous NO-synthesis may be essential for limiting reperfusion injury.

The reduction of reperfusion injury by L-arginine can be explained by an increase of postischemic myocardial blood flow (Table 5.1) and decrease of neutrophil adherence. In contrast, L-NAME led to a significant decrease of myocardial blood flow. Whereas L-arginine significantly improved the endothelium-dependent vasodilatation after infusion of acetylcholine (30 μg/min), L-NAME significantly impaired myocardial blood flow (Fig. 5.3). The endothelium-independent vasodilatation after infusion of sodium-nitroprussid (200 μg/min) was not influenced by altered NO-synthesis. Although systolic and diastolic function and baseline MBF were similar after 24 hours of reperfusion in all groups, endothelium-dependent vasodilatation was still significantly better in the L-arginine than in the control group and both showed a better endothelium-dependent vasodilatation than the L-NAME group indicating a persisting beneficial effect of NO-synthesis on endothelial function during reperfusion (Fig. 5.3).

Histologic findings revealed a slight edema and inflammatory perivascular infiltrate composed predominantly of polymorphonuclear neutrophils and lymphocytes in all treated transplanted hearts in comparison with the native hearts of the recipients after 60 minutes of reperfusion. While, however, only sporadic adherent neutrophils and scare cardiac infiltration were found in the L-arginine group, the control- and the L-arginine + L-NAME group showed an increased infiltration of neutrophils. These findings were more profound in the L-NAME group where also extended infiltrated areas were found including not only the perivascular but the cardiac muscle tissue. After 24 hours, no differences were observed between the groups also in comparison to the native hearts.

Reduction of Reperfusion Injury by Nitric Oxide: Potential Mechanisms

The exact mechanism by which endogenous NO synthesis has a beneficial effect on reperfusion injury remains controversially discussed. One possible mechanism would be through increased postischemic coronary blood flow,[19-21] assuming that endothelial NO release is increased by supplying more substrate for NO production.[19] Endothelium derived NO is a potent vasodilator and exerts its effect by stimulating soluble guanylate cyclase in the

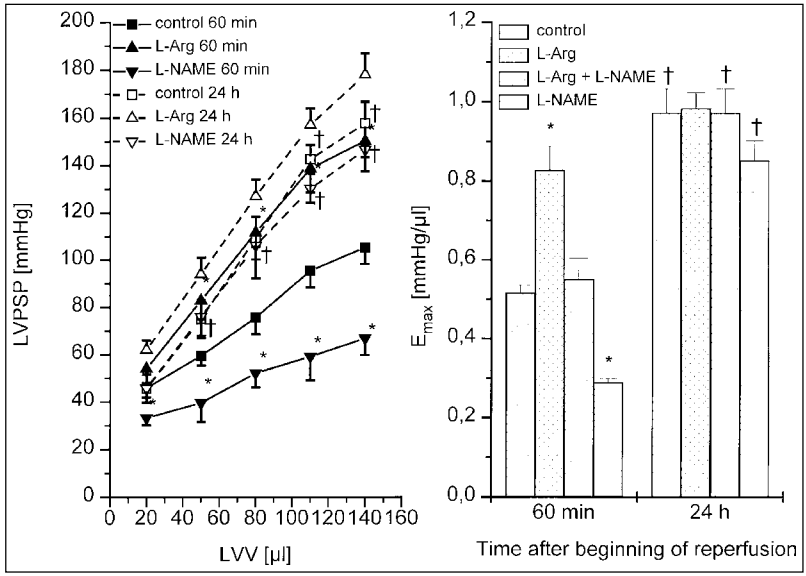

Fig. 5.1. Left ventricular systolic pressure-volume relationships (left panel) and the slope of the left ventricular systolic pressure-volume relationships (E_{max}, right panel) in the heterotopically transplanted rat heart after 60 minutes and 24 hours of reperfusion. L-Arg, L-arginine, L-NAME, nitro-L-arginine methyl ester, LVPSP, left ventricular peak systolic pressure, LVV left ventricular volume. All values are given as mean ± SEM. *p < 0.05 all groups vs. control at a given time point, Üp < 0.05 24 hours vs. 60 minutes.

underlying vascular smooth muscle cells, thereby elevating intracellular levels of cGMP and inducing relaxation of vascular smooth muscle.[22] The significantly higher postischemic myocardial blood flow after L-arginine and the significantly lower postischemic myocardial blood flow after L-NAME in our preparation would support this hypothesis. These results are consistent with the study of Hiramatsu et al[19] who observed a significant increase of coronary blood flow and subsequent improvement of left ventricular functional recovery after cold ischemia in blood perfused isolated lamb hearts if L-arginine was supplemented during the first 20 minutes of reperfusion. In the same study, L-NAME resulted in a significant decrease of postischemic coronary blood flow and left ventricular functional recovery. Amrani et al[23] showed a 31% loss of postischemic coronary flow by application of L-NMMA, another inhibitor of NO-synthesis, after four-hour cold ischemia in oxygenated, Krebs-Henseleit, buffer-perfused, isolated rat hearts. Other studies also showed a protective effect of NO-synthesis regarding functional recovery[11,24] and postischemic blood flow[11] in the setting of regional ischemia and reperfusion. Pinsky et al[25] demonstrated a significantly higher graft survival and better functional recovery assessed by a semiquantitative scoring system after application of the exogenous NO-donor nitroglycerin in heterotopic transplants after 12 hours of ischemia.

NO is not only a vasodilator but also inhibits interaction between the vessel wall and circulating blood elements.[26-28] It has been shown that neutrophil-endothelial interaction plays a crucial role in reperfusion injury. Incited by the paracrine factors released by the endothelium, the neutrophils begin to generate oxygen derived free radicals as well as proteases which compound the endothelial injury and induce myocyte necrosis. In addition, the cytokines released from the activated neutrophils induce the expression of other adhesion molecules (e.g., ICAM-1, E-selectine) that sustain the inflammatory process.[29] Recent

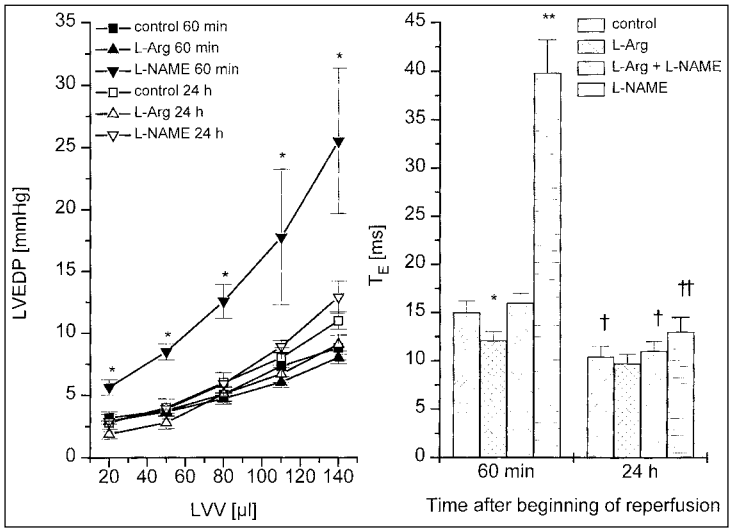

Fig. 5.2. Left ventricular end-diastolic pressure-volume relationships in the heterotopically transplanted rat heart after 60 minutes and 24 hours of reperfusion (left panel). The changes of myocardial relaxation (T_E, isovolumic relaxation constant) are shown on the right panel. L-Arg, L-arginine, L-NAME, nitro-L-arginine methyl ester, LVEDP, left ventricular end-diastolic pressure, LVV left ventricular volume. All values are given as mean ± SEM. *p < 0.05 all groups vs. control at a given time point, †p < 0.05 24 hours vs. 60 minutes.

Table 5.1. Parameters of the coronary circulation

	Control	L-Arg	L-NAME	L-NAME+L-Arg
MBF [ml min^{-1} g^{-1}]				
60 minutes	1.9 ± 0.2	3.6 ± 0.4#	1.2 ± 0.1#	2.0 ± 0.2
24 hours	3.4 ± 0.3*	3.9 ± 0.4	3.0 ± 0.2*	3.8 ± 0.3*
CVR [U/g]				
60 minutes	47 ± 6	25 ± 3#	75 ± 7#	52 ±8
24 hours	36 ± 9*	27 ± 10	34 ± 4*	28 ± 5*

L-Arg, L-arginine, L-NAME, nitro-L-arginine methyl ester. MBF, myocardial blood flow, CVR, coronary vascular resistance. All values are given as mean ± SEM at an intraventricular volume of 80 µl. *p < 0.05 24 hours vs. 60 minutes; #p < 0.05 vs. control

studies[24,25,30,31] have shown that modulation of the NO-pathway may influence neutrophil-mediated reperfusion injury. Pinsky et al[25] and Pabla et al[31] demonstrated a histologic evidence of decreased neutrophil adhesion under enhanced and increased neutrophil adhesion under attenuated NO-synthesis. In a previous study of Pabla et al[11] cardiac myeloperoxidase activity, an index of neutrophil accumulation was significantly lower in NO-donor treated animals after regional ischemia and reperfusion. Furthermore, decreased expression of endothelial and soluble adhesion molecules could be found after treatment with NO-donor.[24,30,32]

NO may also play a direct role by generation of radicals. NO itself is a primary radical species and is inactivated by superoxide radicals.[33,34] Conversely, NO may neutralize superoxide radicals.[35] Decreased endothelial

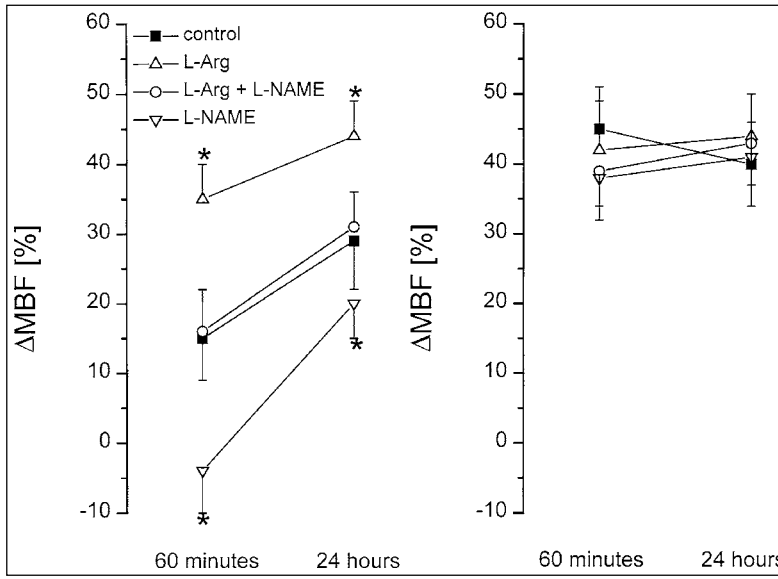

Fig. 5.3. Myocardial blood flow response after application of the endothelium-dependent vasodilator acetylcholine (left panel) and the endothelium-independent vasodilator sodium nitroprussid (right panel). L-Arg, L-arginine, L-NAME, nitro-L-arginine methyl ester. All values are given as mean ± SEM. *p < 0.05 all groups vs. control at a given time point. Endothelium-dependent vasodilatation significantly (p < 0.05) improved in all treatment groups after 24 hours in comparison to 60 minutes of reperfusion (not indicated in the graph).

production of NO with L-NAME infusion may therefore lead to an enhanced generation of free radicals and increases endothelial and myocardial injury during reperfusion. On the other hand, L-arginine may reduce free radical mediated injury. Siegfried et al[13] described cardioprotection and attenuation of endothelial dysfunction by organic nitric oxide donors in myocardial ischemia-reperfusion. They hypothesized that NO may quench other free radicals, particularly superoxide radicals and this may contribute to its protective effect. In contrast, some authors suggested that NO has a detrimental influence during ischemia and reperfusion,[14,15,36] primarily due to generation of peroxynitrite from superoxide anion and NO. Normally, free NO is catabolized by binding with hemoglobin to form nitrosylhemoglobin which is subsequently oxidized to form nitrate and to a lesser content nitrite in a relatively slow reaction.[37] However, NO also may form peroxynitrite as described above in a rapid diffusion limited reaction.[38] Although under normal conditions only a small amount of superoxide is permitted to react with NO because of an approximately 100-fold higher presence of superoxide dismutase, it is possible that under ischemia/reperfusion, increased concentrations of superoxide anion and decreased concentrations of superoxide dismutase allow the generation of peroxynitrite in a significant amount. Maulik et al[39] suggested that NO plays a significant role in transmembrane signaling via cGMP by opposing the effects of phosphodiesterases by inhibiting the ischemia/reperfusion-induced phosphodiesteratic break down. They postulated an on- and off-NO-signaling which is linked to its free radical chemistry.

The relative importance of different possible effects of NO remains unclear during reperfusion. Several authors underline, that the beneficial effects of NO are primarily due to the modulation of postischemic blood flow.[11,19,23,40,41] The fact that NO showed a protection against ischemia-reperfusion injury in crystalloid perfused heart preparations, where neutrophil-mediated actions of NO

seem to be of minor importance, would support this hypothesis. In contrast, in a recent study[31] NO or L-arginine had only a cardioprotective effect if polymorphonuclear leukocytes were present underlining the importance of NO-mediated inhibition of neutrophil-endothelial interaction. Despite a decreased postischemic blood flow, Naseem et al[15] reported better functional recovery after blocking of NO-synthesis by attenuated free radical generation. Takeuchi et al[42] found in crystalloid perfused isolated rabbit hearts that L-arginine had no effect on postischemic myocardial blood flow, but significantly decreased functional recovery after normoterm no-flow ischemia. They suggested that NO has a direct detrimental action by inhibition of mitochondrial respiration or accumulation of intracellular calcium during reperfusion. A possible explanation for the conflicting results regarding the role of NO during reperfusion may be that NO may have an adverse effect on ischemia-reperfusion injury depending on the time of application: the blocking of NO-synthesis was shown to be beneficial during ischemia and NO-donation may improve functional recovery during reperfusion.[14,16] Furthermore, the applied doses of NO-blockers/donors may also play a role in the net effect of these drugs.[14,16] In vasoactive doses, the net effect of NO-donors is probably dominated by the vasodilatative property of NO. In nonvasoactive doses, a protective role of endogenous NO-synthesis was shown only during reperfusion, while during ischemia had a negative effect.[16]

The role of different NOS isoforms in reperfusion injury may also explain at least partly the conflicting results. While eNOS is calcium dependent and is usually constitutively present in predominantly in endothelial cells but also in myocardial cells, iNOS is calcium-independent and is expressed in various types of cells including vascular smooth muscle cells, myocardial cells and macrophages after stimulation with cytokines and polysaccharides.[43] Maintained production of NO by eNOS may be protective, whereas overproduction of NO by activated iNOS may induce tissue damage. In a former study,[44] we observed a clear and marked expression of eNOS in endothelial cells and no expression of iNOS in native fresh hearts by immunohistology. The semiquantitative analysis of the immunohistologic stainings showed a significant decrease of eNOS after 60 minutes of deep hypothermia and 60 minutes of reperfusion whereas the decrease was less pronounced in the control hearts and more pronounced in the L-NAME-treated group. On the other hand iNOS was expressed in all ischemic groups without any differences between the groups. This is in agreement with the study of Wang et al[45] who found after ischemia-reperfusion, that tissue activity of eNOS in L-arginine treated hearts was approximately the same as in nonischemic hearts while control vehicle treated hearts showed a significant decrease of eNOS activity. Interestingly, application of a blocker of NO-synthesis, L-NNA did not cause any further changes in eNOS activity. This phenomenon is most likely, because ischemia and reperfusion reduced eNOS activity to such a degree that addition of NO-blocker did not further inhibit the NOS activity. In contrast to Wang et al,[45] who subjected the hearts to a 30-minute global warm ischemia, in our previous[40,44] studies, after deep hypothermia and reperfusion eNOS activity was probably moderately impaired, and therefore blocking of NO-synthesis by L-NAME may have led to a further decrease of eNOS activity. Consistent with the report of Wang et al, the fact that iNOS expression was almost similar in all groups suggests that effects of modulation of NO synthesis are carried out primarily by eNOS mediated NO-synthesis in this setting. However, under certain circumstances, when eNOS activity or L-arginine concentration is low, the blocking of NO-synthesis may be beneficial by inhibiting iNOS mediated NO-synthesis and subsequent free radical generation. eNOS also may generate free radicals if its substrate-L-arginine has a low concentration. Therefore, at low concentrations of L-arginine, blockers of NO-synthesis may reduce free radical generation by eNOS itself.[45]

The preservation of eNOS activity after L-arginine treatment may be also an explana-

tion for the persisting beneficial effects of NO-synthesis on endothelial function after 24 hours. A rapid infusion of L-arginine during the first minutes of reperfusion may not only increase NO-synthesis by increasing concentrations of its substrate but by preserving eNOS activity. Even if no differences were found between the groups regarding eNOS expression by semiquantitative immunohistology, preserved eNOS activity during the early reperfusion phase may be responsible for better endothelial function after 24 hours of reperfusion. Schnabel et al[46] showed in a recent ultrastructural study in human transplant biopsy specimens that while myocyte ultrastructural integrity recovers within 60 minutes of reperfusion, ultrastructural regeneration of the endothelium lasts days up to one week. Under these aspects, endothelial protection by substituting NO during reperfusion may be essential for preserving endothelial function.

In summary, substitution of NO-donors or -precursors presents a new concept of cardioprotection against ischemia-reperfusion injury during cardiac surgery in two terms. First, NO-donors act beneficially primarily during the reperfusion phase[14,16] especially in case of hypothermic ischemia. In contrast to other cardioprotection concepts which focus on ischemic preservation, the use of NO-donors aims to reduce reperfusion injury. Second, this concept provides a complex integrative cardioprotection at both the myocardial and the vascular level. The latter seems to be very important, since intact vascular function may play a key role in adequate myocardial recovery during reperfusion.

References

1. Argano V, Galinanes M, Edmondson S. Effects of cardioplegia on vascular function and the 'no reflow' phenomenon after ischemia and reperfusion: Studies in the isolated blood perfused rat heart. J Thorac Cardiovasc Surg 1996; 111:432-42.
2. Murphy CO, Pan-Chih, Gott JP et al. Coronary microvascular reactivity after ischemic cold storage and reperfusion. Ann Thorac Surg 1997; 63:20-7.
3. Galinanes M, Saldanha C, Kato H et al. Vascular and contractile function and tissue metabolites after prolonged hypothermic ischemia and reperfusion: Comparison of single- versus multi-dose infusions with two cardioplegic solutions in blood-perfused neonatal pig hearts. J Mol Cell Cardiol 1995; 27:1915-30.
4. Cocks TM, Angus JA, Campbell GR. Release and properties of endothelium-derived relaxing factor (EDRF) from endothelial cells in culture. J Cell Physiol 1985; 123:310-20.
5. Schulz R, Nava E, Moncada S. Introduction and potential relevance of a Ca2+-independent nitric oxide synthase in the myocardium. Br J Pharmacol 1992; 105:575-80.
6. Schulz R, Smith JA, Lewis MJ et al. Nitric oxide synthase in cultured endocardial cells of the pig. Br J Pharmacol 1991; 104:21-24.
7. Kelm M, Schrader J. Control of coronary tone by nitric oxide. Circ Res 1990; 66: 1561-75.
8. Pohl U, Lamontagne D. Impaired tissue perfusion after inhibition of endothelium derived nitric oxide. Basic Res Cardiol 1991; 86: (Suppl 2) 97-105.
9. Lefer AM, Tsao PS, Lefer DJ et al. Role of endothelial dysfunction in the pathogenesis of reperfusion injury after myocardial ischemia. FASEB J 1991; 5:2029-34.
10. Kurose I, Wolf R, Grisham MB et al. Modulation of ischemia/reperfusion-induced microvascular dysfunction by nitric oxide. Circ Res 1994; 74:376-82.
11. Pabla R, Buda AJ, Flynn DM et al. Intracoronary nitric oxide improves postischemic coronary blood flow and myocardial contractile function. Am J Physiol 1995; 269: H1113-1121.
12. Adams MR, Jessup W, Hailstones D et al. L-arginine reduces human monocyte adhesion to vascular endothelium and endothelial expression of cell adhesion molecules. Circulation 1997; 95:662-8.
13. Siegfried MR, Erhardt J, Rider T et al. Cardioprotection and attenuation of endothelial dysfunction by organic nitric oxide donors in myocardial ischemia reperfusion. J Pharm Exp Ther 1992; 260:668-75.
14. Schulz R, Wambolt R. Inhibition of nitric oxide synthesis protects the isolated working rabbit heart from ischemia-reperfusion injury Cardiovasc Res 1995; 30:432-9.
15. Naseem SA, Kontos MC, Rao PS et al. Sustained Inhibition of nitric oxide by NG-nitro-L-arginine improves myocardial function following ischemia/reperfusion in isolated perfused rat heart. J Mol Cell Cardiol 1995; 27:419-26.

16. Depré C, Vanoverschelde JL, Goudemant JF et al. Protection against ischemic injury by nonvasoactive concentrations of nitric oxide synthase inhibitors in the perfused rabbit heart. Circulation 1995; 92:1911-18.
17. Aukland K, Bower BF, Berliner RW. Measurement of local blood flow with hydrogen gas. Circ Res 1964; 14:164-87.
18. Hamano K, Ohmi M, Esato K et al. Myocardial tissue blood flow in allotransplanted rat heart with a special reference to acute rejection. J Heart Transplant 1989; 8:48-52.
19. Hiramatsu T, Forbess JM, Miura T et al. Effects of L-arginine and L-nitro-arginine-methyl ester on recovery of neonatal lamb hearts after cold ischemia. J Thorac Cardiovasc Surg 1995; 109:81-7.
20. Furchgott RF. Role of endothelium in response of vascular smooth muscle. Circ Res 1983; 53:557-73.
21. Amezcua JL, Palmer RMJ, De Souza BM et al. Nitric oxide synthesized from L-arginine regulates vascular tone in the coronary circulation of the rabbit. Br J Pharmacol 1990; 97:1119-24.
22. Moncada S, Palmer RMJ, Higgs EA. Nitric oxide: Physiology, pathophysiology and pharmacology. Pharmacol Rev 1991; 43:109-42.
23. Amrani M, Chester AH, Jakyakumar CJ et al. L-arginine reverses low coronary reflow and enhances postischemic recovery of cardiac mechanical function. Cardiovasc Res 1995; 30:200-4.
24. Engelman DT, Watanabe M, Maulik N et al. L-arginine reduces endothelial inflammation and myocardial stunning during ischemia/reperfusion. Ann Thorac Surg 1995; 60:1275-81.
25. Pinsky DJ, Oz MC, Koga S et al. Cardiac preservation is enhanced in a heterotopic rat transplant model by supplementing the nitric oxide pathway. J Clin Invest 1994; 93:2291-97.
26. Kubes P, Suzuki M, Granger DN. Nitric oxide: An endogenous modulator of leukocyte adhesion. Proc Natl Acad Sci USA. 1991;88:4651-5.
27. Bath PMW, MacGregor GA. Spontaneous nitric oxide donors inhibit monocyte chemotaxis and increase intracellular cGMP concentration. J Vasc Res 1992; 29:170.
28. Moilanen E, Vuorinen P, Kankaareranta H et al. NO donors inhibit human neutrophil activation in vivo [Abstract], J Vasc Res 1992; 29:170.
29. Cooke JP, Tsao PS. Cytoprotective effects of nitric oxide Circulation 1993; 88:2451-4.
30. MA XL, Weyrich AS, Lefer DJ et al. Diminished basal nitric oxide release after myocardial ischemia and reperfusion promotes neutrophil adherence to coronary endothelium Circ Res 1993; 72:403-12.
31. Pabla R, Buda AJ, Flynn DM et al. Nitric oxide attenuates neutrophil-mediated contractile dysfunction after ischemia and reperfusion. Circ Res 1996; 78:65-72.
32. Gauthier TW, Davenpeck KL, Lefer AM. Nitric oxide attenuates leukocyte-endothelial interaction via P-selectin in splanchnic ischemia reperfusion. Am J Physiol 1994; 267 G562-8.
33. Gryglewski RJ, Palmer RMJ, Moncada S. Superoxide anion is involved in the breakdown of endothelium-derived relaxing factor. Nature 1986; 320:454-6.
34. Rubanyi GM, Vanhoutte PM. Superoxide anions and hypoxia inactivate endothelium-derived relaxing factor. Am J Physiol 1986; 250:H822-7.
35. Rubanyi GM, Ho EH, Cantor EH et al. Cytoprotective function of nitric oxide: Inactivation of superoxide radicals produces by human leukocytes. Biochem Biophys Res Commun 1991; 181:1392-7.
36. Patel VC, Yellon DM, Singh KJ et al. Inhibition of nitric oxide limits infarct size in the in situ rabbit heart. Biochem Biophys Res Commun 1993; 194:234-8.
37. Craven PA, DeRubertis FR. Restoration of the responsiveness of purified guanylate cyclase to nitrosoguanidine, nitric oxide and related activators by heme and heme proteins: Evidence for the involvement of the paramagnetic nitrosyl-heme complex in enzyme activation. J Biol Chem 1978; 253:8433-43.
38. Beckman JS, Ischiropoulos H, Zhu L et al. Kinetics of SOD and iron catalyzed nitration of phenolics by peroxynitrite. Arch Biochem Biophys 1992; 298:438-45.
39. Maulik N, Engelmann DT, Watanabe M et al. Nitric oxide signaling in ischemic heart. Cardiovasc Res 1995; 30:593-601.
40. Szabó G, Bährle S, Dengler TJ et al. Reduction of reperfusion injury after heart transplantation by the nitric oxide donor L-arginine. Langebecks Arch Chir I Forumband 1997:7-10.
41. Beresewitz A, Karwatowska-Prokopzuk E, Lewartowski B et al. A protective role of nitric oxide in isolated ischemic/reperfused rat heart. Cardiovasc Res 1995; 30:1001-8.
42. Takeuchi K, McGowan FX, Danh HC et al. Direct detrimental effects of L-arginine upon ischemia-reperfusion injury to the myocardium. J Mol Cell Cardiol 1995; 27:1405-14.
43. Ursell PC, Mayes M. Anatomic distribution of nitric oxide synthase in the heart. Int J Cardiol 1995; 50:217-23.
44. Szabó G, Bährle S, Dengler TJ et al. L-arginine reduces reperfusion injury and coronary endothelial dysfunction in a rat heart transplantation model. [Abstract] J Mol Cell Cardiol 1997.

45. Wang QD, Morcos E, Wiklund P et al. L-arginine enhances functional recovery and Ca^{2+}-dependent nitric oxide synthase activity after ischemia and reperfusion in the heart. J Cardiovasc Pharmacol 1997; 29:291-6.

46. Schnabel PA, Lange R, Amann K et al. Ultrastructural integrity predicts the incidence of acute rejection during the first year after HTX. [Abstract] J Heart Lung Transplant 1997; 16:S45.

CHAPTER 6

Sodium-Proton Exchange Inhibition as a Novel Strategy for Myocardial Protection

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Ongoing developments in cardiac surgery, like surgery on the beating, warm heart in minimal invasive coronary bypass grafting, the increasing incidence of cardiac surgery in the elderly as well as in neonates and the increasing number of complex interventions, imply a continuous request for better myocardial protective techniques. The common challenge for these techniques however remains the damaging effects of acute myocardial ischemia. Because the sequence of events during acute myocardial ischemia had been the subject of many studies in the past, it became more and more clear that a disturbance of cellular ionic homeostasis is the initial trigger of the ultimate damage.

One of the most important early mechanisms of ionic disturbances is the activation of the sodium-proton exchange system.[1] The inhibition of this system during ischemia and reperfusion diminishes both Na^+ and Ca^{++} overload and potentially prevents or delays myocardial damage.[2-9]

In this chapter, the idea to use a selective inhibition of the sodium-proton exchange system is propagated as a novel approach for protection against ischemia-induced myocardial damage. For this purpose, HOE642 (4-isopropyl-3-methylsulfonyl-benzoylguanidinmethanesulfonat) (Hoechst, Frankfurt, Germany), a new, selective, and potent sodium-proton exchange inhibitor with good tolerability and kinetic properties was chosen.

First of all we will describe more in detail the theoretical background of sodium-proton exchange inhibition in myocardial ischemia-reperfusion. Then we will provide experimental evidence for the protective properties of inhibiting this exchange system during and after ischemia.

The drug HOE 642 however was never tested clinically so that a preliminary clinical trial was mandatory. We performed a small prospective randomized double blind study, first of all to assess safety and secondly to search for some efficacy in terms of improved recovery of function after complex cardiac surgery in man. We will present these data here, bearing clearly in mind that efficacy of sodium-proton exchange inhibition in the clinical setting still needs to be proven.

Sodium-Proton Exchange Inhibition in Cardiac Ischemia-Reperfusion

In the last decade it has become clear, that most pathophysiological processes in cardiac ischemia and reperfusion are connected to a derangement of cellular ion homeostasis. The characterization of various ion transport systems has led to the identification of three effects, which seem to play key roles in the

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impairment of ischemic and reperfused tissue. These interdependent effects are decreased intracellular pH (pH_i), intracellular Na^+ (Na^+_i) overload and intracellular Ca^{++} (Ca^{++}_i) overload.

The interdependence between intracellular acidosis and elevated Na^+_i and Ca^{++}_i concentrations is based on the activity of certain ion transport systems. Increased activity of the Na^+ coupled Cl^-/HCO_3^- exchange and an excessive activation of Na^+/H^+ exchange (NHE) by a decrease of pH_i lead to a significant elevation of Na^+ influx into the endangered tissue. As long as sufficient ATP is available, the intruding Na^+ ions can be transported against the Na^+ gradient into the extracellular space by the Na^+/K^+ ATPase. But eventually, decreasing energy stores and elevated Na^+ influx result in a marked increase of the concentration of Na^+_i. Since intracellular Na^+ and Ca^{++} concentrations are linked by a $3Na^+/Ca^{++}$ exchange, the elevation of Na^+_i finally causes intracellular Ca^{++} overload, which induces cardiac arrhythmias,[10,11] contracture, stunning and necrosis.

Already in 1984 it has been suggested by Frelin and co-workers,[12] that the NHE could play an important role in this chain of pathophysiological events. While the regulation of pH_i seems to be dominated by HCO_3^- dependent systems under normal conditions, it has been claimed that the NHE causes a major part of the Na^+ influx in cardiac ischemia and reperfusion. More recently, this point of view has been supported by studies showing a significant reduction of Na^+ and Ca^{++} overload and impressive cardioprotective effects with inhibitors of the Na^+/H^+ exchanger.[9,13] Altogether this points to a crucial involvement of this ion transport system in the mechanisms of ischemic and reperfusion injuries.

In the following, this chapter surveys the observed effects of NHE inhibitors on intracellular Na^+ (Na^+_i), Ca^{++} (Ca^{++}_i), pH (pH_i) and metabolism in cardiac tissue, as well as on ischemic and reperfusion injuries. Furthermore it tries to evaluate the potential clinical use of specific NHE inhibitors in indications related to cardiac ischemia and reperfusion.

There are at present at least five different NHE subtypes (NHE 1-5).[14,15] NHE inhibitors as amiloride derivatives, as well as HOE 694 and HOE 642 (cariporide) are to different degrees preferential inhibitors of the NHE subtype 1, the so called housekeeper, which seems to be predominant in cardiac tissue. Whenever the NHE is mentioned in the following, the involvement of this subtype is implicated.

Effects on Intracellular Na^+

An increase of Na^+_i is well documented in "chemically ischemic" or hypoxic myocardial cells and in ischemic or hypoxic hearts. It has become clear, that the increase of Na^+_i results from an imbalance between Na^+ influx and Na^+ extrusion in ischemic cells. On the one hand, Na^+ influx is increased by an activation of pH regulating ion transport systems as the NHE and possibly the Na^+-HCO_3^- symport and the Na^+ dependent Cl-/HCO_3^- exchange. On the other hand, the extrusion of Na^+ by the Na^+/K^+ ATPase decreases according to energy depletion. It has been claimed that the NHE is responsible for a substantial part of Na^+ influx under these conditions, while another part of Na^+ influx goes through the HCO_3^- dependent systems and Na^+ channels. Recent results indicate that the balance between NHE activity and Na^+-HCO_3^- symport activity seems influenced by alpha adrenergic receptors.[16] The release of alpha adrenergic agents, as it occurs in myocardial ischemia, is expected to shift transport activity towards the NHE system. This would mean that Na^+ influx through the NHE might be even more important in the living organism than in isolated heart models, which are removed from their normal systemic neuronal and hormonal influences.

Whatever the balance between different Na^+ transport systems in the ischemic myocardium might be, it has been demonstrated that Na^+ overload is reduced by NHE inhibitors. Observations made in hypoxic or "chemically ischemic" myocardial cells have been confirmed by a growing number of Na^+ NMR measurements in ischemic hearts.

While this effect of NHE inhibitors in cardiac ischemia seems quite clear, it is more difficult to judge their influence on Na^+_i during reperfusion. In reperfusion, after a transient increase, Na^+_i rapidly decreases, possibly as fast as the cells begin to recover from energy depletion. The rate of Na^+_i decrease depends on the relation between Na^+ influx and Na^+ extrusion, mainly by Na^+/K^+ ATPase. Since the NHE is excessively activated in reperfusion, it might be expected that NHE inhibitors would facilitate the recovery from Na^+_i overload. Up to now, this has been shown in isolated myocardial cells under rather artificial conditions and in the reperfused heart. In general it has been claimed that protective effects of NHE inhibitors in cardiac reperfusion result from a reduction of Na^+_i overload.

Effects on Intracellular Ca^{++}

It is the connection between Na^+_i levels and Ca^{++}_i levels which is thought to make Na^+_i overload harmful for cardiac tissue. In cardiac cells, Ca^{++}_i is significantly affected by the activity of a $3Na^+/Ca^{++}$ exchanger. This exchanger depends on the Na^+ gradient to extrude Ca^{++} from the cell. If this gradient is diminished by increasing Na^+_i, the $3Na^+/Ca^{++}$ exchanger fails and Ca^{++}_i is going to rise. It has been shown in numerous studies with myocardial cells and isolated hearts that in ischemia the increase of Na^+_i is paralleled by an increase Ca^{++}_i. As it would be expected, not only Na^+_i overload but also ischemic Ca^{++}_i overload is substantially reduced by NHE inhibitors.

It has been observed in myocardial cells that there is a rapid and transient decrease of free Ca^{++}_i at the onset of reperfusion. It is assumed that this fast decrease is due to an uptake of Ca^{++} ions in intracellular stores. The overall cellular Ca^{++} content is reduced more slowly via the $3Na^+/Ca^{++}$ exchanger after restoration of the Na^+ gradient. If the restoration of the gradient fails because of an imbalance between Na^+ influx and Na^+ extrusion, Ca^{++}_i stores are kept in an overloaded unstable state. They release Ca^{++} ions and cause repetitive Ca^{++} oscillations, which finally kill the reperfused cells These detrimental Ca^{++} oscillations can be reduced by NHE inhibitors.[17]

Effects on Intracellular pH and Ischemic Metabolism

In ischemia, anaerobic metabolism and hydrolysis of ATP cause an accumulation of intracellular protons, and thus, an activation of pH regulating ion transport systems as the NHE. In lack of proton clearance the extracellular pH (pH_o) tends to drop in parallel with pH_i in the ischemic tissue. It has been demonstrated that NHE activity is decreased by extracellular acidosis. At a pH_o of about 6.3, which can be reached within 10-15 min in ischemic hearts, the NHE seems to be quiescent. The activity of the NHE in myocardial ischemia might be limited to this first period.

Since protons are extruded by the active NHE, it could be assumed, that the decrease of pH_i was accelerated by NHE inhibitors. This has not, as yet, been confirmed by NMR studies in isolated hearts. In general the drop of pH_i was not influenced, or only slightly influenced by NHE inhibitors. However, it has been demonstrated that in the presence of the α-1 agonist phenylephrine NHE inhibition caused a faster drop of pH_i in isolated rat hearts. Since there is a massive release of catecholamines in cardiac ischemia/reperfusion in animals and humans, it might well be that this study was more significant for the situation in the living organism, than other experiments with isolated hearts which were lacking catecholamins in the perfusion solution.

In spite of a reduction of anaerobic metabolism by NHE inhibitors, high energy phosphates (HEP) have been found conserved or unchanged in ischemic cardiac tissue. This strongly suggests, that the consumption of HEP has been decreased. NHE inhibitors might cause this effect by diminishing ischemic Na^+_i overload and thereby reducing Na^+/K^+ATPase activity.

In cardiac reperfusion, a rapid restoration of pH_i to normal values has been observed. Together with elevated Ca^{++}_i or with Ca^{++} oscillations this fast increase of pH_i might be the major cause of reperfusion damage. Since

the coupling of Ca^{++} ions to contractile elements and other Ca^{++} induced reactions is suspended under acidotic conditions, elevated Ca^{++}_i becomes especially serious when pH_i is restored. The fate of reperfused myocardium might be decided by the balance between decreasing Ca^{++}_i and increasing pH_i. It seems quite clear that the crucial period affecting the ultimate course of these events is within the first minutes or even seconds of reperfusion. Acidic reperfusion solutions showed marked protective effects during the first two minutes of reperfusion. Thereafter, perfusion at normal pH was no longer harmful.[18]

The NHE system, which is excessively activated in reperfusion, seems to have a significant part in pH recovery. Measurements of pH_i with fluorescent dyes in isolated myocytes have shown that pH recovery is delayed by NHE inhibitors. In most NMR studies there are rather large intervals of two or three minutes between pH measurements which are not suitable to evaluate rapid pH changes during reperfusion. Nevertheless, there are studies showing that pH recovery is slowed down by NHE inhibitors in reperfused hearts. It is probable, that this effect of NHE inhibitors adds to the protective reduction of Ca^{++}_i overload in ischemia and reperfusion, by making elevated Ca^{++}_i less harmful.

Cardioprotective Effects

The reduction of Ca^{++}_i overload and the desensitization of cardiac cells to elevated Ca^{++}_i are paralleled by outstanding cardioprotective effects of NHE inhibitors. These have been demonstrated with amiloride and numerous derivatives including ethylisopropyl amiloride (EIPA), dimethyl amiloride (DMA), methylisobutyl amiloride (MIBA), hexamethylene amiloride (HMA) and with the more specific NHE 1 inhibitors HOE 694[8] and cariporide.[19] There are only a few studies which did not demonstrate protective effects of NHE inhibitors. When looked at in detail, it can be seen that they were possibly performed under conditions where the NHE system was not significantly activated. Imai and coworkers did not see any effect of EIPA in low flow ischemia in rat hearts.[20] In these experiments, the ischemic insult was so mild, that there was hardly any decrease of ATP and pH_i and consequently no activation of the exchanger. Duff and coworkers[21] did not find antiarrhythmic effects of EIPA in a postinfarction model in dogs under conditions where the exchanger should be quiescent.

However, in acute ischemia and reperfusion where the NHE is excessively activated, it seems that all the typical injuries as arrhythmias, contracture, stunning, ultrastructural damage and cardiac necrosis are ameliorated or prevented by NHE inhibitors.

A prevention of arrhythmias has been observed in isolated hearts and in animals of different species with amiloride derivatives, HOE 694 and cariporide. Ventricular fibrillation, which seems connected to Ca^{++}_i overload, can be abolished by NHE inhibitors in ischemia and reperfusion. This has also been shown in pigs, which are known to be highly vulnerable to rhythm disturbances and sudden cardiac death.

Numerous investigators report a substantial reduction of ischemic and postischemic contracture, an improvement of cardiac performance, and an amelioration of myocardial stunning caused by NHE inhibitors. In several studies, a corresponding prevention of ultrastructural cellular damage was also observed. Other experiments show a diminished release of intracellular enzymes in cardiac ischemia and reperfusion, indicating a reduction of cellular necrosis. A very substantial reduction of infarct size has been demonstrated in rats, rabbits and pigs.

For some time, there has been some controversy as to whether cardioprotection by NHE inhibitors was limited to reperfusion or would also occur under ischemic conditions where the NHE might be blocked by low pH_o. It has become clear, however, that the reported reduction of ischemic Ca^{++}_i overload is accompanied by a prevention of arrhythmias, contracture and myocardial damage during ischemia. It has been shown that there are protective effects of NHE-inhibitors, if the compound is given only during reperfusion.

In our laboratory a study was performed on isolated blood perfused rabbit hearts[7] that gives an example for the full scope of cardioprotection caused by NHE inhibition in ischemia/reperfusion. In this study, the hearts were subjected to 45min of warm ischemia followed by 1h of reperfusion. They served either as controls or were treated with the NHE-1 inhibitor HOE 694 during ischemia and reperfusion or only during reperfusion. The pretreatment prevented the contracture which was observed in controls nearly totally and markedly improved the performance of the hearts on reperfusion. The reperfusion treatment reduced contracture on reperfusion and slightly improved the performance of the rabbit hearts (see Fig. 6.1). In addition, in this study, biopsies were taken from the hearts and evaluated by electron microscopy. The results showed that pretreatment with the NHE inhibitor nearly totally prevented ultrastructural damage, accumulation of Ca^{++} in the mitochondria and mitochondrial damage. Treatment on reperfusion only was still protective, but not as effective as pretreatment.

In another experimental study from our laboratory,[22] we assessed the effects of HOE 642, on myocardial function after transplantation of canine brain-dead and nonbrain-dead donor hearts preserved for 4 hours. Four groups were studied: brain-dead donors; nonbrain-dead donors; brain-dead donors and recipients treated with HOE 642 (2 mg/kg); and treated nonbrain-dead donors and recipients. Donor hearts were stored in hyperkalemic cardioplegic solution. At the end of 60 minutes reperfusion after transplantation, pressure-volume curves were constructed. Biopsies were analyzed histologically and ultrastructurally. Afterwards, weaning from CPB was accomplished.

When both donor and recipient are treated with this inhibitor both in the brain death and nonbrain death group, significantly lower myocardial stiffness is shown after transplantation than in the untreated group (see Fig. 6.2). However, there were no significant differences for the LV systolic performance and myocardial water content among the groups.

Myocardial stiffness is known to be influenced by myocardial contracture and/or edema.[23-28] In this study, significantly different stiffness coefficients without difference in myocardial water content among the groups suggest that the improvement in myocardial compliance in the treated groups is mainly affected by myocardial contracture.

All groups showed successful weaning from CPB without inotropic support such as dopamine and/or dobutamine and well-preserved ultrastructure without irreversible damage.

In a subsequent experimental study in dogs we extended the donor heart storage time to 24 hours (Flameng et al, unpublished results). Then the hearts were orthotopically transplanted and reperfused for one hour on CPB. Pressure volume relations were studied during CPB using a balloon inserted in the left ventricle. It was found that, when the donor as well as the recipient was treated with HOE 642, myocardial compliance was significantly improved and ultrastructure of the myocardium remained intact. All hearts stored cold for 24 hours after hyperkalemic cardioplegic arrest could be weaned from CPB with stable hemodynamics and a normal cardiac index, provided donor and recipient were treated with the sodium proton exchange inhibitor (HOE 642 at a dose of 2 mg/kg body weight). Compliance, myocardial structure and functional outcome after transplantation were significantly better than those in control experiments without drug treatment.

We concluded that the use of this inhibitor might improve the current myocardial preservation technique for transplantation.

Preliminary Clinical Experience with Sodium-Proton Exchange Inhibition

In this pilot study, a relatively small number of patients is included. Nevertheless the group of patients represents a rather uniform pathology : all patients had a combination of aortic valve disease and coronary artery disease of such an extent that surgical correction is required. This implies a combination of left

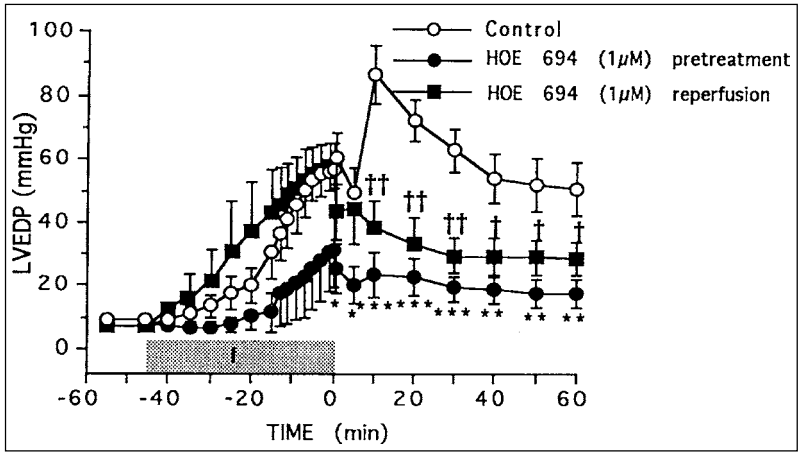

Fig. 6.1. Line plot shows effect of (3-methylsulfonyl-4-piperidinobenzoyl)guanidine methanesulfonate (HOE 694) on left ventricular end-diastolic pressure (LVEDP) during 45 minutes of ischemia and 1 hour of reperfusion. LVEDP is plotted as a function of time. Zero time is the beginning of reperfusion. Bars represent SEM. *p < .05, **p < .01, ***p < .001 control vs HOE 694 pretreatment, +p < .05, ++ p < .01 control vs HOE 694 reperfusion. This phenomenon is largely suppressed by HOE 694, even when given only during reperfusion. (Reprinted with permission from: Circulation 1994; 89:2787-2798).

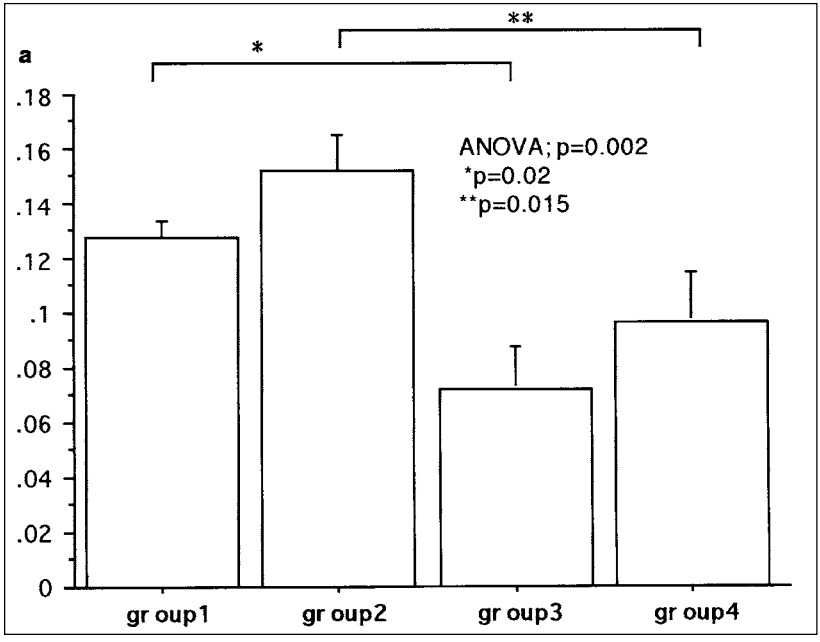

Fig. 6.2. LV stiffness coefficients (a); There were significantly different SC's between group 1 and group 3 (0.127 ± 0.006 vs 0.072 ± 0.016; p = 0.02), and between group 2 and group 4 (0.152 ± 0.013 vs 0.096 ± 0.019; p = 0.015). Data are means with SEM as error bar (Reprinted with permission from: Cardiovascular Surgery 1998; (6)67-75.

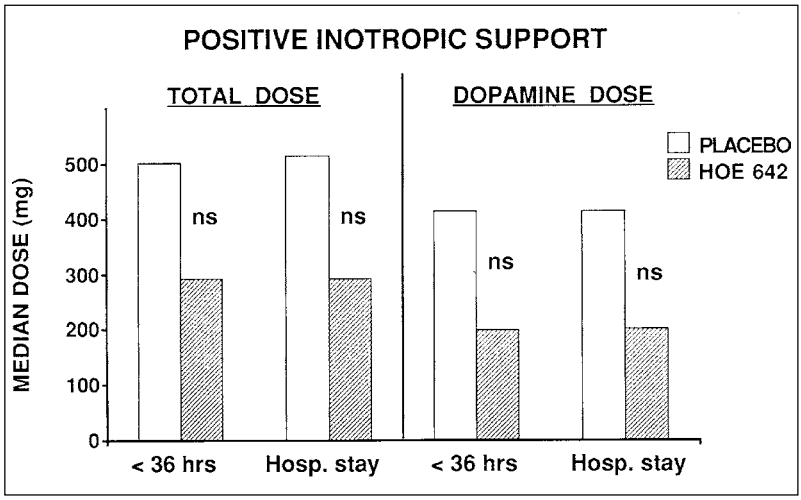

Fig. 6.3. Effect of HOE 642 on the need for positive inotropic support after weaning from cardiopulmonary bypass. The right panel represents the median values of the total dose of dopamine (in mg) given during the first 36 hours after weaning and during the whole hospital stay. In the left panel the total dose of positive inotropic medication is represented during these time intervals. (ns: $p > 0.05$, placebo versus HOE 642).

ventricular hypertrophy and impaired myocardial blood supply. On top of this cardiac pathology, a period of aortic cross clamping and consequently of acute ischemia renders these hearts more vulnerable and make them a good model to test myocardial protection techniques.

Twenty patients undergoing aortic valve replacement combined with coronary artery bypass grafting were included in this prospective randomized double blind study. The patients were either pretreated with HOE 642 (40 mg I.V. over 10 minutes) or placebo intravenously before going on cardiopulmonary bypass. The study end-points were 1) safety assessment and 2) efficacy of treatment in terms of improvement of hemodynamics after weaning from bypass, the need for positive inotropic support, release of cardiac enzymes and clinical outcome. The results of the study were published previously in detail.[29] The twenty patients were prospectively randomized into 2 groups of 10 patients each. They were 13 males and 7 females. Mean age was 67 years with a range of 44-77 years.

From the point of safety assessment, the frequency of possibly treatment related adverse effects until 8 weeks after discharge was registered. The investigators mentioned 5 features as possibly related to treatment but none of them however was statistically significantly related to treatment ($p > 0.05$).

Efficacy assessment was done by evaluation of hemodynamic parameters during weaning from cardiopulmonary bypass, parameters of myocardial damage, clinical outcome and the need for positive inotropic support.

Hemodynamic parameters were studied 5 minutes before starting cardiopulmonary bypass and every 5 minutes after weaning, up to 45 minutes after cessation of bypass. None of these parameters showed any difference between placebo and HOE 642 treatment. Also transesophageal echocardiography revealed no differences in the value for end diastolic and end systolic dimensions between groups ($p > 0.05$). Also pulmonary and systemic vascular resistance did not differ between groups ($p > 0.05$).

The dose of positive inotropic medication was calculated in mg for each drug (dopam-

ine, dobutamine or noradrenaline) during the first 36 hours and during the total hospital stay. The dose of noradrenaline was multiplied by 20 to obtain an equal reflection of drug potency when the total dose of positive inotropic medication was calculated. The results are shown in Figure 6.3. In the right panel only the median dose of dopamine is given as well for the first 36 hours period as for the total duration of hospital stay. The need for dopamine is lower in the HOE 642 treated group, although statistical significance is not reached ($p > 0.05$). The total dose of positive inotropic drugs (left panel) provides approximately the same distribution, indicating that the majority of patients received only dopamine.

None of the patients showed any evidence of myocardial infarction nor life threatening arrhythmias during the intra- or postoperative phase. The release of CK-MB during the first 24 hours postoperatively is very small and did not differ significantly ($p > 0.05$) between treatment groups.

There was no hospital mortality in the study population. The length of stay on the intensive care unit and the hospital stay was not different between the two groups ($p > 0.05$).

This preliminary study showed that the drug has no adverse effects at the dose used and that the need for positive inotropic support was less in the group of patients receiving the Na^+/H^+ exchange inhibitor.

These data suggest that further clinical evaluation of this type of pharmacological protection is meaningful.

References

1. Karmazyn M, Moffat MP. Role of Na+/H+ exchange in cardiac physiology and pathophysiology; mediation of myocardial reperfusion injury by the pH paradox. Cardiovasc Res 1993; 27:915-924.
2. Scholz W, Albus U. Na+/H+ exchange and its inhibition in cardiac ischemia and reperfusion. Basic Res Cardiol 1993; 88:443-455.
3. Karmazyn M, Ray M, Haist JV. Comparative effects of Na+/H+ exchange inhibitors against cardiac injury produced by ischemia/reperfusion hypoxia/reoxygenation, and the calcium paradox. J Cardiovasc Pharmacol 1993; 21:172-178.
4. Karmazyn M. Na+/H+ exchange inhibitors reverse lactate-induced depression in postischemic ventricular recovery. Br J Pharmacol 1993; 108:50-56.
5. Hata K, Takasago T, Saeki A et al. Stunned myocardium after rapid correction of acidosis-increased oxygen cost of contractility and the role of the Na+/H+ exchange system. Circ Res 1994; 74:794-805.
6. Harada K, Johnson RG, Grossman W et al. Acidemia and hypernatremia enhance postischemic recovery of excitation-contraction coupling. Circ Res 1994; 74:1197-1209.
7. Hendrikx M, Mubagwa K, Verdonck F et al. New Na+/H+ exchange inhibitor HOE 694 improves postischemic function and high-energy phosphate resynthesis and reduces Ca^{++} overload in isolated perfused rabbit heart. Circulation 1994; 89:2787-2798.
8. Scholz W, Albus U, Lang J et al. HOE 694, a new Na+/H+ exchange inhibitor and its effects in cardiac ischemia. Br J Pharmacol 1993; 109:562-568.
9. Scholz W, Albus U, Linz W et al. Effects of Na^+/H^+ exchange inhibitors in cardiac ischemia. J Moll Cell Cardiol 1992; 24: 731-740.
10. Coetzee WA, Opie LH. Effects of components of ischemia and metabolic inhibition on delayed afterpolarization in guinea pig papillary muscle. Circulat Res 1987; 61: 157-165.
11. Shen AC, Jennings RB. Myocardial calcium and magnesium in acute ischemic injury. Am J Pathol 1972; 67:417-440.
12. Frelin C, Vigne P, Ladzdunski M. The role of the Na^+/H^+ exchange system in cardiac cells in relation to the control of internal Na^+ concentration. J Biol Chem 1984; 259: 8880-8885.
13. Anderson SE, Murphy E, Steenbergen C et al. Na-H exchange in myocardium: effects of hypoxia and acidification Na and Ca. Am J Physiol 1990; 259:C940-C948.
14. Orlowsky J, Kandasamy RA, Shull GE. Molecular cloning of putative members of the Na/H exchanger gene family. J Biol Chem 1992; 267:9331-9339.
15. Klanke CA, Su YR, Callen DF et al. Molecular cloning and physical and genetic mapping of a novel human Na^+/H^+ exchanger (NHE5/SLC9A5) to chromosome 16q22.1. Genomics 1995; 25:615-622.
16. Lagadic Gossmann D, Vaughn-Johns RD Coupling of dual acid extrusion in the guinea pig isolated ventricular myocyte to alpha 1 and beta adreno receptors. J Physiol London 1993; 464:49-73.

17. Ladilov Y, Siegmund B, Piper HM. Blockade of the Na^+/H^+ exchanger protects against reoxygenation induced hypercontracture in Ca^{++} overloaded cardiomyocytes. Europ J Physiol 1994; 426:R117.
18. Ibuki C, Hearse DJ, Avkiran M. Mechanisms of antifibrillatory effect of acidic reperfusion: role of perfusate bicarbonate concentration. Am J Physiol 1993; 264:H783-H790.
19. Scholz W, Albus U, Lang HJ et al. Protective effects of HOE 642, a selective sodium-hydrogen exchange subtype 1 inhibitor, on cardiac ischaemia and reperfusion. Cardiovascular Research 1995; 29:260-268.
20. Imai S, Shi AY, Ishibashi T, Nakazawa N. Na^+/H^+ exchange is not operative under low flow ischemic conditions. J. Mol Cell Cardiol 1991; 23:505-517.
21. Duff HJ, Lester WM, Rahmberg M. Amiloride, antiarrhythmic and electrophysiological activity in the dog. Circulation 1988; 78:1469-1477.
22. Kim Y-I, Herijgers P, Van Lommel A et al. Na^+/H^+ Exchange Inhibition Improves Post-transplant Myocardial Compliance in 4-Hour Stored Donor Hearts. Cardiovascular Surgery 1997; (6):67-75.
23. Bethencourt D, Laks H. Importance of edema and compliance changes during 24 hours of preservation of the dog heart. J Thorac Cardiovasc Surg 1981; 81:440-449.
24. Burt JM, Copeland JG. Myocardial function after preservation for 24 hours. J Thorac Cardiovasc Surg 1986; 92:238-246.
25. Cross CE, Rieben PA, Salisbury PF. Influence of coronary perfusion and myocardial edema on pressure-volume diagram of left ventricle. Am J Physiol 1961; 201:102-108.
26. Hsu DT, Weng Z-C, Nicolosi AC et al. Quantitative effects of myocardial edema on the left ventricular pressure-volume relation. Influence of cardioplegia osmolarity over two hours of ischemic arrest. J Thorac Cardiovasc Surg 1993; 106:651-657.
27. Lazar HL, Haasler GB, Sponitz WD et al. Compliance, mass, and shape of the canine left ventricle after global ischemia analyzed with two-dimensional echocardiography. J Surg Res 1985; 39:199-208.
28. Amirhamzeh MMR, Jia CX, Starr JP et al. Diastolic function in the heterotopic rat heart transplant model-Effect of edema, ischemia, and rejection. J Thorac Cardiovasc Surg 1994; 108:928-937.
29. Flameng W. Clinical experience with HOE 642. A pilot study. Proceedings Symposium "Organ Preservation in Heart Transplantation". Berlin: Deutsches Herzzentrum 1996; in press.

Chapter 7

Metabolic Support for the Heart During Ischemia and Reperfusion:
Role of Amino Acids

Heinrich Taegtmeyer and Torsten Doenst

In reviewing amino acid metabolism of the heart during ischemia and reperfusion it is important to address a few principles of energy substrate metabolism first. The healthy human heart has a very high rate of energy turnover. Like all living cells, cardiac myocytes need the energy captured in ATP to power their essential functions. The bulk of it is derived from oxidative phosphorylation of ADP, which, in turn, is fueled by oxidation of substrates such as fatty acids, glucose, glycogen, lactate, and certain amino acids. The normal human heart, which weighs about 300 g, consumes about 5 kg of ATP in the course of 24 hours, i.e., 17 times its own weight![1] Under normal conditions most of the energy for contraction is derived from the oxidation of long-chain fatty acids,[2] but inotropic stimulation results in the preferential oxidation of glycogen.[3] The heart oxidizes amino acids only when their plasma concentrations are very high.[4]

Ischemia alters substrate metabolism and impairs energy transfer. With severe ischemia fatty acid oxidation ceases, while glucose metabolism continues. Because heart muscle, like all living tissues, contains only very small amounts of ATP and phosphocreatine, this "energy reserve" is exhausted within seconds after the interruption of blood supply. This extreme situation is modified by substrate level phosphorylation of ADP from the metabolism of endogenous substrates. These substrates are glycogen and, as will be discussed below, certain amino acids. The breakdown of glycogen provides glycolytic substrate for essential cell functions, especially for support of ion pumps.[5,6] The role of the amino acids in the metabolic response to myocardial ischemia[7,8] is still not very well defined. There is, however, intriguing evidence that amino acids modify the response to myocardial ischemia and improve the recovery of function.[9,10] The biochemical basis for this phenomenon can only be understood in the wider context of some of the principles of energy transfer.

Energy for Contraction: Cycles Improve Efficiency

The term "oxidative phosphorylation of ADP" is an insufficient description of the process of myocardial energy production. Linked to oxidative metabolism is not only the production of protons (or reducing equivalents) in the citric acid cycle and other oxidative pathways, but also the maze of highways and byways of intermediary metabolism.

Intermediary metabolism of energy providing substrates forms the link between gene expression on the one hand and contractile function of the heart on the other hand.[11] Energy substrate metabolism is complex and highly regulated.[12] Heart muscle is capable of

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using energy-providing substrates interchangeably and has therefore been called an "omnivore".[13] There is growing recognition for the concepts that the regulation of substrate flux is shared by several of the pathway enzymes[14,15] and that contractile function is optimal only when fatty acids and carbohydrates are oxidized simultaneously.[2]

We have recently drawn attention to the importance of moiety-conserved cycles for the efficient transfer of energy in heart muscle.[16] A moiety-conserved cycle is defined as a regularly returning sequence of reactions, in which the concentrations of the key components (moieties) neither increase nor decrease. Amino acids play an important role in either maintaining or restoring moiety-conserved cycles.

The rhythmic nature of contraction and relaxation of heart muscle is a palpable manifestation of cyclic processes as integral part of normal physiology. Cycles can be grouped into cycles of energy provision (e.g., the circulation itself) and into cycles of energy consumption (e.g., the actin-myosin crossbridge formation and its release). Many intermediary metabolites that serve as vehicles for energy transfer are cycled and recycled as well. Best known among them are the intermediates of the Krebs citric acid cycle. As it has been pointed out by Krebs himself, the evolutionary advantage of such cycles is that they make efficient use of resources.[17] The principle is easily understood if one compares two different types of locomotion.[16] A bicyclist covers nearly four times the distance of a runner for the same amount of energy expended. The only difference between the two is the interposition of two wheels (i.e., two moiety-conserved cycles) between the body and the ground.

Myocardial ischemia results in a collapse of most moiety-conserved cycles, and the heart has to rely on less efficient linear pathways for energy production. A frequently quoted example is the energy yield of 36 moles of ATP per mole of glucose oxidized compared to 2 moles of ATP per mole of glucose converted to lactate. As we will show below, restoration of oxidative metabolism with reperfusion also requires the restoration of moiety-conserved cycles, a process termed "anaplerosis". The amino acids glutamate, aspartate, isoleucine, and valine are able to function as anaplerotic substrates for the citric acid cycle (see below).

Amino Acids: Cycling In and Out of Proteins

Although this review emphasizes the metabolism of amino acids, it is important to remember that amino acids are first and foremost the building blocks for proteins, as amino acids cycle in and out of proteins (Fig. 7.1). The incorporation of specific amino acids into specific proteins of the heart is governed by the transcription and translation of specific genes on the chromosomes of the nucleus. While the details of gene expression and protein synthesis are increasingly well understood, the same cannot be said for protein degradation.[12] Both processes require energy in the form of ATP[18] and oxygen deprivation inhibits both protein synthesis and degradation.[7] Studies with tracers and inhibitors of either protein synthesis or degradation have shown that under steady state conditions a large portion of the amino acids is recycled between synthesis and degradation. Furthermore, branched chain amino acids (e.g., leucine) are thought to regulate protein turnover.[19]

Some amino acids are neither synthesized nor degraded by heart muscle. These compounds are frequently used as tracers for protein turnover.[19] The rates of protein turnover in the heart are often underestimated. The principle of the "dynamic nature of body constituents"[20] is perhaps best illustrated in the heart, which regenerates itself completely once every thirty days.[21]

Amino Acid Metabolism in Normal Heart

The important role of amino acids in energy substrate metabolism is also often overlooked. Heart muscle contains the enzymes required for the synthesis and degradation of most, but not all, amino acids. Oxidation of branched chain amino acids proceeds rapidly

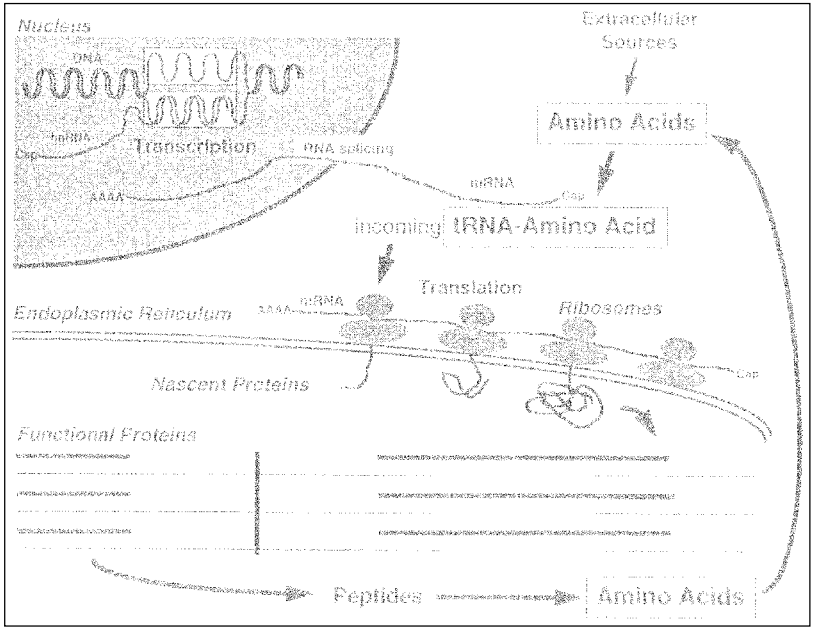

Fig. 7.1. A simple scheme which emphasizes the importance of amino acids as building blocks for myocardial proteins. Gene transcription in the nucleus is followed by translation of activated amino acids (tRNA-amino acid) into protein. Sets of three nucleotides (codons) in an mRNA are translated into amino acids in the course of protein synthesis. Major steps include RNA transcription from DNA, RNA processing in the nucleus (RNA splicing) and RNA translation on ribosomes. The "dynamic nature of body constituents" is best illustrated by the cycling of amino acids between protein synthesis and degradation. The cycling of amino acids in and out of proteins requires biological energy in the form of ATP.

(and in preference to other substrates) when their plasma level is high.[22,23]

Furthermore, the amino acids aspartate and glutamate also constitute an integral part in the transport of reducing equivalents across the inner mitochondrial membrane for the oxidation of cytosolic NADH by the mitochondrial electron transport chain.[24] In the malate-aspartate shuttle cytosolic NADH is oxidized by the reduction of oxaloacetate to malate. Malate enters the mitochondrial matrix, where it is oxidized to oxaloacetate, which is transaminated with glutamate to form alpha-ketoglutarate and aspartate. Aspartate and alpha-ketoglutarate leave the mitochondrion. Transamination of these metabolites regenerates oxaloacetate and glutamate in the cytosol, and the net effect is the transfer of hydrogen ions across the mitochondrial membrane. Net forward flux through the malate-aspartate shuttle involves the concerted activity of a unidirectional glutamate-aspartate exchanger and a reversible alpha-ketoglutarate-malate exchanger.[25]

The dynamic nature of amino acid metabolism and the turnover of amino acids is underscored by the participation of amino acids as intermediates of many metabolic pathways. Experimentally, isotopic enrichment of the amino acid glutamate from ^{13}C-labeled exogenous substrates is used as a "lap counter" for the citric acid cycle[26] and sophisticated models have been developed to determine the origin and fate of specific substrates by NMR spectroscopy.[27,28] The same technique lends itself to the analysis of the fate of dicarboxy-acids during ischemia and reperfusion.[29]

The main features of myocardial amino acid metabolism are listed in Table 7.1 and illustrated in Figure 7.2. First, we draw

Table 7.1. Main features of myocardial amino acid metabolism

1.	Amino acids and myocardial energy metabolism
	Source of acetyl-CoA
	Precursors of citric acid cycle intermediates
	Shuttle of reducing equivalents across the inner mitochondrial membrane
2.	Amino acids and myocardial ammonia metabolism
	Glutamine synthesis
	Glutamate synthesis

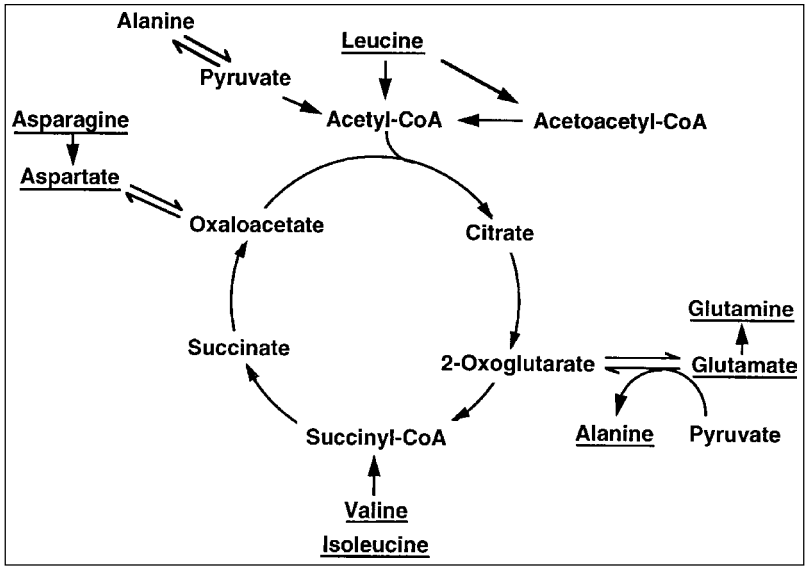

Fig. 7.2. Salient features of anaerobic amino acid metabolism in heart muscle. Enhanced rates of glycolysis result in the accumulation of lactate, alanine, succinate, and CO_2, as well as the depletion of glutamate and alpha-ketoglutarate. Substrate level phosphorylation occurs at two sites by mechanisms indicated on the scheme. See text for further discussion.

attention to amino acids as intermediates of myocardial energy metabolism. Secondly, we draw attention to amino acids as an integral part of myocardial ammonia metabolism, as it has been demonstrated in studies using [^{13}N]ammonia, which is avidly extracted by the myocardium and retained as glutamine.[30] The positron-emitting tracer [^{13}N]ammonia is commonly used as tracer for myocardial blood flow.

Amino acids which are metabolized to acetyl-CoA (Fig. 7.2) may become an efficient source of energy and compete with other substrates for the fuel of respiration. A case in point is the amino acid leucine. Leucine is transaminated, decarboxylated, and further metabolized in heart muscle to yield both acetyl-CoA and acetoacetyl-CoA. The high K_m of branched-chain amino transferase for leucine assures that leucine is oxidized only when present in high plasma concentrations ($\approx$ 2.5 μmol/L), as it is the case after a protein-rich meal. Early studies of cardiac substrate metabolism, using coronary sinus catheterization to measure A-V differences, suggest that as much as 40% of the fuel for respira-

tion can be supplied by amino acids.[4] Leucine oxidation by heart muscle has been demonstrated by several investigators.[22,31,32] The physiologic importance of the control of amino acid metabolism by K_m seems evident. Since dietary protein and amino acids cannot be stored in major quantities, amino acids ingested in the form of protein are distributed in the blood stream, and degradation of any excess amino acids takes precedence over the degradation of carbohydrate and fat.[33]

Amino acids which may be metabolized to citric cycle intermediates are, in addition to leucine, alanine, glutamate, aspartate, valine, and isoleucine (Fig. 7.2). The oxidation of glutamate by heart muscle homogenates and mitochondria[34,35] is thought to be of particular importance. Because heart muscle does not contain the enzyme glutamate dehydrogenase, glutamate is transaminated to alanine and alpha-ketoglutarate in an anaplerotic reaction of the citric acid cycle. A similar transamination process occurs with the conversion of aspartate to alanine and oxaloacetate.

Amino Acid Metabolism in Oxygen-Deprived Heart

Ischemia and reperfusion alter the pattern of myocardial amino acid metabolism in several ways. First, the efficient transfer of energy through moiety-conserved cycles is replaced by less efficient linear pathways. Secondly, amino acids are no longer oxidized to acetyl-CoA. Third, alanine and succinate accumulate, while glutamate is catabolized (Fig. 7.3). This short pathway supports the substrate-level phosphorylation of GDP.

When we discovered alanine accumulation in ischemic heart muscle, we postulated that alanine was, like lactate, a product of anaerobic glucose metabolism.[36] Indeed, in patients with coronary artery disease alanine release paralleled the release of lactate and was accompanied by increased utilization of glutamate.[37] We thought of alanine as a marker for ischemia, however, quantitatively much less pyruvate is converted to alanine than to lactate,[7] and rigorous testing of this hypothesis has never been carried out.

An important aspect of anaerobic amino acid metabolism is the conversion of glutamate to succinate.[8] As already shown, this pathway is linked to substrate-level phosphorylation in part of the citric acid cycle. Unlike glycolysis, glutamate utilization can be augmented by a simple mass action effect and this pathway may represent a source of energy during O_2 deprivation. Sanborn et al[38] studied the mechanism of glutamate conversion to succinate and found that in oxygen deprived papillary muscles of the rabbit decarboxylation of alpha-ketoglutarate continued under hypoxic conditions, whereas complete oxidation of alpha-ketoglutarate was inhibited. More recent work by Lazar et al[9] and by Bittle and Shine[39] demonstrated the beneficial effect of glutamate on cardiac function when glutamate was added to the perfusion medium of reversibly ischemic, reperfused rabbit heart. In an effort to quantitate the amount of ATP production from the conversion of glutamate to succinate, Wiesner et al[40] measured succinate production from other amino acids in regional ischemia in dog hearts. The investigators calculated that glutamate accounted for 20% of the ATP generated during the ischemic period. Although exogenous supplementation of glutamate leads to an increased uptake,[41] the amount of ATP production from glutamate metabolism during ischemia when glutamate was supplemented is not known.

A second aspect of anaerobic amino acid metabolism is the conversion of aspartate to fumarate. Fumarate may be metabolized to succinate by the enzyme fumarate reductase regenerating FAD^+ and ATP in the process.[42] However, the concept of anaerobic ATP production through reduction of fumarate to succinate has been questioned based on insignificant reduction of fumarate during ischemia.[43]

Other concepts on the mechanism of protection against ischemic damage by amino acids include the maintenance of glycolytic activity during ischemia by regeneration of cytosolic NAD^+ through the malate aspartate shuttle or by the reduction of feedback inhibition of glycolysis by lactate since part of the pyruvate is transaminated to alanine and does not enter the lactate pool.[44]

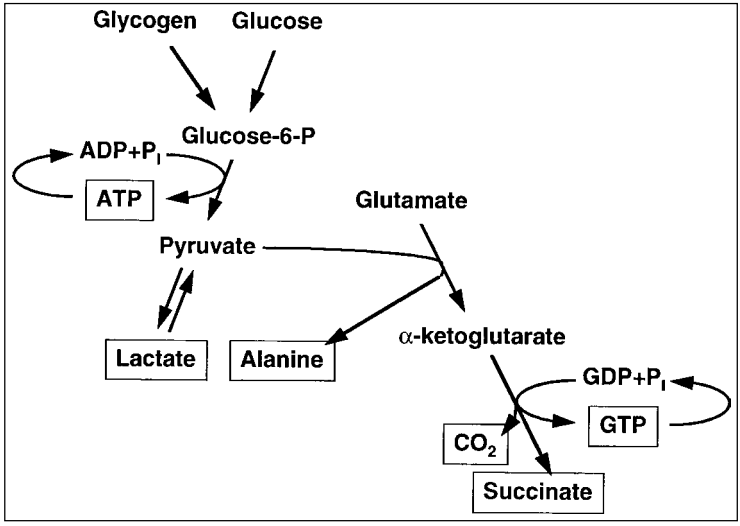

Fig. 7.3. Amino acids as precursors or products of citric acid cycle intermediates. The amino acids glutamate, valine, isoleucine, and aspartate may be important carbon sources for the replenishment of the citric acid cycle upon reperfusion after ischemia. See text for further discussion.

All findings indicate that carbohydrate and amino acid metabolism in oxygen-deprived heart muscle are linked through the transamination of pyruvate with glutamate and the resultant formation of alanine and succinate. Since the conversion of glutamate to succinate occurs in the mitochondrial matrix, it appears that the matrix enzymes still function during brief periods of oxygen deprivation. As will be discussed further below, the advanced hypotheses have to be viewed with caution because they depend on proper functioning of the malate-aspartate shuttle which may be impaired in its activity during ischemia and reperfusion.[45]

Amino Acids as Substrates for Metabolic Support During Reperfusion

There is good experimental evidence that amino acids are capable of decreasing the amount of injury caused by ischemia or reperfusion or both, although the results are not all consistent.[32] Some of the discrepancies may be explained by the different models used by different investigators. Beneficial effects can be observed when the perfusion medium is supplemented before, during, and after ischemia[39,44,46] or during reperfusion alone.[9,46] Most of the studies have focused on aspartate and glutamate, because these substrates are the key amino acids of the malate-aspartate shuttle, an important transport mechanism for the transfer of cytosolic reducing equivalents in to the mitochondrial matrix, and because glutamate is readily transaminated to alpha-ketoglutarate, a citric acid cycle intermediate (see "anaplerosis" above).

Several concepts have been advanced on the mechanism of the protective effects of amino acids and their metabolites during reperfusion. First, as already described above, maintenance of anaerobic glycolysis and ATP generation through substrate level phosphorylation in the formation of succinate reduce injury during ischemia thereby improving recovery during reperfusion.[8,9,40] Secondly, the replenishment of the intermediates of the malate-aspartate shuttle (e.g., glutamate, which is depleted during ischemia) during reperfusion promotes oxidative metabolism, specifically glucose oxidation.[46] Third, aspartate gives rise to ATP when converted to suc-

cinate.[42] Fourth, fumarate, the integral intermediate in the conversion of aspartate to succinate during ischemia, may also function as free radical scavenger during reperfusion and contributes to Ca^{2+} transport.[47] The first three concepts reflect the rationale behind the addition of aspartate and glutamate to cardioplegia (see below). The fourth concept was the basis for a study on neonate piglets, where the hearts were arrested for 120 min with blood cardioplegia supplemented with fumarate.[47] The investigators found full recovery of contractile function during reperfusion in the group with fumarate supplementation compared to approximately 70% of recovery of function in the hearts without fumarate addition.

However, all the suggested mechanisms depend more or less on the exchange of metabolites across the inner mitochondrial membrane through the malate-aspartate shuttle. This aspect may render the advanced mechanisms only hypothetical, since Lewandowski et al[45] demonstrated that the bidirectional part of the malate-aspartate shuttle (malate/alpha-ketoglutarate exchanger) is reduced in its activity during ischemia and reperfusion in rabbit heart. This decreased activity of the malate-aspartate shuttle may contribute to a persisting proton accumulation in the reperfused, "stunned" myocardium[48] and may explain why in our hands glutamate failed to improve contractile function during and following ischemia in isolated rabbit hearts perfused with glucose.[32] Thus, while some experimental results suggest protective effects of amino acids against ischemic injury, the mechanisms for this protective effect are not yet understood. An important step in the validation of the above discussed mechanisms would be the demonstration of normal or increased activity of the malate-aspartate shuttle in the setting where amino acid supplementation leads to reduction in ischemic injury and improvement of postischemic function. For the systemic application of glutamate in patients to improve recovery in postischemic cardiac function, one has to bear in mind that high concentrations of glutamate result in neurotoxicity.[49,50] This effect of glutamate may limit the clinical use of this amino acid to the enrichment cardioplegia.

Substrate Enhancement of Cardioplegic Solutions by Amino Acids

The observation that amino acids reduce ischemic injury in vitro has led to the next logical step, the addition of various amino acids to cardioplegic solutions for the protection of ischemic heart muscle in vivo. Myocardial utilization of amino acids, especially of glutamate, has been demonstrated clinically in patients during aorto-coronary bypass surgery.[51] Most studies showed evidence for reduced ischemic injury with amino acid supplementation in cardioplegia (mainly blood cardioplegia).[39,47,52,53] Rosenkranz et al[54] demonstrated an additive benefit of glutamate and aspartate supplementation of blood cardioplegia on postreperfusion ventricular function in adults (Fig. 7.4). These findings are consistent with the concepts of anaerobic ATP generation through two different pathways, both resulting in the production of succinate (see Fig. 7.3 and the above). However, the importance of these pathways has never been demonstrated under the conditions tested, and like in the experimental data in vitro, the results of other studies argue against a benefit of amino acids in cardioplegic solutions.[55,56]

Perhaps the most striking observations in the clinical setting of myocardial stunning after cardioplegia are those of Beyersdorf et al.[57] The investigators used glutamate/aspartate-enriched blood cardioplegia during the coronary revascularization procedure in patients with perioperative sudden death. Despite the grim prognosis in this group of patients, 78% of them recovered completely and were discharged with significantly improved ejection fractions (Fig. 7.5). Still, the results need to be viewed with caution. The obvious difficulties in obtaining a control group for this complex clinical study did not allow conclusions whether the excellent outcome was due to or at least influenced by amino acid addition.

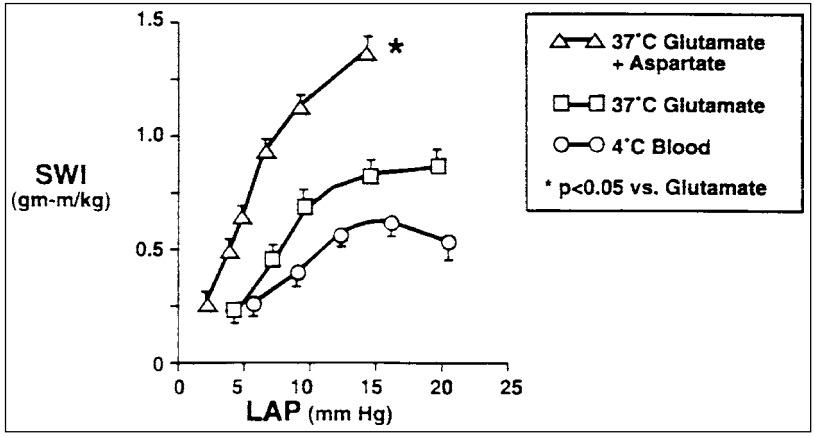

Fig. 7.4. Benefit of glutamate and aspartate enrichment of blood cardioplegia on postreperfusion ventricular function in adults. LAP, left atrial pressure; SWI, stroke work index. Reprinted with permission from Rosenkranz ER, Okamoto F, Buckberg GD et al. Safety of prolonged aortic clamping with blood cardioplegia: Aspartate enrichment of glutamate-blood cardioplegia in energy-depleted hearts after ischemic and reperfusion injury. J Thorac Cardiovasc Surg 1986; 91:428-435.

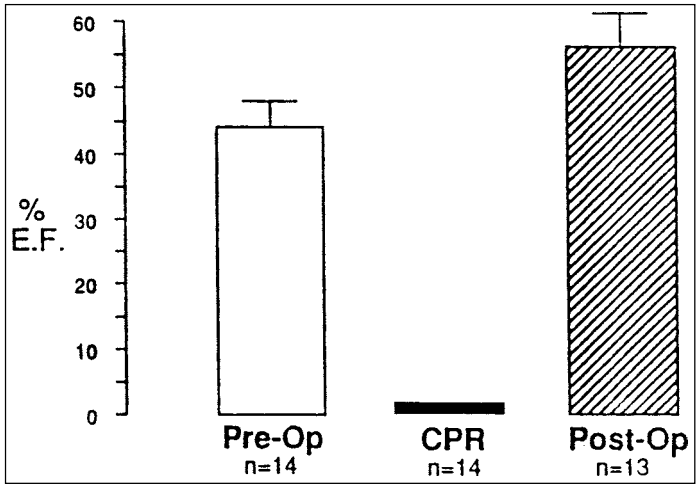

Fig. 7.5. Global ejection fraction (EF) of the left ventricle before (Pre-Op) and after (Post-Op) operation and during cardiopulmonary resuscitation (CPR). Note a) the moderate reduction of EF before operation and before intractable ventricular fibrillation occurred (open bar), b) the significant reduction of EF in those patients with preoperative or postoperative arrest (filled bar), and c) significant improvement ($p<0.05$) of EF postoperatively (hatched bar). Reprinted with permission from Beyersdorf F, Kirsh M, Allen BS. Warm glutamate/aspartate-enriched blood cardioplegic solution for perioperative sudden death. J Thorac Cardiovasc Surg 1992; 104:1141-1147.

It is possible that the inconsistencies observed in the different studies are due to different experimental designs. It appears necessary to further define indications for amino acid enrichment of cardioplegic solutions for the use in human heart. A better understanding of the underlying mechanisms is therefore of paramount importance. It should, however, be noted that none of the studies demonstrated any detrimental effects of amino acid enrichment of cardioplegia.

Conclusions and Outlook

Amino acids are the building blocks of myocardial proteins. Certain amino acids are also important intermediates of energy substrate metabolism in aerobic and anaerobic heart. The conversion of glutamate to succinate during oxygen deprivation is coupled to substrate-level phosphorylation of GDP to GTP. The conversion of aspartate to succinate via fumarate reductase has also been suggested as a source of anaerobic energy. Because amino acids appear to improve ischemia tolerance in vitro, solutions fortified with amino acids have been successfully employed for cardioplegia and reperfusion in vivo. Although some reports record spectacular results and no adverse effects of amino acid infusions have been observed, the well known neurotoxicity of glutamate may limit the unrestricted use of this amino acid in patients with ischemic heart disease.

Acknowledgments

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References

1. Apstein CS, Taegtmeyer H. Glucose-insulin-potassium in acute myocardial infarction. Circulation 1997; 96:1074-1077.
2. Taegtmeyer H, Hems R, Krebs HA. Utilization of energy providing substrates in the isolated working rat heart. Biochem J 1980; 186:701-711.
3. Goodwin GW, Ahmad F, Doenst T et al. Energy provision from glycogen, glucose and fatty acids upon adrenergic stimulation of isolated working rat heart. Am J Physiol 1998; in press.
4. Bing RJ. The metabolism of the heart. Harvey Lect 1955; 50:27-70.
5. McDonald T, MacLeod D. Metabolism and the electrical activity of ventricular muscle. J Physiol (Lond) 1973; 229:559-582.
6. Xu KY, Zweier JL, Becker LC. Functional coupling between glycolysis and sarcoplasmic reticulum Ca^{2+} transport. Circ Res 1995; 77:88-97.
7. Taegtmeyer H, Ferguson A, Lesch M. Protein degradation and amino acid metabolism in autolyzing rabbit myocardium. Exp Mol Pathol 1977; 26:52.
8. Taegtmeyer H. Metabolic responses to cardiac hypoxia: Increased production of succinate by rabbit papillary muscles. Circ Res 1978; 43:808-815.
9. Lazar HL, Buckberg GD, Manganaro AJ et al. Reversal of ischemic damage with amino acid substrate enhancement during reperfusion. Surgery 1980; 88:702-709.
10. Pisarenko O, Solomatina E, Studneva I et al. Protective effect of glutamic acid on cardiac function and metabolism during cardioplegia and reperfusion. Basic Res Cardiol 1983; 78:534-543.
11. Taegtmeyer H, Goodwin GW, Doenst T et al. Substrate metabolism as a determinant for postischemic functional recovery of the heart. Am J Cardiol 1997; 80:3A-10A.
12. Taegtmeyer H. Energy metabolism of the heart: From basic concepts to clinical applications. Curr Prob Cardiol 1994; 19:57-116.
13. Taegtmeyer H, Roberts AFC, Rayne AEG. Energy metabolism in reperfused rat heart: Return of function before normalization of ATP content. J Am Coll Cardiol 1985; 6:864-870.
14. Kashiwaya Y, Sato K, Tsuchiya N et al. Control of glucose utilization in working perfused rat heart. J Biol Chem 1994; 269(41):25502-25514.
15. Srere P. Complexities of metabolic regulation. Trends in Biochem Sci 1994; 19:519-520.
16. Taegtmeyer H, Villalobos DH. Metabolic support for the postischaemic heart. Lancet 1995; 345:1552-1555.
17. Baldwin JE, Krebs HA. The evolution of metabolic cycles. Nature 1981; 291:381-382.
18. Lesch M, Taegtmeyer H, Peterson M et al. Studies on the mechanism of the inhibition of myocardial protein synthesis during oxygen deprivation. Am J Physiol 1976; 230: 120-126.

19. Morgan H, Rannels D, McKee E. Protein Metabolism of the Heart. In: Berne R, ed. Handbook of Physiology: The Cardiovascular System: The Heart. Washington, D.C.: American Physiology Society, 1979:845-871.
20. Schoenheimer R. The Dynamic State of Body Constituents. Cambridge, MA: Harvard University Press, 1942.
21. Morgan H, Neely J. Metabolic Regulation and Myocardial Function. In: Hurst JW, ed. The Heart. 6th ed. New York: McGraw-Hill, 1986:85-100.
22. Ichihara K, Neely J, Siehl D et al. Utilization of leucine by working rat heart. Am J Physiol 1980; 239:E430-E436.
23. Bing R, Siegel A, Ungar I et al. Metabolism of the human heart. 2. Studies on fat, ketone and amino acid metabolism. Am J Med 1954; 16:504-515.
24. Safer B, Smith C, Williamson J. Control of the transport of reducing equivalents cross the mitochondrial membrane in perfused rat heart. J Mol Cell Cardiol 1971; 2:111-124.
25. LaNoue K, Schoolwerth A. Metabolite transport in mitochondria. Annu Rev Biochem 1979; 48:871-922.
26. Malloy C, Sherry A, Jeffrey F. Carbon flux through citric acid cycle pathways in perfused heart by ^{13}C NMR spectroscopy. FEBS Lett 1987; 212:58-62.
27. Chance E, Seeholzer S, Kobayashi K et al. Mathematical analysis of isotope labeling in the citric acid cycle with applications to ^{13}C NMR studies in perfused rat hearts. J Biol Chem 1983; 258:13785-13794.
28. Jeffrey F, Diczku V, Sherry A et al. Substrate selection in the isolated working rat heart: Effects of reperfusion, afterload, and concentration. Basic Res Cardiol 1995; 90:388-396.
29. Brainard JR, Hutson JY, Hoekenga DE et al. Ordered synthesis and mobilization of glycogen in the perfused heart. Biochem J 1989; 28:9766-9772.
30. Schelbert H, Phelps M, Hoffman E. Regional myocardium blood flow, metabolism, and function assessed noninvasively with positron emission tomography. Am J Cardiol 1980; 46:1269-1276.
31. Tischler ME, Goldberg AL. Leucine degradation and release of glutamine and alanine by adipose tissue. J Biol Chem 1980; 255: 8074-8081.
32. Taegtmeyer H, Russell R. Glutamate metabolism in rabbit heart: Augmentation by ischemia and inhibition with acetoacetate. J Appl Cardiol 1987; 2:231-249.
33. Krebs HA. Some aspects of the regulation of fuel supply in omnivorous animals. Adv Enzyme Regul 1972; 10:397-420.
34. Borst P, Slater E. The oxidation of glutamate by rat heart sarcosomes. Biochim Biophys Acta 1960; 41:170-171.
35. Krebs H, Bellamy D. The interconversion of glutamic acid and aspartic acid in respiring tissues. Biochem J 1960; 75:523-529.
36. Taegtmeyer H, Peterson MB, Ragavan VV et al. De novo alanine synthesis in isolated oxygen-deprived rabbit myocardium. J Biol Chem 1977; 252:5010-5018.
37. Mudge G, Mills R, Taegtmeyer H et al. Alterations of myocardial amino acid metabolism in chronic ischemic heart disease. J Clin Invest 1976; 58:1185-1192.
38. Sanborn T, Gavin W, Berkowitz S et al. Augmented conversion of aspartate and glutamate to succinate during anoxia in rabbit heart. Am J Physiol 1979; 237:H535-H541.
39. Bittl J, Shine K. Protection of ischemic rabbit myocardium by glutamic acid. Am J Physiol 1983; 245:H406-H412.
40. Wiesner R, Deussen A, Borst M et al. Glutamate degradation in the ischemic dog heart: Contribution to anaerobic energy production. J Mol Cell Cardiol 1989; 21:49-59.
41. Dinkelborg LM, Kinne RKH, Grieshaber MK. Transport and metabolism of L-glutamate during oxygenation, anoxia, and reoxygenation of rat cardiomyocytes. Am J Physiol 1996; 270:H1825-H1832.
42. Wilson MA, Cascarano J. The energy yielding oxidation of NADH by fumarate in submitochondrial particles of rat tissues. Biochim Biophys Acta 1970; 216:54-62.
43. Peuhkurinen K, Takala T, Nuutinen E et al. Tricarboxylic acid cycle metabolites during ischemia in isolated perfused rat heart. Am J Physiol 1983; 244:H281-H288.
44. Pisarenko OI, Studneva IM, Shulzhenko VS et al. Substrate accessibility to cytosolic aspartate aminotransferase improves posthypoxic recovery of isolated rat heart. Biochem Mol Medicine 1995; 55:138-148.
45. Lewandowski ED, Yu X, LaNoue KF et al. Altered metabolite exchange between subcellular compartments in intact postischemic rabbit hearts. Circ Res 1997; 81:165-175.
46. Shug AL, Madsen D, Dobbie R et al. Protection of mitochondrial and heart function by amino acids after ischemia and cardioplegia. Life Sci 1994; 54:557-567.
47. Pearl JM, Hiramoto J, Laks H et al. Fumarate-enriched blood cardioplegia results in complete functional recovery of immature myocardium. Ann Thorac Surg 1994; 57: 1636-1641.

48. Marban E, Kitakaze M, Koretsune Y et al. Quantification of $[Ca^{2+}]_i$ in perfused hearts. Critical evaluation of the 5F-BAPTA and nuclear magnetic response method as applied to the study of ischemia and reperfusion. Circ Res 1990; 66:1255-1267.
49. Bittigau P, Ikonomidou C. Glutamate in neurologic diseases. J Child Neurol 1997; 12: 471-485.
50. Olney JW. Brain lesions, obesity, and other disturbances in mice treated with monosodium glutamate. Science 1969; 164:719-721.
51. Svedjeholm R, Ekroth R, Joachimsson PO et al. Myocardial uptake of amino acids and other substrates in relation to myocardial oxygen consumption four hours after cardiac operations. J Thorac Cardiovasc Surg 1991; 101:688-694.
52. Svedjeholm R, Hakanson E, Vanhanen I. Rationale for metabolic support with amino acids and glucose-insulin-potassium (GIK) in cardiac surgery. Ann Thor Surg 1995; 59 (Suppl 2):S15-S22.
53. Kjellman U, Björk K, Ekroth R et al. Alpha-ketoglutarate for myocardial protection in heart surgery. Lancet 1995; 345:552-553.
54. Rosenkranz ER, Okamoto F, Buckberg GD et al. Safety of prolonged aortic clamping with blood cardioplegia: Aspartate enrichment of glutamate-blood cardioplegia in energy-depleted hearts after ischemic and reperfusion injury. J Thorac Cardiovasc Surg 1986; 91:428-435.
55. Asai T, Grossi EA, LeBoutillier MR et al. Resuscitative retrograde blood cardioplegia. Are amino acids or continuous warm techniques necessary? J Thorac Cardiovasc Surg 1995; 109:242-248.
56. Crooke GA, Harris LJ, Grossi EA et al. Role of amino acids and enhancement. Cardioplegia in routine myocardial protection. J Thorac Cardiovasc Surg 1993; 106:497-501.
57. Beyersdorf F, Kirsh M, Allen BS. Warm glutamate/aspartate-enriched blood cardioplegic solution for perioperative sudden death. J Thorac Cardiovasc Surg 1992; 104:1141-1147.

Chapter 8

Metabolic and Antioxidant Support with Amino Acids

Oleg I. Pisarenko

Hypothermic hyperkalemic cardioplegia is currently the preferred method of myocardial preservation for the performance of cardiac operations. At present research efforts of many laboratories focus on approaches to enhanced myocardial protection by various pharmacological agents. During the past 15 years amino acids are being extensively studied among other natural metabolites potentially capable to attenuate ischemic and reperfusion injury. A minor significance of amino acids as energy producing substrates is generally accepted since less than 5% of oxygen consumed by the heart is used for their oxidation.[1] Although amino acids are involved in intracellular metabolism and operating specific transport systems of the plasma membrane, they do not affect cardiac function under normal conditions.[2] However, there is a growing body of evidence that certain of them may be vital for myocardial function and survival during ischemia/reperfusion stress. In this respect glutamate (Glu), aspartate (Asp), taurine (Tau), branched chain amino acids (BCAA), histidine (His) and methionine (Met) seem to be the most important. Mechanisms of their action, in conditions relevant to cardiac surgery, are briefly discussed in this chapter.

Glutamate and Aspartate

The first evidence of Glu importance for ischemic myocardium was observed more than 20 years ago in clinical studies showing that hearts of patients with coronary artery disease extracted more L-glutamate than did hearts of individuals with normal coronary arteries.[3] Later enhanced myocardial extraction of L-^{13}N-Glu in the areas with a reduced blood flow was confirmed in human heart by positron-emission tomography.[4] Together with Asp, myocardial Glu content dramatically decreases during periods of ischemia and hypoxia. It was shown that catabolism of both amino acids may be coupled with anaerobic and aerobic energy formation.

Anaerobic Action

The key enzymes controlling the entry of these amino acids into the energy generating pathways are cytosolic alanine and aspartate aminotransferases, leading to formation of 2-oxoglutarate and oxaloacetate.[5] Oxidation of 2-oxoglutarate to succinate in mitochondria leads to substrate phosphorylation of GDP in the succinic thiokinase reaction and then formation of ATP by the nucleoside diphosphate kinase. Oxaloacetate can be converted to fumarate in successive reactions catalyzed by cytosolic malate dehydrogenase and mitochondrial fumarase. Further NADH-dependent reduction of fumarate to succinate is coupled with ATP formation in complex 1 of the respiratory chain. Probably, electron transfer from NADH to fumarate occurs from NADH dehydrogenase through ubiquinone to succinate dehydrogenase.[6] Both succinate-producing routes may operate with the common mitochondrial NAD^+/NADH pair to

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maintain the redox potential of the cell unchanged.[7] Succinate is the end product of anaerobic metabolism of Glu and Asp and does not undergo further conversions in the absence of oxygen.

Glu and Asp may also exert stimulatory effects on anaerobic glycolysis. Conversion of Asp to oxaloacetate and then to malate consumes cytosolic NADH in the malate dehydrogenase reaction, thus, relieving glycolytic flux through the glyceraldehyde 3-phosphate dehydrogenase.[8] Additionally, transamination of glycolytic pyruvate with glutamate may reduce lactate accumulation in myocardial tissue and diminish inhibition of glycolysis at the lactate dehydrogenase step. Intracellular acidosis favors this reaction, since K_m of alanine aminotransferase for pyruvate decreases with decreasing pH.[9] Alternatively, transport of Glu and malate into mitochondria may stimulate operation of mitochondrial part of the malate-aspartate shuttle. Dismutation of malate to oxaloacetate and succinate in oxygen-deprived rat heart mitochondria[7] provides the formation of reducing equivalents and their prompt removal by the mitochondrial fumarate reductase.[10] According to this hypothesis, dehydration of malate to fumarate may serve as an alternative electron acceptor promoting deinhibition of glycolysis.

Coupling of Glu and Asp degradation with the energy-producing pathways in the cytosol and mitochondria is well-documented in studies with labeled amino acids and intermediates of the tricarboxylic acid cycle and presented in Figure 8.1. Importance of transamination, as a necessary step for further energy production, was justified by elimination of the protective effect of Glu by adding aminooxyacetate, an inhibitor of mitochondrial and cytosolic transaminases.[11,12] Numerous animal experiments testify that exogenous Glu and Asp improve myocardial tolerance to ischemia and enhance postischemic functional recovery via stimulation of anaerobic and aerobic energy formation. These observations provided a solid rationale for the use of these compounds as beneficial additives to cardioplegic solutions. Both amino acids separately enhance the protection afforded by blood and crystalloid cardioplegia.[13-16] Moreover, aspartate enrichment of Glu-blood cardioplegia further improves myocardial protection.[17] Beneficial effects of Glu/Asp-blood cardioplegia are not confined to adult hearts. Abundant experimental data obtained by Buckberg and colleagues clearly demonstrate advantages of amino acid-enriched blood cardioplegia in immature hearts.[18] Addition of Glu or Asp to crystalloid cardioplegic solutions can also prolong the safe limits for cold storage techniques used in cardiac transplantation.[19-21]

Aerobic Action

Noteworthy, exogenous Glu and Asp promote recovery of oxidative metabolism after periods of ischemic arrest restoring depleted intracellular pools of tricarboxylic acid cycle intermediates and amino acids.[11,12,15,19,22] Replenishment of these reactants is of critical importance for operation of the tricarboxylic acid cycle[23] and malate-aspartate shuttle, which transfers reducing equivalents of NADH from the cytosol to the respiratory chain of mitochondria.[24] Further, these amino acids take part in binding of ammonia excess in glutamine and asparagine synthesis. Cytotoxic effects of ammonia, a by-product of adenine nucleotide degradation, are associated with the inhibition of isocitrate dehydrogenase and activation of mitochondrial pyrophosphatase catalyzing degradation of NADPH to nicotinamide.[25] They may impede coordination of tricarboxylic acid cycle activity with electron transfer in mitochondrial respiratory chain.[23] Correction of these metabolic disorders may reduce the impact of reperfusion injury after cardiac surgery and, therefore, incidences of postoperative myocardial stunning. In turn, providing higher cytosolic concentrations of ATP and ATP/ADP ratios in compartments close to ion pumps and myofibrils, Glu and Asp supplementation favors cellular membrane integrity, maintenance of ionic gradients, and removal and binding of Ca^{2+} by myofibrils.[26] Recent studies suggest that supplementing the prime of cardiopulmonary bypass circuit with Glu and Asp attenuates lipid peroxidation and myocardial dysfunction after reoxygenation.

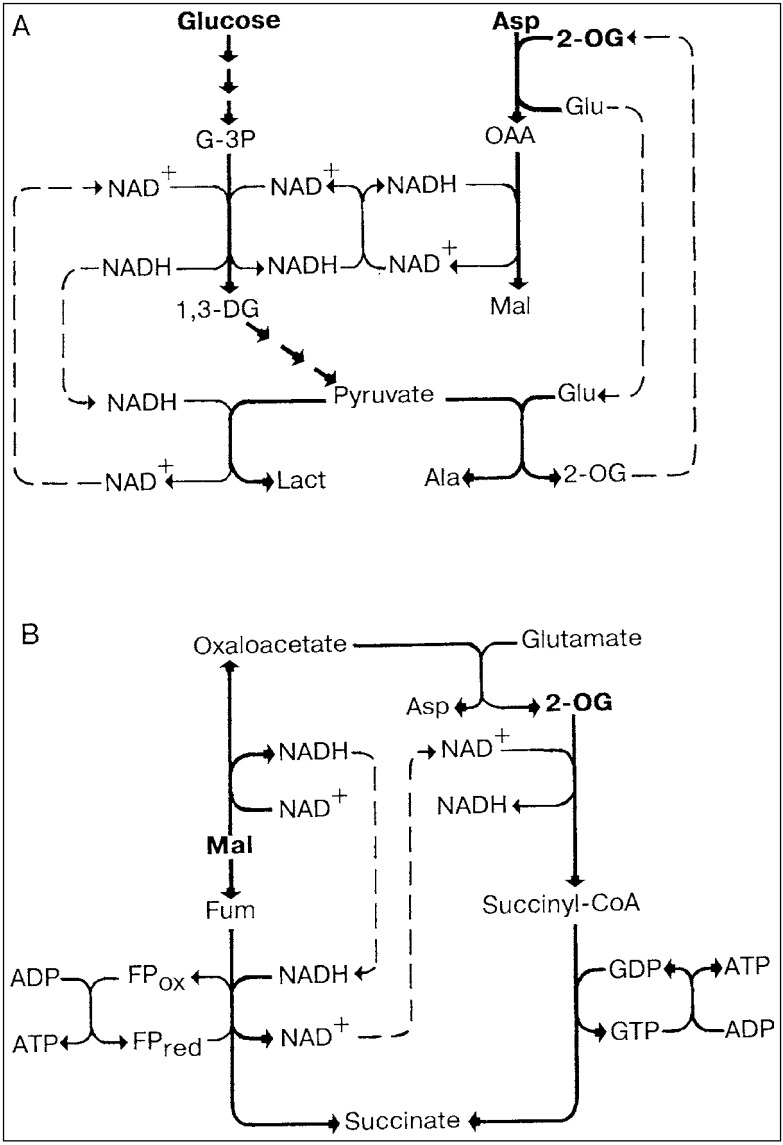

Fig. 8.1. Stimulation of anaerobic energy formation in cytosol (A) and mitochondria (B) by Glu and Asp supplementation. G-3P, glyceraldehyde 3-phosphate; 1,3-DG, 1,3-diphosphoglycerate; Mal, malate; Fum, fumarate; FP, flavoprotein of the succinate dehydrogenase complex in the oxidized (ox) or reduced (red) form.

This effect may be attributed to amino acid inhibition of L-arginine uptake by myocardial cells leading to subsequent reduction of •NO formation.[27]

In agreement with these findings, there are a number of experimental reports demonstrating superior recovery of high-energy phosphates, stroke volume index and compliance after cardioplegic arrest followed by reperfusion or reoxygenation with Glu and Asp.[16-18,28,29] In experimental coronary artery occlusion, the intravenous infusion of Glu and

Asp may reduce infarct size due to improvement of the myocardial metabolism stimulating reparative processes.[30,31] Additionally, the intravenous compositions containing both amino acids exhibit salutary effects on the contractility of viable remote myocardium in coronary occlusion[32] implying that metabolic support of cardiomyocytes can be helpful before coronary artery bypass grafting.

Clinical Experience

In routine cardiac surgical practice, the main interest concerning Glu and Asp supplementation is focused on enhancement of myocardial protection during the periods of aortic cross-clamping. As a rule, separate or combined enrichment of blood and crystalloid cardioplegia with these amino acids improves postoperative hemodynamic recovery even in high-risk coronary patients and enhances restoration of myocardial energy metabolism in congenital surgical patients.[13,33,34] Antiischemic effects of Glu were also observed in the preoperative setting. Thus, intravenous administration of Glu to patients with coronary artery disease was associated with increased tolerance to ischemia in regard to chest pain and electrographic changes during exercise testing and pacing.[35]

In the early postoperative period, blood-born amino acids and endogenous intracellular constituents are appeared to be the preferable substrates oxidized by the heart, while uptake of free fatty acids and carbohydrates is significantly compromised.[36] It is very likely that under these conditions, augmented myocardial consumption of amino acids, and first of all Glu, precedes an adequate recovery of aerobic metabolism. The high fractional extraction of Glu exhibits a consistent correlation to its arterial plasma level.[37] These interesting observations suggest that a relative shortage of myocardial glutamate following cardiac arrest[38] persists during the first hours after unclamping of the aorta and, therefore, substantiate administration of exogenous Glu after operations. Accordingly, positive effects of postoperative Glu infusion on myocardial metabolism were revealed in patients treated with dopamine because of cardiac failure. They were associated with an increased Glu myocardial uptake, a change from lactate release to its uptake, a cessation of ammonia release and simultaneous improvement of hemodynamic performance.[39]

Other Amino Acids

Histidine

The idea for using His as a protective agent against myocardial ischemia/reperfusion injury was developed from several studies demonstrating a lesser degradation of Ca^{2+}-ATP activity of sarcoplasmic reticulum, reduction of sarcolemmal Na^{+}-K^{+}-ATPase activity, inhibition of lipid peroxidation and delayed genesis of arrhythmias in isolated hearts challenged with singlet oxygen generation in the presence of this amino acid.[40-42] Additionally, His is a well-known scavenger of $OH^{\bullet}$ radicals.[43]

The use of high His concentrations (150-200 mM) in cardioplegic solutions provides superior intracellular buffer capacity to stimulate anaerobic energy production during oxygen deprivation. His-buffered hearts display effective recovery of cardiac function coincident with more normal pH and P_{CO2} in the coronary sinus effluent at the termination of ischemia.[44] The rationale for this type of solution is 2-fold. His exerts the maximal buffering near the pH of neutrality, since pK_a of its imidasole group is 7.0 at 25°C. In contrast to bicarbonate buffer solutions, the buffering capacity of His does not depend upon the partial pressure of CO_2 which may dramatically change during global myocardial ischemia. Due to these cardioprotective effects His containing Bretschneider's cardioplegic solutions have a sound reputation in clinical and experimental practice for long years.[45]

Branched Chain Amino Acids

A mixture of leucine, isoleucine and valine added to crystalloid cardioplegic solutions maintains myocardial ATP at higher levels during experimental ischemia.[46] This effect is accompanied by a reduced release of adenine

nucleotide catabolites from the heart and better sarcolemmal integrity during the early phase of reperfusion.[47] The mechanisms of BCAA action on myocardial energetics remain obscure.

Increased extracellular concentrations of these amino acids facilitate their uptake by cardiomyocytes.[48] There are experimental facts suggesting that the reduced intracellular pH during myocardial ischemia favors the transport of BCAA from cytosol into the mitochondrial matrix, where they are transaminated.[49] These reactions yield branched chain 2-oxoacids and Glu, which is a powerful antiischemic agent itself. However, further catabolism of BCAA is probably inhibited, since it involves steps catalyzed by NAD^+ and FAD-dependent dehydrogenases and by ATP-linked carboxylases which are blocked under ischemia.[50]

It has been claimed that after cardioplegic arrest, increased intracellular concentrations of BCAA stimulate formation of acetyl-CoA and succinyl-CoA depleted during ischemia, thus enhancing recovery of oxidative phosphorylation on reperfusion.[47] As Glu and Asp, BCAA are the only exogenous substrates extracted by the human heart early after cardiac surgery. Myocardial uptake of these amino acids correlates positively with their arterial concentrations and oxygen consumption.[37,51] This suggests that the accessibility of these substrates to myocytes may be one of the factors determining postischemic recovery of energy metabolism. Other potential benefits of increased intracellular levels of BCAA are usually attributed to the promotion of protein synthesis and reduction of proteolysis.[52] Further clinical studies investigating the metabolic and functional effects of BCAA in postoperative settings are, therefore, warranted.

Taurine

This is the most abundant amino acid of the mammalian heart comprising about 50% of the total free amino acid pool.[53] Although its physiological function remains undefined, Tau exhibits an extensive cardiovascular pharmacology as an antiarrhythmic agent,[54] a modifier of Ca^{2+} fluxes through the sarcolemma[55] and a membrane stabilizer.[56]

The normal heart maintains Tau content in cytosol by several selective transport mechanisms, so that intracellular concentration of this free amino acid is extremely high (20-40 mM).[57] However, lowered Tau contents have been reported in cardiomyopathies induced by hypoxia and ischemia[58] and in hearts subjected to calcium paradox.[59] Recent assessment of Tau release into the interstitial fluid by microdialysis technique revealed its potential significance as a marker of myocardial cell injury in experimental coronary occlusion.[60] Profound Tau losses were associated with irreversible damage of myocytes and accompanied by increased plasma levels of creatine phosphokinase MB fraction. The loss of myocardial Tau and other α-amino acids was also observed in patients undergoing cold cardioplegia with St. Thomas' Hospital cardioplegic solution.[61] Accordingly, postoperatively increased Tau levels were found in the blood of patients subjected to major surgical procedures.[62] As a rule, correction of Tau depletion results in improvement of myocardial damage.[59] Franconi et al[63] found a good positive relation between intracellular and extracellular concentrations of Tau, suggesting that administration of high doses of Tau increases its content in the myocardium. In this regard, the addition of Tau to cardioplegic and reperfusate solutions or in organ storage media may be a worthwhile measure to prevent the loss of α-amino acid pool and disturbances of energy metabolism.[64,65]

Tau has been shown to possess antioxidant activity suppressing lipoperoxide formation and membrane phospholipid degradation, which could also account for its cardioprotective action. This "membrane-stabilizing" effect is accompanied by a lesser release of myocardial reduced glutathione, creatine phosphokinase and diminished depletion of ATP.[66,67] A free-radical scavenging action of Tau was observed in patients during myocardial revascularization. Reduction of reperfusion injury due to preoperative intravenous infusion of Tau was associated with reduced lipoperoxidation and significantly lesser dam-

age of cardiomyocyte ultrastructure assessed in biopsy samples.[68] Surprisingly, despite abundance of promising experimental data the relative benefit of myocardial support with Tau has not been properly evaluated in cardiac surgery.

Methionine and Cysteine

Met deficiency is mainly associated with disturbances in lipid metabolism and myocardial lesions, since this essential amino acid is required for transmethylation of membrane phospholipids.[69,70] In patients with coronary artery disease, Met potentiates the hemodynamic effects of acutely administrated intravenous nitroglycerin.[71] This observation supports the concept that nitroglycerin action requires sulfhydril groups (SH) to form S-nitrosothiols in vascular smooth muscle. These, in turn, activate guanilate cyclase, which stimulates the production of cyclic GMP, which is known to mediate vasodilatation.[72] Met contains no SH groups, but it is converted to Cys, SH-amino acid and, thus, may provide additional formation of S-nitrosothiols leading to increased cyclic GMP levels.

As a powerful $OH^{\bullet}$ radical scavenger,[73] Met is effective in reducing myocardial damage induced by oxygen free radical formation following ischemia and reperfusion. Thus, addition of Met to the St. Thomas' Hospital cardioplegic solution enhances postischemic functional recovery and cell membrane integrity in the isolated working heart model of cardiopulmonary bypass and ischemic cardiac arrest.[74] As Met is not freely permeable through the cell membrane,[75] its scavenging effect may be mediated extracellularly, on cell surfaces and in the capillary endothelium. There are several reports of Met ability to prevent changes in myofibrillar ATPase activities induced by hypochlorous acid (HOCl) and H_2O_2.[76,77] This effect, associated with a lesser oxidation of myofibrillar SH groups, suggests that Met can also protect the basic contractile machinery of the myocardial cell against the adverse influence of oxygen free radicals.

Like Met, Cys potentiates vasodilating effect of nitroglycerin through formation of S-nitrosothiols which activate guanilate cyclase.[78] Cys reverses structural changes of mitochondrial membranes and restores the aortic flow in rat heart perfused with uncouplers of oxidative phosphorylation.[79] This effect is attributed to preventing interaction of uncouplers with SH and amino groups at the inner mitochondrial membrane which results in reduction of a depletion of high energy phosphates. There were no attempts to enhance postischemic recovery of cardiac function and metabolism using Cys as a donor of SH groups in cardioplegic and reperfusate solutions.

Conclusion

Under conditions relevant to cardiac surgery, mechanisms of action of the majority of amino acids, except Glu and Asp, have not yet been thoroughly elucidated. Presumably, they are closely linked with adaptive derangement of myocardial metabolism induced by ischemic and reperfusion injury. As seen, amino acid intervention may affect intermediary and energy metabolism, ion homeostasis, scavenging oxygen-derived free radicals, and subsequently membrane integrity and myocardial performance. In view of such multifactorial effect on the state of myocardial metabolism, it seems logical to use these naturally-occurring compounds in various settings of cardiac surgery. Further studies are needed to delineate the optimal regimens of myocardial treatment with individual amino acids or their combination.

References

1. Neely JR, Morgan HE. Relationship between carbohydrate and lipid metabolism and energy balance of heart muscle. Ann Rev Physiol 1974; 36: 413-459.
2. Opie LH. Fuels. In: The Heart. Physiology, Metabolism, Pharmacology and Therapy. New York: Gruine & Stratton Ltd., 1984: 111-117.
3. Mudge GH, Mills RM, Taegtmeyer H et al. Alterations of myocardial amino acid metabolism in chronic ischemic heart disease. J Clin Invest 1976; 58:1185-1192.

4. Knapp WH, Helus F, Ostertag H et al. Uptake and turnover of L-[^{13}N]glutamate in the normal heart and in patients with coronary artery disease. Eur J Nucl Med 1982; 7: 211-215.
5. Sanborn T, Gavin W, Berkowitz S et al. Augmented conversion of aspartate and glutamate to succinate during anoxia in rabbit heart. Am J Physiol 1979; 273: H535-H541.
6. Haas DW. Phosphorylation coupled to the oxidation of NADH by fumarate in digitonin fragments of beef heart mitochondria. Biochim Biophys Acta 1964; 92: 433-439.
7. Pisarenko OI, Khlopkov VN, Ruuge EK. A ^{1}H-NMR study of succinate synthesis from exogenous precursors in oxygen-deprived rat heart mitochondria. Biochem Internat 1986; 12:145-153.
8. Wright G. Amino acids in the treatment of ischemic heart disease. J Mol Cell Cardiol 1985; 17:441-443.
9. Owen TG, Hochachka PW. Purification and properties of Dolphin muscle aspartate and alanine transaminases and their possible roles in the energy metabolism of diving mammals. Biochem J 1974; 143:541-553.
10. Hohl CH, Oestreich R, Rosen P et al. Evidence for succinate production by reduction of fumarate during hypoxia in isolated adult rat heart cells. Arch Biochem Biophys 1987; 259:527-535.
11. Rau EE, Shine KI, Douglas AM et al. Enhanced mechanic recovery of anoxic and ischemic myocardium by amino acid reperfusion. Am J Physiol 1979; 236:H873-879.
12. Pisarenko OI, Solomatina ES, Studneva IM et al. Protective effect of glutamic acid on cardiac function and metabolism during cardioplegia and reperfusion. Basic Res Cardiol 1983; 78:534-543.
13. Pisarenko OI, Portnoy VF, Studneva IM et al. Glutamate-blood cardioplegia improves ATP preservation in human myocardium. Biomed Biochim Acta 1987; 46:499-504.
14. Weldner PW, Myers JL, Miller CA et al. Improved recovery of immature myocardium with L-glutamate blood cardioplegia. Ann Thorac Surg 1993; 55:102-105.
15. Engelman RM, Dodds WA, Rousou JH et al. Myocardial high-energy phosphate replenishment during ischemic arrest: Aerobic versus anaerobic metabolism. Ann Thorac Surg 1982; 33:453-458.
16. Lazar HL, Buckberg GD, Mangarano AJ et al. Myocardial energy replenishment and reversal of ischemic damage by substrate enhancement of secondary blood cardioplegia with amino acids during reperfusion. J Thorac Cardiovasc Surg 1980; 80:350-359.
17. Rosenkranz ER, Okamoto F, Buckberg GD et al. Safety of prolong aortic clamping with blood cardioplegia. III. Aspartate enrichment of glutamate-blood cardioplegia in energy-depleted hearts after ischemic and reperfusion injury. J Thorac Cardiovasc Surg 1986; 91:428-435.
18. Kofsky E, Julia P, Buckberg GD et al. Studies of myocardial protection in the immature heart. V. Safety of prolonged aortic clamping with hypocalcemic glutamate/aspartate blood cardioplegia. J Thorac Cardiovasc Surg 1991; 101:33-43.
19. Pisarenko OI, Rosenfeldt FL, Lagley L et al. Differing protection with aspartate and glutamate cardioplegia in the isolated rat heart. Ann Thorac Surg 1995; 59:1541-1548.
20. Haan CK, Lazar HL, Rivers S et al. Improved myocardial preservation during cold storage using substrate enhancement. Ann Thorac Surg 1990; 50:80-85.
21. Choong YS, Gavin JB. L-Aspartate improves the functional recovery of explanted hearts stored in St. Thomas' Hospital cardioplegic solution at 40°C. J Thorac Cardiovasc Surg 1990; 99:510-517.
22. Bittl JA, Shine K. Protection of ischemic rabbit myocardium by glutamic acid. Am J Physiol 1983; 245:H406-412.
23. Williamson JR, Ford C, Illingworth J et al. Coordination of citric acid cycle activity with electron transport flux. Circ Res 1976; 38 (Suppl 1):39-48.
24. Digerness SB, Reddy W. The malate-aspartate shuttle in heart mitochondria. J Mol Cell Cardiol 1976; 8:779-785.
25. Katunuma N, Okada M, Nishii Y. Regulation of the urea cycle and TCA cycle by ammonia. Adv Enzyme Reg 1966; 4:317-336.
26. Humphrey SM, Holliss DG, Seelye RN. Adenine pool catabolism in the ischemic, the calcium-depleted and substrate-free anoxic isolated rat heart: Relationship to contracture development. J Mol Cell Cardiol. 1984; 16:1127-1136.
27. Morita K, Ihnken K, Buckberg GD et al. Studies of hypoxemic/reoxygenation injury: Without aortic clamping. VIII. Counteraction of oxidant damage by exogenous glutamate and aspartate. J Thorac Cardiovasc Surg 1995; 110:1228-1234.
28. Haas GS, DeBoer LWV, O'Keefe DD et al. Reduction of postischemic myocardial dysfunction by substrate repletion during reperfusion. Circulation 1984; 70 (Suppl 1):65-74.
29. Rousou JA, Engelman RM, Anisimowicz L et al. Metabolic enhancement of myocardial preservation during cardioplegic arrest. J Thorac Cardiovasc Surg 1986; 91:270-276.

30. Engelman RM, Rousou JA, Flack JE et al. Reduction of infarct size by systemic amino acid supplementation during reperfusion. J Thorac Cardiovasc Surg 1991; 101:855-859.
31. Singh J, Garg D, Chugh K et al. Effect of aspartate and glutamate on experimental myocardial infarction in rats. Indian J Exper Biol 1989; 27:621-624.
32. Beyersdorf F, Acar C, Buckberg GD et al. Studies on prolonged acute regional ischemia. V. Metabolic support of remote myocardium during left ventricular power failure. J Thorac Cardiovasc Surg 1989; 98:567-579.
33. Beyersdorf F, Kirsh M, Buckberg GD et al. Warm glutamate/aspartate-enriched blood cardioplegic solution for perioperative sudden death. J Thorac Cardiovasc Surg 1992; 104:1141-1147.
34. Rosenkranz ER, Buckberg GD, Laks H et al. Warm induction of cardioplegia with glutamate-enriched blood in coronary artery patients with cardiogenic shock who are dependent on inotropic drugs and intra-aortic balloon support. J Thorac Cardiovasc Surg 1983; 86:507-518.
35. Thomassen A, Nielsen TT, Bagger JP et al. Antiischemic and metabolic effects of glutamate during pacing in patients with stable angina pectoris secondary to either coronary artery disease or syndrome X. Am J Cardiol 1991; 68:291-295.
36. Svedjeholm R, Hakason E, Vanhanen I. Rationale for metabolic support with amino acids and glucose-insulin-potassium (GIK) in cardiac surgery. Ann Thorac Surg 1995; 59:S15-S22.
37. Svedjeholm R, Ekroth R, Joachimsson PO et al. Myocardial uptake of amino acids and other substrates in relation to myocardial oxygen consumption four hours after cardiac surgery. J Thorac Cardiovasc Surg 1991; 101:688-694.
38. Pisarenko OI, Studneva IM, Portnoy VF et al. Ischemic heart arrest: Nitrogenous metabolism in energy-depleted human myocardium. Eur Surg Res 1987; 19:329-336.
39. Pisarenko OI, Lepilin MG, Ivanov VE. Cardiac metabolism and performance during L-glutamic acid infusion in postoperative cardiac failure. Clin Sci 1986; 70:7-12.
40. Kusama, Bernier M, Hearse DJ. Photoactivation of porphyrins: Studies of reactive oxygen intermediates and arrhythmogenesis in the aerobic rat heart. Cardiovasc Res 1990; 24:676-682.
41. Kukreja RC, Kearns AA, Zweier JL et al. Singlet oxygen interaction with Ca^{2+}-ATPase of cardiac sarcoplasmic reticulum. Circ Res 1991; 69:1003-1014.
42. Misra BR, Misra HP. Vasoactive intestinal peptide, a singlet oxygen quencher. J Biol Chem 1990; 265:15371-15374.
43. Zs-Nagy I, Floyd RA. Hydroxyl free radical reactions with amino acids and proteins: Studies by electron spin resonance spectroscopy and spin-trapping. Biochim Biophys Acta 1984; 790:238-250.
44. Heinemeyer D, Belles G, Stapenhorst K. Intracellular pH measurement during cardiac arrest in ventricular myocardium by Bretschneider's cardioplegic solution HTK and St. Thomas' Hospital solution with and without procaine. Thorac Cardiovasc Surg 1987; 35:48-52.
45. Bretschneider HJ. Myocardial Protection. Thorac Cardiovasc Surg 1980; 28:295-302.
46. Schwalb H, Kushnir T, Navon G et al. The protective effect of enriched branched chain amino acid formulation in the ischemic heart: A phosphorus-31 nuclear magnetic resonance study. J Mol Cell Cardiol 1987; 19:991-998.
47. Schwalb H, Izhar U, Yaroslavsky E et al. The effect of amino acids on the ischemic heart. J Thorac Cardiovasc Surg 1989; 98:551-556.
48. Schwartz RG, Barret EJ, Francis CK et al. Regulation of myocardial amino acid balance in conscious dog. J Clin Invest 1985; 75: 1204-1211.
49. McMillin JB. Branched chain amino acid perfusion. J Thorac Cardiovasc Surg 1989; 98:632-638.
50. Pisarenko OI, Solomatina ES, Studneva IM. The role of amino acid catabolism in the formation of the tricarboxylic acid cycle intermediates and ammonia in anoxic rat heart. Biochim Biophys Acta 1986; 885:154-161.
51. Hallhagen S, Svedjeholm R, Ekroth R et al. Effects of insulin on myocardial uptake of branched chain amino acids soon after cardiac operations. J Thorac Cardiovasc Surg 1992; 103:98-107.
52. Tischler ME, Cammisa H. Metabolism of protein, amino acids, and glucose and their response to insulin in atria and cardiac myocytes of traumatized rats. Metabolism 1984;33:515-520.
53. Jacobsen JG, Smith LH. Biochemistry and physiology of taurine and taurine derivatives. Physiol Rev 1968; 48:421-511.
54. Huxtable RJ, Sebring LA. Cardiovascular action of taurine. In: Kiriyama K, Huxtable RJ, Iwata H, ed. Sulfur amino acids: Biochemical and clinical aspects. New York: Alan R. Liss, 1983: 5-37.
55. Chovan J, Kulakowski E, Benson B et al. Taurine enhancement of calcium binding to rat heart sarcolemma. Biochim Biophys Acta 1979; 551:129-136.

56. Huxtable RJ, Sebring LA. Towards an unifying theory for the action of taurine. Trends Pharm Sci 1986; 7:481-485.
57. Huxtable RJ, Bressler R. Elevation of taurine in human congestive heart failure. Life Science 1974; 4:1353-1359.
58. Crass MF, Lombardini JB. Release of taurine from the oxygen-deficient perfused heart. Proc Soc Exper Biol Med 1978; 157:486-488.
59. Kramer J, Chovan J, Schapper S. The effects of taurine on calcium paradox and ischemic heart failure. Am J Physiol 1981; 240: H238-246.
60. Holmgren A, Anderson B, Haggmark S et al. Monitoring of myocardial ischemic and reperfusion damage. A microdialysis study with taurine as a marker. J Mol Cell Cardiol 1997; 29:A89 (Abstract).
61. Chapman RA, Suleiman MS, Rodrigo GS. Intracellular taurine, intracellular sodium and defense against cellular damage. In: Earm, Y.E. & Noble, D. eds, Cardiac Ion Channels and Effects of Taurine on the Heart. Boston: Kluwer Academic Publishers, 1993: 73-92.
62. Lombardini JB, Bricker DL. Effects of cardiovascular surgery on blood concentrations of taurine and amino acids. Proc Soc Exper Biol Med 1981; 167:498-505.
63. Franconi F, Martini F, Stendardi I et al. Effect of taurine on calcium level and contractility in guinea pig ventricular strips. Biochem Pharmacol 1982; 31:3181-3186.
64. Earm YE, Ho WK, So IS et al. Effect of taurine on the activation of background current in cardiac myocytes. In: Earm YE and Noble D eds. Cardiac Ion Channels and Effects of Taurine on the Heart. Boston: Kluwer Academic Publishers 1993: 132-140.
65. Kapelko VI, Pukhova TM, Lakomkin VL et al. Modification of reperfusate formulae for ischemic heart. Cardiology (Russ) 1993; 7:246-251.
66. Pasantes-Morales H, Wrigh CE, Gaull GE. Taurine protects lymphoblastoid cells from iron-ascorbate induced damage. Biochem Pharmacol 1985; 34:2205-2207.
67. Ohta H, Azuma J, Awata N et al. Mechanism of the protective action of taurine against isoprenaline induced myocardial damage. Cardiovasc Res 1988; 22:407-413.
68. Milei J, Ferreira R, Llesuy S et al. Reduction of reperfusion injury with preoperative rapid intravenous infusion of taurine during myocardial revascularization. Am Heart J 1992; 123:339-345.
69. Lovenberg W, Yomori Y. Nutritional factors and cardiovascular disease. Clin Exp Hyperten 1984; A6:417-426.
70. Panagia V, Okumura K, Makino N. et al. Stimulation of Ca^{2+} pump in heart sarcolemma by phosphatidylethanolamine N-methylation. Biochim Biophys Acta 1986; 856:383-387.
71. Levi WS, Katz RJ, Ruffalo RL et al. Potentiation of the hemodynamic effects of acutely administered nitroglycerin by methionine. Circulation 1988; 78:640-645.
72. Ignarro LJ, Lippton H, Edwards JC et al. Mechanisms of vascular smooth muscle relaxation by organic nitrates, nitrites, nitroprusside and nitric oxide: Evidence for the involvement of S-nitrosothiols as active intermediates. J Pharmacol Exp Ther 1981; 218:739-745.
73. Blasig IE. Effects of troxerutin and methionine on spin trapping of free oxy-radicals. Biomed Biochim Acta 1988; 47:252-255.
74. Chambers DJ, Astras G, Takahashi A et al. Free radicals and cardioplegia: organic antioxidants as additives to the St. Thomas' Hospital cardioplegic solution. Cardiovasc Res 1989; 23:351-358.
75. Halliwell B. Free radicals, oxygen toxicity and aging. In: Age Pigments, Shoal, RS, ed. Amsterdam: Elsevier/North Holland Biomedical Press 1981: 1-62 .
76. Kukreja RC, Weaver AB, Hess ML. Stimulated human neutrophils damage cardiac sarcoplasmic reticulum function by generation of oxidants. Biochim Biophys Acta 1989; 990:198-205.
77. Suzuki S, Kaneko M, Chapman DC et al. Alterations in cardiac contractile proteins due to oxygen free radicals. Biochim Biophys Acta 1991; 1074:95-100.
78. Laustiola K, Vuorinen P, Vapaatalo H et al. Effects of cysteine and nitroglycerin on bovine heart guanilate cyclase and on tissue cyclic GMP and lactate of rat atria. Eur J Pharmacol 1983; 91:301-304.
79. Viet P, Fuchs J, Zimmer G. Uncoupler- and hypoxia-induced damage in the working rat heart and its treatment. I. Observation with uncouplers of oxidative phosphorylation. Basic Res Cardiol 1985; 80:107-115.

CHAPTER 9

Methods to Reduce Ischemia/ Reperfusion Injury—PICSO

Günter Steurer, Katharina Palisek and Werner Mohl

Ischemia/Reperfusion Injury

Enormous advances in surgical, pharmacological, and interventional techniques resulting in early restoration of infarct artery patency significantly improved outcome in patients with acute coronary syndromes.[1] However, time-consuming preparations in patients with acute myocardial ischemia scheduled for emergency bypass surgery, prolonged interventional procedures in patients with complex coronary lesions, and early re-establishment of coronary blood flow in acute myocardial infarction by means of thrombolytic agents or primary angioplasty may result in myocardial tissue damage partly related to reperfusion of oxygenated blood into ischemia-damaged myocardial areas.[2] Myocardial stunning,[3] ventricular arrhythmias,[4] or even lethal cell injury in the sequel of successful reperfusion therapy[5] were observed and confirmed the clinical relevance of reperfusion injury in acute myocardial infarction.

Coronary reperfusion within a critical period is essential for survival of ischemic myocardium. In experimental conditions coronary occlusion followed by reperfusion revealed progression of irreversible injury of ischemic myocardium from the endocardium toward the subepicardium with potentially salvageable myocardium for up to 3-6 hours.[6] The course of events is similar in man depending on degree of coronary stenosis, size of the ischemic area, size and number of pre-existing collateral vessels, and hemodynamic complications. Early reperfusion damages vascular endothelium and capillary structures of the coronary microcirculation by formation of oxygen-derived free radicals, activation of the complement system and disturbance of the calcium homeostasis.[7] Increasing evidence of reperfusion-induced myocardial damage in routine revascularization therapy of infarct patients stimulated interest in therapy strategies to attenuate myocardial ischemia/reperfusion injury in acute myocardial ischemia.

As a rule the more time elapsed the higher is the danger of formation of oxygen-derived radicals leading to changes in microcirculation as shown by Komamura et al.[8] In this trial a decrease of coronary vein flow during reperfusion after myocardial infarction was shown, demonstrating again that arterial early reperfusion does not necessarily salvage myocardium. As a conclusion, time should be the determining factor whether to choose arterial reperfusion, as after acute vessel occlusion or ischemia in the cath lab, or venous retrograde reperfusion when the exact time of the occlusion is unknown.

Coronary Sinus Interventions

Coronary sinus techniques comprising the methods of retroinfusion,[9] synchronized retroperfusion (SRP),[10-13] selective suction and retroperfusion (SSR),[14] intermittent coronary

Ischemia-Reperfusion Injury in Cardiac Surgery, edited by Friedhelm Beyersdorf.

sinus occlusion (ICSO),[15-17] and pressure-controlled intermittent coronary sinus occlusion (PICSO)[18-30] are based on the experimental studies of Pratt in the isolated heart[31] to maintain residual myocardial blood flow via the coronary venous system. The coronary venous vasculature, a dense meshwork with numerous interconnections, has three different drainage systems, the coronary sinus, the lymphatic system, and the Thebesian system. The different compartments are connected with each other and create a vast and normally unused volume capacity. This capacitance and the numerous interconnections such as arteriovenous, lymphatic, and venovenous anastomoses provide the essential basis and the mechanism of all coronary sinus interventions.

Evidence of beneficial effects of coronary sinus interventions in experimental ischemia and clinical studies renewed interest in coronary sinus interventions to protect ischemic myocardium from irreversible damage due to ischemia/reperfusion injury in patients with acute ischemia by extension of the filling capacity of the smallest cardiac veins (SCV) and through improvement of myocardial perfusion. Today's role of coronary sinus intervention in the therapy of coronary artery disease is based on the theory of temporary protection of jeopardized myocardium in patients with acute coronary syndromes undergoing early revascularization procedures.

Pressure-Controlled Intermittent Coronary/Sinus Occlusion (PICSO)

The technique of PICSO developed by Mohl and coworkers[19] is based on a forced redistribution of venous blood flow into the coronary beds in the setting of coronary artery occlusion to reduce infarct size, improve regional myocardial function, and enhance washout of ischemic metabolic byproducts (Fig. 9.1). The PICSO device is composed of a balloon-tipped catheter positioned in the orifice of the coronary sinus by means of fluoroscopy, a pneumatic pump device, and a decentral process computer connected by a four knots Field Bus Controller Area Network (CAN). The knot "user interface" is a standard industry personal computer, the knot "master" is a microcontroller system connected to an instrumentation amplifier linked to a pressure sensor, the knot "electrocardiogram (ECG)" is a serial linked I/O (SLIO) controller connected to an ECG amplifier, and the knot "balloon" is a SLIO controller connected to an amplifier linked to a sensor measuring the balloon pressure and to a motor balloon pump.

The PICSO system redistributes coronary venous blood flow by changes in pressure gradients throughout the coronary venous system in the setting of coronary artery occlusion (Fig. 9.2). The pump automatically inflates/deflates the balloon according to the occlusion/release timing obtained from the closed-loop feedback algorithm of the system (Fig. 9.3). During balloon inflation there is a slow increase in systolic and diastolic coronary sinus pressure with the effect of redistribution of the coronary venous blood flow from normal to underperfused, ischemic areas (redistribution period). Gradual rise of systolic coronary sinus pressure to a plateau, and a concomitant increase of systolic pressure in the perfusion area of the infarct-related coronary artery, results in exchange of toxic metabolic products (equilibrium period). The occlusion period is automatically terminated when the exponential curve fitted to the systolic coronary sinus pressure peaks 95% of its predicted plateau value. In healthy controls, plateau values of 60-80 mmHg are usually observed, and occlusion times approximately range from 6-10 seconds. In patients with myocardial infarction, due to the loss of myocardial contractility, however, slope of the peak values will be drastically reduced resulting in decreased plateau values and substantially prolonged occlusion times. Following termination of the PICSO cycle by deflation of the balloon, coronary sinus pressure abruptly decreases and coronary sinus flow is released, facilitating an enhanced drainage of the venous system and reestablishing flow currents that promote washout of accumulated toxic wastes in the ischemic area (washout phase). After a release period of approximately 6-7 heart beats and

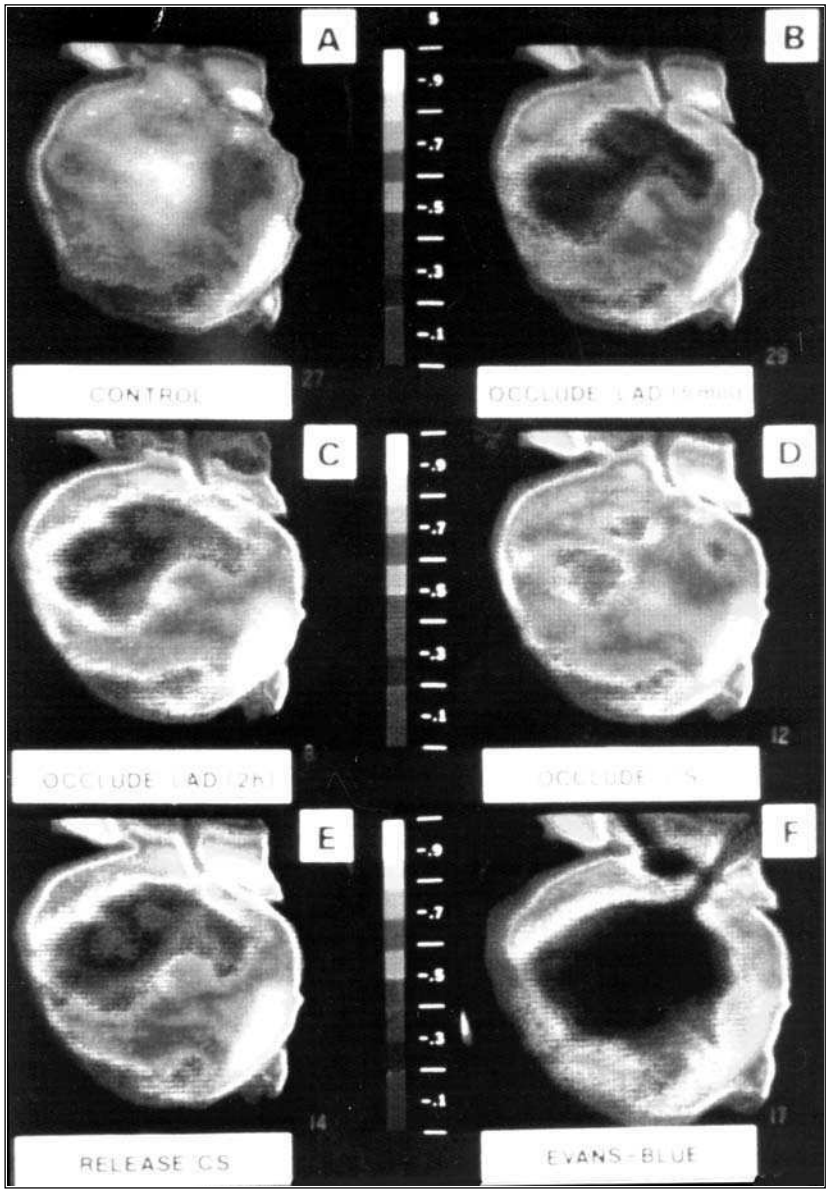

Fig. 9.1. Illustration of a thermographic study of retrograde myocardial blood supply during coronary sinus intervention in experimental ischemia. Note the disappearance of the dark color during coronary sinus occlusion (D) determining improved perfusion. During coronary sinus release (E) washout takes place (recurrence of dark color).

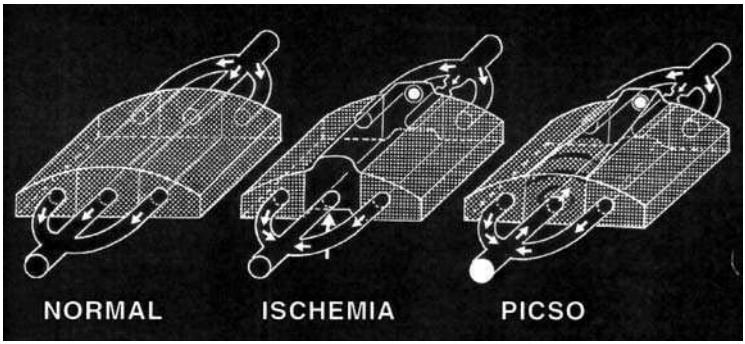

Fig. 9.2. Schematically illustration of myocardial blood supply in normal conditions, during acute ischemia, and during coronary sinus intervention (PICSO).

complete return of coronary sinus pressure to baseline levels, the balloon is automatically inflated in order to initiate the next PICSO cycle. The intermittent nature of the inflation/deflation cycle prevents potential complications of hemorrhage, edema, thrombosis, arrhythmias, or conduction disturbances in spite of an individual peak coronary sinus pressure of 60 mmHg or more.

Experimental Studies

In experimental myocardial infarction ICSO and PICSO demonstrated a significant reduction of infarct size compared to controls (Fig. 9.4). In 31 mongrel dogs with experimental infarction due to ligation of the left anterior descending coronary artery (LAD) for 6 hours Mohl et al[20] showed a significant increase of myocardial salvage by PICSO compared to controls. In 41 dogs subject to a 3 hours occlusion of the proximal LAD Ciuffo et al[16] showed a significant reduction of ischemic necrosis and significant increase of myocardial perfusion in the ICSO therapy group compared to controls. In 22 dogs subject to 3 hours ligation of the LAD followed by 8-12 days or reperfusion Guerci et al[26] showed a significant increase of myocardial salvage in the ICSO therapy group compared to controls. In 22 mongrel dogs with LAD occlusion for 3 hours followed by 3 hours of reperfusion Jacobs et al[23] showed a significantly enhanced myocardial salvage of ischemic myocardium in the PICSO group compared to controls. In two experimental occlusion/reperfusion models, however, PICSO[15] and ICSO,[25] respectively, did not significantly reduce infarct size or afford myocardial protection. The negative results of these trials could be related to the insufficient increase of systolic coronary sinus pressure during coronary sinus intervention and inadequate individual adaptation of the PICSO algorithm.

Recent experimental studies on pigs have shown that PICSO effectively reduced both, infarct size and myocardium at risk after coronary artery occlusion.[27-29] In addition to reduction of infarct size in experimental myocardial infarction a significant improvement of regional ischemic myocardial function due to temporary intravascular balloon occlusion of the proximal LAD by PICSO has been demonstrated.[21]

Clinical Studies

Safety and efficacy of PICSO was evaluated in a randomized trial of 30 patients with coronary artery disease undergoing bypass surgery.[30] To analyze changes of sectional and segmental wall motion during extracorporeal circulation intraoperative two-dimensional echocardiography was performed from the short-axis cross-sectional view. PICSO was started after aortic declamping and continued for one hour during early reperfusion. Although sectional wall motion did not sig-

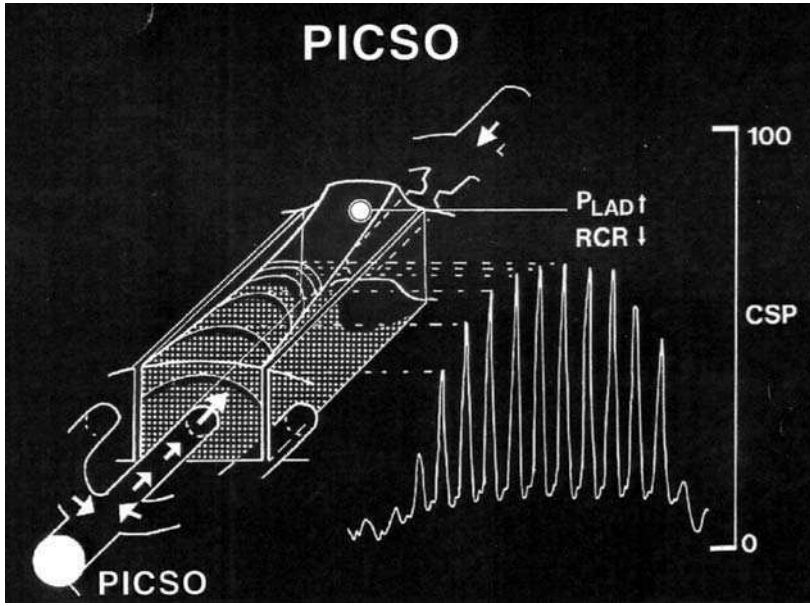

Fig. 9.3 Schematic illustration of a PICSO cycle during acute ischemia and depicts the increase in venous perfusion in relation to the coronary sinus pressure.

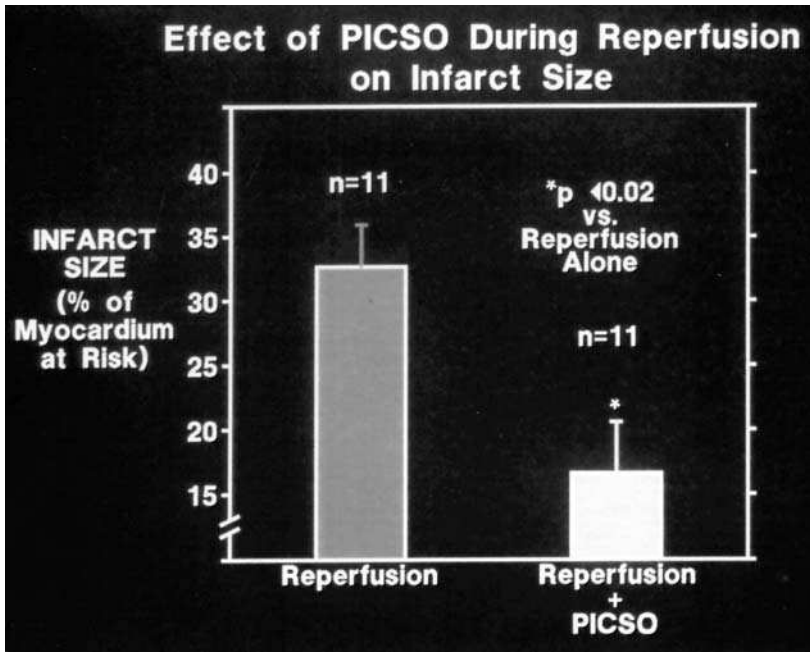

Fig. 9.4. Summary of the effects of PICSO and ICSO in experimental reperfused myocardial infarction.

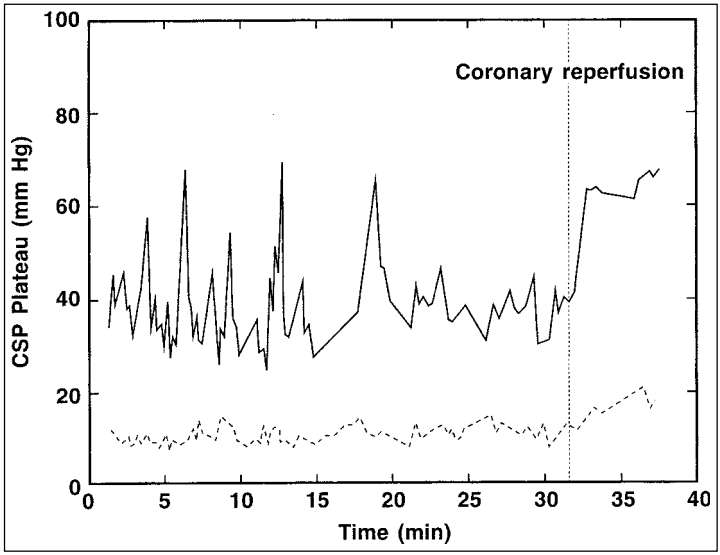

Fig. 9.5. Illustration of the time course of the systolic (solid line) and diastolic (dashed line) coronary sinus pressure (CSP) plateau values during intraoperative PICSO treatment in one patient.

nificantly change, hypokinetic segments were preserved better in the PICSO therapy group compared to placebo coronary sinus intervention. Coronary artery bypass grafting was performed, and PICSO was applied after reopening of the aorta while the patient was still on cardio-pulmonary bypass. Note the sudden increase of the systolic plateau during opening of the bypass graft (Fig. 9.5).

In patients with anterior acute myocardial infarction treated with thrombolytic therapy implementation of ICSO was obtained in 12 patients and compared 12 patients treated with thrombolysis alone.[18] In the absence of adverse effects ICSO therapy slightly improved enzymatic infarct size, myocardial lactate metabolism, left ventricular wall motion, and scintigraphically documented perfusion defects compared to controls.

Future Directions

Based on the positive effects of PICSO and ICSO in experimental myocardial ischemia and clinical studies randomized clinical studies are elaborated to evaluate the safety and efficacy of PICSO in patients with unstable angina or emerging myocardial infarction, during high-risk interventional procedures, and during cardiac arrest in bypass surgery.

References

1. Grover A, Rihal CS. The importance of early patency after acute myocardial infarction. Current Opinion in Cardiology 1995; 10: 361-366.
2. Braunwald E. Myocardial reperfusion, limitation of infarct size, reduction of left ventricular dysfunction, and improved survival. Should the paradigm be expanded? Circulation 1989; 79:441-444.
3. Bolli R. Myocardial stunning in man. Circulation 1992; 86:1671-1691.
4. Kusama Y, Bernier M, Hearse DJ. Exacerbation of reperfusion arrhythmias by sudden oxidant stress. Circulation Research 1990; 67:481-489.
5. Ambrosio G, Becker LC, Hutchins GM et al. Reduction in experimental infarct size by recombinant human superoxide dismutase: Insights into the pathophysiology of reperfusion injury. Circulation 1986; 74:1424-1433.
6. Reimer KA, Lowe JE, Ramussen MM et al. The wave front phenomenon of ischemia cell death: Myocardial infarct size vs. duration of coronary occlusion in dogs. Circulation 1977; 56:786-794.

7. Steurer G, Yang P, Rao V et al. Acute myocardial infarction, reperfusion injury, and intravenous magnesium therapy: Basic concepts and clinical implications. Am Heart J 1996; 132:478-482.
8. Komamura K, Kitakaze M, Nishida K et al. Progressive decreases in coronary vein flow during reperfusion in acute myocardial infarction: Clinical documentation of the no reflow phenomenon after successful thrombolysis. J Am Coll Cardiol 1994; 24(2): 370-377.
9. Bolling SF, Flaherty JF, Bulkley BH. Improved myocardial preservation during global ischemia by continuous retrograde coronary sinus perfusion. J Thorac Cardiovasc Surg 1983; 86:659.
10. Meerbaum S, Lang TW, Osher JV et al. Diastolic retroperfusion of acutely ischemic myocardium. Am J Cardiol 1976; 37: 588-598.
11. Farcot JC, Meerbaum S, Lang TW et al. Am J Cardiol 1978; 41:1191-1201.
12. Gore JM, Weiner BH, Benotti JR et al. Preliminary experience with synchronized coronary sinus retroperfusion in humans. Circulation 1986; 74:381-388.
13. Nanto S, Nishida K, Hirayama A et al. Supported angioplasty with synchronized retroperfusion in high risk patients with left main trunk or near left main trunk obstruction. Am Heart J 1993; 125:301-309.
14. Boekstegers P, Peter W, Degenfeld G et al. Preservation of regional myocardial function and myocardial oxygen tension during acute ischemia in pigs: Comparison of selective synchronized suction and retroinfusion of coronary veins to synchronized coronary venous retroperfusion. J Am Coll Cardiol 1994; 23:459-469.
15. Zalewski A, Goldberg S, Slysh S et al. Myocardial protection via coronary sinus intervention: Superior effects of arterialization compared with intermittent occlusion. Circulation 1985; 71:1215-1223.
16. Ciuffo AA, Guerci AD, Halperin G et al. Intermittent obstruction of the coronary sinus following coronary ligation in dogs reduces ischemic necrosis and increases myocardial perfusion. The coronary sinus. Steinkopff, Darmstadt 1984; 454-64.
17. Komamura K. Human experience with intermittent coronary sinus occlusion in acute myocardial infarction. Int Working Group on CSI, Newsletter 1987; 1:9-10.
18. Komamura K, Mishima M, Kodama K. Preliminary clinical experience with intermittent coronary sinus occlusion in combination with thrombolytic therapy in acute myocardial infarction. Jap Circ J 1989; 53:1152-1163.
19. Mohl W. The development and rationale of pressure controlled intermittent coronary sinus occlusion: A new approach to protect ischemic myocardium. Wi Kli Wo 1984; 96:205-209.
20. Mohl W, Glogar DH, Mary H et al. Reduction of infarct size induced by pressure-controlled intermittent coronary sinus occlusion. Am J Cardiol 1984; 53:923-928.
21. Mohl W, Punzengruber C, Moser. Effects of pressure-controlled intermittent coronary sinus occlusion on regional ischemic myocardial function. J Am Coll Cardiol 1985; 5:939-947.
22. Mohl W, Glogar D, Kenner T. Enhancement of washout induced by PICSO in the canine and human heart. In: W Mohl, W Wolner, and D Glogar, eds. The coronary sinus. New York: Springer-Verlag; 1984:537-548.
23. Jacobs SK, Faxon DP, Coats WD et al. Pressure-controlled, intermittent coronary sinus occlusion (PICSO) during reperfusion markedly reduces infarct size. Clin Res 1985; 33:197A.
24. Jacobs SK, Faxon DP, Coats WD et al. The effect of pressure controlled intermittent coronary sinus occlusion during reperfusion. In: W Mohl, ed. Clinics of CSI. Darmstadt: Steinkopff; 1986:345-347.
25. Diltz EA, Mames RN, Lee JW et al. Intermittent coronary sinus occlusion does not reduce infarct size or ischemic dysfunction in an occlusion/reperfusion model. Circulation 1988; 72(supp III):120.
26. Guerci AD, Ciuffo AA, DiPaula AF et al. Intermittent coronary sinus occlusion in dogs: reduction of infarct size 10 days after reperfusion. JACC 1987; 9:1075-1081.
27. Lazar HL, Rajaii A, Roberts AJ. Reversal of reperfusion injury after ischemic arrest with pressure-controlled intermittent coronary sinus occlusion. J Thorac Cardiovasc Surgery 1988; 95:637-42.
28. Lazar HL, Haan CK, Yang X et al. Reduction of infarct size with coronary venous retroperfusion. Circulation 1992; 96(suppII): 352-357.
29. Lazar HL, Trenor P, Rivers S et al. Combining percutaneous bypass with coronary retroperfusion limits myocardial necrosis. Ann Thorac Surg 1995; 59:373-378.
30. Mohl W, Simon P, Neumann F et al. Clinical evaluation of pressure-controlled intermittent coronary sinus occlusion: Randomized trial during coronary artery surgery. Ann Thorac Surg 1988; 46:192-201.
31. Pratt FH. The nutrition of the heart through the vessels of Thebesius and coronary veins. Am J Physiol 1893; 1:86-103.

CHAPTER 10

Ischemic Preconditioning—from Bench to Bedside

Torsten Doenst and Heinrich Taegtmeyer

Discovery, Definition, and Capabilities

Traditional ways to improve ischemia tolerance in patients with obstructive coronary artery disease include pharmacological and mechanical interventions. Recently, ischemic preconditioning has emerged as a third modality. Observations in the laboratory have developed into a strategy for the protection of the ischemic heart which may prove to be of clinical usefulness. Ischemic preconditioning was first described more than a decade ago as a phenomenon that increases myocardial tolerance against ischemic injury by pre-exposure of the heart to one or more brief episodes of ischemia.[1] In the original work, Murry et al[1] found a 75% reduction in infarct size caused by a 40 min occlusion of a coronary artery when the occlusion was preceded by four episodes of 5 min ischemia and 5 min of reperfusion (Fig. 10.1). In the following years the use of the term ischemic preconditioning has been extended to include endpoints other than reduction of infarct size. Ischemic preconditioning was shown to protect against postischemic contractile dysfunction[2-4] (Fig. 10.2), postischemic dysrhythmia,[5,6] autonomic nerve dysfunction,[7] vascular dysfunction,[8,9] generation of free oxygen radicals,[10,11] and myocardial stunning[12] (Table 10.1).

The phenomenon could be observed in all animal species studied in both in vivo and in vitro preparations, and with global as well as regional ischemia. Most studies have used as experimental models, dogs,[1,13,14] pigs,[15-17] rabbits,[2,18-21] or rats,[4,7,10,22] preconditioning effects in man.[22-26,27] It is of interest that the protective effects of ischemic preconditioning can also be induced by nonischemic interventions such as hypoxia,[28] rapid pacing,[29] or pharmacological agents[30,31] e.g., adenosine agonists[32] (Table 10.1). Ischemic preconditioning has also been observed in patients undergoing angioplasty. It was noted that balloon inflations with occlusion of the vessel subsequent to the first occlusion were associated with a reduction of ST-segment elevation, with reduced lactate release into the coronary sinus, and with less severe pain of angina pectoris.[22,23,33] More recent reports suggest that preinfarction angina may have a protective effect.[26,34-36] The suggested presence of ischemic preconditioning in patients with coronary artery disease is also supported by the observation that patients with a transmural myocardial infarction and a history of angina within 7 days prior to infarction have a lower morbidity and a better clinical outcome than patients without preinfarction angina.[36]

Limitations of Ischemic Preconditioning

Despite the described pluripotency of ischemic preconditioning, the efficacy of the intervention is limited by a number of factors.

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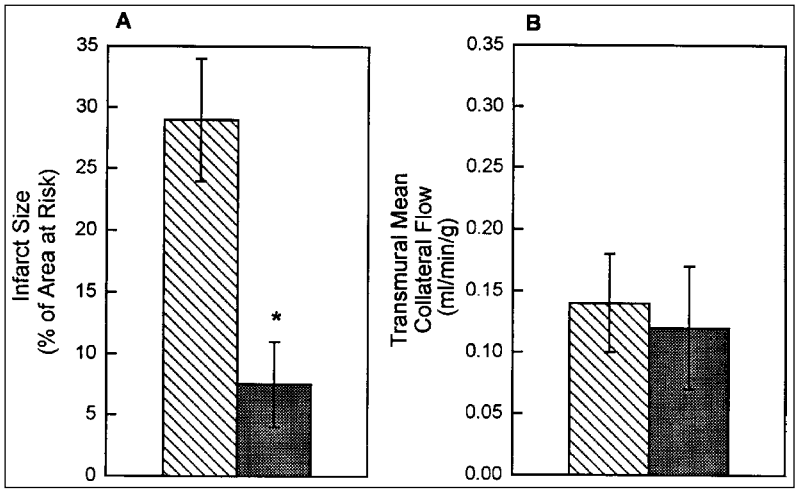

Fig. 10.1. Reduction of infarct size by ischemic preconditioning without a change in collateral flow. Ischemic preconditioning with four cycles of 5 min ischemia followed by 5 min of reperfusion (■) resulted in a 75% reduction of infarct size after a 40 min sustained occlusion of the circumflex artery compared to nonpreconditioned control hearts (▨ , panel A). At the same time collateral flow was unaltered (panel B). Mean ± SEM, * $p < 0.001$ compared to Control. Reproduced, with permission from Murry et al. Circulation 1986; 74:1124-1136.

The adaptive response of the heart to the preconditioning stimulus occurs quickly (within 2-15 min) and is relatively short-lived. If the duration of reperfusion between preconditioning and sustained ischemia exceeds 120 min (in dogs, 60 min in rats) the protective effects are largely lost. Furthermore, if the duration of sustained ischemia exceeds 60-180 min no protective effects can be detected.[6,37-39]

Recent evidence suggests the existence of a "second window of protection", a slower adaptive response where protective effects are detectable 24h after the preconditioning stimulus.[40] The mechanisms involved in this late protection appear to involve the regulation of gene expression, especially the expression of a group of stress proteins termed heat shock proteins.[40,41]

Another limitation is the poor reproducibility of results. Despite keeping to the described time frames several investigators did not find protection by ischemic preconditioning with protocols that have been shown as protective.[42-44] Different protocols also produce conflicting results. It has been reported that in the rat heart three cycles of preconditioning ischemia/reperfusion are necessary to reduce infarct size,[45] while others have shown before that one cycle protects as good against infarction as 12 cycles.[46] Liu and Downey[45] compared a protocol with three cycles of 5 min ischemia and 5 min of reperfusion with a protocol with one cycle of 5 min ischemia and 10 min of reperfusion and concluded that the rat heart has a higher threshold of preconditioning than other animal species. On the contrary, we[4,47] and others[48] have found that only one cycle of ischemic preconditioning (5 min of ischemia with 10 min of reperfusion) is necessary in the isolated rat heart to improve postischemic recovery of contractile function[4,47] or to reduce infarct size.[48] Thus, it seems difficult to establish thresholds of ischemic preconditioning for individual animal species, although species differences may be of importance in situations where several mediators elicit the same response (see section 4a).

The literature also contains controversy about the response of the contractile apparatus of preconditioned hearts during ischemia. Both the delay and early onset of ischemic

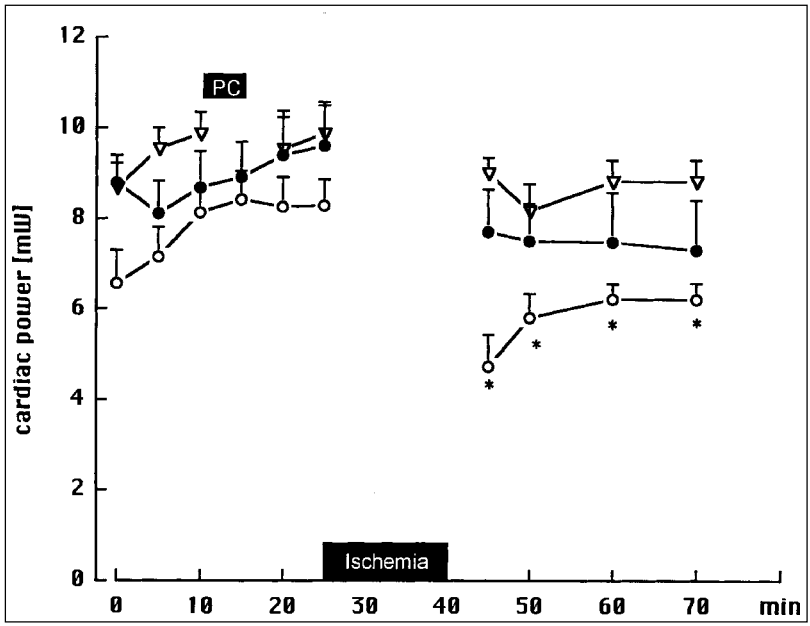

Fig. 10.2. Effects of 15 min ischemia on the recovery of contractile function of isolated working rat hearts perfused with Krebs-Henseleit buffer containing glucose (10 mM). Control hearts (○), ischemically preconditioned hearts ( ), hearts from fasted animals where lactate and insulin were added to the perfusate (●). Note, that both ischemic preconditioning as well as the metabolic interventions resulted in an almost complete recovery of function. Mean ± SEM, * $p < 0.05$ compared with last measurement before ischemia. Reproduced, with permission from Doenst et al. Am. J. Physiol. 1996; 270:H1607-H1615.

Table 10.1. Ischemic preconditioning

A) Ways to assess the effects on the myocardium

- Reduction in infarct size[1,48,134]
- Improvement in recovery of postischemic contractile function[2,4,42,50,135]
- Reduction of postischemic dysrhythmia[5]
- Reduction in nerve dysfunction[7,136]
- Reduction of the generation of free oxygen radicals[10]
- Reduction of the endothelial damage[8]
- Reduction of stunning[12]

B) Ways to induce the effects

- Ischemia[1,4,38]
- Hypoxia[28]
- Rapid pacing[29]
- Transient substrate withdrawal[95,137]
- Pharmacological interventions[30,32]
- Cyclic variations of coronary flow[138]

contracture as well as both decreased and increased end diastolic stiffening during ischemia have been reported.[4,49-52] In contrast, there is no disagreement on the ability of preconditioning to improve postischemic cardiac function.

Similar discrepancies can be found throughout the literature on preconditioning. These discrepancies may possibly be explained by the different animal models and animal species used as well as the different degrees of "inadvertent" preconditioning[35] during the preparation of the model (cannulation of hearts,[53,54] surgery,[35] or cardiopulmonary bypass[54]).

Effects of Ischemic Preconditioning on Cardiac Metabolism

Since the protection afforded by ischemic preconditioning is short-lived it seems reasonable to assume that acute adaptive responses of the metabolism, or of the regulation of ion homeostasis, or of both, are involved in the mechanism of ischemic preconditioning. Quite a few such responses have been identified and are summarized in Table 10.2. Three metabolic concepts emerged from these findings. Those are described in detail below and they are included in Table 10.3 and the schematic of Figure 10.3.

Preservation of ATP—Conservation of the Moieties

The first difference observed between the ischemic metabolism of preconditioned and nonpreconditioned hearts was the preservation of the ATP pool in preconditioned hearts.[55-57] This discovery was already made before ischemic preconditioning got its name.[56] The authors proposed that the tissue ATP content could be further depleted if an ischemic episode was divided into several short periods of ischemia interrupted by a short episode of reperfusion. The hypothesis was based on the observation that during a long period of ischemia ATP depletion occurred in an exponential fashion. The results were unexpected. If hearts were subjected to 40 min of total ischemia in 8 episodes of 5 min ischemia, interrupted by short reperfusion periods, the tissue ATP content at the end of ischemia was significantly higher than in hearts subjected to one episode of 40 min ischemia. Five years later the same laboratory demonstrated that this strategy was also capable of reducing infarct size.[1]

The preservation of ATP during ischemia in preconditioned hearts has since been confirmed by many others.[50,57,58] The mechanism for the "ATP-sparing effect"[57,59] is still a matter of debate. A reduction in energy demand or an inhibition of the mitochondrial F_0F_1 ATPase, which hydrolyzes ATP during ischemia,[60] have been proposed as plausible mechanisms. However, it is important to remember that contractile function is supported by the turnover and not the tissue content of ATP. Myocardial ATP and phosphocreatine stores would be used up within seconds if regeneration did not occur. Therefore, it is reasonable to ask whether the term "ATP-sparing" is not misleading, and whether the estimation of ATP turnover from measurements of tissue ATP content at selected time points[61] is reliable. The preserved ATP levels at the end of ischemia may either reflect a high degree of ATP production or a decrease in ATP demand. The contradictory findings on the glycolytic rate during ischemia (the main provider of ATP in the absence of oxygen) in preconditioned hearts make it impossible to reach a firm conclusion at this time.

We have demonstrated that the depletion of ATP during ischemia is independent of postischemic functional recovery as long as the adenine nucleotide pool remains large enough to maintain sufficient ATP turnover upon reoxygenation.[62,63] The preservation of the key components of metabolism (moieties), e.g., ATP, and adenine nucleotides is a fundamental principle of metabolism[63] which can also be incorporated into the concepts governing the mechanism of ischemic preconditioning.

Table 10.2. Documented effects of ischemic preconditioning on metabolism, contractile function, and ion homeostasis

Preservation of ATP[1,139]
Inhibition of mitochondrial F_0F_1-ATPase[60]
Depletion of glycogen[48,65]
Reduction in lactate accumulation[48,50,139]
Attenuation of acidosis[48,57,67,73]
Decreased accumulation of long chain acyl-carnitine[138]
Decreased generation of oxygen-derived free radicals[10]
Increased glycolytic rate[18]
Decreased glycolytic rate[48,50,57,73,140]
Decreased and delayed ischemic contracture[10,50]
Increased and early onset of ischemic contracture[4,49,51]
Protection against stunning[12,141]
Reduction of Ca^{2+} and Na^+ overloading[91,92]
Inhibition of Na^+/H^+ exchange[91,92]

Table 10.3. Suggested mechanisms of ischemic preconditioning: metabolic concepts

Approach	Mechanism of Action	Pro	Con
- ATP-preservation	preservation of energy charge	Murry et al 1986,1990[1,57] Jennings et al 1991[142] Yellon et al 1993[24] Abd-Elfattah et al 1995[55]	
	- due to inhibition of F_0F_1 ATPase	Vuorinen et al 1995[60]	Kobara et al 1996[140]
- Glycogen depletion	reduction of H^+ and lactate accumulation	Asimakis et al 1992[50] Wolfe et al 1993[48] Reimer et al 1994[61] Barbosa et al 1996[65]	Schaefer et al 1995[67] Asimakis 1996[74] Doenst et al 1996,1997[4,47] King and Opie 1996[143]
- Reduced anaerobic glycolysis	reduction of H^+ and lactate accumulation	Murry et al 1990[57] Asimakis et al 1992[50] Wolfe et al 1993[48] Finegan et al 1995[73] Schaefer et al 1995[67]	Fralix 1992[144] Janier et al 1994[18] Doenst et al 1996,1997[4,47]
- Inhibition of Ca^{2+} channels	avoid Ca^{2+} overloading	Steenbergen et al 1993[91] Zucchi et al 1995[145]	
- Inhibition of Na^+/H^+ exchange	avoid Na^+/Ca^{2+} overloading	Fralix et al 1993[146] Steenbergen et al 1993[91] Bugge and Ytrehus 1995[147]	Ramasamy et al 1995[92]

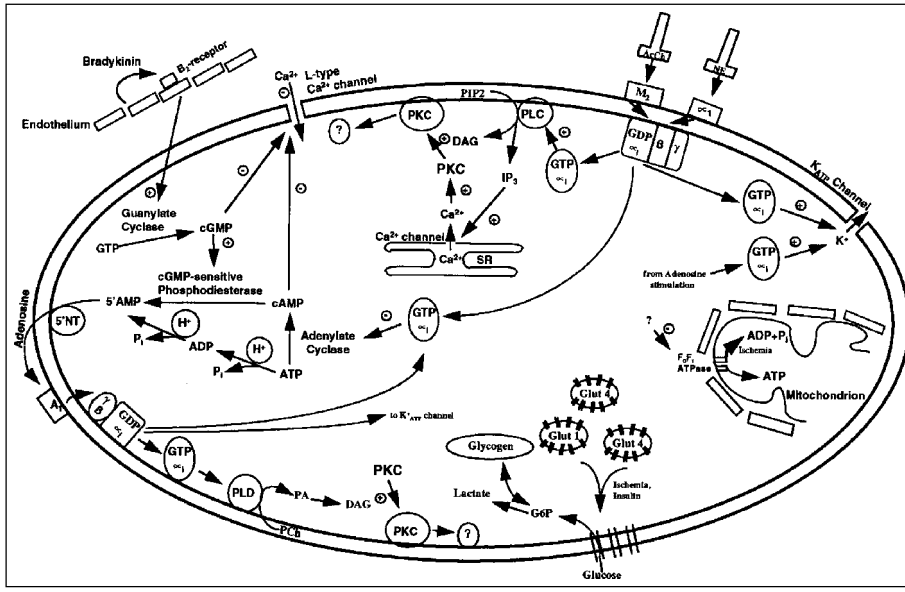

Fig. 10.3. Schematic of the signal transduction cascades possibly involved in the mechanism of ischemic preconditioning. Several sequences are included: preconditioning ischemia leads to the release of adenosine, bradykinin, noradrenaline, and acetylcholine. Most of these mediators activate a Gi-protein which can have several effects. Adenosine A_1-receptor activation leads to activation of phospholipase D (PLD) which gives rise to phosphatidic acid (PA) from phosphatidylcholine (PCh). PA is metabolized to diacyl-glycerol (DAG), which is the primary activator of protein kinase C (PKC). The muscarinic M_2-receptors and the alpha-adrenergic receptor lead to the activation of phospholipase C (PLC) which gives rise to DAG and inositol-1,4,5,-trisphosphate (IP_3) from phosphinositolphosphate (PIP2). IP_3 leads to the release of Ca^{2+} from the sarcoplasmic reticulum (SR) which also activates PKC. G-proteins also reduce the sensitivity of K^+-channels for ATP. A reduction in cellular ATP would result in the opening of the channel and a shortening of the action potential. Finally, G_i proteins reduce the activity of adenylate cyclase activity which suppresses cAMP production. Stimulation of the bradykinin receptor (B_2-receptor) causes an increase in intracellular cGMP which activates a cGMP-sensitive phosphodiesterase. Both the reduction in cAMP and the increase in cGMP cause an inhibition of the L-type Ca^{2+}-channel and thereby reduce Ca^{2+} overloading. Preconditioning ischemia also leads to translocation of glucose transporters to the cell membrane increasing the glucose transport capacity. Inhibition of the F_oF_1-ATPase which is responsible for a major part of ischemic ATP breakdown may also account for the ATP-sparing effect.

The Glycogen Hypothesis—An Epiphenomenon?

The observation that ischemic preconditioning depletes preischemic glycogen content, reduces lactate accumulation, and attenuates intracellular acidosis led to a reexamination of the hypothesis that H^+ could replace Ca^{2+} on the myofilaments[64] which would result in impaired contractile function and ischemic injury. Wolfe and his co-workers[48,65] demonstrated a positive correlation between preischemic tissue glycogen and postischemic infarct size. They suggested that preischemic glycogen depletion by preconditioning-ischemia reduced substrate availability for anaerobic glycolysis during ischemia, thus reducing lactate and H^+ production, and thereby attenuating acidosis. At least three observations argue against this hypothesis.

First, we demonstrated in the isolated working rat heart model that the protection afforded by ischemic preconditioning is independent from the preischemic glycogen content.[4,47] We also demonstrated that lactate accumulation does not affect postischemic

recovery of function. Secondly, if glycogen depletion were involved in the mechanism of ischemic preconditioning, depletion of preischemic glycogen without ischemia (e.g., by anoxia, or by substrate-free perfusion) should also result in protection. We[66] and others[67] were unable to confirm this hypothesis in isolated rat hearts.

In contrast, we demonstrated that raising glycogen before the onset of ischemia was beneficial for the return of postischemic contractile function[4,68-70] and that the addition of lactate or lactate and insulin to the perfusate before or after ischemia improved postischemic recovery.[4,66] The third argument against the glycogen hypothesis is the fact that pharmacological preconditioning does not deplete preischemic glycogen.[71] We therefore conclude that glycogen depletion through ischemic preconditioning appears to be an epiphenomenon.

Glycolysis—A Major Player in Ischemic Preconditioning?

The majority of the studies investigating glucose metabolism in preconditioned hearts suggest that the glycolytic rate during sustained ischemia is decreased which results in less lactate and proton production implementing attenuation of acidosis for the protective effects of ischemic preconditioning.[48,50,57,67,72-74]

In contrast, one group of investigators[18] demonstrated increased glycolytic flux during ischemia after preconditioning in a model of low-flow ischemia, and another group reports an inverse relationship between the rate of glycolysis and ischemic injury.[75] The anaerobic production of cytosolic ATP during ischemia has been linked to the maintenance of ion-pump activity in the sarcolemma for the preservation of cellular integrity.[76] In addition, adenosine increases glycolytic flux through an A_1-receptor-dependent pathway.[77] The same receptor may be involved in the transfer of the ischemic preconditioning stimulus to the cellular level,[43] (see below).

It is difficult to resolve this controversy. Low-flow ischemia differs from total ischemia. Assessment of glycolytic rates during total ischemia relies on indirect measures, such as glycogen depletion or lactate accumulation, or both, and does not provide changes as a function of time or actual rates.

However, if the rate of anaerobic glycolysis is reduced in preconditioned hearts, so is ATP production. Preservation of ATP is therefore only possible if energy consumption is decreased before ATP is depleted. Possible mechanisms could include an inhibition of the mitochondrial F_0F_1 ATPase[60] (a major culprit for ischemic ATP hydrolysis) or an early reduction of contractile function. Another possibility is an increased rate of anaerobic glycolysis maintaining residual ATP generation.[76] The latter proposal is opposed by the majority of studies reporting decreased lactate production and attenuation of acidosis during ischemia.[35,50,67,72-74] Thus, there appears to be some intervening influence to decrease contractile function before total ATP depletion. This influence is even more necessary in the setting of ischemic preconditioning if anaerobic glycolysis is indeed decreased (the major source of ATP production during ischemia) and ATP levels in the cell are maintained. In this context, reduction of lactate accumulation and attenuation of acidosis may just be the consequence of reduced energy demand.

Delivery of Preconditioning—How Is Protection Mediated?

Despite extensive investigation of signal transduction pathways and other possible mediators, the exact mechanisms underlying ischemic preconditioning remain elusive. Several hypotheses have been advanced.

At least one of the mechanisms of ischemic preconditioning can be triggered by the interaction between endogenous factors produced within the heart and cardiac myocytes. Otherwise, the efficacy of ischemic preconditioning could not be explained in isolated hearts?[4,19,47,73] In addition, it may well be that remotely produced factors activate the same or a similar response in the heart.[78, 79] McClanahan et al[79] demonstrated that a prior episode of renal ischemia and reperfusion reduced myocardial infarct size in rabbits.

Several endogenous ischemic byproducts have been identified which may have the potential to mediate protection against ischemic injury of the heart, whether in a paracrine or an endocrine fashion. These mediators generally exert their effects through the interaction with specific receptors and the activation of transduction cascades. The most widely discussed candidates are adenosine[12,17,19,31,38,60,80] and noradrenaline.[21,81-84] Other mediators include bradykinin,[85,86] prostanoids,[5,29] endorphins,[87] acetylcholine,[30,88] and oxygen derived free radicals.[20,57] The "delivery systems" associated with the individual receptors are believed to be protein kinase C,[89] the ATP sensitive K^+ channel,[90] the Na^+H^+ exchanger,[91,92] and the L-type Ca^{2+} channel.[93,94] Figure 10.3 summarizes the most commonly advanced hypotheses into a simple schematic. In this scheme adenosine and noradrenaline are endogenous mediators. Protein kinase C and K^+ ATP channels are possible effectors distal to the receptor. The concepts are discussed in detail in the following section. Please refer to Table 10.4 and Figure 10.3 for summary information.

Two Possible Endogenous Mediators of Protection

Adenosine—Sufficient but not Mandatory for Preconditioning

The increase in tissue AMP during ischemia causes an increased production of adenosine through 5' nucleotidase. The generation of adenosine takes place within minutes and its release during preconditioning reperfusion with subsequent activation of A_1 or A_3 or both adenosine receptors is thought to be involved in the mechanism of ischemic preconditioning.[4,15,19,95,96] Evidence for this hypothesis was provided by experiments where selective A_1 receptor agonists (but not A_2 receptor agonists) could mimic the reduction in infarct size,[38,97] while the addition of adenosine receptor blockers (8-p-sulphophenyl theophylline or PD115199) prevented the infarct size reduction normally observed with ischemic preconditioning[80] (Fig. 10.4). Adenosine may exert its cardioprotective effects in several ways: 1) by activation of ATP sensitive K^+ channels[98] (see below), 2) by translocation of protein kinase C[89] which is believed to be initiated by activation of phospholipase D[99, 100] (see below), 3) by inhibition of catecholamine action through activation of G_i proteins and reduction of adenylate cyclase activity,[101] 4) by the reduction of norepinephrine release from sympathetic nerve endings[102] (in contrast to a_1 adrenergic receptor hypothesis, see below), 5) by blockade of cardiomyocyte L-type Ca^{2+} channels,[93,94] 6) by the stimulation of glucose uptake and utilization.[77] At this time, it is unclear which of these mechanisms are predominant. Since adenosine release during reperfusion ceases after the first cycle, a logical problem emerges when adenosine release is implicated in the mechanism of preconditioning in a model where several cycles of preconditioning/reperfusion are necessary for protection. This effect can be observed in pigs[103] and rats.[45] Hence, it is not surprising that certain studies were not able to confirm the adenosine hypothesis,[104-106] and some investigators even question whether adenosine is involved in the mechanism of ischemic preconditioning.[107,108]

An explanation for these discrepancies may come from the different animal species used or may be provided by the threshold hypothesis of Downey and his colleagues.[45,71] According to this hypothesis, the stimulus provided by adenosine release may not be sufficient to induce the protective effects and may require supplementation by other mediators. Vice versa, if the adenosine stimulus is blocked, e.g., through an A_1-receptor blocker, the stimulation of the protective effects through alternative mediators has to be increased or protection will not be detectable. In conclusion, endogenous adenosine appears to be sufficient but not mandatory for the induction of ischemic preconditioning.

Noradrenaline and the α_1-Receptor—Independent from Adenosine?

Other investigators have demonstrated that ischemic preconditioning may be mediated by increased release of norepinephrine from autonomic nerve terminals.[21,81,82] On the basis of selective α_1-adrenergic blockade and

Table 10.4. Suggested mechanisms of ischemic preconditioning: Pharmacological agents, receptors, and intracellular mediators

Pharmacological Agent	Receptor	Mechanism of Action	Pro	Con
- Adenosine:	A_1 receptor A_3 receptor	activation of G_i protein PKC-translocation K^+_{ATP} channel-opening	Kirsch et al 1990[98] Liu et al 1991-1994[19,80] Downey et al 1993[38] Lasley, Mentzer 1993[148] Burns et al 1995[54] Kerensky et al 1995[32] Schulz et al 1995[31] Woolfson et al 1995[96] Auchampach, Gross 1993[119]	Cave et al 1993[106] Hale et al 1993[105] Hendrikx et al 1993[104] Li and Kloner 1993[108] Liu and Downey 1993[149] Armstrong et al 1994[95] Yabe et al 1995[28]
- Noradrenaline:	α_1 receptor	translocation of PKC	Banerjee et al 1993[81] Mitchell et al 1995[83] Toombs et al 1993[21] Tsuchida et al 1994[84] Burns et al 1995[54] Kitakaze et al 1995[82]	Richardt et al 1987[102] Thornton et al 1993[123] Bugge, Ytrehus 1995[150] Weselcouch et al 1995[151]
- Bradykinin:	BK_2 receptor Nitric oxide	translocation of PKC	Vegh et al 1994, 1995[5,29] Wall et al 1994[86]	Bugge, Ytrehus 1996[152]
- Prostanoids:	Nitric oxide		Vegh et al 1990[153]	Weselcouch et al 1995[154] Woolfson et al 1995[96]
- Endothelin:		translocation of PKC	Wang et al 1996[155]	
- Acetylcholine	M_2-receptor	activation of G_i proteins	Yao and Gross 1993[30] Hendrikx et al 1993[156] Przyklenk et al 1995[88]	
- Morphine	opiate receptor	activation of K^+_{ATP} channel	Schultz et al 1995,1996[87,157]	
- Angiotensin II	ATII receptor	translocation of PKC	Liu et al 1995[158]	
- K^+_{ATP} channel-openers	direct activation of K^+_{ATP} channel		Auchampach et al 1991,1992[121,122] Cole et al 1993[3] Steenbergen et al 1993[91] Schulz et al 1994[15] Tomai et al 1994[22] Mizumura et al 1995[159] Hu et al 1996[112]	Kitzen et al 1992[124] Grover et al 1993[160] Thornton et al 1993[123] Yabe et al 1995[28]
- PKC activators	direct translocation and activation of PKC		Liu et al 1994[161] Speechly-Dick et al 1994[117] Ytrehus et al 1994[89] Mitchell et al 1995[83] Hu et al 1996[112] Kitakaze et al 1996[162]	Armstrong et al 1994[95] Vogt et al 1994[118] Przyklenk et al 1995[14]

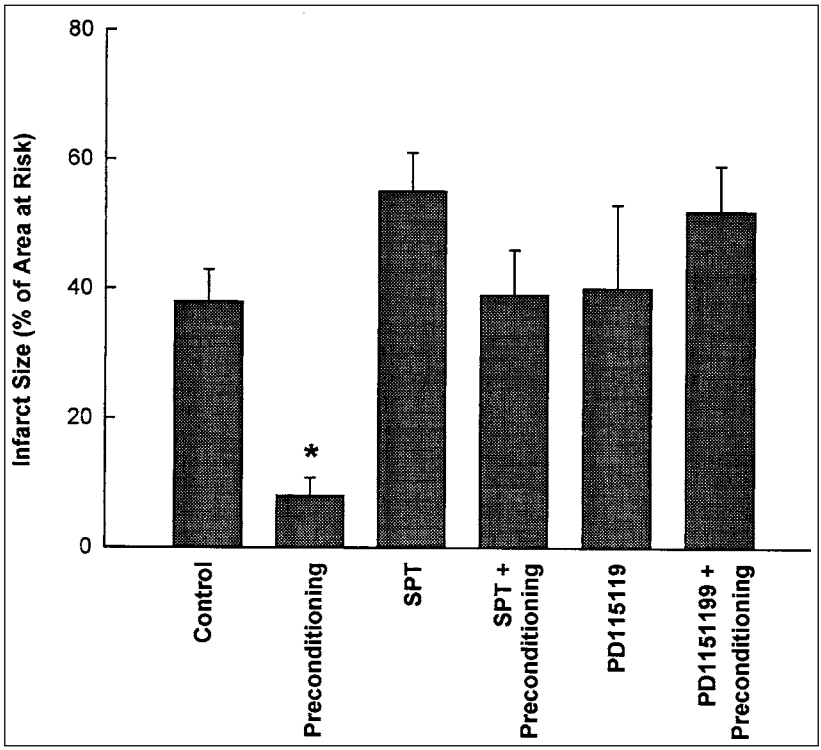

Fig. 10.4. Prevention of the preconditioning-induced reduction in infarct size by the adenosine receptor antagonists 8-p-sulphophenyl theophylline (SPT) and PD115199 in rabbit hearts subjected to a 30 min occlusion of the left coronary artery followed by three hours of reperfusion. Preconditioning was induced by a single cycle of 5 min ischemia and 10 min of reperfusion. * $p < 0.001$ compared to Control. Reproduced with permission from Liu et al. Circulation 1991; 84:350-356.

stimulation experiments it was concluded that ischemic preconditioning is mediated through an α_1-adrenergic mechanism.[81] The activation of α_1-adrenergic receptors leads to the activation of phospholipase C, which in turn leads to transient increases in diacylglycerol (DAG) and inositol-1,4,5-trisphosphate (IP_3).[109,110] DAG is the primary physiological activator of protein kinase C, a serine-threonine kinase implicated in the preconditioning effects[83,111] (see below and Fig. 10.3).

Some investigators suggest that the α_1-adrenoceptor is still capable of reducing infarct size despite the presence of an adenosine receptor blocker.[84] Thus, it appears that both adenosine receptors and α_1-adrenoceptors share certain parts of the same transduction cascade but are able to activate them independently.

Two Possible Effectors Distal to the Receptors

Protein Kinase C— The Common Denominator

Protein kinase C (PKC) is a promiscuous enzyme which is considered to be involved in most of the transduction cascades implicated with ischemic preconditioning.[83,89,112] PKC belongs to the serine threonine kinase family. Its 11 or more isoforms are associated with a variety of receptors and physiological effectors.[113] PKC participates in the regulation of ion homeostasis, vascular tone, myocyte contractility,

gene expression, hypertrophy, and in phosphorylation of specific cellular proteins.[111,113] The a, d, and e isoforms are predominant in the heart.[83,114] Upon stimulation of e.g., adenosine A_1-receptors, α_1-adrenoceptors, or during ischemia, DAG and IP_3 are formed resulting in the activation and translocation of protein kinase C to the myocyte membrane.[19,83,115] An as yet unidentified, membrane bound target protein has been proposed to undergo phosphorylation by PKC, thereby inducing the protective effects of ischemic preconditioning.[19,89]

However, support for the concept that PKC translocation by ischemic preconditioning is responsible for the reduction of infarct size is mainly indirect. In rat and rabbit hearts as well as in rabbit myocyte preparations pretreatment with PKC blockers (staurosporin, polymyxin B, or calphostin C) abolishes the protection of ischemic preconditioning.[19,83,89,95,116,117] Vice versa, the activation of PKC with potent PKC activators (4b-phorbol, 12-myristate, or 13-acetate) mimics the cardioprotective effects of preconditioning.[19,83,89,95,116,117] Mitchell et al[83] demonstrated, with the use of isoform specific antibodies to PKC in the rat heart, that ischemic preconditioning causes a translocation of the d-isoform to the sarcolemma and of the e-isoform to the nucleus. The authors proposed the d-isoform to be involved in the acute protection afforded by ischemic preconditioning.

In dog hearts, a short period of ischemia induced no significant translocation of PKC.[14] In addition, not all the pharmacological evidence has been supportive of the PKC translocation hypothesis.[14,118] The issue is further complicated by the demonstration that blockers of protein kinase C (bisimdolyl, maleimide, or staurosporin) elicit their own effects limiting infarct size.[118]

Thus, if PKC is involved in preconditioning, the end effector is still unknown. Activation of the K^+_{ATP} channel may be a possibility (see below). An alternative hypothesis has been suggested by Kitakaze et al[82] who suggested that the activation of PKC precedes and is responsible for the release of adenosine through activation of ecto-5'nucleotidase and its translocation to the cell membrane.

K^+_{ATP}—A Possible End Effector of the Transduction Cascades

The ATP sensitive K^+-channel is one of the most abundant ion channels on the sarcolemma. Studies on ischemic preconditioning suggest that activation of the K^+_{ATP} channel in myocardial fibers limits the detrimental effects of ischemia, probably because of the shortening of the action potential duration, which in turn results in a reduction in both Ca^{2+} inflow and ATP depletion.[3,90,91,119,120] The mechanism by which the activation of K^+_{ATP}-channels during a brief ischemic period protects the myocardium during subsequent ischemic episodes is unknown, but it has been shown in a number of studies and animal species that the inhibition of channel opening (e.g., with glibenclamide[90,119]) also abolished the protective effects afforded by ischemic preconditioning. Vice versa, the stimulation of the channel opening was able to mimic preconditioning.[121,122]

Although several investigations do not support the hypothesis of an involvement of the K^+_{ATP} channel in the mechanism of ischemic preconditioning,[123,124] recent studies suggest a role for K^+_{ATP} channels in preconditioning of human myocardium.[22,112] Hu et al[112] linked the activation of PKC in human and rabbit myocytes to the opening of ATP-sensitive K^+ channels. In their study, protein kinase C reduced the channels' sensitivity for ATP rendering it open under conditions of decreasing ATP.

Preconditioning Without Preconditioning?

The search for a common mechanism underlying the phenomenon of ischemic and pharmacological preconditioning is complicated further by observations that simple metabolic or nutritional changes, such as fasting in vivo, can increase ischemia tolerance in vitro to the same extent as ischemic preconditioning.[4] As shown in Figure 10.2, the addition of lactate and insulin to the perfusion medium of hearts from fasted animals improved postischemic cardiac function to the same extent as ischemic preconditioning. The two experi-

mental groups shared two other findings which were different from the control group. First, we observed a reduction of the release of the ischemically accumulated lactate. We suggested that the ability to oxidize lactate during the reperfusion period is a strong indicator for postischemic return of function. Secondly, we observed a biphasic behavior of glucose uptake in the reperfusion period, measured with the positron-emitting glucose tracer analogue [^{18}F]-2-deoxy-2-fluoroglucose (FDG). An initially high uptake rate was followed by a suppression in the late phase. In both, ischemically preconditioned hearts and hearts subjected to the metabolic manipulations, there was an early increase in glucose uptake compared to control, suggesting an early need for glucose substrate. Other reports add support to this hypothesis. Insulin like growth factor (IGF_2)[114] as well as the calcitonin gene-related peptide[125] mimic the protective effects of ischemic preconditioning. Both agents are known for their stimulating effect on glucose uptake. Ischemia also results in the translocation of GLUT-1 and GLUT-4 transporters to the plasma membrane.[126] The protective role of increased glucose uptake is not new, but it is often overlooked that glucose uptake, and not its supply, is the limiting factor.

Clinical Aspects—Implications for the Cardiac Surgeon

Ischemic preconditioning has also been documented in the human heart.[22-26] It can be observed in infarct patients with preceding angina, in patients undergoing coronary angioplasty, as well as in patients undergoing surgery with cardiopulmonary bypass and hypothermic ischemic arrest of the myocardium. One of the most persuasive evidences for the occurrence of ischemic preconditioning in human heart is presented by Yellon et al.[24] In this study patients undergoing coronary artery bypass grafting were subjected to cross-clamp fibrillation for 10 min with or without two 3 minute episodes of prior aortic cross clamping. Biopsies taken during the surgery showed significantly higher ATP tissue contents at the end of the 10 min cross-clamping episode when patients were subjected to the two 3 min episodes of prior cross-clamping. See, however, our earlier reservations about ATP levels as markers for ischemic injury.

The protective effects induced by ischemic preconditioning seem to be additive to those afforded by cardioplegia or hypothermia or both.[127-131] The additive effects rank from overcoming the lack of protection by cardioplegia due to impaired delivery of cardioplegia,[130] through reversing the detrimental effects of aprotinin on the ischemic myocardium,[131] to the better recovery of the implanted, ischemically preconditioned, donor hearts.[128]

Some investigators, however, suggested from their experiments on sheep hearts that the use of cardiopulmonary bypass alone is stimulus enough to elicit the preconditioning effects.[54] The authors compared infarct size of preconditioned and nonpreconditioned hearts with or without the use of cardiopulmonary bypass.

It is certainly desirable to "bottle" the different variants of protection afforded by the mechanisms of ischemic preconditioning. We hope that further research in this field will lead to the development of safe drugs mimicking the effect of preconditioning.[34,99] However, characterization of the exact mechanism is a prerequisite to achieve this goal.

Conclusions

Ischemic preconditioning protects the myocardium from ischemic injury by probably more than one mechanism. One of the mechanisms involves the release of adenosine or noradrenaline during preconditioning reperfusion and subsequent translocation of PKC and opening of K^+_{ATP} channels. It appears that simple metabolic interventions induce protection of the myocardium similar to ischemic preconditioning. Conventional metabolic interventions such as the administration of glucose, insulin, and potassium may be the most valuable for clinical use at the moment because their safety has been documented.[132,133] The exploitation of ischemic

preconditioning for clinical practice will only be possible when the mechanisms of ischemic preconditioning are fully understood.

Acknowledgments

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References

1. Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: A delay of lethal cell injury in ischemic myocardium. Circulation 1986; 74:1124-1136.
2. Cohen MW, Liu GS, Downey JM. Preconditioning causes improved wall motion as well as smaller infarcts after transient coronary occlusion in rabbits. Circulation 1991; 84:341-349.
3. Cole WC. ATP-sensitive K^+ channels in cardiac ischemia: An endogenous mechanism for protection of the heart. Cardiovasc Drugs Ther 1993; 7 Suppl 3:527-537.
4. Doenst T, Guthrie PH, Chemnitius J-M et al. Fasting, lactate, and insulin improve ischemia tolerance: A comparison with ischemic preconditioning. Am J Physiol 1996; 270: H1607-H1615.
5. Vegh A, Papp JG, Parratt J. Attenuation of the antiarrhythmic effects of ischemic preconditioning by blockade of bradykinin B2 receptors. Br J Pharmacol 1994; 113:1167-1172.
6. Parratt JR. Protection of the heart by ischemic preconditioning: Mechanisms and possibilities for pharmacological exploitation. Trends Pharmacol Sci 1994; 15:19-25.
7. Seyfarth M, Richardt G, Mizsnyak A et al. Transient ischemia reduces norepinephrine release during sustained ischemia. Neural preconditioning in isolated rat heart. Circ Res 1996; 78:573-580.
8. DeFily DV, Chilian WM. Preconditioning protects coronary arteriolar endothelium from ischemia-reperfusion injury. Am J Physiol 1993; 265:H700-H706.
9. Jerome SN, Akimitsu T, Gute DC et al. Ischemic preconditioning attenuates capillary no-reflow induced by prolonged ischemia and reperfusion. Am J Physiol 1995; 268:H2063-H2067.
10. Tosaki A, Cordis GA, Szerdahelyi P et al. Effects of preconditioning on reperfusion arrhythmias, myocardial functions, formation of free radicals, and ion shifts in isolated ischemic/reperfused rat hearts. J Cardiovasc Pharmacol 1994; 23:365-373.
11. Turrens JF, Thornton J, Barnard ML et al. Protection from reperfusion injury by preconditioning hearts does not involve increased antioxidant defenses. Am J Physiol 1992; 262:H585-H589.
12. Ogawa T, Miura T, Shimamoto K et al. Activation of adenosine receptors before ischemia enhances tolerance against myocardial stunning in the rabbit heart. J Am Coll Cardiol 1996; 27:225-233.
13. Murry CE, Richard VJ, Jennings RB et al. Myocardial protection is lost before contractile function recovers from ischemic preconditioning. Am J Physiol 1991; 260:1796-1804.
14. Przyklenk K, Sussman MA, Simkhovich BZ et al. Does ischemic preconditioning trigger translocation of protein kinase C in the canine model? Circulation 1995; 92:1546-1557.
15. Schulz R, Rose J, Heusch G. Involvement of activation of ATP-dependent potassium channels in ischemic preconditioning in swine. Am J Physiol 1994; 267:H1341-H1352.
16. Schulz R, Post H, Sakka S et al. Intraischemic preconditioning. Increased tolerance to sustained low-flow ischaemia by a brief episode of no-flow ischaemia without intermittent reperfusion. Circ Res 1995; 76:942-950.
17. Schott RJ, Rohmans S, Brau ER et al. Ischemic preconditioning reduces infarct size in swine myocardium. Circ Res 1990; 66: 1133-42.
18. Janier MF, Vanoverschelde JL, Bergmann SR. Ischemic preconditioning stimulates anaerobic glycolysis in the isolated rabbit heart. Am J Physiol 1994; 267:H1353-H1360.
19. Liu GS, Richards SC, Olsson RA et al. Evidence that the adenosine A3 receptor may mediate the protection afforded by preconditioning in the isolated rabbit heart. Cardiovasc Res 1994; 28:1057-1061.
20. Tanaka M, Fujiwara H, Yamasaki K et al. Superoxide dismutase and N-2-mercaptopropionyl glycine attenuate infarct size limitation effect of ischemic preconditioning in the rabbit. Cardiovasc Res 1994; 28:980-986.
21. Toombs CF, Witte AL, Shebuski RJ. Ischemic preconditioning fails to limit infarct size in reserpinized rabbit myocardium: Implications of norepinephrine release in the preconditioning effect. Circulation 1993; 88:2351-2358.

22. Tomai F, Crea F, Gaspardone A et al. Ischemic preconditioning during coronary angioplasty is prevented by glibenclamide, a selective ATP-sensitive K^+ channel blocker. Circulation 1994; 90:700-705.
23. Deutsch E, Berger M, Kussmaul WG et al. Adaptation to ischemia during percutaneous transluminal coronary angioplasty: Clinical, hemodynamic, and metabolic features. Circulation 1990; 82(2044-2051).
24. Yellon DM, Alkhulaifi AM, Pugsley WB. Preconditioning the human myocardium. Lancet 1993; 342:276-277.
25. Vaitkus PT, Miller JM, Buxton AE et al. Ischemia-induced changes in human endocardial electrograms during percutaneous transluminal coronary angioplasty. Am Heart J 1994; 127:1481-1490.
26. Ottani F, Galvani M, Ferrini D et al. Prodromal angina limits infarct size. A role for ischemic preconditioning. Circulation 1995; 91:1667-1668.
27. Bolli R, Leesar M, Stoddard M. Ischemic preconditioning during coronary angioplasty. J Am Col Cardiol 1997; 29(2):469.
28. Yabe K, Nasa Y, Takeo S. Hypoxic preconditioning in isolated rat hearts: Noninvolvement of activation of adenosine A1 receptor, G_i protein, and ATP-sensitive K^+ channel. Heart & Vessels 1995; 10:294-303.
29. Vegh A, Kaszala K, Papp JG et al. Bradykinin, a trigger for delayed pacing-induced preconditioning (abstract). J Mol Cell Cardiol 1995; 27:A279.
30. Yao Z, Gross GJ. Role of nitric oxide, muscarinic receptors, and the ATP-sensitive K^+ channel in mediating the effects of acetylcholine to mimic preconditioning in dogs. Circ Res 1993; 73:1193-1201.
31. Schulz R, Rose J, Post H et al. Involvement of endogenous adenosine in ischemic preconditioning in swine. Pflugers Arch-Eur J Physiol 1995; 430:273-282.
32. Kerensky RA, Kutcher MA, Braden GA et al. The effects of intracoronary adenosine on preconditioning during coronary angioplasty. Clin Cardiol 1995; 18:91-96.
33. Heibig J, Bolli R, Harris S. Initial coronary occlusion improves tolerance to subsequent prolonged balloon inflations. Cath Cardiovasc Diag 1989; 16:99-102.
34. Kloner RA, Przyklenk K, Shook T et al. Clinical aspects of preconditioning and implications for the cardiac surgeon. J Card Surg 1995; 10 (4 Suppl):369-375.
35. Kloner RA, Przyklenk K, Whittaker P et al. Preconditioning stimuli and inadvertent preconditioning. J Mol Cell Cardiol 1995; 27:743-747.
36. Anzai T, Yoshikawa T, Asakura Y et al. Preinfarction angina as a major predictor of left ventricular function and long-term prognosis after a first Q wave myocardial infarction. J Am Coll Cardiol 1995; 26:319-27.
37. Alkhulaifi AM, Pugsley WB, Yellon DM. The influence of the time period between preconditioning ischemia and prolonged ischemia on myocardial protection. Cardioscience 1993; 4:163-169.
38. Downey JM, Liu GS, Thornton JD. Adenosine and the anti-infarct effects of preconditioning. Cardiovasc Res 1993; 27:3-8.
39. Van Winkle D, Thornton J, Downey D et al. The natural history of preconditioning: Cardioprotection depends on the duration of transient ischemia and time to subsequent ischemia. Coron Art Dis 1991; 2:613-619.
40. Marber MS, Latchman DS, Walker JM et al. Cardiac stress protein elevation 24 hours after brief ischemia or heat stress is associated with resistance to myocardial infarction. Circulation 1993; 88:1264-1272.
41. Knowlton AA, Brecher P, Apstein CS. Rapid expression of heat shock protein in the rabbit after brief cardiac ischemia. J Clin Invest 1991; 87:139-147.
42. Volovsek A, Subramanian R, Reboussin D. Effects of duration of ischemia during preconditioning on mechanical function, enzyme release and energy production in the isolated working rat heart. J Mol Cell Cardiol 1992; 24:1011-1019.
43. Lawson CS, Downey JM. Preconditioning: State of the art myocardial protection. Cardiovasc Res 1993; 27:542-550.
44. Yang XM, Arnoult S, Tsuchida A et al. The protection of ischemic preconditioning can be reinstated in the rabbit heart after the initial protection has waned. Cardiovasc Res 1993; 27:556-558.
45. Liu Y, Downey JM. Ischemic preconditioning protects against infarction in rat heart. Am J Physiol 1992; 263:H1107-H1112.
46. Li GC, Vasquez JA, Gallagher KP et al. Myocardial protection with preconditioning. Circulation 1990; 82:609-619.
47. Doenst T, Guthrie PH, Taegtmeyer H. Ischemic preconditioning in rat heart: No correlation between glycogen content and return of function. J Mol Cell Biochem 1997:in press.
48. Wolfe CL, Sievers RE, Visseren FLJ et al. Loss of myocardial protection after preconditioning correlates with the time course of glycogen recovery within the preconditioned segment. Circulation 1993; 87:881-892.

49. Kolocassides KG, Galinanes M, Hearse DJ. Dichotomy of ischemic preconditioning: Improved postischemic contractile function despite intensification of ischemic contracture. Circulation 1996; 93:1725-1733.
50. Asimakis GK, Inners-McBride K, Medellin G et al. Ischemic preconditioning attenuates acidosis and postischemic dysfunction in isolated rat hearts. Am J Physiol 1992; 263: H887-H894.
51. Cave AC, Horowitz GL, Apstein CS. Can ischemic preconditioning protect against hypoxia-induced damage? Studies of contractile function in isolated perfused rat hearts. J Mol Cell Cardiol 1994; 26:1471-1486.
52. Ladilov Y, Siegmund B, Balser C et al. Simulated ischemia increases the susceptibility of rat cardiomyocytes to hypercontracture. Circ Res 1997; 80(1):69-75.
53. Minhaz U, Koide S, Shohtsu A et al. Perfusion delay causes unintentional ischemic preconditioning in isolated heart preparation. Basic Res Cardiol 1995; 90:418-423.
54. Burns PG, Krukenkamp IB, Caldarone CA et al. Does cardiopulmonary bypass alone elicit myoprotective preconditioning? Circulation 1995; 92 (9 Suppl):II447-II451.
55. Abd-Elfattah AS, Ding M, Wechsler AS. Intermittent aortic crossclamping prevents cumulative adenosine triphosphate depletion, ventricular fibrillation, and dysfunction (stunning): Is it preconditioning? J Thorac Cardiovasc Surg 1995; 110:328-339.
56. Reimer KA, Hill ML, Jennings RB. Prolonged depletion of ATP and adenine nucleotide pool due to delayed resynthesis of adenine nucleotides following reversible myocardial ischemia injury in dogs. J Mol Cell Cardiol 1981; 13:229.
57. Murry CE, Richard VJ, Reimer KA et al. Ischemic preconditioning slows energy metabolism and delays ultrastructural damage during a sustained ischemic episode. Circ Res 1990; 66:913-931.
58. Downey JM. Ischemic preconditioning: Nature's own cardioprotective intervention. Trends Cardiovasc Med 1992; 2:170-176.
59. Van der Heide R, Hill M, Steenbergen C et al. Effect of reversible ischemia on mitochondrial ATPase activity in canine myocardium. Circulation 1991; 84:192.
60. Vuorinen K, Ylitalo K, Peuhkurinen K et al. Mechanisms of ischemic preconditioning in rat myocardium. Roles of adenosine, cellular energy state, and mitochondrial F1F0-ATPase. Circulation 1995; 91:2810-2818.
61. Reimer KA, Vander Heide RS, Jennings RB. Ischemic preconditioning slows ischemic metabolism and limits myocardial infarct size. Ann N Y Acad Sci 1994; 723:99-115.
62. Taegtmeyer H, Roberts AFC, Rayne AEG. Energy metabolism in reperfused rat heart: Return of function before normalization of ATP content. J Am Coll Cardiol 1985; 6:864-870.
63. Taegtmeyer H, Goodwin GW, Doenst T et al. Substrate metabolism as a determinant for postischemic functional recovery of the heart. Am J Cardiol 1997:in press.
64. Katz AM, Hecht HH. The early "pump" failure of the ischemic heart. Am J Med 1969; 47:497-502.
65. Barbosa V, Sievers RE, Zaugg CE et al. Preconditioning ischemia time determines the degree of glycogen depletion and infarct size reduction in rat hearts. Am Heart J 1996; 131:224-230.
66. Goodwin GW, Taegtmeyer H. Metabolic recovery of the isolated working rat heart after brief global ischemia. Am J Physiol 1994; 267:H462-H470.
67. Schaefer S, Carr LJ, Prussel E et al. Effects of glycogen depletion on ischemic injury in isolated rat hearts: Insights into preconditioning. Am J Physiol 1995; 268:H935-H944.
68. Schneider CA, Nguyên VTB, Taegtmeyer H. Feeding and fasting determine postischemic glucose utilization in isolated working rat hearts. Am J Physiol 1991; 260:H542-H548.
69. Schneider CA, Taegtmeyer H. Fasting in vivo delays myocardial cell damage after brief periods of ischemia in the isolated working rat heart. Circ Res 1991; 68:1045-1050.
70. Lagerstrom CF, Walker WE, Taegtmeyer H. Failure of glycogen depletion to improve left ventricular function of the rabbit heart after hypothermic ischemic arrest. Circ Res 1988; 63:81-86.
71. Downey JM, Cohen MV. Preconditioning: Markers vs. epiphenomena. In: Heusch G, Schulz R, eds. New Paradigms of Coronary Artery Disease. New York: Springer, 1996: 153-155.
72. Liedtke AJ, Renstrom B, Nellis SH et al. Mechanical and metabolic functions in pig hearts after 4 days of chronic coronary stenosis. J Am Col Cardiol 1995; 26:815-825.
73. Finegan BA, Lopaschuk GD, Gandhi M et al. Ischemic preconditioning inhibits glycolysis and proton production in isolated working rat hearts. Am J Physiol 1995; 269: H1767-H1775.
74. Asimakis G. Myocardial glycogen depletion cannot explain the cardioprotective effects of ischemic preconditioning in the rat heart. J Mol Cell Cardiol 1996; 28:563-570.
75. Vanoverschelde JL, Janier MF, Bakke JE et al. Rate of glycolysis during ischemia determines extent of ischemic injury and functional recovery after reperfusion. Am J Physiol 1994; 267:H1785-H1794.

76. Weiss J, Hiltbrand B. Functional compartmentation of glycolytic versus oxidative metabolism in isolated rabbit heart. J Clin Invest 1985; 75:436-447.
77. Wyatt DA, Edmunds MC, Rubio R et al. Adenosine stimulates glycolytic flux in isolated perfused rat hearts by A_1 adenosine receptors. Am J Physiol 1989; 257:H1952-H1957.
78. Przyklenk K, Bauer B, Ovize M et al. Regional ischemic 'preconditioning' protects remote virgin myocardium from subsequent sustained coronary occlusion. Circulation 1993; 87:893-899.
79. McClanahan TB, Nao BS, Wolke LJ et al. Brief renal occlusion and reperfusion reduces myocardial infarct size in rabbits. The FASEB Journal 1993; 7:682.
80. Liu GS, Thornton J, Van Winkle DM et al. Protection against infarction afforded by preconditioning is mediated by A_1 adenosine receptors in rabbit heart. Circulation 1991; 84:350-356.
81. Banerjee A, Locke-Winter C, Rogers KB et al. Preconditioning against myocardial dysfunction after ischemia and reperfusion by an α_1-adrenergic mechanism. Circ Res 1993; 73:656-670.
82. Kitakaze M, Hori M, Morioka T et al. Alpha 1-adrenoceptor activation increases ecto-5'-nucleotidase activity and adenosine release in rat cardiomyocytes by activating protein kinase. Circulation 1995; 91:2226-2234.
83. Mitchell MB, Meng X, Ao L et al. Preconditioning of isolated rat heart is mediated by protein kinase C. Circ Res 1995; 76:73-81.
84. Tsuchida A, Liu Y, Liu GS et al. alpha 1-adrenergic agonists precondition rabbit ischemic myocardium independent of adenosine by direct activation of protein kinase C. Circ Res 1994; 75:576-585.
85. Miura T. Adenosine and bradykinin: Are they independent triggers of preconditioning. In: Heusch G, Schulz R, eds. New Paradigms of Coronary Artery Disease. New York: Springer, 1996:138-140.
86. Wall TM, Sheehy R, Hartman JC. Role of bradykinin in myocardial preconditioning. J Pharmacol Exp Therapeutics 1994; 270: 681-689.
87. Schultz JE, Hsu AK, Gross GJ. Morphine mimics the cardioprotective effect of ischemic preconditioning via a glibenclamide-sensitive mechanism in the rat heart. Circ Res 1996; 78:1100-1104.
88. Przyklenk K, Kloner RA. Low-dose i.v. acetylcholine acts as a "preconditioning-mimetic" in the canine model. J Card Surg 1995; 10 (Suppl 4):389-395.
89. Ytrehus K, Liu Y, Downey JM. Preconditioning protects ischemic rabbit heart by protein kinase C activation. Am J Physiol 1994; 266:H1145-H1152.
90. Gross GJ, Auchampach JA. Blockade of ATP-sensitive potassium channels prevents myocardial preconditioning in dogs. Circ Res 1992; 70:223-233.
91. Steenbergen C, Perlman ME, London RE et al. Mechanisms of preconditioning: Ionic alterations. Circ Res 1993; 72:112-125.
92. Ramasamy R, Liu H, Anderson S et al. Ischemic preconditioning stimulates sodium and proton transport in isolated rat hearts. J Clin Invest 1995; 96:1464-1472.
93. Eckert R, Utz J, Trautwein W et al. Involvement of intracellular Ca^{2+} release mechanism in adenosine-induced cardiac Ca^{2+} current inhibition. Surgery 1993; 114:334-342.
94. Schrader J, Rubio R, Berne RM. Inhibition of slow action potentials of guinea pig atrial muscle by adenosine: A possible effect on Ca^{2+} influx. J Mol Cell Cardiol 1975; 7: 427-433.
95. Armstrong S, Downey JM, Ganote CE. Preconditioning of isolated rabbit cardiomyocytes: Induction by metabolic stress and blockade by the adenosine antagonist SPT and calphostin C, a protein kinase C inhibitor. Cardiovasc Res 1994; 28:72-77.
96. Woolfson RG, Patel VC, Neild GH et al. Inhibition of nitric oxide synthesis reduces infarct size by an adenosine-dependent mechanism. Circulation 1995; 91:1545-1551.
97. Tsuchida A, Miura T, Miki T et al. Role of adenosine receptor activation in myocardial infarct size limitation by ischemic preconditioning. Cardiovasc Res 1992; 26:456-461.
98. Kirsch GE, Codina J, Birnbaumer L et al. Coupling of ATP-sensitive K+ channels to A1 receptors by G proteins in rat ventricular myocytes. Am J Physiol 1990; 259:H820-H826.
99. Cohen MV, Downey JM. Myocardial preconditioning promises to be a novel approach to the treatment of ischemic heart disease. Ann Rev Medicine 1996; 47:21-29.
100. Cohen M, Liu Y, Liu G et al. Phospholipase D plays arole in ischemic preconditioning in rabbit heart. Circulation 1996; 94:1713-1718.
101. Dobson JGJ. Mechanism of adenosine inhibition of catecholamine-induced responses in heart. Circ Res 1983; 52:151-160.
102. Richardt G, Waas W, Kranzhofer R et al. Adenosine inhibits exocytotic release of endogenous noradrenalin in rat heart: A protective mechanism in early myocardial ischemia. Circ Res 1987; 61:117-123.
103. Sack S, Mohri M, Arras M et al. Ischemic preconditioning—time course of renewal in the pig. Cardiovasc Res 1993; 27:551-555.

104. Hendrikx M, Toshima Y, Mubagwa K et al. Improved functional recovery after ischemic preconditioning in the globally ischemic rabbit heart is not mediated by adenosine A1 receptor activation. Bas Res Cardiol 1993; 88:576-593.
105. Hale SL, Bellows SD, Hammerman H et al. An adenosine A1 receptor agonist, R(-)-N-(2-phenylisopropyl)-adenosine (PIA), but not adenosine itself, acts as a therapeutic preconditioning-mimetic agent in rabbits. Cardiovasc Res 1993; 27:2140-2150.
106. Cave CC, Collis CS, Downey JM et al. Improved functional recovery by ischemic preconditioning is not mediated by adenosine in the globally ischemic isolated rat heart. Cardiovasc Res 1993; 27:663-668.
107. Li Y, Kloner RA. Adenosine deaminase inhibition is not cardioprotective in the rat. Am Heart J 1993; 126:1293-1298.
108. Li Y, Kloner RA. The cardioprotective efforts of ischemic preconditioning are not mediated by adenosine receptors in rat hearts. Circulation 1993; 87:1642-1648.
109. Fedida D, Braun AP, Giles WR. Alpha 1-adrenoceptors in myocardium: Functional aspects and transmembranes signaling mechanisms. Physiol Rev 1993; 73:469-487.
110. Terzic A, Puceat M, Vassort G et al. Cardiac alpha 1-adrenoceptors: An overview. Pharmacol Rev 1993; 45:147-175.
111. Nishizuka Y. Intracellular signaling by hydrolysis of phospholipids and activation of protein kinase C. Science 1992; 1992: 607-614.
112. Hu K, Duan D, Li GR et al. Protein kinase C activates ATP-sensitive K^+ current in human and rabbit ventricular myocytes. Circ Res 1996; 78:492-498.
113. Johnson JA, Mochly-Rosen D. Inhibition of the spontaneous rate of contraction of neonatal cardiac myocytes by protein kinase C isozymes. A putative role for the epsilon isozyme. Circ Res 1995; 76:654-663.
114. Schaper W. Ischemic preconditioning, remembrances of things past and future. Bas Res Cardiol 1996; 91:8-11.
115. Strasser RH, Braun-Dullaeus R, Walendzik H et al. Alpha 1-receptor-independent activation of protein kinase C in acute myocardial ischemia. Mechanisms for sensitization of the adenylyl cyclase. Circ Res 1992; 70:1304-1312.
116. Li Y, Kloner RA. Cardioprotective effects of ischemic preconditioning can be recaptured after they are lost. J Am Coll Cardiol 1994; 23:470-474.
117. Speechley-Dick ME, Mocanu MM, Yellon DM. Protein kinase C. Its role in ischemic preconditioning in the rat. Circ Res 1994; 75:586-590.
118. Vogt AM, Barancik M, Weihrand D et al. Protein kinase C inhibitors reduce infarct size in pig hearts in vivo. Circulation 1994; 90:I647.
119. Auchampach JA, Gross GJ. Adenosine A1 receptors, KATP channels, and ischemic preconditioning in dogs. Am J Physiol 1993; 264:H1327-H1336.
120. Noma A. ATP-regulated K^+ channels in cardiac muscle. Nature 1983; 305:147-148.
121. Auchampach JA, Maruyama M, Cavero I et al. The new K^+ channel opener Aprikalim (RP 52891) reduces experimental infarct size in dogs in the absence of hemodynamic changes. J Pharmacol Exp Ther 1991; 259:961-967.
122. Auchampach JA, Grover GJ, Gross GJ. Blockade of ischemic preconditioning in dogs by the novel ATP dependent potassium channel antagonist sodium 5-hydroxydecanoate. Cardiovasc Res 1992; 26:1054-1062.
123. Thornton JD, Daly JF, Cohen MV et al. Catecholamines can induce adenosine receptor-mediated protection of the myocardium but do not participate in ischemic preconditioning in the rabbit. Circ Res 1993; 73: 649-655.
124. Kitzen J, McCallum J, Harvey C et al. Potassium channel activators cromakalim and celikalim (WAY-120-491) fail to decrease myocardial infarct size in the anesthetized canine. Pharmacology 1992; 45:71-82.
125. Li YJ, Xiao ZS, Peng CF et al. Calcitonin gene-related peptide-induced preconditioning protects against ischemia-reperfusion injury in isolated rat hearts. Eur J Pharmacol 1996; 311:163-167.
126. Young LH, Renfu Y, Russell R et al. Low-flow ischemia leads to translocation of canine heart GLUT-4 and GLUT-1 glucose transporters to the sarcolemma in vivo. Circulation 1997; 95:415-422.
127. Ogino H, Smolenski RT, Zych M et al. Influence of preconditioning on rat heart subjected to prolonged cardioplegic arrest. Ann Thorac Surg 1996; 62:469-474.
128. Karck M, Rahmanian P, Haverich A. Ischemic preconditioning enhances donor heart preservation. Transplantation 1996; 62:17-22.
129. Menasche P, Kevelaitis E, Mouas C et al. Preconditioning with potassium channel openers. A new concept for enhancing cardioplegic protection? J Thor Cardiovasc Surg 1995; 110:1606-1613.
130. Galinanes M, Argano V, Hearse DJ. Can ischemic preconditioning ensure optimal myocardial protection when delivery of cardioplegia is impaired? Circulation 1995; 92 (Suppl): II389-II394.

131. Bukhari EA, Krukenkamp IB, Burns PG et al. Does aprotinin increase the myocardial damage in the setting of ischemia and preconditioning? Ann Thorac Surg 1995; 60: 301-310.
132. Sodi-Pallares D, Bisteni A, Medrano G et al. The polarizing treatment of acute myocardial infarction. Dis Chest 1963; 43:424-432.
133. Gradinak S, Coleman GM, Taegtmeyer H et al. Improved cardiac function with glucose-insulin-potassium after coronary bypass surgery. Ann Thorac Surg 1989; 48:484-489.
134. Iwamoto T, Bai X-J, Downey HF. Preconditioning with supply-demand imbalance limits infarct size in dog heart. Cardiovasc Res 1993; 27:2071-2076.
135. Ovize M, Kloner RA, Przyklenk K. Stretch preconditions canine myocardium. Am J Physiol 1994; 266:H137-H146.
136. Shiki K, Hearse DJ. Preconditioning of ischemic myocardium: Reperfusion-induced arrhythmias. Am J Physiol 1987; 253:H1470-H1476.
137. Goto M, Tsuchida A, Liu Y et al. Transient inhibition of glucose uptake mimics ischemic preconditioning by salvaging ischemic myocardium in the rabbit heart. J Mol Cell Cardiol 1995; 27:1883-1894.
138. Simkhovich BZ, Hale SL, Ovize M et al. Ischemic preconditioning and long-chain acyl carnitine in the canine heart. Cor Art Disease 1993; 4:387-392.
139. Jennings RB, Murry CE, Reimer KA. Energy metabolism in preconditioned and control myocardium: Effect of total ischemia. J Mol Cell Cardiol 1991; 23:1449-1458.
140. Kobara M, Tatsumi T, Matoba S et al. Effect of ischemic preconditioning on mitochondrial oxidative phosphorylation and high energy phosphates in rat hearts. J Mol Cell Cardiol 1996; 28:417-428.
141. Sun JZ, Tang XL, Knowlton AA et al. Late preconditioning against myocardial stunning. An endogenous protective mechanism that confers resistance to postischemic dysfunction 24 h after brief ischemia in conscious pigs. J Clin Invest 1995; 95:388-403.
142. Jennings R, Murry C, Reimer K. Preconditioning myocardium with ischemia. Cardiovasc Drugs Ther 1991; 5:933-938.
143. King L, Opie L. Does preconditioning act by glycogen depletion in the isolated rat heart? J Mol Cell Cardiol 1996; 28:2305-2321.
144. Fralix TA, Steenbergen C, London RE et al. Metabolic substrates can alter postischemic recovery in preconditioned ischemic heart. Am J Physiol 1992; 263:C17-C23.
145. Zucchi R, Ronca-Testoni S, Yu G et al. Postischemic changes in cardiac sarcoplasmic reticulum Ca channels. A possible mechanism of ischemic preconditioning. Circ Res 1995; 76:1049-1056.
146. Fralix TA, Murphy E, London RE et al. Protective effects of adenosine in the perfused rat heart: Changes in metabolism and ion homeostasis. Am J Physiol 1993; 264:C986-C994.
147. Bugge E, Ytrehus K. Inhibition of sodium-hydrogen exchange reduces infarct size in the isolated rat heart—a protective additive to ischemic preconditioning. Cardiovasc Res 1995; 29:269-274.
148. Lasley R, Mentzer R. Pertussis toxin blocks adenosine A1 receptor mediated protection of the ischemic heart. J Mol Cell Cardiol 1993; 25:815-821.
149. Liu Y, Downey J. Preconditioning against infarction in the rat heart does not involve a pertussis toxin sensitive G protein. Cardiovasc Res 1993; 27:608-611.
150. Bugge E, Ytrehus K. Ischemic preconditioning is protein kinase C dependent but not through stimulation of alpha adrenergic or adenosine receptors in the isolated rat heart. Cardiovasc Res 1995; 29:401-406.
151. Weselcouch E, Baird A, Sleph P et al. Endogenous catecholamines are not necessary for ischemic preconditioning in the isolated perfused rat heart. Cardiovasc Res 1995; 29: 126-132.
152. Bugge E, Ytrehus K. Bradykinin protects against infarction but does not mediate ischemic preconditioning in the isolated rat heart. J Mol Cell Cardiol 1996; 28:2333-2341.
153. Vegh A, Szekeres L, Parrat J. Protective effects of preconditioning on the ischemic myocardium involve cyclo-oxygenase products. Cardiovasc Res 1990; 24:1020-1022.
154. Weselcouch EO, Baird AJ, Sleph P et al. Inhibition of nitric oxide synthesis does not affect ischemic preconditioning in isolated perfused rat hearts. Am J Physiol 1995; 268:H242-H249.
155. Wang P, Gallagher K, Downey J et al. Pretreatment with endothelin-1 mimics ischemic preconditioning against infarction in isolated rabbit heart. J Mol Cell Cardiol 1996; 28:577-588.
156. Hendrikx M, Toshima Y, Mubagwa K et al. Muscarinic receptor stimulation by carbachol improves functional recovery in isolated, blood perfused rabbit heart. Cardiovasc Res 1993; 27:980-989.
157. Schultz JE, Rose E, Yao Z et al. Evidence for involvement of opioid receptors in ischemic preconditioning in rat hearts. Am J Physiol 1995; 268:H2157-H2161.

158. Liu Y, Tsuchida A, Cohen MV et al. Pretreatment with angiotensin II activates protein kinase C and limits myocardial infarction in isolated rabbit hearts. J Mol Cell Cardiol 1995; 27:883-892.
159. Mizumura T, Nithipatikom K, Gross GJ. Bimakalim, an ATP-sensitive potassium channel opener, mimics the effects of ischemic preconditioning to reduce infarct size, adenosine release, and neutrophil function in dogs. Circulation 1995; 92:1236-1245.
160. Grover G, Dzwonczyk S, Sleph P et al. The ATP-sensitive potassium channel blocker glibenclamide (glyburide) does not abolish preconditioning in isolated ischemic rat hearts. J Pharmacol Exp Ther 1993; 265: 559-564.
161. Liu Y, Cohen MV, Downey JM. Chelerythrine, a highly selective protein kinase C inhibitor, blocks the anti-infarct effect of ischemic preconditioning in rabbit hearts. Cardiovasc Drugs Ther 1994; 8:881-882.
162. Kitakaze M, Node K, Minamino T et al. Role of activation of protein kinase C in the infarct size-limiting effect of ischemic preconditioning through activation of ecto-5'-nucleotidase. Circulation 1996; 93:781-791.

Chapter 11

Away from Ischemic Preconditioning and Towards Pharmacological Preconditioning

Louis P. Perrault and Philippe Menasché

Abstract

Endogenous myocardial protection refers to the natural defense mechanisms available to the heart to withstand an ischemic injury. So far these mechanisms have been shown to encompass two phenomena most likely interrelated: ischemic preconditioning and stress protein synthesis. Ischemic PC can be defined as the adaptive mechanism induced by a brief period of reversible ischemia increasing the heart's resistance to a subsequent longer period of ischemia. Two different time frames are defined for preconditioning, one early or classical preconditioning and one late also called the second window of preconditioning. The therapeutic exploitation of these natural adaptive mechanisms in cardiac surgery is an appealing prospect. Preconditioning could be used before aortic cross-clamping to enhance the current methods of myocardial protection. Two major conclusions emerge from the bulk of experimental data on preconditioning. First, the adaptive phenomenon reduces infarct size after regional ischemia in animal preparations across a wide variety of species but its effects on arrhythmias and on preservation of function after global ischemia are less consistent. This is relevant to cardiac surgery where postbypass pump failure is more often due to stunning than to discrete necrosis. Second, regardless of the various components of the intracellular signaling pathway elicited by the preconditioning stimulus, it seems that a major mechanism by which this pathway leads to a cardioprotective effect is a slowing of ATP depletion during the protracted period of ischemia. If the latter is true, then it can reasonably be predicted that this energy-sparing effect may become redundant to that of cardioplegia. These problems emphasize the importance of identifying the mechanisms underlying endogenous myocardial protection in an attempt to pharmacologically duplicate the protective action of ischemically induced preconditioning. Numerous triggers have been identified as being able to elicit and reproduce the effects of ischemic preconditioning. The role of ATP-sensitive potassium channels as mediators of the cardioprotective effects of preconditioning has been largely based on the observations that these effects could be duplicated by potassium channel openers (PCO) whereas they were abolished by potassium channel blockers. PCO given before potassium arrest have the ability to enhance functional recovery during reperfusion and may be independent of the temperature of the cardioplegia administered. Currently the application of these drugs is hampered by the fact that the anti-ischemic effects are obtained at much higher doses than those required for relaxation

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of smooth muscle and thus could cause important hemodynamic effects due to vasodilatation before the appearance of the cardioprotective effects. The modalities of pharmacologically-induced preconditioning, although intellectually appealing, remain to be determined including the choice of the optimal agents and regimens. The only clinical situation in which the ischemic preconditioning remains logical would be "off-pump" minimally invasive cardiac surgery as the occlusion of the target vessel during construction of the distal anastomosis most closely mimics the experimental scenario that has turned out to result in reduction of regionally-induced ischemic damage.

Endogenous myocardial protection mechanisms are available to the heart to withstand an ischemic injury. So far clinical and basic research have shown that these mechanisms include two phenomena most likely interrelated: ischemic preconditioning and stress protein synthesis. Ischemic preconditioning is the adaptive mechanism induced by a brief period of reversible ischemia increasing the heart's resistance to a subsequent longer period of ischemia. Two different time frames are defined for preconditioning, one early (or "classical" preconditioning) which involves activation of various membrane receptors and one late (termed the second window of preconditioning) which is related to changes in gene expression leading to the synthesis of cardioprotective stress proteins.[1,2] Exploitation of these natural adaptive mechanisms in cardiac surgery is an appealing prospect since preconditioning in one form or another could be used during aortic cross-clamping or local control of target vessels to optimize the efficacy of current methods of myocardial protection. However, major questions have to be answered before preconditioning can be used appropriately in patients undergoing heart surgery. Are these endogenous protection mechanisms relevant to surgically-induced myocardial ischemia? How can they be applied during open-heart procedures and which situations and patients are they best indicated for? Data concerning ischemic preconditioning in the early phase will first be addressed before discussing pharmacological mediators of endogenous myocardial protection.

Clinical Trials: An Apparent Discrepancy

Clinically, the first use of preconditioning (PC) has been reported by Alkulaifi and co-workers[2] using conservation of adenosine triphosphate (ATP) as the major end point. Twenty patients were randomised to either preconditioning by two 3-minute periods of cross-clamping separated by 2 minutes of reperfusion prior to an ischemic insult of 10 minutes ischemia and ventricular fibrillation done at a temperature of 37°C. The ten controls underwent 10 minutes of cross-clamping with fibrillation without preconditioning. The major finding of this study was that preconditioning slowed the rate of ATP depletion to such an extent that at the end of the 10 minute period, the preconditioned group had a significantly higher ATP content than controls. The authors should be credited for demonstrating the adaptive response of the human heart to an ischemic stress. However, a major limitation of this study was that the normothermic ventricular fibrillation technique studied is not commonly used in many centers (where it is usually employed with a moderate degree of hypothermia) and likely resulted in extensive myocardial injury which provided room for improvement from the ischemic stimulus used as preconditioning.

Thus to assess whether this type of cardioprotective intervention would be relevant to cardioplegic arrest, we[4] studied the effects of ischemic preconditioning (achieved with 3 minutes of aortic cross-clamping with 2 minutes of reperfusion before the onset of cardioplegic arrest under cardiopulmonary bypass support) before continuous retrograde normothermic blood cardioplegia in twenty patients undergoing coronary artery bypass operations. Ten case-matched patients serving as controls, underwent a 5-minute period of bypass before the induction of cardioplegic arrest. At the end of arrest, the release of CK-MB from the myocardium (calculated as the difference between coronary sinus and

radial artery values) was markedly greater than in controls (5.7 ± 1.7 ng/ml vs. 1.9 ± 1.1 ng/ml, p=0.05). The transmyocardial lactate gradient was shifted towards production in the PC group (+0.22 ± 0.13 μmol/L) and towards extraction in the control group (-0.06 ± 0.21 μmol/L). The lack of additional protection conferred by ischemic preconditioning was further confirmed by the absence of difference in postarrest myocardial levels of ribonucleic acid messengers coding for the cardioprotective heat shock proteins (HSP) 70 between the two groups. Although, there were no PC-related adverse clinical events, this trend towards increased initial ischemic insult cannot be dismissed, suggesting that a brief prearrest period of aortic occlusion largely offsets, under the conditions studied, its putative cardioprotective action. Since then, similar results showing a detrimental effect of ischemic preconditioning before normothermic aerobic arrest have been shown by Kaukoranta and associates[5] with preconditioned patients showing a greater release of creatine kinase MB isoenzymes and troponin T indicating a greater amount of tissue injury compared with nonpreconditioned controls.

The apparent discrepancy in the results obtained with either noncardioplegic and cardioplegic techniques may be explained by two basic observations. First, preconditioning reduces infarct size but has little effect on stunning and beneficial effects are only detectable in situations of unprotected ischemia. Indeed, only a few studies have concomitantly assessed function and infarct size to shed light on this observation.[6] Such studies have conclusively demonstrated that preconditioning improves postischemic myocardial function by a reduction of the size of the infarct and not by decreasing the amount of stunning of reversibly injured myocardium. Moreover, this conclusion applies both to regional ischemia models such as obtained through transient occlusion of a coronary artery as well as to global ischemia induced by a period of aortic cross-clamping.[7,8] Indirect evidence of this also comes from Qiu's study performed in a conscious porcine model in which preconditioning significantly reduced the size of infarction secondary to a 40 minute period of occlusion but did not reduce either infarct size nor improve regional function when reproduced 24 hours later.[9] Similar results were obtained recently in our laboratory on an isolated buffer-perfused rabbit heart preparation. Sixty minutes of normothermic hyperkalemic arrest caused only minimal necrosis (< 10% of the left ventricle) as assessed by triphenyltetrazolium staining after 60 minutes of reperfusion. Ischemic preconditioning with 5 minutes of zero-flow ischemia followed by 5 minutes of reperfusion before cardioplegic arrest failed to enhance recovery of myocardial function or coronary flow compared with nonpreconditioned hearts. Conversely, a similar preconditioning stimulus improved functional recovery after 45 minutes of unprotected global ischemia[11] but infarct size was not measured. We duplicated this protocol and showed that 45 minutes of unprotected global ischemia caused a massive infarction of the left ventricle thereby lending room for preconditioning to reduce the extent of necrosis and hence functional recovery. These data suggest that the pertinence of preconditioning in routine cardiac surgery may be limited since postbypass failure is more often secondary to global reversible dysfunction than to discrete necrosis.[12]

An important observation is that most experimental studies having documented the salutary effects of preconditioning have done so in models of unprotected ischemia while a number of studies have shown that the benefits of preconditioning are lost when some protective strategies are added such as hypothermia or cardioplegic arrest.[13] One such study is that of Cleveland et al[14] which showed that preconditioning improved mechanical recovery of human right atrial trabeculae exposed to hypoxia at normothermia but was lost under conditions of hypothermia. This can be explained by the fact that regardless of the precise mechanisms by which preconditioning exerts its protective effect, slowing of the decay of high energy phosphates and glycogen depletion, which leads to slower lactate accumulation, during the early phase of sustained ischemia, appears to be important

phenomena.[15,16] It is therefore plausible that these energy sparing effects and prevention of intracellular acidosis may become redundant to that of cardioplegia. This hypothesis is confirmed by the observation, made in isolated rat hearts subjected to 35 minutes of global ischemia, that both ischemic preconditioning and cardioplegia independently improved functional recovery compared to control hearts but these effects were not additive.[17] Moreover, combination of preconditioning with magnesium based cardioplegia fails to improve results over that obtained with cardioplegia alone.[18] For these reasons, it is conceivable that preconditioning may enhance the effects of normothermic ventricular fibrillation used by Alkhulaifi[3] which is expected to cause a major decline in myocardial ATP levels and intracellular pH. In confirmation of this, such preservation of ATP was lost in a study by the same investigators when preconditioning was used before induction of ventricular fibrillation at lower temperatures.[19] In the same line of reasoning, the satisfactory outcomes reported in patients undergoing coronary artery bypass surgery under intermittent aortic cross-clamping and ventricular fibrillation may be explained by the fact that cumulative myocardial injury by successive no-flow intervals could be limited by the initial period of aortic occlusion acting as a preconditioning stimulus.[20]

Possible Clinical Indications of Preconditioning in Cardiac Surgery

From the above data, it results that preconditioning may be indicated electively in certain situations when the potential exists for suboptimal myocardial protection and consequently and increased risk of myocardial infarction and it could be anticipated that the extent of the infarct will be reduced by preconditioning and serve to preserve function. This scenario has been investigated in experimental studies in which preconditioning conferred additional protection to that of hypothermia and cardioplegia when ischemic times were prolonged[21-23] and when distribution of cardioplegic solutions were suboptimal due to proximal occlusions of coronary arteries.[24] It is likely that in these situations, preconditioning reduces the amount of necrosis, as shown by lower postischemic creatine kinase release, resulting from suboptimal protection. Thus in clinical practice, high risk situations that could benefit from preconditioning may include 1) extensive coronary disease with poor collaterals that increases the risk of cardioplegia maldistribution even with the use of combined antegrade/retrograde perfusion, 2) severe left ventricular hypertrophy, 3) anticipated long ischemic times including those of cardiac allografts are exposed to during cold storage, 4) the senescent myocardium, more prone to developing calcium overload injury,[25] although the capacity of the aged heart for becoming preconditioned remains unclear.[26,27] One final situation in which preconditioning may find a niche is, 5) beating heart minimally invasive coronary artery bypass operations where the necessary control and occlusion of target vessels sometimes results in distal ischemia, not unlike the experimental models of regional ischemia in which the infarct-limiting effect of preconditioning has been best demonstrated. Potential benefits notwithstanding, the clinical use of a potentially deleterious ischemic stimulus is unappealing hence the importance of identifying the mediators of this endogenous adaptive phenomenon to best exploit it therapeutically.

Pharmacological Preconditioning

According to the commonly accepted scheme, the preconditioning ischemia activates various membrane receptors, including those for adenosine, catecholamines, acetylcholine, bradykinin and opioids. The respective role of these receptors may vary among species as exemplified by the adenosine receptor which mediates preconditioning in rabbit, dog, swine and human but not rats and the precise level of activation of preconditioning triggers required to reach the effective threshold for cardioprotection may also vary.[28] Stimulated receptors then initiate an intracellular signaling pathway leading to activation of protein kinase

C (PKC) although its predominant role remains questionable considering other kinases such as mitogen-activated protein kinases (MAP kinases) may also be involved.[29] Evidence supports that activation of PKC leads to its translocation from the cytosol to the membrane where it phosphorylates substrate proteins that ultimately increase the resistance to ischemia. The end-effectors of the preconditioning pathway are not complementaly characterized but the most likely candidate is the ATP-dependent potassium channel, supported by evidence in rabbits that PKC can activate potassium channels at physiological levels of ATP.[30] It is generally accepted that opening of the potassium channels shortens the duration of the action potential and reduces calcium influx and the attendant tissue damage. This hypothesis has been challenged recently by data showing that the potassium channel opener bimakalim induces cardioprotection without modifying the duration of the action potential,[31] raising the possibility of alternate protective mechanisms either at the intracellular level (in mitochondrial membranes) and/or extracellularly by preventing adhesion of neutrophils.[32] Another way for preconditioning to reduce acidosis during ischemia could be through decreasing the sodium/proton exchange[18] leading to a lesser rise in intracellular sodium and secondarily a reduced rise in intracellular calcium through preservation of sarcolemmal sodium/potassium ATPase.[33] Of great interest is that all end-effectors under consideration share the common property of reducing intracellular accumulation of calcium and ultimately enhancing tissue viability. This framework is convenient for classifying interventions targeted at pharmacological induction of preconditioning in the myocardium.

Interventions Targeted at the Triggers

The most widely used trigger has been adenosine as stimulation of adenosine A1 receptors have been successful in duplicating the cardioprotection conferred by ischemic preconditioning except in rats. Data in human myocardium is more dismal and based on isolated in vitro preparations of human atrial trabeculae or cultured ventricular myocytes. Recently, clinically relevant data has been reported by Lee et al[34] which showed that infusion of adenosine prior to cardiopulmonary bypass improved postoperative ventricular function in patients under CABG. However a period of drug-free intervening reperfusion, necessary for qualifying as preconditioning rather than pretreatment was not used, and could have falsely improved the extent of recovery.[35] A more convincing study published by Leesar and co-workers[36] showed that the intracoronary infusion of adenosine 10 minutes before percutaneous transluminal coronary angioplasty rendered the myocardium significantly more resistant to subsequent ischemia induced by successive balloon inflations. In the surgical context, however, the use of adenosine can be fraught with several problems including down-regulation of the receptors with uncoupling of the intracellular signaling pathway as well as systemic vasodilatation and subsequent hypotension. It remains to be determined whether these potential side effects will be eliminated by the more selective A1 agonists currently under development.

Activation of α1-adrenergic receptors[37] for induction of preconditioning is another interesting possibility as agonists of such receptors are available for human use such as phenylephrine. The clinical applicability of this approach remains unproven which holds true for stimulation of bradykinin receptors which may mediate the antiarrythmic effects of preconditioning through a pathway related to nitric oxide.[38]

Interventions Targeted at the Mediators

A number of PKC agonists have been studied and shown to reproduce the cardioprotective effect of ischemic preconditioning but they remain solely mechanistic tools because of their tumor-promoting effects. Recent evidence seems to suggest that calcium chloride could effectively precondition the

heart through a PKC-dependent mechanism and could perhaps become a useful drug if the benefits and safety of an increase in inotropism before going on bypass is established.

Interventions Targeted at the End-Effectors

Since potassium channel openers duplicate the cardioprotective effects induced by preconditioning, the ATP-dependent potassium channel is currently considered one of the major effectors of the signaling pathway.[40] This has been shown to be true in numerous species as well as in man as demonstrated by protection of human right atrial trabeculae against hypoxic injury by preconditioning induced by hypoxia or cromakalim[41] and by the absence of such a protective effect in trabeculae from diabetic patients on potassium-blocking hypoglycemic drugs.[42] Several reports have also documented the capacity of potassium channel openers to enhance functional recovery at reperfusion when given before standard depolarized arrest[43-45] but again some of these agents were given as pretreatment rather than true preconditioning. We have shown in an isolated model rat heart model that the protective effects of ischemic preconditioning (achieved by 5 minutes of zero-flow ischemia followed by 5 minutes of reperfusion before arrest) on post cardioplegia systolic and diastolic function after 45-minute normothermic potassium arrest could be duplicated by nicorandil (10 mM/L) given as a true preconditioning regimen.[46] In addition, both forms of preconditioning similarly lengthened the time before peak contracture and its magnitude. Glibenclamide, a potassium channel blocker, completely abolished the cardioprotective effects of nicorandil preconditioning confirming its action through modulation of potassium channels, whereas it only partially blunted those of ischemic preconditioning suggesting that other end-effectors may be involved, at least in the rat heart. Similar results were obtained with the more commonly used conditions of hypothermic cardioplegic arrest.[47] The mechanism by which myocardial cells retain the memory of the transient exposure to potassium channel openers before arrest is unclear but may include a lowering of the threshold for potassium channels opening favoring a greater activation during the subsequent period of global ischemia.[48] Unfortunately, the clinical usefulness of these drugs in clinical cardiac surgery is currently hampered by their sole enteral route of administration. Furthermore, antiischemic effects related to potassium channel opening can only be obtained at doses also causing smooth muscle cell relaxation because of their poor cardiac selectivity and may cause important hemodynamic changes before the occurrence of cardioprotective effects.[49]

Confounding Factors in Cardiac Surgery

During cardiac operations, numerous pre and intraoperative confounding factors may skew the preconditioning effect and should be controlled for in both experimental and clinical studies. These include, in particular, the use of opioids agonists, aprotinin and cardiopulmonary bypass itself which may all have preconditioning effects.

In the rat heart, opioid receptor stimulation by morphine results in a reduction in infarct size similar to that produced by ischemic preconditioning and is blocked by glibenclamide suggesting its mediation by potassium channel opening.[50] We have specifically identified the δ-opioid receptor as a target for pharmacological interventions duplicating the effects of preconditioning in a rabbit model of prolonged cold storage (unpublished observation).

Preconditioning has been shown to decrease the increase in infarct size caused by aprotinin in a sheep model of regional ischemia.[51] Should these observations be confirmed in the clinical setting, preconditioning could be useful in patients requiring aprotinin therapy.

Finally, the same group of investigators has also shown that cardiopulmonary bypass itself could act as a preconditioning stimulus through the stimulation of release of membrane activating mediators like adenosine and

catecholamines.[52] This hypothesis is based on the observation that a period of cardiopulmonary bypass could duplicate the reduction in infarct size achieved by short bursts of preconditioning and was abolished by adenosine and α-1 adrenoreceptors antagonists. If this hypothesis is correct, it would imply that, in any clinical study of preconditioning, "control" patients might be already preconditioned which could lessen the likelihood of demonstrating an added benefit from any superimposed intraoperative preconditioning intervention.

In conclusion, preconditioning is an extremely effective mechanism for limiting ischemia-induced cell necrosis and, consequently, preserving myocardial function. As no current methods of cardioplegia is perfect, additional protection could be obtained in selected cases through the use of preconditioning. This requires, however, the characterization of the endogenous mediators of this phenomenon to develop new agents or exploit current drugs to duplicate the cardioprotective effects of preconditioning. The clinical evaluation of these agents may be difficult considering the numerous confounding factors. Nevertheless, this should not deter us from applying continued research efforts for understanding and exploiting therapeutically this endogenous adaptive defense system which has proven so far to be one of the most effective infarct-limiting strategies available.

References

1. Parratt JR. Protection of the heart by ischaemic preconditioning: Mechanisms and possibilities for pharmacological exploitation. TiPS 1994; 15:19-25.
2. Marber MS, Heads RJ, Yellon DM. Stress proteins, heat stress and myocardial protection. In: Przyklenk K, Kloner RA, Yellon DM eds. Ischemic preconditioning: The concept of endogenous cardioprotection. Boston: Kluwer Academic Publishers 1994:105-23.
3. Alkhulaifi AM, Yellon DM, Pugsley WB. Preconditioning the human heart during aorto-coronary bypass surgery. Eur J Cardio-Thorac Surg 1994; 8:270-276.
4. Perrault LP, Menasché P, Bel A et al.Ischemic preconditioning in cardiac surgery: A word of caution. J Thorac Cardiovasc Surg 1996; 112:1378-86.
5. Kaukoranta P, Lepojärvi MPK, Ylitalo KV et al. Normothermic retrograde blood cardioplegia with or without preceding ischemic preconditioning. Ann Thorac Surg 1997; 63: 1268-74.
6. Bolling SF, Olszanski DA, Childs KF et al. Stunning, preconditioning, and functional recovery after global myocardial ischemia. Ann Thorac Surg 1994; 58:822-7.
7. Ovize M, Kloner RA, Przyklenk K. Preconditioning and myocardial contractile function. In: Przyklenk K, Kloner RA, Yellon DM. eds. Ischemic preconditioning: The concept of endogenous cardioprotection. Boston: Kluwer Academic Publishers, 1994: 41-60.
8. Iles RW, Wright JK, Inners-McBride K et al. Ischemic preconditioning improves preservation with crystalloid cardioplegia. Ann Thorac Surg 1994; 58:1481-5.
9. Qiu Y, Tang XL, Park SW et al. The early and late phases of ischemic preconditioning. A comparative analysis of their effects on infarct size, myocardial stunning, and arrhythmias in conscious pigs undergoing a 40-minute coronary occlusion. Circ Res 1997; 80:730-42.
10. Faris B, Peynet J, Wassef M et al. Failure of preconditioning to improve postcardioplegia stunning of minimally infarcted hearts. Ann Thorac Surg (accepted for publication).
11. Hendricx M, Toshima Y, Mubagwa K et al. Improved functional recovery after ischemic preconditioning in the globally ischemic rabbit heart is not mediated by adenosine A_1 receptor activation. Bas Res Cardiol 1993; 88:576-93.
12. Kloner RA, Przyklenk K, Kay GL. Clinical evidence for stunned myocardium after coronary artery bypass surgery. J Card Surg 1994; 9[Suppl]:397-402.
13. Valen G, Takeshima S, Vaage J. Preconditioning improves cardiac function after global ischemia, but not after cold cardioplegia. Ann Thorac Surg 1996; 62:1397-403.
14. Cleveland JC Jr., Meldrum DR, Rowland RT et al. Preconditioning and hypothermic cardioplegia protect human heart equally against ischemia. Ann Thorac Surg 1997; 63:147-52.
15. Kida M, Fujiwara H, Ishida M et al. Ischemic preconditioning preserves creatine phosphate and intracellular pH. Circulation 1991; 84:2495-503.
16. Murry CE, Richard VJ, Reimer KA et al. Ischemic preconditioning slows energy metabolism and delays ultrastructural damage during a sustained ischemic episode. Circ Res 1990; 66:913-31.
17. Kolocassides KG, Galinanes M, Hearse DJ. Ischemic preconditioning, cardioplegia or both? J Mol Cell Cardiol 1994; 26:1411-4.

18. Steenbergen C, Perlman ME, London RE et al. Mechanism of preconditioning; ionic alterations. Circ Res 1993; 72:112-25.
19. Di Salvo C, Hemming A, Jenkins D et al. Can the human myocardium be preconditioned with ischaemia under hypothermic conditions? Proceedings of the 9th annual meeting of the European Association for Cardio-thoracic Surgery, Paris, 25-27 September 1995; 324 (abstract).
20. Abd-Elfattah AS, Ding M, Wechsler AS. Intermittent aortic cross-clamping prevents cumulative adenosine triphosphate depletion, ventricular fibrillation, and dysfunction (stunning): Is it preconditioning? J Thorac Cardiovasc Surg 1995; 110:328-39.
21. Cave AC, Hearse DJ. Ischaemic preconditioning and contractile function: Studies with normothermic and hypothermic global ischaemia. J Mol Cell Cardiol 1992; 24: 1113-23.
22. Ogino H, Smolenski RT, Zych M et al. Influence of preconditioning on rat heart subjected to prolonged cardioplegic arrest. Ann Thorac Surg 1996; 62:469-74.
23. Karck M, Rahmanian P, Haverich A. Ischemic preconditioning enhances donor heart preservation. Transplantation 1996; 62:17-22.
24. Galinanes M, Argano V, Hearse DJ. Can ischemic preconditioning ensure optimal myocardial protection when delivery of cardioplegia is impaired? Circulation 1995; 92 (suppl II):II-389-94.
25. Tsukube T, McCully JD, Federman M et al. Developmental differences in cytosolic calcium accumulation associated with surgically induced global ischemia: Optimization of cardioplegic protection and mechanism of action. J Thorac Cardiovasc Surg 1996; 112:175-84.
26. Burns PG, Krukenkamp I, Caldarone CA et al. Is the preconditioning response conserved in senescent myocardium? Ann Thorac Surg 1996; 61:925-9.
27. Tani M, Suganuma Y, Hasegawa H et al. Changes in ischemic tolerance and effects of ischemic preconditioning in middle-aged rat hearts. Circulation 1997; 95:2559-66.
28. Goto M, Liu Y, Yang XM et al. Role of bradykinin in protection of ischemic preconditioning in rabbit hearts. Circ Res 1995; 77:611-21.
29. Brooks G, Hearse DJ. Role of protein kinase C in ischemic preconditioning: Player or spectator? Circ Res 1997; 79:627-30.
30. Light P, Sabir AA, Allen BG et al. Protein kinase C-induced changes in the stochiometry of ATP binding activate cardiac ATP-sensitive K^+ channels. Circ Res 1996; 79:399-406.
31. Yao Z, Gross GJ. Effect of the K_{ATP} channel opener bimakalim on coronary blood flow, monophasic action potential duration, and infarct size in dogs. Circulation 1994; 89: 1769-75.
32. Akimitsu T, Gute DC, Korthuis RJ. Ischemic preconditioning attenuates postischemic leukocyte adhesion and emigration: Role of adenosine and ATP-sensitive potassium channels. Circulation 1994; 90:(suppl I):I-476 (abstract).
33. Nawada R, Murakami T, Iwase T et al. Inhibition of sarcolemmal Na^+, K^+-ATPase activity reduces the infarct size-limiting effect of preconditioning in rabbit hearts. Circulation 1997; 96:599-604.
34. Lee HT, LaFaro RJ, Reed GE. Preconditioning of human myocardium with adenosine during open-heart surgery. J Card Surg 1995; 10:665-76.
35. Lasley RD, Noble MA, Konyn PJ et al. Different effects of an adenosine A_1 analogue and ischemic preconditioning in isolated rabbit hearts. Ann Thorac Surg 1995; 60:1698-703.
36. Leesar MA, Stoddard M, Ahmed M et al. Preconditioning of human myocardium with adenosine during coronary angioplasty . Circulation 1997; 95:2500-7.
37. Banerjee A, Winter-Locke C, Rogers KB et al. Preconditioning against myocardial dysfunction after ischemia and reperfusion by an a_1-adrenergic mechanism. Circ Res 1993; 73:656-70.
38. Vegh A, Szekeres L, Parratt JR. Protective effects of preconditioning on the ischaemic myocardium involve cyclo-oxygenase products. Cardiovasc Res 1990; 24:1020-3.
39. Meldrum DR, Cleveland JC Jr., Sheridan BC et al. Cardiac preconditioning with calcium: Clinically accessible myocardial protection. J Thorac Cardiovasc Surg 1996; 112:778-86.
40. Gross GJ, Auchampach JA. Blockade of ATP-sensitive potassium channels prevents myocardial preconditioning in dogs. Circ Res 1992; 70:223-33.
41. Speechly-Dick ME, Grover GJ, Yellon DM. Does ischemic preconditioning in the human involve protein kinase C and the ATP-dependent K^+ channel? Studies of contractile function after simulated ischemia in an atrial in vitro model. Circ Res 1995; 77:1030-5.
42. Cleveland JC, Meldrum DR, Cain BS et al. Oral sulfonylurea hypoglycemic agents prevent ischemic preconditioning in human myocardium. Two paradoxes revisited. Circulation 1997; 96:29-32.
43. Grover GJ, Sleph PG. Protective effect of K_{ATP} openers in ischemic rat heart treated with a potassium cardioplegic solution. J Cardiovasc Pharmacol 1995; 26:698-706.

44. Pignac J, Bourgouin J, Dumont L. Cold cardioplegia and the K^+ channel modulator aprikalim (RP 52891): Improved cardioprotection in isolated ischemic rabbit hearts. Can J Physiol 1994; 72:126-32.
45. Sugimoto S, Puddu PE, Monti F et al. Pretreatment with the adenosine triphosphate-sensitive potassium channel opener nicorandil and improved myocardial protection during high-potassium cardioplegic hypoxia. J Thorac Cardiovasc 1994; 108:455-66.
46. Menasché P, Kevelaïtis E, Mouas C et al. Preconditioning with potassium channel openers: A new concept for enhancing cardioplegic protection? J Thorac Cardiovasc Surg 1995; 110:1606-14.
47. Menasché P, Mouas C, Grousset C. Is potassium channel opening an effective form of preconditioning before cardioplegia? Ann Thorac Surg 1996; 61:1764-8.
48. Shigematsu S, Sato T, Abe T et al. Pharmacological evidence for the persistent activation of ATP-sensitive K^+ channels in early phase of reperfusion and its protective role against myocardial stunning. Circulation 1995; 92:2266-75.
49. Grover GJ. Protective effects of ATP sensitive potassium channel openers in models of myocardial ischaemia. Cardiovasc Res 1994; 28:778-82.
50. Schultz JE, Hsu AK, Gross GJ. Morphine mimics the cardioprotective effect of ischemic preconditioning via a glibenclamide-sensitive mechanism in the rat heart. Circ Res 1996; 78:1100-4.
51. Bukhari EA, Krukenkamp I, Burns PG et al. Does aprotinin increase the myocardial damage in the setting of ischemia and preconditioning ? Ann Thorac Surg 1995; 60:307-10.
52. Burns PG, Krukenkamp IB, Caldarone CA et al. Does cardiopulmonary bypass alone elicit myoprotective preconditioning? Circulation 1995; 92(supplII):II-447-51.

Section III:
Intravenous Metabolic Support

Chapter 12

Intravenous Metabolic Support with GIK (Glucose-Insulin-Potassium) and Amino Acids in Cardiac Surgery

Rolf Svedjeholm

Myocardial preservation in cardiac surgery has evolved rapidly during the last decades. Some authors of this book have made major contributions to this development. A variety of methods to protect the heart are available and complex procedures can be safely performed. Inappropriate protection of the heart during aortic cross-clamping is now an uncommon cause of *major* myocardial damage. A recent survey at our institution showed that PMI (perioperative myocardial infarction) in association with valvular surgery was rare.[1] When PMI occurred in these cases, the myocardial damage was usually caused by evident technical problems. Thus, PMI in our hands was a significant problem in association with surgery for ischemic heart disease (despite shorter cross-clamp times). Furthermore, in this setting PMI was mainly associated with unstable angina (preoperative ischemia) or poor conditions for revascularization. The role of preoperative ischemia as a risk factor for PMI has been documented by Slogoff and Keats in a classic paper.[2] Moreover, postoperative ischemia after coronary artery bypass surgery is common and may also play a role for the development of PMI.[3] Postischemic myocardial metabolic derangement that is further aggravated by the systemic neuroendocrine stress response to operative trauma may, together with reperfusion injury, cause cardiac failure.[4] In light of this, we believe that additional measures to improve outcome and reduce permanent myocardial damage in cardiac surgery should focus on the preoperative and the postoperative phase of CABG surgery. Furthermore, efforts should be instituted to reduce reperfusion injury and minimize permanent myocardial damage in long standing or severe myocardial ischemia.

The basic principles to address myocardial ischemia imply improving the ratio between oxygen delivery and myocardial oxygen demand. If, however, ischemia is severe enough to cause cardiac failure, or if it is followed by cardiac failure after revascularization due to stunning or myocardial damage, the treatment traditionally employed (inotropic drugs) is less than ideal for the ischemic or postischemic heart. Traditional treatment of cardiac failure with inotropic drugs improves the hemodynamic state, but at the price of a marked increase in myocardial expenditure.[5-8] In animal experiments adrenergic drugs aggravate ischemia and precipitate myocardial infarction.[9] Inotropes have also been shown to cause de-energization of the myocardium, and if instituted prematurely it may imply that the condition of the heart is aggravated by further metabolic derangement.[8] The detrimental effects of premature use of inotropic drugs for weaning from CPB have been documented in animals.[10] Therefore, alternative measures that

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can enhance myocardial recovery and function without putting further strain on the heart are desirable. It is with this perspective intravenous metabolic support will be addressed in the following presentation.

A brief review of myocardial metabolism in association with ischemia and cardiac surgery will be presented. After that, the current state of metabolic support with GIK (glucose-insulin-potassium) and amino acids will be surveyed. Finally, a preliminary report on our clinical experience with metabolic support will be given.

Myocardial Substrate Metabolism

The myocardial ATP content is limited and, therefore, the myocytes must continually resynthesize high-energy phosphates to maintain contractile function and cellular viability.[11] To cope with this the heart can use a variety of substrates for energy production.[12] The choice in a given situation is normally determined by the availability of substrates. In the fasting state arterial levels of FFA (free fatty acids) are elevated and FFA oxidation then dominates. After a carbohydrate meal a shift toward glucose oxidation occurs, and during vigorous exercise lactate may become the dominating fuel. In conditions of starvation or diabetic ketosis ketones may account for a significant amount of myocardial energy production. Cardiac function is not affected by the specific substrate utilization under normal circumstances.[12] However, in conditions where myocardial perfusion is, or has been inadequate, the preference of substrates may obtain functional significance.

Myocardial ischemia enhances the use of glucose (glycolysis) and the use of amino acid metabolites in the Krebs cycle.[12,17] Ischemic heart disease in humans is characteristically associated with an increased myocardial uptake of glutamate.[13,14,18] Glutamate facilitates glycolysis and provides an alternative anaerobic pathway for regeneration of high-energy phosphates by substrate level phosphorylation in the Krebs cycle.[17,19] These changes serve as adaptive measures to endure myocardial ischemia and to facilitate postischemic recovery. The role of these substrates is illustrated by the importance of the myocardial glutamate/aspartate pool and myocardial glycogen content for myocardial recovery after ischemia.[20,23] Furthermore, administration of glucose or glutamate enhances myocardial tolerance to ischemia, improves the metabolic and functional recovery of the postischemic heart and reduces the size of myocardial infarct in experimental coronary ligation.[4,6,24,28] In contrast to the beneficial effects of carbohydrates and amino acids, FFA metabolism may be harmful in association with myocardial ischemia. High plasma levels of FFA impede myocardial uptake of glucose.[12] Under normal conditions FFA metabolism requires 12% more oxygen per mole ATP produced, compared with glucose utilization.[12] In conditions associated with high levels of circulating catecholamines, such as trauma or treatment with adrenergic drugs, the oxygen-wasting effect of FFA metabolism is markedly aggravated.[29] Accumulation of intracellular FFA metabolites may exert toxic effects and high levels of circulating FFA depress myocardial function and increase the incidence of arrhythmias during ischemia.[30,33]

Oxidative metabolism which normally accounts for more than 90% of myocardial energy production[11] is impeded after severe ischemia in animal experiments.[34,36] Loss of Krebs cycle intermediates during ischemia has been proposed as an explanation to this phenomenon.[36,38] Another mechanism that may contribute is inactivation of pyruvate dehydrogenase enzyme during ischemia.[6,39] This postischemic mitochondrial dysfunction seems to occur also in humans after cardiac operations. Teoh et al demonstrated zero oxidation of FFA during early reperfusion utilizing crystalloid cardioplegia, and minimal oxidation after blood cardioplegic arrest.[40] Svensson et al could not demonstrate significant myocardial uptake of FFA or carbohydrates in the first hours after coronary surgery.[41] After cardiac operations with limited ischemic insult these metabolic abnormalities are minor.[42] However, when postischemic metabolic derangement occurs the conditions for recovery are unfa-

vorable because of the systemic neuroendocrine response elicited by cardiac surgery.[43] Krebs cycle intermediates are replenished primarily by amino acids and carbohydrate substrates.[37,44] As the systemic neuroendocrine stress response is associated with elevated FFA levels and a state of insulin resistance, the myocardial uptake of carbohydrate substrates is impeded.[45] Thus, the relative importance of amino acids for the recovery of myocardial oxidative metabolism is enhanced in this setting.[46] Accordingly, myocardial uptake of glutamate has been shown to precede the recovery of oxidative metabolism after CABG.[43] A relative shortage of glutamate in this setting is suggested by the high fractional extraction rate of glutamate from plasma (Fig. 12.1).[43,46]

Metabolic Intervention

The ideal condition for metabolic intervention is a state of functional disorder secondary to a correctable metabolic abnormality. This may be exemplified by coma secondary to severe hypoglycemia. In this condition the administration of a small amount of glucose will have dramatic effects. In conditions where there is no lack of glucose, the addition of glucose will not affect brain function. If we were to treat all comatose patients with glucose, the results would soon become discouraging. This may serve as a simple and illustrative example of the importance of delineating what we are treating. Since the heart can use a wide range of substrates, it implies that under many conditions the addition of extra substrates may have little influence on the metabolic and hemodynamic function. Therefore, it would be surprising to find one substrate that in every experimental condition improved the metabolic and hemodynamic function of the heart.[47] Our knowledge about the precise indications and dosages of substrates still is limited. It is only in the last decade that we have began to understand the transport mechanisms of glucose and amino acids across the cell membrane of the cardiomyocytes. In vivo and in vitro experiments on animal hearts have contributed to our knowledge by permitting the purification of experimental conditions. They allow sophisticated analyses that are not possible to perform in humans. However, these studies have limitations, not only due to species differences, but also because some of them are conducted under unphysiological conditions and sometimes with irrelevant dosages. In vitro studies cannot take systemic interactions or neuroendocrine responses into account. Furthermore, important interactions between different substrates and metabolites may be overlooked. However, the number of studies concerning myocardial metabolism and metabolic intervention in humans are still few. Lack of commercial interest and the tradition regarding therapeutic policies have not helped to promote metabolic studies nor the introduction of metabolic treatment. With guidance of the knowledge gained from animal studies we have to identify metabolic states in humans that may be accessible to metabolic interventions. From such studies metabolic interventions that are appropriate regarding indication, choice of substrate and dosage can be evolved. So far, the limited data available show that metabolic trials in humans that have tried to define the rationale for the treatment, and proved that the metabolic objectives were achieved, have been successful also regarding variables of clinical interest.

GIK (Glucose-Insulin-Potassium)

Basic research suggests that glucose is beneficial to the ischemic myocardium and may influence the preservation of mechanical function, structure, histology and ionic balance.[48] The glucose for glycolysis originates from both breakdown of myocardial glycogen stores and the uptake of glucose from blood.[49] Myocardial glucose uptake is stimulated during ischemia by translocation of glucose transporters from the intracellular pool to the sarcolemmal membrane.[49,51] However, in severe ischemia glucose uptake may decrease due to inadequate glucose delivery or accumulation of protons and lactate.[12,49]

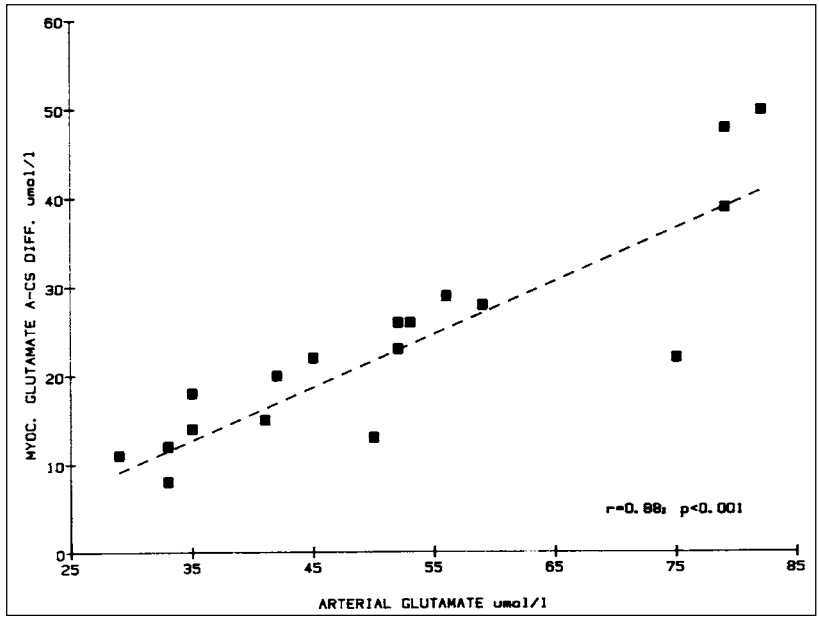

Fig. 12.1. Relationship between arterial plasma concentration of glutamate and the arterial-coronary sinus plasma concentration difference of glutamate in 18 patients 4 to5 hours after coronary surgery. Each *square* denotes one patient. Reprinted with permission from: Svedjeholm et al. J Thorac Cardiovasc Surg 1991; 101:688-94. © Mosby-Year Book Inc.

Myocardial uptake of glucose is not dependent on insulin. However, insulin stimulates glucose uptake by translocation of glucose transporters to the sarcolemmal membrane and possibly by improving their capacity.[52] Plasma FFA level is reduced and myocardial glycogen synthesis is promoted.[6,23] The effects of ischemia and insulin on glucose uptake seem to be additive.[50] Furthermore, insulin activates hexokinase and causes transfer of hexokinase to the mitochondrial membrane, thus increasing phosphorylation of free glucose in the cytoplasm.[53] It has also been proposed that insulin may stimulate the pyruvate dehydrogenase enzyme and thereby enhance Krebs cycle metabolism after ischemia.[6,39]

The need for insulin in non-diabetics is debatable. Endogenous insulin production responds to changes in nutritional state and activity of other hormones. Furthermore, the myocardial sensitivity to insulin may increase as an adaptive measure, as demonstrated in patients with severe valvular aortic stenosis.[54] However, the reverse—a state of insulin resistance—is observed after trauma and other conditions that activate the neuro-endocrine system.[45,55] Therefore, the need for exogenous insulin to achieve metabolic effects with GIK treatment may be expected to be most pronounced in states of relative insulin deficiency, such as diabetes mellitus, surgical stress and other states of insulin resistance.

As substrate levels normally play a role for the substrate preference by the heart, blood glucose level obviously has to be considered.[56] After cardiac surgery, when systemic glucose uptake is severely restricted in spite of using high insulin doses, systemic glucose uptake can be further enhanced by increasing blood glucose.[57]

Interactions between substrates have to be considered. High FFA levels impede myocardial glucose uptake and this is at least partly caused by inward translocation of glucose transporters.[50] Myocardial uptake of glutamate correlates with myocardial uptake of glucose

and other carbohydrate substrates in coronary patients.[14] This may, however, merely reflect the substrate preference of the ischemic heart. Exposure of the isolated heart to pyruvate and lactate leads to a reduction in glucose uptake.[58]

In the preoperative setting most of the interest concerning GIK has focused on myocardial glycogen content. Experimental data suggest that elevation of myocardial glycogen content improves tolerance to ischemia.[12,59] Preoperative GIK given during preoperative fasting has been shown to enhance myocardial glycogen level. This was associated with improved myocardial preservation and postoperative course.[21-23] However, the value of preoperative GIK is attenuated by the fact that fasting per se promotes maintenance of myocardial glycogen. This is due to the inhibition of glycolysis caused by the elevated plasma FFA in the fasting state.[60] The impact of preoperative GIK may be expected to be most pronounced in energy depleted hearts.

Whereas small doses of insulin and glucose seem to have been adequate during preoperative fasting,[21-23,60] little is known about the requirements of insulin and glucose in the early stages of surgery.[61-63] Recent data (unpublished) suggest that insulin resistance and impeded glucose uptake is an issue of concern primarily during the early postoperative period. In the anesthetized patient we found substantial myocardial uptake of glucose during early reperfusion. In the early postoperative setting the rationale for GIK treatment is more evident. A pronounced insulin resistance and a markedly impeded uptake of glucose have been demonstrated. Whereas moderate doses of insulin may suffice to reduce plasma FFA, insulin doses up to 1 IU/kg/hour may be required to achieve full metabolic effects early after cardiac surgery.[41,45,55,64] Several studies on GIK have failed to take the insulin resistance caused by the postoperative neuroendocrine stress response into account. It appears that studies on prebypass GIK have been successful when a sustained postoperative metabolic effect could have been expected, either due to high doses of insulin given prebypass or by treatment extended into the postoperative course.[61,65,66] The administration of small doses of insulin and glucose from the induction of anesthesia to the start of CPB in elective CABG failed to influence the postoperative hemodynamic state or the release of cardiac enzymes compared with controls (16 patients in each group of whom 75% received counteracting treatment with dopamine).[63] Inasmuch as preoperative fasting per se promotes maintenance of myocardial glycogen, stable coronary heart disease may not be the ideal condition for studies on prophylactic GIK treatment.[60] In patients undergoing urgent CABG due to unstable angina an improved hemodynamic recovery and clinical outcome have been demonstrated.[66] In the postoperative setting positive hemodynamic effects have been obtained with supra-physiological doses of insulin.[67,68] The hemodynamic effects of very large doses of insulin though, are caused by insulin per se. Insulin has both vasodilative and inotropic properties according to animal experiments.[69,70] In humans the vasodilative effect has been demonstrated, while the inotropic effect has been more difficult to evaluate.[71,72]

Gradinac et al demonstrated favorable effects of postoperative GIK treatment in patients requiring IABP (intra-aortic balloon pump).[73] The early hemodynamic recovery was improved, the time on IABP was shortened and the requirement for inotropic drugs reduced. These results were achieved in spite of moderate doses of insulin (0.08 IU/kg/hour for up to 48 hours) not known to exert any hemodynamic effects. Therefore, it is conceivable that the results obtained were due to metabolic effects. As this study lacked a metabolic correlate, we do not know to what extent the metabolic objectives were achieved. It is possible that larger doses of insulin would have amplified the impact of GIK treatment.

The metabolic effects of *high-dose GIK* on the heart, have been demonstrated in patients after CABG surgery. Svensson et al observed a significantly improved myocardial uptake of carbohydrate substrates one hour after surgery.[41,45] Furthermore, even in patients treated with dopamine the addition of high-dose insulin induced a shift of energy substrate preference toward carbohydrate oxidation at the

expense of FFA. The hemodynamic efficacy of dopamine was improved by high-dose GIK without further increasing myocardial oxygen demand.[6]

It is important to consider that the rate of glucose uptake, glycogen synthesis and breakdown, and the rate of glycolysis and glucose oxidation are controlled at multiple sites. In postischemic hearts, high rates of glycolysis and low rates of glucose oxidation (due to inhibition of pyruvate dehydrogenase by FFA metabolism) may lead to an uncoupling of glycolysis from glucose oxidation. The consequent production of protons can contribute to ischemic injury and myocardial dysfunction.[49] Adrenergic inotropic drugs may by their metabolic actions be expected to aggravate this metabolic condition. Accordingly, elevation of plasma FFA and a myocardial release of pyruvate correlating with myocardial oxygen demand was found during dopamine stimulation in nondiabetic patients after CABG. The addition of high-dose GIK to dopamine reverted this relationship to an uptake of pyruvate which correlated positively with myocardial oxygen demand.[6] Therefore, it appears that pyruvate oxidation was stimulated. Whether this beneficial effect was caused by the reduction of plasma FFA or by direct intracellular action of insulin on the pyruvate dehydrogenase enzyme, or both, remains to be clarified.[6,39] Also in the diabetic heart a defect in pyruvate oxidation rather than a defect in glucose uptake has been demonstrated during dobutamine stimulation.[74] In acute myocardial infarction it has been demonstrated that insulin-glucose infusion followed by a multidose insulin regime improves one-year survival in diabetics patients.[75] It is plausible that GIK treatment will prove to be of particular benefit in diabetic patients undergoing cardiac surgery.

The introduction of GIK in clinical practice has been offset by a reputation of conflicting data in the medical literature. However, the number of inconclusive trials in the literature are surprisingly few compared with the number of papers demonstrating beneficial effects of GIK for the ischemic and postischemic heart. In recent years beneficial effects of glucose metabolites, such as lactate and pyruvate, on postischemic recovery have also been reported.[8,76,77] In an extensive review Stanley recently concluded that metabolic interventions aimed at enhancing glucose utilization and pyruvate oxidation at the expense of FFA oxidation is a valid therapeutic approach to the treatment of myocardial ischemia.[53] The introduction of pharmacologic agents which enhance full glucose oxidation at the expense of FFA may create the commercial interest necessary to achieve a widespread use of metabolic therapies.[53]

Amino Acids

Amino acids are not quantitatively important substrates for myocardial energy production. However, increasing evidence suggest that amino acids are *qualitatively* important for the intermediary metabolism of the cardiomyocytes.[15,19,37,44] Their importance is further enhanced during ischemia and after ischemia.[15-17,78-80] Amino acids associated with the malate-aspartate shuttle (glutamate, aspartate, arginine, ornithine) are of particular importance in this respect.[17,19] The effects of these amino acids are partly related to their role in the malate-aspartate shuttle, transporting reducing equivalents across the mitochondrial membrane, regulating the NAD/NADH balance in the cytosol of the cells, and thereby enhancing anaerobic glycolysis during ischemia. Furthermore, glutamate and aspartate contribute to an alternative anaerobic pathway for regeneration of high-energy phosphates, by substrate level phosphorylation in the Krebs cycle. Glutamate also improves the clearance of lactate and NH_3 excess, by taking part in the reactions involving transamination of pyruvate to alanine and of glutamate to glutamine. During reperfusion glutamate and aspartate serve as substrates for the replenishment of Krebs cycle intermediates lost during ischemia.[15-17,20,36,78-81]

BCAA (branched chain amino acids—leucine, isoleucine, valine) are with glutamate the amino acids that are preferentially taken up by the heart after CABG surgery.[46,82] Based on a study using mixed amino acid solutions

enriched with BCAA it was suggested that BCAA improve the metabolic and functional recovery of the heart after ischemia.[83] However, the relative impact of BCAA compared with other amino acids contained in the solution may be difficult to evaluate. Other investigators suggest that increased intracellular concentrations of BCAA stimulate formation of acetyl-coenzyme and succinyl-CoA and, thus, recovery of oxidative metabolism.[84] On the other hand, it is uncertain whether exogenous administration alone would influence myocardial BCAA uptake after CABG, as no relationship between plasma levels of BCAA and myocardial uptake has been found.[46,82] Thus, the role of BCAA in this setting remains to be clarified. Other amino acids of interest for myocardial protection and metabolism in association with ischemia include taurine, methionine, cysteine and histidine. A comprehensive review on this subject has been given by Pisarenko.[84]

Of the amino acids associated with the malate-aspartate shuttle, glutamate and aspartate have by far gained the greatest attention as regards the heart and metabolic intervention. Arginine has received attention recently mainly due to its metabolism to nitric oxide.[85,86]

In cardiac surgical practice most of the interest concerning amino acids has focused on their use as additives in cardioplegic and reperfusion solutions. The glutamate and aspartate enrichment of these solutions relies on basic metabolic research and studies on myocardial preservation in animal models. These studies show that glutamate and aspartate improve myocardial tolerance to ischemia, and enhance metabolic and functional recovery of the heart after ischemia.[15,17,36,87-92] The relative clinical impact of substrates in cardioplegic/reperfusion solutions remains to be clarified in humans. Improved postoperative hemodynamic recovery when applied to high-risk coronary patients and an improved myocardial ATP maintenance in congenital surgical patients have been suggested.[93-96] It has also been demonstrated that alpha-ketoglutarate, a metabolite of glutamate, improves myocardial preservation in humans undergoing CABG surgery when added to blood cardioplegia.[97] Based on the knowledge about amino acid transporters that has emerged there is reason to question whether cardioplegic solutions are ideal for delivery of amino acids to the heart. Glutamate and aspartate are transported into muscle cells by Na^+-dependent and Na^+-independent systems.[98] Suleiman has suggested that delivery of glutamate and aspartate by cardioplegia could be self defeating due to the anticipated effect on the amino acid transporters.[99] Myocardial loss of glutamate has now been demonstrated both after crystalloid cardioplegic arrest and during cold blood cardioplegia.[97,99,100] This may explain some conflicting data regarding amino acid enrichment of cardioplegic solutions.[101,102] However, it cannot yet be excluded that loss of glutamate is attenuated by glutamate enrichment of the cardioplegic solution. Furthermore, plasma levels of glutamate and aspartate are markedly increased during reperfusion and these amino acids will be available to the postischemic heart.[103]

Intravenous infusions offer an alternative to amino acid enhancement of cardioplegic solutions. As glutamate is reported to improve myocardial tolerance to ischemia in coronary patients, intravenous glutamate administration offers the prospect of improving myocardial protection during the pre- and postoperative periods of CABG surgery.[25,104] Furthermore, glutamate and aspartate may eventually play a role in the treatment of myocardial infarcts as the infusion of glutamate and aspartate reduces the size of myocardial infarcts in experimental coronary artery occlusion.[27,105] Moreover, infusion of these amino acids together with GIK had salutary effects on the contractility of viable remote myocardium in experimental coronary occlusion.[106] This is a significant finding, since failure of remote myocardium to sustain the compensatory contractility required to maintain cardiac output may play an important role in the development of cardiogenic shock.

Due to the importance of glutamate for the recovery of oxidative metabolism after ischemia[36,80] and the shortage of glutamate

demonstrated early after coronary surgery, a rationale for metabolic intervention is provided in this setting.[46,82] Postoperative glutamate infusion has been shown to improve the metabolic and functional state of the heart after routine CABG and in postoperative cardiac failure (Figs. 12.2 and 12.3).[26,107] Glutamate has no inotropic effects per se. Therefore, it is conceivable that the hemodynamic effects during postoperative treatment were due to the improved metabolic state of the heart. In keeping with this, the hemodynamic impact of postoperative glutamate infusion seems to be related to the magnitude of metabolic derangement before treatment.

In animal experiments glutamate and aspartate have additive effects on myocardial recovery after ischemia.[108] Based on data from the isolated rat heart it has been claimed that aspartate may be more important than glutamate for the recovery of myocardial metabolism after cardioplegic arrest.[109] However, in humans early after CABG we were unable to detect any beneficial effect of aspartate on myocardial metabolism or function (unpublished). Intravenous aspartate infusion caused an increase of myocardial aspartate uptake but considerable interactions with glutamate compatible with competitive inhibition of glutamate uptake were observed. These observations agree with findings in the rat heart where aspartate and glutamate compete for the same Na^+ dependent amino acid carrier.[110] In humans the arterial plasma aspartate level is very low and mainly glutamate is taken up by the ischemic and postischemic heart, suggesting a physiologically more important role for glutamate.[13,14,46,82] Current knowledge, thus, indicates that glutamate is of particular importance for the ischemic and postischemic human heart, whereas similar data for aspartate is lacking.

Dosage and Toxicity of Glutamate

In rodents exogenous administration of glutamate is associated with dose dependent neurotoxicity.[111] In primates glutamate does not pass the blood brain barrier and the administration of glutamate has, therefore, not been shown to cause cerebral damage.[112] The possible influence of extracorporeal circulation on the blood brain barrier has to be considered in cardiac surgery. However, extensive experience with amino acid enhanced blood cardioplegia is not reported to be associated with an increased incidence of cerebral complications.[113] On the other hand, glutamate causes a transient but unpleasant side effect known as the "Chinese restaurant" syndrome. It is considered to be caused by stimulation of the peripheral nervous system, and may be related both to individual susceptibility and dosage of glutamate.[114,115]

A high turnover of glutamate in the human body is suggested by the rapid decrease of arterial levels after discontinuation of glutamate infusion.[26] If arterial levels play an important role for adverse reactions, it implies that the time during which glutamate is administered may be more important than the amount given. Accordingly, Thomassen observed short-lasting symptoms compatible with the "Chinese restaurant syndrome" in a patient with coronary artery disease receiving an intravenous injection of only 2.5 mg/kg BW. The arterial plasma levels reported were only moderately increased, but the peak levels may not have been caught due to rapid clearance of glutamate.[25] Adverse effects were not seen in patients receiving 1.5 mg/kg BW intravenously or 40 mg/kg BW orally.[104]

Excess amounts of glutamate seem to have been employed in cardioplegic and reperfusion solutions.[103] In anesthetized patients the issue of transient adverse effects is of little significance and explains why the issue of dosages has received limited attention. However, potential applications for intravenous glutamate infusions include awake patients during the pre- and postoperative course, and patients with acute myocardial ischemia in the coronary care unit. Therefore, establishing a dosage of glutamate that would supply the needs of the heart with a minimum risk of side effects is desirable.

After CABG surgery myocardial glutamate uptake is closely related to arterial plasma levels.[43,46] However, due to the high capacity of the heart to extract glutamate, a moderate

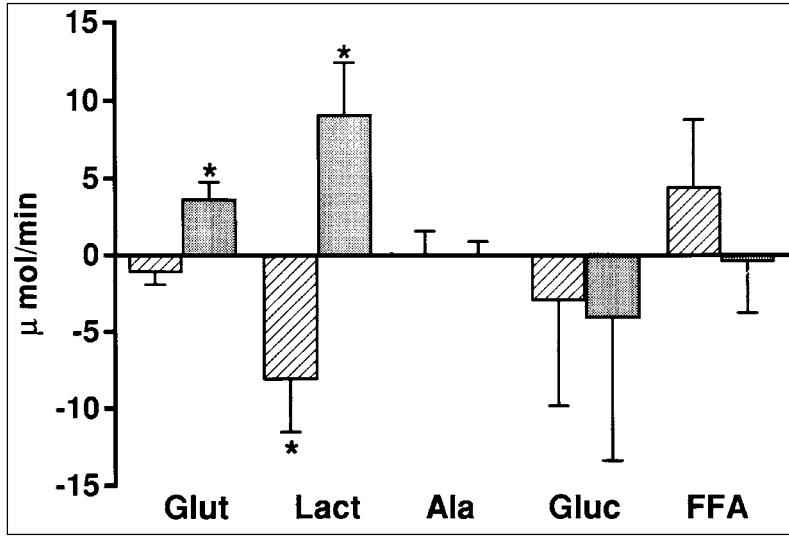

Fig. 12.2. Change in myocardial flux of substrates in controls and patients treated with intravenous glutamate infusion (early after elective CABG) from the basal state to 60 minutes (mean ± SEM). Hatched bars = Control group. Grey bars = Glutamate group. Glut = glutamate, Lact = lactate, Ala = alanine, Gluc = glucose and FFA = free fatty acids. * Indicate statistically significant differences compared with the basal state, * $p<0.05$, ** $p<0.01$. Reprinted with permission from: Svedjeholm et al, J Thorac Cardiovasc Surg 1996; 112:1468-77. © Mosby-Year Book Inc.

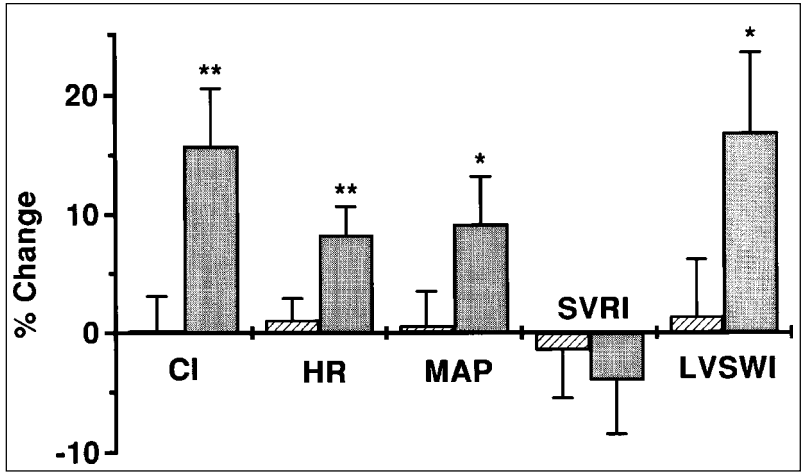

Fig. 12.3. Change in hemodynamic state in controls and patients treated with intravenous glutamate infusion (early after elective CABG) from the basal state to 60 minutes expressed in percentages (mean ± SEM). Hatched bars = Control group. Grey bars = Glutamate group. CI = cardiac index, HR = heart rate, MAP = mean arterial pressure, SVRI = systemic vascular resistance index, LVSWI = left ventricular stroke work index. * Indicate statistically significant differences compared with the basal state, * $p<0.05$, ** $p<0.01$. Reprinted with permission from: Svedjeholm et al, J Thorac Cardiovasc Surg 1996; 112:1468-77. © Mosby-Year Book Inc.

increase of arterial glutamate sufficed to optimize myocardial glutamate uptake after elective CABG. A significant increase in the myocardial uptake of glutamate was observed during glutamate infusion but increasing arterial (whole blood) levels by more than 100-200 mmol/L was not associated with a further substantial increment in myocardial glutamate uptake (Fig. 12.4). Glutamate infusion caused a dose dependent linear increase of arterial glutamate levels and an infusion rate of 30-40 mg/kg BW/hour may be sufficient to supply the needs of the heart (unpublished data). The adequacy of this dosage remains to be confirmed in high-risk patients.

Recently published data show the pH-dependence of L-glutamate transport in sarcolemmal vesicles from rat heart. Intravesicular acidosis enhanced Na^+-dependent glutamate transport, whereas acidification of the incubation medium enhanced Na^+-independent uptake of glutamate.[110] Therefore, acidosis in ischemic heart regions could explain the increased uptake of glutamate observed in ischemic and postischemic hearts.[13,14,46,82,107,112,116] It remains to be clarified to what extent myocardial glutamate uptake is governed by myocardial requirements (acidosis) and to what extent it may be limited by saturable carriers.

Clinical Experience with Metabolic Support in Cardiac Surgery

The majority of patients undergoing cardiac surgery will do well independent of the treatment given postoperatively. Therefore, it is tempting to consider the hazards associated with adrenergic stimulation of the heart to be of theoretical interest only. Paradoxically overuse of inotropes may give an impression of efficiency that may be lacking when these drugs are really "needed". Many patients are treated with inotropes because of low blood pressure caused by low systemic vascular resistance or hypovolemia. Furthermore, hearts with benign causes of cardiac failure, such as residual depressant effect of cardioplegia or limited ischemic insult, may be expected to respond better to inotropic stimulation than seriously injured hearts. In cardiac failure caused by severe myocardial ischemia or evolving myocardial infarction the use of inotropic drugs can be detrimental. Basic science has demonstrated that adrenergic stimulation aggravates ischemia and precipitates myocardial infarction.[9] Inotropes have also been shown to cause de-energization of the myocardium, and if instituted prematurely it may imply that the condition of the heart is aggravated by further metabolic derangement. [8]

However, as the clinical usefulness of alternative treatments other than mechanical circulatory support has not been demonstrated, conventional pharmacological treatment has prevailed in clinical practice. It has been assumed that inotropic drugs are required to treat myocardial stunning and to avoid detrimental effects of hypoperfusion. These issues will be challenged below by a metabolic strategy that implies reliance on metabolic measures for the enhancement of myocardial recovery.

Metabolic Strategy

Parallel to metabolic studies, our research group has applied metabolic treatment in clinical practice, to gain experience and to find out to what extent metabolic support can replace traditional treatment of cardiac failure. As data from metabolic studies have emerged and our experience has increased, a metabolic strategy has gradually evolved. This strategy is compiled of metabolic, pharmacological and physiological principles that have been found to enhance myocardial tolerance to ischemia and to promote recovery after ischemia. Metabolic support found to be adequate regarding dosage and choice of substrates has been chosen and modified to achieve metabolic effects with stable hemodynamic conditions. Therefore, the metabolic strategy includes *general measures* in all patients to reduce the metabolic stress on the heart, and *metabolic support* in selected cases. Contrary to common belief we have found that these principles can replace traditional treatment as the main therapy in the postoperative setting after cardiac surgery.

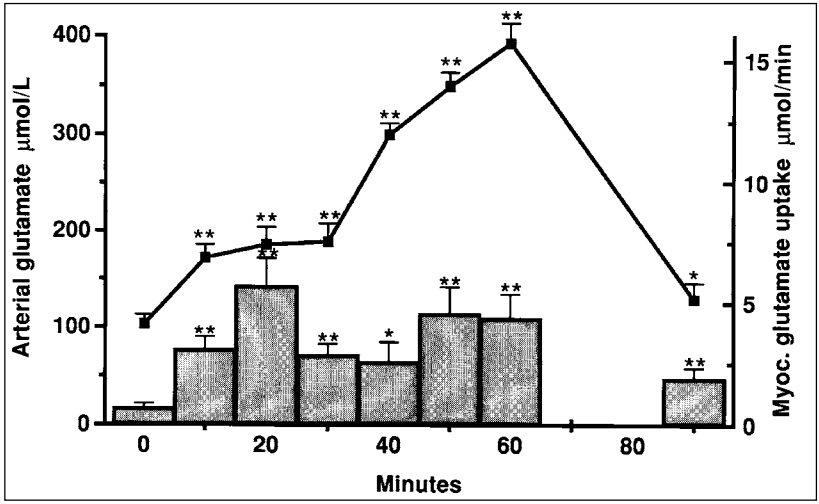

Fig. 12.4. Myocardial glutamate uptake (µmol/min) [bars] and arterial glutamate level during intravenous glutamate infusion early after CABG (µmol/L) [straight line + squares] according to whole blood analyses (Mean ± SEM). * Indicate statistically significant differences compared with the basal state, * $p < 0.05$, ** $p < 0.01$. Reprinted with permission from: Svedjeholm et al, J Thorac Cardiovasc Surg 1996; 112:1468-77. ©Mosby-Year Book Inc.

General Measures

The general measures include conventional pharmacological treatment of myocardial ischemia and unconventional measures to reduce myocardial energy expenditure and systemic oxygen demand.[117] In patients with cardiac failure care is taken to reduce strain on the heart by reducing systemic oxygen demand, which is, in fact the main determinant of cardiac output. Mixed venous oxygen saturation (SvO_2) reflects the balance between cardiac output and systemic oxygen consumption and indicates whether cardiac output can adequately provide the peripheral tissues with oxygen. This physiological approach toward the treatment of postoperative cardiac failure helps to avoid overtreatment, that is, stimulating cardiac output more than necessary. In patients with signs of inadequate hemodynamics, systemic oxygen demand is lowered by sedatives and muscle relaxants. Inotropic drugs increase myocardial energy expenditure directly by increasing myocardial oxygen demand and indirectly by increasing systemic oxygen demand.[6,7] The negative effects of inotropic drugs may be expected to be particularly pronounced during early reperfusion where myocardial energy reserve may be limited and in impending or evolving myocardial infarction. Accordingly, Lazar showed detrimental effects of premature use of inotropic drugs for weaning from CPB.[10] Inotropic drugs for weaning from CPB are therefore avoided or the use of them delayed. As cardiac failure is usually apparent on weaning from CPB, prolonged unloading of the heart on CPB and metabolic support are used to enhance myocardial recovery. Volume work by the heart (in favor of pressure work) is promoted by after load reduction with nitroprusside. In patients with severe postoperative cardiac failure a systolic blood pressure of 80-100 mm Hg is accepted if urinary output is adequate and the patient has no critical arterial stenosis. If SvO_2 or urinary output suggests that cardiac output is inadequate, inotropes are used in low dosages. A mechanical assist device is preferred in favor of increasing the dose of inotropic drugs such as dobutamine above 4-5 µg/kg/min.

Metabolic Support

Metabolic support has been used in selected patients and included the use of glutamate

infusion and high-dose GIK (glucose-insulin-potassium). Although theoretically attractive, the role of prophylactic treatment with GIK or glutamate in cardiac surgery remains to be clarified. At present preoperative GIK is considered in energy depleted hearts. As glutamate improves myocardial tolerance to ischemia in coronary patients during exercise tests and pacing we have used glutamate preoperatively in severely unstable patients.[25,104] In contrast, the rationale for postoperative metabolic treatment in cardiac surgery has been more clearly defined. First the metabolic abnormalities and the state of insulin resistance have been demonstrated.[41,45,82] The desired metabolic effects, and positive hemodynamic effects, have been achieved with glutamate and high-dose GIK.[6,26,41,45] For treatment of cardiac failure after CABG the combined use of glutamate and GIK seems advisable by addressing the metabolic consequences of both myocardial ischemia and the systemic neuroendocrine stress response.[4] However, it remains to be shown that the combined use of glutamate and GIK provides additional metabolic benefit.

Glutamate Infusion

Glutamate infusion has been used preoperatively and during the early stages of surgery to increase myocardial tolerance to ischemia in severely unstable patients. As glutamate enhances postischemic recovery of myocardial metabolism it has also been used to treat or prevent cardiac failure on weaning from CPB. According to data that we are about to publish an infusion rate of 30-40 mg/kg/hour should be adequate in most patients to optimize myocardial uptake of glutamate. In clinical practice an infusion rate of 1.5 ml/kg/hour of a 0.125M solution has been employed and has so far not been associated with any ill effects. The total volume infused has usually been 250 ml, however, in exceptional cases the infusion has been continued to a total volume of 500 ml. As we have sometimes experienced that the patients "sag" a little after stopping glutamate, we now reduce the infusion rate to half the original when 1/2 hour remains according to the original infusion rate.

High-Dose GIK (Glucose-Insulin-Potassium)

GIK (glucose-insulin-potassium) treatment has mainly been used to treat or prevent cardiac failure on weaning from CPB and in the early postoperative setting. The rationale in this setting is to counteract the negative effects of systemic trauma metabolism on the heart; reduce plasma FFA, give the heart carbohydrate substrates and enhance full glucose oxidation.[43] Due to the insulin resistance after cardiac surgery, high doses of insulin may be required to achieve these objectives.[41,43,45] We have employed a high-dose GIK regime that will ensure maximal metabolic effects in most patients, without having the pronounced vasodilative properties of the "hemodynamic doses" of insulin described in previous studies.[72,118] The reason is that after load reduction with nitroprusside is easier to control in critically ill patients. Moderate hyperglycemia is employed as systemic glucose uptake after cardiac surgery can be further enhanced (in spite of high plasma insulin) by increasing blood glucose up to 10 μmol/L.[57]

In clinical practice a 30% glucose solution, supplemented by 10 μmol Mg and 40 mmol of phosphate/1000 ml, is infused at a rate of 60-100 ml/hour. We have found it advisable to start with approximately 1ml/kg/hour. The infusion rate is determined by regular checks of s-glucose to achieve a stable blood glucose level between 7-12 μmol/L. We prefer to keep blood glucose just below 10 μmol/L.

Insulin of a fast-acting type (Actrapid Novo) is infused at a rate of 1 IU/kg/hour for 6 hours. A bolus dose of 25 IU is injected i.v. after 5 minutes to achieve an early steady state metabolic effect. After the insulin infusion has been completed, the glucose infusion is maintained for another 8-18 hours, the infusion rate gradually decreased according to blood glucose level. This treatment is a hyperinsulinemic glucose clamp, therefore, blood glucose levels in the short perspective are influenced only by the rate of glucose infusion. Consequently, the high-dose GIK treatment is easy to manage and stable glucose levels are routine. However, this treatment requires careful attention and the glucose

infusion should not be stopped until the effect of insulin has subsided.

Potassium is infused separately but s-potassium is allowed to decrease to 3.5 μmol/L to avoid rebound hyperkalemia at the discontinuation of insulin infusion.

As the treatment proceeds, some degree of vasodilation will develop in most patients. Nitroprusside is discontinued and in approximately one third of the cases small to moderate doses of a counteracting drug (angiotensin II or noradrenaline) has to be instituted.

Metabolic Support in Severe Cardiac Failure

Initially metabolic support was used mainly in patients with severe cardiac failure to gain experience and to find out to what extent metabolic support can be used to replace inotropes and mechanical assist devices.[119] In 16 (3.1%) out of 515 patients cardiac failure on weaning from CPB (cardiopulmonary bypass) was considered severe enough to have justified the use of IABP treatment. The average systolic arterial blood pressure (SAP) was 54 ± 19 mmHg when the decision to start metabolic support was taken. The condition of most patients was too poor to allow further hemodynamic measurements or blood sampling. The first SVO_2 and cardiac index (CI) obtained were on average 45 ± 7% and 1.6 ± 0.4 L/min/m^2 respectively. These patients were treated by unloading of the heart with prolonged CPB and metabolic support with glutamate and high-dose GIK.

Thirteen out of 16 patients could be weaned from CPB after an estimated average prolongation of CPB with 75 minutes. A rapid improvement of hemodynamic performance was seen in the first hour after weaning from CPB, and almost full recovery within 6 hours (Fig. 12.5). The average dobutamine dose in the first 6 hours was 2.2 ± 1.8 μg/kg/min. Three patients failed to wean from CPB and required a mechanical assist device. None of these patients, however, showed any signs of reversible cardiac failure and they subsequently died. One further patient died of sepsis one week postoperatively. Thus, with a questionmark for one patient, metabolic support was associated with a 100% success rate in reversible cardiac failure. It may be argued that it was time and prolonged unloading of the heart by CPB rather than metabolic treatment that improved the condition of the hearts. However, there is no contradiction between the use of prolonged CPB and metabolic support. Other investigators have achieved encouraging results in critically ill patients after cardiac surgery employing the principles of substrate enhancement and unloading of the heart.[93] Both principles enhance the recovery of myocardial metabolism. In experimental coronary ligation the beneficial effects of GIK and IABP on myocardial metabolism are additive.[120] We have found substantial uptake of both glucose and glutamate in the anesthetized patient during CPB (unpublished). Metabolic support, however, may enhance the speed of recovery by improving myocardial uptake of glucose and glutamate and by reducing plasma FFA. Furthermore a sustained effect is acquired by continuing metabolic support into the early postoperative course when the consequences of the neuro-endocrine stress response may become more obvious. The hemodynamic recovery within six hours compared with the 2-3 days generally required with conventional mechanical assist devices is in keeping with this assumption.[73,121]

These results should not be interpreted as though avoidance of IABP or other mechanical assist devices is a major objective of the metabolic strategy. Unloading of the heart is an attractive mode of treatment from a metabolic point of view.[122,123] In fact due to this early encouraging experience we have broadened the indications for both metabolic support and mechanical assist devices to reduce the need for inotropic drugs further.

Metabolic Strategy in Surgery for Ischemic Heart Disease

Data from 785 consecutive patients operated by our group for ischemic heart disease (745 CABG and 40 CABG + valve procedures) according to the metabolic strategy are currently undergoing analysis (unpublished). The average age was 65 years and the proportion of females was 20%. 229 patients had unstable

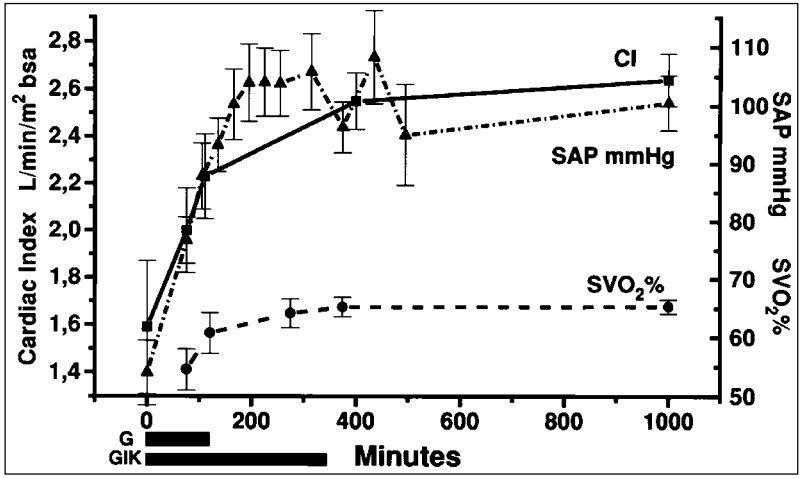

Fig. 12.5. Hemodynamic recovery in patients with severe cardiac failure on weaning from CPB treated with intravenous glutamate and high-dose GIK (n=13). The change in systolic arterial blood pressure (SAP, n=13; right axis) and cardiac index (CI, n=7; left axis) after institution of metabolic support and the change in mixed venous oxygen saturation (SVO2, n=13; right axis) after weaning from CPB. Data are presented as mean ± SEM. The horizontal bars depict the time used for metabolic treatment. G = glutamate, GIK = high-dose Glucose-Insulin-Potassium. Reprinted with permission from: Svedjeholm et al, Ann Thorac Surg 1995; 59:S23-30. © Elsevier Science Inc.

angina (29.2%) and 108 patients (13.8%) had severely compromised left ventricular function preoperatively (ejection fraction ≤ 0.35). Overall 96% of the patients were weaned from CPB without inotropic drugs. In Table 12.1 the use of dobutamine and metabolic support from 1991-1995 is shown. Despite an increasing proportion of high risk patients, the need for inotropes for weaning from CPB was gradually abolished as the use of metabolic support increased. This was not achieved at the price of a poorer hemodynamic state according to SvO_2 measurements on arrival to ICU. However, in the ICU a small dose of dobutamine to stimulate urinary output was later instituted in 18% of the patients overall. The average dose of dobutamine when used was 2.2 ± 1.4 μg/kg/min. The maximum dobutamine dose in an individual patient was 5.7 μg/kg/min and during the last two-year period no patient received a dobutamine dose exceeding 5 μg/kg/min. PDA inhibitors were used in 0.5% and mechanical assist devices in 1.4% of the cases. One patient required dialysis because of postoperative renal failure (0.1%). No patient developed evident signs of intestinal ischemia. Average stay in the ICU was 1.6 days and the median time on the ventilator 8 hours. The 30-day mortality was 0.9%.

Inotropic drugs improve cardiac output and thus can contribute to termination of CPB. However, there are to our knowledge no evidence that these drugs improve outcome in cardiac surgery. After cardiac surgery inotropic drugs cause a marked increase in myocardial oxygen demand and aggravate the metabolic condition of the heart.[6] From cardiology practice we know that reduction of adrenergic stress with beta-blockers reduces mortality in myocardial infarction.[124] There is no reason to believe that a myocardial infarct in association with cardiac surgery would differ in this respect. A high incidence of myocardial ischemia and myocardial infarction has been reported when inotropes are used to terminate CPB.[125] Another negative aspect is that adrenergic drugs also increase the metabolic rate in the peripheral tissues.[6,7] As the main purpose of the heart is to supply the periph-

Table 12.1. Changes in some risk groups and trends in treatment from 1991 to 1995 in our clinical experience with the metabolic strategy (n=785 patients).

Year	Age ≥ 70	EF ≤ 0.35	CABG + valve	Inotropes for weaning from CPB	SvO_2% on arrival to ICU	Dobutamine μg/kg/min 0-6 hrs	Dobutamine μg/kg/min 6-24 hrs	Dobutamine 5 μg/kg/min	Glutamate	GIK
1991	25.9%	6.9%	1.7%	6%	66.5 ± 7.6	2.4 ± 1.5	1.8 ± 1.4	0.9%	1.7%	3.4%
1992	29.9%	10.4%	1.7%	5.7%	67.2 ± 7.0	2.2 ± 1.5	2.2 ± 1.2	0.6%	12.6%	5.7%
1993	34.0%	15.2%	5.0%	6.6%	66.7 ± 7.3	2.4 ± 1.0	2.4 ± 1.6	1.0%	16.0%	8.0%
1994	37.8%	17.0%	6.9%	0.5%	66.3 ± 8.4	1.6 ± 1.1	2.3 ± 0.8	0	21.4%	16.8%
1995	35.5%	19.0%	10.3%	0	67.6 ± 7.9	1.3 ± 1.5	2.3 ± 1.4	0	30.8%	25.2%

From left to right the table shows the proportion of patients aged 70 years or older, the proportion of patients with preoperatively compromised left ventricular function, the proportion of combined CABG + valve procedures, the proportion of patients weaned from CPB with inotropes, average SvO_2 on arrival to ICU (intensive care unit), the average dobutamine dose when used during the first 0-6 hours and 6-24 hours after surgery respectively, the proportion of patients receiving dobutamine doses exceeding 5 mg/kg/min and finally the proportion of patients treated with glutamate and GIK respectively. Results are presented in percentages or mean ± SD.

eral tissues with oxygen and substrates, this therapy is to some extent self defeating. It has been claimed that phosphodiesterase inhibitors are less noxious to the diseased heart, but this remains to be proved. In chronic congestive heart failure these drugs have been associated with a significant increase in overall mortality.[126] Although most patients tolerate inotropes, there is now reason to believe that they may have a detrimental influence on outcome in a subset of patients with cardiac failure caused by a serious ischemic insult or evolving myocardial infarction.

One further disadvantage of inotropic treatment is that the natural course of myocardial recovery is concealed by the effects of these drugs. As down regulation of beta receptors in the myocardium occurs the drug requirements are increased. Deteriorations in the hemodynamic condition are therefore frequently addressed by increasing the dosage of inotropic drugs. This implies that identification of other causes for deterioration may be delayed or even overlooked. By employing the metabolic strategy we have improved our understanding of the natural postoperative course.

Since, this is a report of clinical experience and not a formal scientific trial, we have no data on the metabolic effects of metabolic support in these patients. However, in previous studies the desired metabolic effects, and positive hemodynamic effects, have been achieved with glutamate and high-dose GIK.[41] After elective CABG, the parallel sequence of metabolic and functional recovery of the heart suggests a casual link between metabolic and contractile dysfunction.[43,26,127] In animal experiments a close relationship between mitochondrial respiration and myocardial function during reperfusion has been demonstrated.[128] It is, therefore, conceivable that the recovery of myocardial function observed during glutamate and high-dose GIK was caused by recovery of myocardial metabolism. The maintenance of intracellular calcium homeostasis and force development by the contractile apparatus are dependent upon the free energy derived from ATP hydrolysis. This energy of hydrolysis is determined by the myocardial phosphorylation potential.[77] It has been demonstrated that pyruvate enhances contractility in stunned myocardium by enhancing myocardial phosphorylation potential.[77] Similar effects may be expected by other substrates that can enhance pyruvate oxidation such as GIK and glutamate.[6,26]

Renal function is a sensitive parameter of the adequacy of hemodynamic treatment. Also, splanchnic circulation may be jeopardized in low output syndrome. In our five-year experience only one patient (0.1%) required dialysis because of postoperative renal failure and we found no obvious case of intestinal ischemia. In contrast, an incidence of renal impairment requiring dialysis of 1.2-2.5% has been reported from comparable centers after adult cardiac surgery.[129,130] However, less pronounced changes of renal function may have to be evaluated to assess the adequacy of hemodynamic treatment. The incidence of postoperative renal failure, defined as a postoperative increase of s-creatinine by 50% or more compared with preoperative values, was 2.8% in our experience. In contrast, a 16% incidence of postoperative renal failure (same definition) after routine CABG was recently reported.[129] Multiple logistic regression analysis singled out the use of adrenergic drugs as the most important determinant for development of postoperative renal failure. It may be argued that it was the need for, rather than the use of these drugs that was the causative factor. However, our experience shows that cardiac failure can be treated with a low incidence of renal problems.[119] If adrenergic drugs are used in high doses, vasoconstrictive properties predominate and renal perfusion may be adversely affected. In contrast, GIK and amino acid infusion may enhance renal perfusion.[131] Also, the negative effects of inotropic drugs on myocardial recovery may contribute to more severe and prolonged states of low output syndrome. Therefore, the choice of treatment may have a decisive influence on maintenance of renal function. A similar link between the use of inotropic drugs and the development of intestinal ischemia may also be suspected. Thus, the use of inotropic adrenergic drugs

may be two-edged, not only from a myocardial point of view.

Several indices of improved outcome (improved hemodynamic recovery, less need for inotropes and IABP, less need for ventilator treatment, fewer serious complications, lower incidence of postoperative arrhythmias, shorter ICU and hospital stay) have been reported with GIK in high risk patients.[23,66,73] Although it remains to be proved that metabolic interventions improve survival in cardiac surgery, metabolic treatment evidently offers several advantages over traditional inotropic treatment, 1) Myocardial metabolic derangement—an important cause of postoperative cardiac failure is treated. Whereas inotropic drugs impede metabolic recovery 2) Glucose and amino acids add energy to the heart whereas inotropes drain energy from the heart 3) Glucose and amino acids increase myocardial tolerance to ischemia whereas inotropes aggravate ischemia and may precipitate myocardial infarction 4) GIK and amino acids are reported to improve renal perfusion whereas adrenergic drugs in high doses impair renal perfusion.

Summary

The availability of different metabolic substrates can evidently influence postischemic functional recovery and the damage incurred during episodes of myocardial ischemia. It is no longer a question of whether metabolic support is of benefit to the heart or not, rather a question of defining the conditions under which metabolic interventions are beneficial. Basic research, studies on human myocardial metabolism after cardiac surgery and available experience of metabolic interventions provide a solid rationale for metabolic support in postoperative cardiac failure. The rationale for metabolic treatment of postoperative cardiac failure is further supported by the drawbacks of traditional treatment. Adrenergic inotropic drugs improve the hemodynamic condition, but at the price of a marked increase in myocardial energy expenditure.[5,6,10] This puts further strain on the already compromised energy metabolism of the heart, and may precipitate myocardial ischemia and infarction.[9] However, as the clinical usefulness of alternative treatments has not been shown, conventional pharmacological treatment has prevailed in clinical practice. Our clinical experience over a five-year period proves that traditional treatment with inotropes can be replaced by metabolic measures, involving metabolic support with glutamate and high-dose GIK in selected patients. Intravenous infusions of glutamate and high-dose GIK have provided a consistently effective alternative in the treatment of postoperative cardiac failure. The overall results have been satisfactory and the low incidence of renal problems has been particularly gratifying. Further studies, though, are needed to delineate the optimal metabolic treatment for postischemic cardiac failure. More important, the role of prophylactic metabolic treatment in cardiac surgery and myocardial ischemia remains to be clarified.

References

1. Svedjeholm R, Dahlin LG, Olin C. Perioperative myocardial infarction (PMI) in cardiac surgery—risk factors and clinical outcome. Brit J Anaesth 1996; 76:(Abstract)
2. Slogoff S, Keats AS. Does perioperative myocardial ischemia lead to postoperative myocardial infarction? Anesthesiology 1985; 62:107-114.
3. Smith RC, Leung JM, Mangano DT. Postoperative myocardial ischemia in patients undergoing coronary artery bypass graft surgery. Anesthesiology 1991; 74:464-473.
4. Svedjeholm R, Håkanson E, Vanhanen I. Rationale for metabolic support with amino acids and glucose-insulin-potassium (GIK) in cardiac surgery. Ann Thorac Surg 1995; 59:S15-S22
5. Fowler MB, Alderman EL, Oesterle SN et al. Dobutamine and dopamine after cardiac surgery: greater augmentation of myocardial blood flow with dobutamine. Circulation 1984; 70 (suppl I):I 103-111.
6. Svedjeholm R, Hallhagen S, Ekroth R et al. Dopamine and High-dose Insulin infusion (Glucose-Insulin-Potassium) after a cardiac operation. Effects on myocardial metabolism. Ann Thorac Surg 1991; 51:262-270.

7. Hiesmayr M, Haider WJ, Grubhofer G et al. Effects of dobutamine versus insulin on cardiac performance, myocardial oxygen demand, and total body metabolism after coronary artery bypass grafting. J Cardiothorac & Vasc Anesth 1995; 9:653-658.
8. Zhou Z, Lasley RD, Hegge JO et al. Myocardial stunning: a therapeutic conundrum. J Thorac Cardiovas Surg 1995; 110:1391-1400.
9. Maroko PR, Kjekshus JK, Sobel BE et al. Factors influencing infarct size following experimental coronary artery occlusions. Circulation 1971; 43:67-82.
10. Lazar HL, Buckberg GD, Foglia RP et al. Detrimental effects of premature use of inotropic drugs to discontinue cardiopulmonary bypass. J Thorac Cardiovasc Surg 1981; 82:18-25.
11. Lewandowski ED, Ingwall JS. The physiological chemistry of energy production in the heart. In: Schlant RC, Alexander RW, eds. Hurst's The Heart. 8 ed. New York: McGraw-Hill, 1994:153-164.
12. Opie L: Carbohydrates and Lipids. In: The Heart. 1 ed. London: Grune & Stratton, 1984:111-135.
13. Mudge GH, Mills RM, Taegtmeyer H et al. Alterations of myocardial amino acid metabolism in chronic ischemic heart disease. J Clin Invest 1976; 58:1185-1192.
14. Thomassen A, Nielsen TT, Bagger JP et al. Myocardial glutamate and alanine exchanges related to carbohydrate metabolism in patients with normal and stenotic coronary arteries. Clin Physiol 1984; 4:425-434.
15. Taegtmeyer H. Metabolic responses to hypoxia; increased production of succinate by rabbit papillary muscles. Circ Res 1978; 43:808-815.
16. Sanborn T, Gavin W, Berkowitz S et al. Augmented conversion of aspartate and glutamate to succinate during anoxia in rabbit heart. Am J Physiol 1979; 237:H535-H541.
17. Rau EE, Shine KI, Gervais A et al. Enhanced mechanical recovery of anoxic and ischemic myocardium by amino acid perfusion. Am J Physiol 1979; 236:H873-H879
18. Pisarenko OI, Baranov AV, Pomerantsev EV et al. Myocardial metabolism of glutamate and left ventricular function in patients with coronary arterial disease. Int J Cardiol 1989; 23:43-52.
19. Safer B. The metabolic significance of the malate-aspartate cycle in heart. Circ Res 1975; 37:527-533.
20. Pisarenko OI, Oleynikov OD, Shulzhenko VS et al. Association of myocardial glutamate and aspartate pool and functional recovery of postischemic heart. Biochem Med Metab Biol 1989; 42:105-117.
21. Berggren H, Ekroth R, Herlitz J et al. Improved myocardial protection during cold cardioplegia by means of increased myocardial glycogen stores. Thorac Cardiovasc Surg 1982; 30:389-392.
22. Lolley DM, Ray JFI, Myers WO et al. Importance of preoperative myocardial glycogen levels in human cardiac preservation. Preliminary report. J Thorac Cardiovasc Surg 1979; 78:678-687.
23. Oldfield GS, Commerford PJ, Opie LH. Effects of preoperative glucose-insulin-potassium on myocardial glycogen levels and on complications of mitral valve replacement. J Thorac Cardiovasc Surg 1986; 91:874-878.
24. Thomassen A, Nielsen TT, Bagger JP et al. Antianginal and cardiac metabolic effects of low-dose glucose infusion during pacing in patients with and without coronary artery disease. Am Heart J 1989; 118:25-32.
25. Thomassen A, Nielsen TT, Bagger JP et al. Antiischemic and metabolic effects of glutamate during pacing in patients with stable angina pectoris secondary to either coronary artery disease or syndrome X. Am J Cardiol 1991; 68:291-295.
26. Svedjeholm R, Vanhanen I, Håkanson E et al. Metabolic and hemodynamic effects of intravenous glutamate infusion early after coronary surgery. J Thorac Cardiovasc Surg 1996; 112:1468-1477.
27. Engelman RM, Rousou JA, Flack JE et al. Reduction of infarct size by systemic amino acid supplementation during reperfusion. J Thorac Cardiovasc Surg 1991; 101:855-859.
28. Maroko PR, Libby P, Sobel BE et al. Effect of Glucose-Insulin-Potassium infusion on myocardial infarction following experimental coronary artery occlusion. Circulation 1972; 45:11601175
29. Simonsen S, Kjekshus JK. The effect of free fatty acids on myocardial oxygen consumption during atrial pacing and catecholamine infusion in man. Circulation 1978; 58: 484-491.
30. Vik-Mo H, Mjos OD. Influence of free fatty acids on myocardial oxygen consumption and ischemic injury. Am J Cardiol 1981; 48: 361-365.
31. Liedtke AJ, Nellis S, Neely JR. Effects of excess free fatty acids on mechanical and metabolic function in normal and ischemic myocardium in swine. Circ Res 1978; 43: 652-661.
32. Henderson AH, Craig RJ, Gorlin R et al. Free fatty acids and myocardial function in perfused rat hearts. Cardiovasc Res 1970; 4: 466-472.
33. Borst P, Loos JA, Christ EJ et al. Uncoupling activity of long-chain fatty acids. Biochim Biophys Acta 1962; 62:509-518.

34. Lucas SK, Kanter KR, Schaff HV et al. Reduced oxygen extraction during reperfusion: A consequence of global ischemic arrest. J Surg Res 1980; 28:434-441.
35. Kanter KR, Glower DD, Schaff HV et al. Mechanism of defective oxygen extraction following global ischemia. J Surg Res 1981; 30:482-488.
36. Lazar HL, Buckberg GD, Manganaro AJ et al. Reversal of ischemic damage with amino acid substrate enhancement during reperfusion. Surgery 1980; 80:702-709.
37. Peuhkurinen KJ. Regulation of the tricarboxylic acid pool size in heart muscle. J Mol Cell Cardiol 1984; 16:487-495.
38. Pisarenko O, Studneva I, Khlopkov V et al. An assessment of anaerobic metabolism during ischemia and reperfusion in isolated guinea pig heart. Biochim Biophys Acta 1988; 934:55-63.
39. Kobayashi K, Neely JR. Effects of ischemia and reperfusion on pyruvate dehydrogenase activity in isolated rat hearts. J Mol Cell Cardiol 1983; 15:359-367.
40. Teoh KH, Mickle DAG, Weisel RD et al. Decreased postoperative myocardial free fatty acid oxidation. J Surg Res 1988; 44:36-44.
41. Svensson S, Svedjeholm R, Ekroth R et al. Trauma metabolism and the heart. Uptake of substrates and effects of insulin early after cardiac operations. J Thorac Cardiovasc Surg 1990; 99:1063-1073.
42. Svedjeholm R, Vanhanen I, Hakanson E et al. Metabolic and hemodynamic effects of intravenous glutamate infusion early after coronary operations. J Thorac Cardiovas Surg 1996; 112:1468-1477.
43. Svedjeholm, R. Myocardial uptake of substrates early after cardiac surgery. A clinical study of metabolic derangement and recovery with special reference to effects of dopamine and insulin. 7-54. 1990. Acta Universitatis Upsaliensis. Thesis/Dissertation
44. Safer B, Williamson JR. Mitochondrial-cytosolic interactions in perfused rat heart. Role of coupled transamination in repletion of citric acid cycle intermediates. J Biol Chem 1973; 248:2570-2579.
45. Svensson S, Ekroth R, Milocco I et al. Glucose and lactate balances in heart and leg after coronary surgery: influence of insulin infusion. Scand J Thorac Cardiovasc Surg 1989; 23:145-150.
46. Svedjeholm R, Ekroth R, Joachimsson PO et al. Myocardial uptake of amino acids and other substrates in relation to myocardial oxygen consumption four hours after cardiac surgery. J Thorac Cardiovasc Surg 1991; 101:688-694.
47. Apstein CS, Gravino FN, Haudenschild CC. Determinants of a protective effect of glucose and insulin on the ischemic myocardium. Effects on contractile function, diastolic compliance, metabolism, and ultrastructure during ischemia and reperfusion. Circ Res 1983; 52:515-526.
48. Rackley CE, Russel RO, Rogers JW et al. Glucose-Insulin-Potassium in acute myocardial infarction. Ann Rev Med 1982; 33: 375-383.
49. Lopaschuk GD, Stanley WC. Glucose metabolism in the ischemic heart [editorial comment]. [Review] [22 refs]. Circulation 1997; 95:313-315.
50. Wheeler TJ, Fell RD, Hauck MA. Translocation of two glucose transporters in heart: effects of rotenone, uncouplers, workload, palmitate, insulin and anoxia. Biochim Biophys Acta 1994; 1196:191-200.
51. McNulty PH, Sinusas AJ, Shi CQ et al. Glucose metabolism distal to a critical coronary stenosis in a canine model of low-flow myocardial ischemia. J Clin Invest 1996; 98: 62-69.
52. Zaninetti D, Greco-Perotto R, Assimacopoulos-Jeannet F et al. Effects of insulin on glucose transport and glucose transporters in rat heart. Biochem J 1988; 250: 277-283.
53. Stanley WC, Lopaschuk GD, Hall JL et al. Regulation of myocardial carbohydrate metabolism under normal and ischaemic conditions. Potential for pharmacological interventions. [Review] [184 refs]. Cardiovasc Res 1997; 33:243-257.
54. Ekroth R, Berggren H, Björntorp J et al. Effect of valvular aortic stenosis on insulin sensitivity. Scand J Thorac Cardiovasc Surg 1982; 16:141-144.
55. Nilsson F, Ekroth R, Milocco I et al. Splanchnic glucose balance and insulin resistance in the early postoperative phase of cardiac surgery. JPEN J Parenter Enteral Nutr 1988; 12:574-578.
56. Zaninetti D, Greco-Perotto R, Jeanrenaud B. Heart glucose transport and transporters in rat heart: regulation by insulin, workload and glucose. Diabetologia 1988; 31:108-113.
57. Nilsson F, Bake B, Berglin E et al. Glucose and insulin infusion directly after cardiac surgery: effects on systemic glucose uptake, catecholamine excretion, O2 consumption, and CO2 production. JPEN J Parenter Enteral Nutr 1985; 9:159-164.
58. Fischer Y, Bottcher U, Eblenkamp M et al. Glucose transport and glucose transporter GLUT4 are regulated by product(s) of intermediary metabolism in cardiomyocytes. Biochemical Journal 1997; 321:629-638.

59. Scheuer J, Stezoski SW. Protective role of increased myocardial glycogen stores in cardiac anoxia in the rat. Circ Res 1970; 27:835-849.
60. Wiese S, Askanazi J. Glucose/Insulin/Potassium therapy: A reevaluation of myocardial benefits during cardiopulmonary bypass. J Cardiothorac Vasc Anesth 1992; 6:517-520.
61. Girard C, Quentin P, Bouvier H et al. Glucose and insulin supply before cardiopulmonary bypass in cardiac surgery: A double-blind study. Ann Thorac Surg 1992; 54:259-263.
62. Haider W, Eckersberger F, Losert U et al. Improved myocardial protection in open heart surgery by massive preoperative insulin supply. Anaesthesist 1982; 31:124-128.
63. Wistbacka JM, Paivi KK, Nuutinen LS. Prebypass glucose-insulin-potassium infusion in elective non-diabetic coronary artery surgery patients. J Cardiothorac Vasc Anesth 1992; 6:517-520.
64. Nilsson F, Ekroth R, Milocco I et al. The effect of insulin on the leg and splanchnic balance of glucose and gluconeogenic substrates after cardiac surgery. Thorac Cardiovasc Surg 1988; 36:343-347.
65. Brodin LA, Dahlgren G, Ekestrom S et al. Influence of glucose-insulin-potassium on left ventricular function during coronary artery bypass grafting. Scand J Thorac Cardiovasc Surg 1993; 27:27-34.
66. Lazar HL, Philippides G, Fitzgerald C et al. Glucose-insulin-potassium solutions enhance recovery after urgent coronary artery bypass grafting. J Thorac Cardiovas Surg 1997; 113:354-60; discussion 360-2.
67. Svensson SE, Berglin-W-OE, Ekroth R, Milocco I et al. Haemodynamic effects of a single large dose of insulin in open heart surgery. Cardiovasc Res 1984; 18:697-701.
68. Haider W, Benzer H, Coraim F et al. Postoperative therapy by means of acute parenteral alimentation (APA) with high doses of insulin and glucose after open heart surgery (author's transl). Anaesthesist 1981; 30:53-63.
69. Lucchesi BR, Median M, Kniffen FJ. The positive inotropic action of insulin in the canine heart. Eur J Pharmacol 1972; 18: 107-115.
70. Reikerås O, Gunnes P, Sörlie D et al. Haemodynamic effects of high doses of insulin during acute left ventricular failure in dogs. Eur Heart J 1985; 6:451-457.
71. Svedjeholm R, Ekroth R, Joachimsson PO et al. High-dose insulin improves the efficacy of dopamine early after cardiac surgery. A study of myocardial performance and oxygen consumption. Scand J Thorac Cardiovasc Surg 1991; 25:215-221.
72. Svensson S, Ekroth R, Nilsson F et al. Insulin as a vasodilating agent in the first hour after cardiopulmonary bypass. Scand J Thorac Cardiovasc Surg 1989; 23:139-143.
73. Gradinac S, Coleman GM, Taegtmeyer H et al. Improved cardiac function with Glucose-Insulin-Potassium after aortocoronary bypass grafting. Ann Thorac Surg 1989; 48:484-489.
74. Hall JL, Stanley WC, Lopaschuk GD et al. Impaired pyruvate oxidation but normal glucose uptake in diabetic pig heart during dobutamine-induced work. Am J Physiol 1996; 271:H2320-H2329.
75. Malmberg K, Ryden L, Efendic S et al. Randomized trial of insulin-glucose infusion followed by subcutaneous insulin treatment in diabetic patients with acute myocardial infarction (DIGAMI study): effects on mortality at 1 year. J Am Coll Card 1995; 26:57-65.
76. Teoh KH, Mickle DAG, Weisel RD et al. Improving myocardial metabolic and functional recovery after cardiac arrest. J Thorac Cardiovasc Surg 1988; 95:788-798.
77. Lasley RD, Bunger R, Zhou Z et al. Metabolically based treatment of stunned myocardium. J Card Surg 1994; 9:469-473.
78. Gailis L, Benmouyal E. Endogenous alanine, glutamate, aspartate and glutamine in the perfused guinea pig heart: effect of substrates and cardioactive agents. Can J Biochem 1973; 51:11-20.
79. Bittl J, Shine K. Protection of ischemic rabbit myocardium by glutamic acid. Am J Physiol 1983; 245:H406-H412
80. Burns AH, Reddy WJ. Amino acid stimulation of oxygen and substrate utilization by cardiac myocytes. Am J Physiol 1978; 235: E461-E466.
81. Pisarenko OI. Regulation of cardiac metabolism by amino acids in ischemia and hypoxia. Sov Med Rev A Cardiol 1989; 2:133-167.
82. Svedjeholm R, Svensson SE, Ekroth R et al. Trauma metabolism and the heart; studies of heart and leg amino acid flux after cardiac surgery. Thorac Cardiovasc Surg 1990; 38:1-5.
83. Schwalb H, Izhar U, Yaroslavsky E et al. The effect of amino acids on the ischemic heart. Improvement of oxygenated crystalloid cardioplegic solution by an enriched branched amino acid formulation. J Thorac Cardiovasc Surg 1989; 98:551-556.
84. Pisarenko OI. Mechanisms of myocardial protection by amino acids: facts and hypotheses. [Review] [88 refs]. Clin & Exper Pharmacol & Physiol 1996; 23: 627-633.

85. Morita K, Sherman MP, Buckberg GD et al. Studies of hypoxemic/reoxygenation injury: Without aortic clamping. V. Role of the L-arginine-nitric oxide pathway: the nitric oxide paradox. J Thorac Cardiovas Surg 1995; 110:1200-1211.
86. Sato H, Zhao ZQ, McGee DS et al. Supplemental L-arginine during cardioplegic arrest and reperfusion avoids regional postischemic injury. J Thorac Cardiovas Surg 1995; 110:302-314.
87. Sanborn T, Gavin W, Berkowitz S et al. Augmented conversion of aspartate and glutamate to succinate during anoxia in rabbit heart. Am J Physiol 1979; 237:H535-H541
88. Haas GS, DeBoer LWV, O'Keefe DD et al. Reduction of postischemic myocardial dysfunction by substrate repletion during reperfusion. Circulation 1984; 70(suppl I):65-74.
89. Lazar HL, Buckberg GD, Manganaro AJ et al. Myocardial energy replenishment and reversal of ischemic damage by substrate enhancement of secondary blood cardioplegia with amino acids during reperfusion. J Thorac Cardiovasc Surg 1980; 80:350-359.
90. Weldner PW, Myers JL, Miller CA et al. Improved recovery of immature myocardium with L-glutamate blood cardioplegia. Ann Thorac Surg 1993; 55:102-105.
91. Engelman RM, Dobbs WA, Rousou JH et al. Myocardial high-energy phosphate replenishment during ischemic arrest: aerobic versus anaerobic metabolism. Ann Thorac Surg 1982; 33:453-458.
92. Kimose HH, Helligso P, Randsbaek F et al. Improved recovery after crystalloid cardioplegia using low-dose glutamate enrichment during reperfusion after aortic unclamping: a study in isolated blood-perfused pig hearts. Thorac Cardiovasc Surg 1996; 44:118-125.
93. Beyersdorf F, Kirsh M, Buckberg GD et al. Warm glutamate/aspartate-enriched blood cardioplegic solution for perioperative sudden death. J Thorac Cardiovasc Surg 1992; 104:1141-1147.
94. Allen BS, Buckberg GD, Schwaiger M et al. Early recovery of regional wall motion in patients following surgical revascularization after eight hours of acute coronary occlusion: Published erratum appears in J Thorac Cardiovasc Surg 1987 Jan;93(1):140. J Thorac Cardiovasc Surg 1986; 92:636-648.
95. Rosenkranz ER, Buckberg GD, Laks H et al. Warm induction of cardioplegia with glutamate-enriched blood in coronary artery patients with cardiogenic shock who are dependent on inotropic drugs and intra-aortic balloon support. J Thorac Cardiovasc Surg 1983; 86:507-518.
96. Pisarenko OI, Portnoy VF, Studneva IM et al. Glutamate-blood cardioplegia improves ATP preservation in human myocardium. Biomed Biochim Acta 1987; 46:499-504.
97. Kjellman UW, Björk K, Ekroth R et al. Addition of alpha-ketoglutarate to blood cardioplegia improves cardioprotection. Ann Thorac Surg 1997; 63:1625-1634.
98. Low SY, Rennie MJ, Taylor PM. Sodium-dependent glutamate transport in cultured rat myotubes increases after glutamine deprivation. FASEB J 1994; 8:127-131.
99. Suleiman MS, Chapman RA. Changes in the principal free intracellular amino acids in the Langendorff perfused guinea pig heart during arrest with calcium-free or high potassium media. Cardiovasc Res 1993; 27:1810-1814.
100. Kimose HH, Ravkilde J, Helligsö P et al. Myocardial loss of glutamate after cold chemical cardioplegia and storage in isolated blood-perfused pig hearts. Thorac Cardiovasc Surg 1993; 41:93-100.
101. Bical OM, Gerhardt MF, Paumier D et al. Effects of two different crystalloid cardioplegic solutions assessed by myocardial pH, tissue lactate content and energy metabolism. Eur J Cardiothorac Surg 1996; 10:417-421.
102. Reed MK, Barak C, Malloy CR et al. Effects of glutamate and aspartate on myocardial substrate oxidation during potassium arrest [see comments]. J Thorac Cardiovas Surg 1996; 112:1651-1660.
103. Wistbacka J-OM, Lepojärvi MVK, Karlqvist KEV et al. Amino acid-enriched glucose-insulin-potassium infusion improves hemodynamic function after coronary surgery. Infusions Ther Transfusions Med 1995; 22:82-90.
104. Thomassen A, Bötker HE, Nielsen TT et al. Effects of glutamate on exercise tolerance and circulating substrate levels in stable angina pectoris. Am J Cardiol 1990; 65:173-178.
105. Singh J, Garg KN, Garg D et al. Effect of aspartate and glutamate on experimental myocardial infarction in rats. Indian J Exp Biol 1989; 27:621-624.
106. Beyersdorf F, Acar C, Buckberg GD et al. Studies on prolonged acute regional ischemia. V. Metabolic support of remote myocardium during left ventricular power failure. J Thorac Cardiovasc Surg 1989; 98:567-579.
107. Pisarenko OI, Lepilin MG, Ivanov VE. Cardiac metabolism and performance during L-glutamic acid infusion in postoperative cardiac failure. Clin Science 1986; 70:7-12.
108. Rosenkranz ER, Okamoto F, Buckberg GD et al. Safety of prolonged aortic clamping with blood cardioplegia. III. Aspartate enrichment of glutamate-blood cardioplegia in energy-depleted hearts after ischemic and reperfusion injury. J Thorac Cardiovasc Surg 1986; 91:428-435.

109. Pisarenko OI, Rosenfeldt FL, Langley L et al. Differing protection with aspartate and glutamate cardioplegia in the isolated rat heart. Ann Thorac Surg 1995; 59:1541-1548.
110. Dinkelborg LM, Kinne RK, Grieshaber MK. Characterization and pH dependence of L-glutamate transport in sarcolemmal vesicles from rat hearts. Am J Physiol 1995; 268: H194-201.
111. Daabees TT, Finkelstein MW, Stegink LD et al. Correlation of glutamate plus aspartate dose, plasma amino acid concentration and neuronal necrosis in infant mice. Food Chem Toxicol 1985; 23:887-893.
112. Thomassen AR. Myocardial uptake and effects of glutamate during non-ischaemic and ischaemic conditions. A clinical study with special reference to possible interrelationships between glutamate and myocardial utilization of carbohydrate substrates. Dan Med Bull 1992; 39:471-488.
113. Loop FD, Higgins TL, Panda R et al. Myocardial protection during cardiac operations: Decreased morbidity and lower cost with blood cardioplegia and coronary sinus perfusion. J Thorac Cardiovasc Surg 1992; 104: 608-618.
114. Schaumburg HH, Byck R, Gerstl R et al. Monosodium L-glutamate: Its pharmacology and role in the "Chinese restaurant syndrome". Science 1969; 163:826-828.
115. Kenney RA, Tidball CS. Human susceptibility to oral monosodium L-glutamate. Am J Clin Nutr 1972; 25:140-146.
116. Knapp WH, Helus F, Ostertag H et al. Uptake and turnover L-(13N)-glutamate in the normal human heart and in patients with coronary artery disease. Eur J Nucl Med 1982; 7:211-215.
117. Håkanson E, Svedjeholm R, Vanhanen I. Physiologic aspects in postoperative cardiac patients. Ann Thorac Surg 1995; 59:S12-4.
118. Svedjeholm R, Ekroth R, Joachimsson PO et al. High dose insulin improves the efficacy of dopamine early after cardiac surgery. A study of myocardial performance and oxygen consumption. Scand J Thorac Cardiovasc Surg 1991; 25:215-221.
119. Svedjeholm R, Huljebrant I, Håkanson E et al. Glutamate and high-dose glucose-insulin-potassium (GIK) in the treatment of severe cardiac failure after cardiac operations. Ann Thorac Surg 1995; 59:S23-S30
120. Pissarek M, Goos H, Nohring J et al. Beneficial effect of combined glucose-insulin-potassium and mechanical support in acute myocardial ischaemia. Biomedica Biochimica Acta 1986; 45:629-636.
121. Creswell LL, Rosenbloom M, Cox JL et al. Intraaortic balloon counterpulsation: patterns of usage and outcome in cardiac surgery patients. Ann Thorac Surg 1992; 54:11-20.
122. Allen BS, Okamoto F, Buckberg GD et al. Reperfusion conditions: critical importance of total ventricular decompression during regional reperfusion. J Thorac Cardiovasc Surg 1986; 92:605-612.
123. Waldenberger FR, Andres J, Flameng W. Effects of unloaded reperfusion on mitochondrial function in the postischemic myocardium. Artif Organs 1994; 18:206-213.
124. Herliz J, Elmfeldt D, Hjalmarson Å et al. Effect of metoprolol on indirect signs of the size and severity of acute myocardial infarction. Am J Cardiol 1983; 51:1282-1288.
125. Dupuis JY, Bondy R, Cattran C et al. Amrinone and dobutamine as primary treatment of low cardiac output syndrome following coronary artery surgery: a comparison of their effects on hemodynamics and outcome. Journal of Cardiothoracic & Vascular Anesthesia 1992; 6:542-553.
126. Andrews R, Cowley AJ. Phosphodiesterase inhibitors. Do the risks outweight the benefits?. [Review] [38 refs]. Drug Safety 1993; 9:404-409.
127. Wesslén, Ö. β_1-blockade early after coronary surgery. A hemodynamic, metabolic and electrophysiologic study with special reference to effects of metoprolol. 7-45. 1990. Acta Universitatis Upsaliensis. Thesis/Dissertation
128. Weinstein ES, Spector ML, Adams EM et al. Mitochondrial and myocardial performance. Response to ischemia and reperfusion. Arch Surg 1986; 121:324-329.
129. Andersson LG, Ekroth R, Bratteby LE et al. Acute renal failure after coronary surgery—a study of incidence and risk factors in 2009 consecutive patients. Thorac Cardiovasc Surg 1993; 41:237-241.
130. Frost L, Pedersen RS, Lund O et al. Prognosis and risk factors in acute, dialysis-requiring renal failure after open-heart surgery. Scand J Thorac Cardiovasc Surg 1991; 25:161-166.
131. Jeppsson A, Ekroth R, Kirno K et al. Insulin and amino acid infusion after cardiac operations: effects on systemic and renal perfusion. J Thorac Cardiovas Surg 1997; 113:594-602.

Section IV:
Surgical Techniques
A. Temperature of Blood Cardioplegia

CHAPTER 13

Cold/Tepid Cardioplegia

Hendrick B. Barner and Andrew C. Fiore

Hypothermia has been an essential component of myocardial protection for cardiac operations since the beginning.[1] The evolution of cardioplegia from crystalloid solutions of intracellular composition to those of extracellular composition and finally to blood based solutions has had the common theme of cardiac and systemic hypothermia.[2]

The focus of many investigators has been that of reducing myocardial metabolism to the lowest possible level during the ischemic interval so that myocardial energy stores (adenosine triphosphate and glycogen) are maintained and tissue acidosis is avoided during the ischemic interval. Myocardial oxygen consumption of the working heart is 10-12 ml/100 g/min and of the nonworking vented heart 6-8 ml/100 g/min.[3] The potassium paralyzed heart has a myocardial oxygen consumption of 0.31 ml/100 g/min at 22°C[3] which is reduced to 0.135 ml/100 g/min at 10-12°C.[4]

The optimal degree of hypothermia for intraoperative myocardial protection has never been determined. Myocardial hypothermia to 0.5°C in the absence of ischemia does not appear to be harmful to the heart.[5] St. Thomas' solution provided optimal protection to the isolated rat heart at 12°C but the advantage over 25°C was not statistically significant.[6,7] Tyers and associates[8] found that myocardial recovery after crystalloid cardioplegia was best when the heart was maintained at 10-15°C, but Flaherty and co-workers found that 10-20°C was no better than 27°C for protecting the myocardium during one hour of crystalloid cardioplegia.[9] Since the introduction of cardioplegia it has been common clinical practice to use systemic hypothermia of 25-30°C and cardioplegia temperature of 4-12°C with topical cardiac cooling to achieve a myocardial temperature of 10-20°C. Many surgeons have believed and practiced the principle that "the colder the heart the better". Thus, the introduction of normothermic (warm) blood cardioplegia in 1989 represented a sudden, dramatic shift in the strategy for intraoperative myocardial protection and has resulted in a reassessment of the need for myocardial and systemic hypothermia during cardioplegia.[10-12] Despite enthusiasm for and success with warm heart operations some clinical and experimental studies have indicated potential limitations of normothermia and it has been proposed that an intermediate temperature (tepid) for the body and the heart may be preferable.[12,13] "Tepid" was defined as a cardioplegia temperature of 29°C which resulted in a myocardial temperature of 28.4°C[13] or 29.2°C[14] while systemic temperature drifted to 33 ± 1°C. Others have considered "tepid" as 32°C for blood cardioplegia and systemic temperature. Therefore, this chapter broadly defines tepid as 28-32°C for cardioplegia, cardiac and systemic temperatures.

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Goals of Cardioplegia

Myocardial Protection

The primary goal of cardioplegia is that of preservation of the heart during conduct of a cardiac operation which requires a quiet field (absence of mechanical activity) and good visualization (blood free). Until recently the major focus has been that of preserving myocyte contractility to prevent pump failure which includes conserving cell energy by reducing metabolism to a low level which allows continued support of vital cell activities such as ion pumping to maintain the internal millieu. New knowledge about endothelial function and its pivotal role in the behavior of the microvasculature as well as reperfusion injury has shifted the investigative focus toward this area. Not only is there current interest in the cardiac and systemic temperature during cardioplegia but also in the effect of cardioplegia on the endothelium. Thus, endothelial preservation may be as important as myocyte preservation.

The excellent clinical results attained with warm blood cardioplegia have suggested that earlier observations on impairment of some cell functions by hypothermia may be more relevant than previously thought. These include reduced: membrane stability;[15] ability to utilize glucose,[16] and fatty acids;[17] mitochondrial generation of adenosine triphosphate[18] leading to depressed cell membrane function;[19] activity of the adenosine triphosphatase system,[20] leading to impaired cell volume regulation[21,22] and decreased ability of the sarcoplasmic reticulum to bind calcium;[23] mitochondrial state respiration and activity of citrate synthetase;[24] control of intracellular pH;[25] and activity of the sarcoplasmic reticulum with regard to calcium uptake.[23,26]

Current Research

Ventricular Function

Reperfusion recovery of global left ventricular function as determined by the slope of the preload recruitable strike work relationship after 30, 60 and 90 minutes of reperfusion was comparable after 90 minutes of single coronary occlusion followed by 60 minutes of continuous retrograde blood cardioplegia at 18°C or 28°C with the same systemic temperature.[27] Systolic ventricular function (maximum elastance) and diastolic function (stress-strain relationship) showed comparable recovery in the same two groups.[27]

Left ventricular stroke work indices were better in the tepid (29°C) than in the cold (4°C) blood cardioplegia groups after intermittent antegrade and continuous retrograde infusion when measured at one and four hours after cardiopulmonary bypass. Following 60 ± 3 minutes (cold) or 54 ± 4 minutes (tepid) of aortic cross clamping.[14]

We measured left ventricular stroke work index after 78 minutes of 4°C or 70 minutes of 29°C intermittent antegrade blood cardioplegia and found that at 12 hours postoperatively tepid cardioplegia was associated with a significantly ($p < .05$) better index and that at 24 hours the stroke work index in the tepid group had exceeded the pre-bypass value ($p < .05$) whereas the cold group had not.[28]

In these same patients we assessed left ventricular function by volume loading after termination of cardiopulmonary bypass and measurement with transesophageal echocardiographic sagittal and transverse images to allow calculation of the ejection fraction which increased significantly over the control value in the tepid group ($p < .03$) but not in the cold group.[28]

Myocardial Metabolism

Oxygen Consumption

During clinical intermittent antegrade combined with continuous retrograde blood cardioplegia myocardial oxygen consumption during antegrade infusion was greater for tepid (29°C) than for cold (9°C) ($p < .05$) cardioplegia (3.8 vs 1.5 ml/min).[14] Immediately after cross-clamp release there was an increase in myocardial oxygen extraction which continued to increase 10 minutes later and 10 minutes after discontinuation of bypass with no difference between the two groups.[14]

We measured myocardial oxygen extraction immediately after cross-clamp release and also found no difference between 78 minutes with 4°C and 70 minutes with 29°C intermittent antegrade blood cardioplegia although both groups had depressed oxygen uptake compared to control.[28] After five minutes of reperfusion oxygen consumption had returned to pre-cross clamp levels in both groups and exceeded these values ten minutes after termination of cardiopulmonary bypass (p=ns).

Myocardial oxygen consumption during the first hour of reperfusion after 90 minutes of single coronary occlusion followed by 60 minutes of continuous retrograde blood cardioplegia at 18°C or 28°C was 5.4 ± 1.4 ml 02/min/100g and 4.7 ± 1.1 ml 02/min/100g (p=ns) respectively.[27]

Lactate Release

During clinical intermittent antegrade and continuous retrograde blood cardioplegia lactate release during antegrade infusion was greater for tepid (29°C) than for cold (9°C) (0.1 vs 0.03 μmol/min, p<.05) hearts.[14] With cross-clamp release there was no difference in lactate production between cold and tepid cardioplegia. Ten minutes later and 10 minutes after bypass was discontinued there was comparable lactate uptake in both groups.

We observed significant lactate washout with cross-clamp release in the tepid group, when comparing 4°C and 29°C intermittent antegrade blood cardioplegia (0.84 ± 014 vs 2.2 ± 1.2 μmol/L, (p=0.0001).[28] Ten minutes later hearts receiving 4°C cardioplegia were extracting lactate while tepid hearts continued to release lactate (p=ns). Ten minutes after discontinuation of cardiopulmonary bypass lactate consumption was present in the tepid group and continued in the cold group (p=ns).

Acid Release

Acid release during intermittent antegrade and continuous retrograde blood cardioplegia was greater for tepid (29°C) than cold cardioplegia (9°C) (p < .05).[14] Immediately after cross-clamp release acid production was similar for tepid and cold hearts but reduced over pre-cross-clamp measurements while 10 minutes later and 10 minutes after discontinuation of bypass acid production was comparable to that measured before aortic clamping.[14]

We found that acid production with cross-clamp release was significantly greater in hearts receiving tepid (29°C) intermittent antegrade blood cardioplegia than in these receiving 4°C. cardioplegia (4.5 ± 0.9 vs 0.46 ± 1.1 μmol/L, p < .05).[28] The cold group released less acid than prior to cross-clamping (control 6.1 ± 0.9 μmol/L, p < .05) whereas the warm group released a comparable amount (5.0 ± 0.6 μmol/L). At subsequent times (5 and 10 minutes after cross-clamp release and 10 minutes after termination of cardiopulmonary bypass) acid release was comparable for each group and not significantly different from control.

Sarcoplasmic Reticulum

Antegrade cold blood (6-10°C) cardioplegia infused intermittently for two hours resulted in impaired sarcoplasmic reticulum calcium uptake (p < .05), and reduced activity of calcium-adenosine triphosphatase (p < .05) compared to continuous normothermic (37°C) blood cardioplegia.[26] This difference only became significant after 60 minutes of reperfusion. The effect of tepid cardioplegia on sarcoplasmic reticulum was not determined.

Myocardial Enzyme Release

The total release of creatinine kinase—myocardial band (CK-MB) in the 48 hours after operation was similar for intermittent antegrade/continuous retrograde 9°C or 29°C blood cardioplegia.[14] We found that total CK-MB release in the 24 hours after operation was greater for 4°C than for 29°C intermittent antegrade blood cardioplegia (1120 ± 141 vs 767 ± 89 U x h/L; p < .04).[28] In a third report peak CK-MB was similar for intermittent antegrade/continuous retrograde blood cardioplegia at 8-10°C (cold) or 32°C (tepid).[29]

Electrical Stability

After continuous blood cardioplegia at 18°C or 28°C there was a trend for more countershocks (1.8 ± 1.2 vs 0.6 ± 0.5, p = 0.07) to restore an organized cardiac rhythm in the colder group.[27] We also found that electrical defibrillation was more common after 4°C blood cardioplegia than after 29°C blood cardioplegia (0.74 ± 0.9 vs 0.08 ± .27, defibrillations per patient p<.001).[28]

Inotropic Support

We observed a greater need for inotropic drug infusion in the postoperative interval in hearts protected with 4°C versus 29°C intermittent antegrade blood cardioplegia (9/25 vs 2/27; p<.05).[28]

Neurologic Dysfunction

Hypothermia has been the traditional means of brain protection during cardiopulmonary bypass since its inception. The current interest in warm heart surgery has prompted reassessment of neurologic function after cardiopulmonary bypass. In some reports maintenance of normothermia during cardiopulmonary bypass has been associated with increased risk of neurologic or neuropsychologic dysfunction[30-32] while others have reported no increased risk of adverse neurologic outcome.[33-35] In one report tepid (32°) cardioplegia and body temperature had the lowest (2%) incidence of abnormal neurologic examinations prompting computed tomographic scanning while normothermia had an intermediate incidence (9.3%) and cold (8°-10°C) had the highest (18.9%) (p=ns).[29] Postoperative cognitive function was similar for patients maintained at 34°-35°C (n=30) or at 28°C (n=24) with both groups receiving intermittent cold (4°C) blood cardioplegia.[36]

The data of Mora et al suggest that if the systemic temperature is maintained at 35°C or more by warming during cardiopulmonary bypass there is an increased risk of neurologic events after coronary bypass grafting.[31] Also this study and the one by Regragui and associates observed a higher incidence of neuropsychologic deficit after normothermic versus tepid or hypothermic cardiopulmonary bypass.[32] To maintain a body temperature of 35°-37°C requires active warming during cardiopulmonary bypass which may achieve a perfusate temperature greater than 37°C and initiate or exacerbate neuronal injury. Temperature management strategy during cardiopulmonary bypass did not influence performance on postoperative neuropsychological tests.[31]

At this time there is not sufficient information to indicate the optimum perfusion temperature to minimize the potential for adverse neurologic outcome. Hyperthermic perfusion (active warming) during cardiopulmonary bypass is inappropriate and a tepid temperature during perfusion may be more promising.[29,31]

Fibrinolytic Potential

Engelman and others determined that there is an increased potential for fibrinolysis in patients receiving warm (37°C) blood cardioplegia as opposed to those receiving tepid (32°C) or cold (20°C) blood cardioplegia.[29]

Summary

Both clinical studies[14,28] observed better left ventricular function after tepid blood cardioplegia; one report was at one and four hours post cardiopulmonary bypass, which were the only intervals studied, and the other at 12 and 24 hours but not at earlier intervals.

The data[14,28] on oxygen consumption, lactate uptake (and release) and acid production suggests that with tepid cardioplegia myocardial metabolic activity is greater than with cold cardioplegia. Intermittent infusion of cardioplegia is associated with greater washout of lactate and acid with reperfusion in the tepid group indicative of greater metabolic (anaerobic) activity, but with rapid return of metabolic activity to control levels with reperfusion.

Only our report[28] found reduced release of CK-MB following operation in the tepid group compared to 4°C cardioplegia. Electrical

stability was greater and the need for inotropic drugs reduced in clinical tepid cardioplegia.[28]

Future Applications

Since 1990 there have been striking changes in the utilization of cardioplegia involving route of delivery (antegrade/retrograde), mode (intermittent/continuous) temperature (tepid/normothermic) and to a lesser degree of composition. Some surgeons have adapted normothermic or mildly hypothermic (tepid) cardioplegia with a parallel systemic temperature. At this time it is unclear whether a consensus will be reached regarding the ideal temperature or optimal management of the other parameters. It is likely that there will be continued assessment of the changes initiated in this interval, with the result that new information will continue to be assimilated into the arena of cardioplegia as we seek to achieve the best possible myocardial preservation with the fewest systemic complications.

New Research

Current interest in the microcirculation and the apparent major role of the endothelium in influencing cardiac tolerance to ischemia/reperfusion will include examination of the role of temperature on endothelial function in relation to cardioplegia. It appears that 4°C blood cardioplegia or crystalloid cardioplegia with albumin is not associated with endothelial injury[37] whereas crystalloid cardioplegia without albumin results in an immediate injury which was reversible in one study.[38]

Normothermic myocardial ischemia (45 minutes) did not result in reduced vascular responses to acetylcholine nor did the addition of one hour of intermittent 4°C antegrade blood cardioplegia, but if either event was followed by reperfusion then vascular relaxation was impaired.[39] Addition of nitric oxide donor agent (SPM-5185) in low (1 μmol/L) or high (10 μmol/L) dose to the blood cardioplegia after 30 minutes of normothermic myocardial ischemia followed by 60 minutes of 4°C intermittent cardioplegic infusion preserved left ventricular function in the high dose group.[40] In coronary arteries isolated from these hearts endothelium-dependent maximum relaxation was completely preserved in the high dose group, 18% impaired in the low dose group and 27% impaired in the control group.[40] Additionally, neutrophil accumulation in the postischemic myocardium was elevated in the control and low dose groups, but significantly reduced in the high dose group. Using a similar model with 90 minutes of regional ischemia but with inhibition of basal endothelial nitric oxide release by adding L-nitro-arginine to the blood cardioplegia there was increased postischemic/reperfusion injury associated with neutrophil adherence.[41] Thus, cold blood cardioplegia does not appear to be associated with endothelial injury nor does continuous antegrade normothermic blood cardioplegia[42] so that it is likely that tepid blood cardioplegia is not harmful to the endothelium.

The optimal temperature for myocardial protection during intermittent cardioplegia and the ideal length of the intervals between infusions must be defined. Other parameters which might influence outcome are the volume of infusate and composition. There is not consensus on the best temperature for central nervous system protection which may prove difficult to determine because of the many variables and the problems inherent in the conduct of randomized clinical trials.

Anticipated Developments

The authors believe that tepid cardioplegia with a comparable systemic temperature will prove advantageous with regard to myocardial protection and rapidity of cardiac and systemic metabolic recovery from cardioplegia and mild hypothermia. Intermittent rather than continuous cardioplegia facilitates conduct of the operation (better visualization). Determining the optimal cardiac and systemic temperature for stroke prevention will be difficult because of the many variables and the great numerical size needed for a randomized study to provide statistically useful data. It seems clear that tepid conduct of the operation

provides some margin of safety should systemic circulation be suddenly and unavoidably interrupted (pump failure) and that the need for hyperthermic perfusion may be less than that required for hypothermic or normothermic conduct of the operation.

References

1. Bigelow RWG, Lindsey WK, Greenwood WF. Hypothermia: Its possible role in cardiac surgery. Ann Surg 1950; 132:849-856.
2. Barner HB. Historical aspects and current review of myocardial protection. in Salerno TA, ed. Warm Heart Surgery, London: Arnold, 1995:1-15.
3. Buckberg GD, Brazier JR, Nelson RL et al. Studies of the effects of hypothermia on regional myocardial blood flow and metabolism during cardiopulmonary bypass. J Thorac Cardiovasc Surg 1977; 73:87-94.
4. Iles RW, Silverman NA, Krukenkamp IB et al. Role of the red cell in oxygenated cardioplegia. FASEB J 1988; 2:A921-927.
5. Shargge BW, Digerness SB, Blackstone EH. Complete recovery of myocardial function following cold exposure. Circulation 1978; 56(suppl II); 2:97-103.
6. Hearse DJ, Stewart DA, Chain CEB. Recovery from cardiac bypass and elective cardiac arrest. Circ Res 1974; 35:448-456.
7. Hearse DJ, Stewart DA, Braimbridge MV. Cellular protection during myocardial ischemia: The development and utilization of a procedure for the induction of reversible ischemic arrest. Circulation 1976; 54: 193-202.
8. Tyers GFO, Williams EH, Hughes HC et al. Effects of perfusion temperature on myocardial protection from ischemia. J Thorac Cardiovasc Surg 1977; 73:766-771.
9. Flaherty JT, Schaff HV, Goldman RA et al. Metabolic and functional effects of progressive degrees of hypothermia during global ischemia. Am J Physiol 1979; 236:H839-H845.
10. Lichtenstein SV, Ashe KA, El Dalati H et al. Warm heart surgery. J Thorac Cardiovasc Surg 1991; 101:269-274.
11. Lichtenstein SV, Abel JG, Salerno TA. Warm heart surgery and results of operation for recent myocardial infarction. N Thorac Surg 1991; 52:455-460.
12. Lichtenstein SV, El Dalati H, Panos A et al. Long cross-clamp times with warm heart surgery. Lancet 1989; 189; 1:144-144.
13. Hayashida N, Ikonomidis JS, Weisel RD et al. The optimal cardioplegic temperature. N Thorac Surg 1994; 58:961-971.
14. Hayashida N, Weisel RD, Shirai T et al. Tepid antegrade and retrograde cardioplegia. Ann Thorac Surg 1995; 59:723-729.
15. McMurchie EJ, Raison JK, Cairncross KD. Temperature-induced phase changes in membranes of heart: A contrast between the thermo response poikilotherms and homothermous. Comp Biochem Physiol (B) 1973; 44:1017-1026.
16. Fuhrman GJ, Fuhrman FA. Utilization of glucose by the hypothermic rat. Am J Physiol 1963; 295:181-183.
17. Teoh KH, Mickle DAJ, Weisel RD et al. Decreased postoperative myocardial fatty acid oxidation. J Surg Res 1988; 44:36-44.
18. Lyons JN, Raison JK. A temperature-induced transition in myocardial oxidation: Contrast between cold and warm blooded animals. Comp Biochem Physiol 1970; 37:405-411.
19. Vary TC, Angelakos ET, Schaffer SW. Relationship between adenine nucleotide metabolism and irreversible ischemic tissue damage in isolated perfused rat heart. Circ Res 1979; 45:218-224
20. Martin DR, Scott DF, Downes GL et al. Primary cause of unsuccessful liver and heart preservation: Cold sensitivity of the Atpase system. Ann Surg 1972; 175:111-117.
21. Leaf A. Cell swelling: A factor in ischemic tissue injury. Circulation 1973; 48:455-458.
22. Macknight AD, Leaf A. Regulation of cellular volume. Physiol Rev 1977; 57:510-573.
23. Hess ML, Warner MF, Robbins AD et al. Postischemic deterioration of sarcoplasmic reticulum: Warm versus cold blood cardioplegia. Ann Thorac Surg 1994; 58:953-60.
24. See YP, Weisel RD, Mickle DAJ et al. Prolonged hypothermic cardiac storage for transplantation: The effects on myocardial metabolism and mitachondrial function. J Thorac Cardiovasc Surg 1992; 104:817-824.
25. Rahn H, Reeves RB, Howell BJ. Hydrogen ion regulation, temperature and evolution. Am Rev Respir Dis 1975; 112:165-172.
26. Liu XL, Engelman RM, Wei Z et al. Postischemic deterioration of sarcoplasmic reticulum: Warm versus cold blood cardioplegia. Ann Thorac Surg 1993; 56:1154-1159.
27. Buffkin BL, Mellitt RJ, Gott JP et al. Aerobic blood cardioplegia for revascularization of acute infarct: Effects of delivery temperature. Ann Thorac Surg 1994; 58:953-960.
28. Fiore AC, Swartz M, Nevett R et al. Tepid blood cardioplegia for elective myocardial revascularization. Ann Thorac Surg 1995; 65:1559-1560.
29. Engelman RM, Pleet AB, Rousou JA et al. What is the best perfusion temperature for coronary revascularization? J Thorac Cardiovasc Surg 1996; 112:1622-1633.

30. Martin TD, Craver JN, Gott JP et al. Prospective, randomized trial of retrograde warm blood cardioplegia: Myocardial benefit and neurologic threat. Ann Thorac Surg 1994; 57:298-304.
31. Mora CT, Henson MB, Weintraub WS et al. The effect of temperature management during cardiopulmonary bypass on neurologic and neuropsychologic outcomes in patients undergoing coronary revascularization. J Thorac Cardiovasc Surg 1996; 112:514-522.
32. Regraqui I, Birdi I, Izzat MB et al. The effects of cardiopulmonary bypass temperature on neuropsychological outcome after coronary artery surgery. A prospective randomized trial. J Thorac Cardiovasc Surg (in press)
33. McLean RF, Wong BI, Naylor CD et al. Cardiopulmonary bypass temperature and central nervous system dysfunction. Circulation 1994; 90:250-255.
34. Warm heart investigators. Randomized trial of normothermic versus hypothermic coronary bypass surgery. Lancet 1994; 343:559-563.
35. Singh AK, Bert AA, Feng WC. Neurologic complications during myocardial revascularization using warm-body, cold-heart surgery. Eur J Cardiothorac Surg 1994; 8:259-264.
36. Plourde G, Leduc AS, Morin JE et al. Temperature during cardiopulmonary bypass for coronary artery operations does not influence postoperative cognitive function: A prospective randomized trial. J Thorac Cardiovasc Surg 1997; 114:123-128
37. Selke FW, Shafique T, Johnson RJ et al. Blood and albumen cardioplegia preserve endothelian-dependent microvascular responses. Ann Thorac Surg 1993; 55:977-985.
38. Nilsson FN, Miller VN, Van Houtte PM et al. Methods of cardiac preservation alter the function of the endothelium in porcine coronary arteries. J Thorac Cardiovasc Surg 1991;102:923-930.
39. Nakanishi K, Zhao ZQ, Vinten-Johansen J et al. Coronary artery endothelial dysfunction after global ischemia, blood cardioplegia and reperfusion. Ann Thorac Surg 1994; 58: 191-199.
40. Nakanishi K, Zhao ZQ, Vinten-Johansen J et al. Blood cardioplegia enhanced with nitric oxide donor SPM-5185 counteracts postischemic endothelial and ventricular dysfunction. J Thorac Cardiovasc Surg 1995; 109: 1146-1154.
41. Sato H, Zhao ZQ, Jordan JE et al. Basal nitric oxide depresses endogenous cardio protection during reperfusion by inhibition of neutrophil-mediated damage after surgical revascularization. J Thorac Cardiovasc Surg 1997; 113:399-409.
42. Lin PJ, Chang CH, Hsiao CW et al. Continuous antegrade warm blood cardioplegia attenuates augmented coronary endothelium-dependent contraction after cardiac global ischemia and reperfusion. J Thorac Cardiovasc Surg 1997; 114:100-108.

CHAPTER 14

Surgical Techniques for Warm Blood Cardioplegia

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Hypothermic techniques of myocardial preservation (crystalloid or blood cardioplegia) have been utilized in recent years and represent an important development in cardiac surgery. It can be stated that results of cardiac surgery are largely due to precision in surgical techniques and myocardial protective strategies. Among strategies available to cardiac surgeons are the use of either crystalloid (delivered cold) or blood (delivered either warm or cold) cardioplegic solutions. The mode of delivery, i.e., antegrade, retrograde, alternately antegrade/retrograde, or simultaneously delivered antegrade/retrograde are all important routes of administration available in any cardiac procedure. The use of intermittent delivery of cardioplegia implies that the heart is subjected to a period of ischemia which, if for short periods of time (< 10 minutes each), may be inconsequential. However, longer periods of aortic clamping and/or the presence of ventricular hypertrophy or acute ischemic syndromes, may lead to an adverse outcome, should the intermittent method be utilized. Warm blood cardioplegia was developed in search of prevention of ischemia during the period of aortic clamping. The implication of the original concept was that the heart would be continuously perfused during the period of aortic clamping.

Conceptual Framework

As mentioned earlier, the conceptual framework of warm blood cardioplegia (WBC) is prevention of ischemia during the period of aortic cross-clamping. Utilized by Gott et al[1,2] who used continuous retrograde warm blood perfusion for aortic valve surgery, the method was further developed by Bomfim et al[3] who administered continuous cold blood cardioplegia and by our group in Toronto who rediscovered the technique, modified it, and applied it clinically.[4-7] Over the years, there have been important developments regarding the technique, some of which are related to the cardioplegia composition, route of administration and changes in the temperature of the cardioplegia. More importantly, for precision during coronary artery surgery, the intermittent method of WBC was reported[8,9] and its efficacy and principles were reported by Deslauriers and her group at the National Research Council[10] Canada. What follows is a current view of the warm cardioplegic method, with emphasis on some of the most important aspects of the technique.

Techniques

Antegrade Warm Blood Cardioplegia (AWBC)

Although there are concerns in the literature regarding the flow distribution of cardioplegia distal to coronary stenoses, AWBC

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is one of the simplest methods of myocardial protection. It can be utilized in all forms of cardiac procedures, including coronary and valvular surgery and other procedures. The current technique is usually via the "miniplegia" route, i.e., from the oxygenator blood is extracted and is infused into the aorta with the aid of a roller pump; the "miniplegia" is simply KCl and Mg, which are infused directly into the cardioplegic line.[11] The advantages of this method is that hemodilution is avoided and higher hemoglobin and oxygen carrying capacity are achieved. When crystalloid solutions are used and mixed with blood in a 4:1, 8:1 or other dilutions, severe hemodilution may occur if the period of aortic clamping is prolonged. Left ventricular distention may be avoided either by intermittently venting the aorta during very short periods of cardioplegic interruptions, or by venting the left heart. Patients with moderate or severe aortic insufficiency pose problems during antegrade cardioplegic delivery as most of the solution goes into the left ventricle via an incompetent aortic valve, leading to inadequate myocardial protection. Furthermore, in coronary artery surgery, the presence of blood in the coronary artery makes visualization at times difficult unless the artery is snared, shunted, or a blower device is used. For these reasons, intermittent warm blood cardioplegia (IWBC) was developed and was shown experimentally[8,10] to be safe. Periods of less than or equal to 10 minutes are used, with reinfusion of cardioplegia at these intervals. This replenishes the energy stores and is well tolerated by the normal heart. Recently, Calafiore et al utilized this method in hypertrophied hearts (aortic stenosis) with excellent results. Due to other technical difficulties such as LV distention during construction of a circumflex anastomosis, the concern of maldistribution of flow and other technical problems during surgery, retrograde continuous warm blood cardioplegia was rediscovered and was applied clinically.

Retrograde Continuous Warm Blood Cardioplegia (RCWBC)

As mentioned, this technique was rediscovered, and it was first utilized by Gott et al.[1,2] The development of the retrograde catheter by Buckberg et al, in which insertion is via the transatrial approach, simplified the method. After aortic clamping, an infusion of antegrade cardioplegia arrests the heart alternatively. Aortic clamping is followed by retrograde delivery of cardioplegia without antegrade infusion. The infusion pressure, measured at the distal port of the coronary sinus catheter, should be < 50 mmHg, which usually corresponds to flows of 250-300 ml/min of cardioplegia. Blood in the operative field is minimized, i.e., when the coronary artery is opened, venous blood exits from the arteriotomy. This is easily controlled by the blower, flowrester or suture devices. Ventricular distention does not occur and the presence of aortic insufficiency does not preclude its use. There have been excellent studies in the literature dealing with flow distribution during RCWBC, including elegant MRI studies in pigs by Deslaurier's group at the National Research Council.[12] In these studies the RV region is not adequately perfused during RCWBC, although there have been no human studies showing that the right ventricle is poorly protected.

Alternate Retrograde/Antegrade Cardioplegia

Elegant studies by Buckberg et al[13] suggest that antegrade versus retrograde cardioplegia perfuses different vascular beds. It is therefore reasoned that for "ideal" cardioplegic protection, the cardioplegia must be administered antegradely followed by the retrograde route. This method was defined for and used by utilizing cold intermittent blood cardioplegia.

Simultaneous Antegrade/ Retrograde Cardioplegia

During a complex cardiac procedure, the aortic clamp was released while retrograde car-

dioplegia was being administered. This lead to the clinical observation that the pressure in the distal port of the coronary sinus catheter did not change. We therefore began to deliver continuously simultaneous antegrade (into the grafts or coronary ostia) and retrograde (into the coronary sinus) cardioplegia during all types of cardiac surgery. Experimental work[14] and clinical data confirmed the safety of the technique, which, in our view, represents one of the most effective method of myocardial protection, addressing all concerns of maldistribution of flow during cardioplegic administration. Again, in all these methods, "miniplegia" is used without any additives such as Asparate/Glutamate, delivered at 37°C and without interruption. Experimental and clinical work[14] attests to the safety and efficiency of this modality of myocardial protection.

Warm Cardioplegia Techniques for Valvular Surgery

Currently we have modified the cardioplegia technique for valvular surgery with or without combined coronary surgery. We simply clamp the aorta and deliver blood under pressure (line resistance 130 mmHg and distal coronary sinus port pressure < 50 mmHg) which usually equates to mean flows of 300 ml/min. The heart beats throughout the operation and, if needed, boluses of KCl are infused for brief periods to ensure continued cardiac arrest. During aortic valvular surgery, after aortic clamping and delivery of blood into the coronary sinus, the aortotomy is performed and a Polystan™ cannula is placed in the left and right coronary ostia and simultaneous coronary sinus and ostia blood flow are established. If the right ostium is small, only the coronary sinus and the left ostium are perfused simultaneously. As each coronary artery bypass is performed, blood flow is delivered into the coronary sinus and into each vein as the distal anastomosis is completed. The whole procedure is performed during a single aortic clamping period. For mitral valve replacement or repair only retrograde perfusion of blood or "miniplegia" is infused while the procedure is performed on a clamped aorta. Despite concerns regarding right ventricular protection in hypertrophied hearts and in patients with pulmonary hypertension, right ventricular failure has not been observed clinically.

Normothermic Perfusion

During the cardiac procedure, the systemic temperature is maintained at normothermia (37°C). This usually requires warming the patient as soon as cardiopulmonary bypass is established. Although there were initial concerns regarding the adverse effects of normothermia on the central nervous system, recent studies have failed to confirm this observation and normothermia appears to be safe.[15] Peripheral dilatation usually occurs and requires administration of alpha constrictors during and sometimes postoperatively. Some believe that the beneficial effects of warm heart surgery are due to normothermic systemic perfusion and its sequellae, i.e., peripheral dilatation. Shivering is avoided postsurgery allowing for more stability hemodynamically and for early extubation of the patient.

Intermittent Techniques of Warm Blood Cardioplegia

These have been championed by Calafiore et al.[8] There is no doubt that for coronary artery surgery this technique has many advantages, such as simplicity, decreased cost, and excellent early results. Although warm blood cardioplegia (WBC) was not designed to be delivered intermittently, i.e., the principle is prevention of ischemia and not to subject the heart to normothermic ischemia, this method is being extensively used in coronary artery surgery in Europe. Its scientific basis has been established[16] but surgeons are still skeptical about the safety of warm ischemia in high risk patients and acute syndromes.

Myocardial Resuscitation

The goal of the WBC method was to allow for maintenance of aerobic metabolism and, hopefully resuscitation of the damaged heart by continuous perfusion of WBC, based on

the principle that only WBC resuscitates the heart. Unfortunately, this has not been confirmed and studies on Asparate/Glutamate in the cardioplegia failed to confirm the incorporation of these additives in the TCA cycle and failure to prove a beneficial effect. This has lead us to abandon the use of these additives in a clinical solution. The idea of resuscitation of the injured heart to our knowledge remains elusive.

Monitoring of Cardioplegia Delivery

Nowhere is monitoring more important than during WBC techniques. As mentioned, the principle of the method is continuous delivery of WBC into the coronary sinus. Failure to monitor flow and/or distal coronary sinus catheter port pressure, may lead to excessive pressure thereby the risk of rupture of the coronary sinus or alternatively, dislodgment of the coronary sinus catheter into the right atrium without warning. This may lead to a false sense of security when in actual fact flow of WBC has been interrupted and the heart is subjected to prolonged periods of normothermic ischemia. Therefore, WBC is more cumbersome to the perfusionist, who must be constantly attendant during the period of aortic clamping, while the surgeon is busy with the technical details of the procedure. This is in contrast with intermittent techniques of cardioplegia delivery, in which case the perfusionist only monitors the time period of the cardioplegic interruption. One of the most important pitfalls of the RCWBC method is coronary sinus injury and catheter displacement leading to normothermic ischemia.

Advantages and Disadvantages of Warm Blood Cardioplegia

Theoretically the single greatest advantage of WBC is to reduce or prevent myocardial ischemia during aortic cross-clamping for performance of cardiac operations. A review of the clinical studies reported over the last 7 years (since the introduction of WBC in 1989) show that opinions vary from no minimal advantage of warm versus cold cardioplegia techniques to actual resuscitation of the myocardium with WBC particularly in shock hearts and in hypertrophic hearts (patients undergoing valve surgery).

Use of retrograde warm blood perfusion of the myocardium was first reported by Gott[2] et al in 1957. This was later replaced by direct coronary ostial perfusion, until crystalloid hyperkalemic arrest and the hypothermic blood cardioplegic techniques become widely used in the 1970s. Warm blood cardioplegia was rediscovered by the group in Toronto and reported in 1989.[16]

It has been shown in a prospective, randomized study of 20 patients undergoing cardiac surgery that oxidative stress is minimized with warm blood cardioplegia,[17] but this reduction in oxidative stress did not correlate with postoperative hemodynamic measurements.

In another study, the beta adrenergic receptor function of the heart was compared in a group of 20 patients, each undergoing CABG with either cold intermittent or intermittent warm cardioplegia.[18] This study showed improved preservation of the autonomic sympathetic function of the heart with warm cardioplegia.

Myocardial ultrastructure was compared in the two cardioplegia groups[19] and was noted that only mild to moderate and reversible ultrastructure changes occur during continuous retrograde warm blood cardioplegia (WBC) and cold blood cardioplegia (CBC) for CABG operation. In another study of ultrastructure, WBC was shown to be superior to CBC techniques.[20]

Numerous clinical studies, both retrospective[8,21-30] and prospective randomized[31-37] have reported that WBC is at least as efficacious or better than CBC techniques for CABG. Of note are the two large prospective, randomized clinical trials comparing WBC versus CBC in patients undergoing CABG.[32,37] The first of these trials[32] was conducted at Emory University in Atlanta and randomized 1001 patients undergoing CABG surgery to either continuous WBC with systemic normothermia or intermittent cold oxygenated, crystal-

loid cardioplegia and moderate systemic hypothermia. The preoperative variables were similar in the two groups. The postoperative results showed that the mortality (warm 1.0% and cold 1.6%), Q wave infarction (warm 1.4%, cold 0.8%) and need for intra-aortic balloon pump (IABP) (warm 1.4% and cold 2.0%) were similar in the 2 groups. Total neurologic events (warm 4.5% and cold 1.4%; $p<0.005$) and perioperative strokes (warm 3.1% and cold 1.0%; $p<0.02$) were significantly higher in the warm group. In the Toronto prospective trial[37] 1732 patients undergoing CABG were randomized to warm and cold blood cardioplegia for myocardial preservation. The baseline demographics were similar. The all-cause mortality was 2.5% in the cold group and 1.4% in the warm group ($p < 0.12$), as was the incidence of nonfatal Q-wave infarction (warm 10.1%, cold 11.1%). In this study, however, there were no differences in the rate of stroke, reoperation for bleeding or tamponade, or sternal complications. They concluded that warm heart surgery is a safe and effective alternative to conventional hypothermic techniques for patients undergoing CABG surgery.

Technical Considerations in WBC

Obviously the most important consideration in any myocardial preservation technique is the ability to perform a perfect anastomosis while performing CABG, which requires a still heart and clear blood vessels. To obtain a still heart in WBC, larger amounts of cardioplegic solution is required to be infused antegrade or retrograde continuously. This resulted in large volume of cardioplegia solution to be delivered resulting in hypervolemia as well as hyperkalemia. These problems were largely resolved with the introduction of "miniplegia" techniques.[11] In this method, blood from the arterial pump is infused into the aortic roof or the coronary sinus, and small amounts of crystalloid solution containing K^+ and Mg^{++} are added to maintain a still heart.

Visual Enhancement During WBC

Three techniques and innovations have been used to improve conditions for coronary anastomoses during CABG surgery with WBC. These include use of Flowresters and use of blowers which blow saline with a mist of O_2 to blow away blood from coronary arteries. The third technique is to use intermittent WBC, where cardioplegia delivery is interrupted for up to 10 minutes while performing the anastomoses. In a series of very elegant articles, Calafiore et al from Chieti, Italy have demonstrated that safety and efficacy of using intermittent WBC[9,28,38] in various cardiac operations. In one study[8] comparing intermittent antegrade WBC (IAWBC) used in the first 250 patients undergoing CABG with this technique to the last 250 patients who had CABG using intermittent antegrade CBC, Calafiore et al showed that the mortality was lower in the warm group (0.8% versus 3.6%; $p < .05$). Further, in-hospital mortality for high risk patients was 0/53 in the warm group compared to 2/28 ($p < 0.05$) in the cold group. Circulatory assistance was needed in 0 versus 4 patients in Group A and B. The rates of postoperative myocardial infarction and strokes were similar in the two groups.

To determine if intermittence in WBC was harmful, the Toronto Warm Heart Investigators studied 720 CABG patients with particular attention to the longest single time off (LTOC) and the time off cardioplegia as percentage of the cross-clamp time (PTOC).[39] Longer LTOC was found to be harmful whereas longer PTOC was protective. It was further shown that repeated interruptions of warm blood cardioplegia are unlikely to lead to adverse clinical results as single interruptions are greater than or equal to 13 minutes.

Inadequate RV Protection with WBC

That the right ventricle is inadequately protected in WBC has been a concern, particularly when the delivery of WBC is through

the retrograde approach via the coronary sinus. However, this has not been found to be a clinical problem. Two studies need be cited in this regard. The first by Menasche et al[21] where 75 patients were carefully studied to determine the RV function after cardiac operation using RWBC. In the first part of this three-part study, they showed that the anaerobic metabolism was similar in RV and LV. In a second part, the RV stroke work index was compared in 15 patients where RWBC was used to case match 15 patients where cold antegrade crystalloid cardioplegia was used. Again the results were similar in the two groups. In part three, function was determined in 30 patients undergoing mitral valve procedures where RV was at higher risk of ischemia (15 each in warm and cold groups). Again there were no differences in postoperative RV function.

The second study was reported by Christakis et al from Toronto.[40] They randomized 52 patients to either intermittent warm or cold blood cardioplegia during CABG surgery. The RV ejection fraction was greater at six hours (warm 0.46 ± 0.06; cold 0.37 ± 0.08; $p < 0.05$) and at 8 hours (warm 0.43 ± 0.08; cold 0.37 ± 0.08; $p < 0.05$) in the warm group. The RV end diastolic volume index was less in the warm group at eight hours postoperatively.

Thus RV dysfunction after WBC is not a clinical problem.

Coronary Sinus Injuries

Coronary sinus injuries during placement of retrograde coronary sinus catheter have been reported by Panos et al.[41] These are of course not related to warm or cold cardioplegia, but to the route of delivery. One must be cautious in placing of these catheters. The catheters should never be pushed forcefully. The guide should be pulled back about a centimeter before advancing the catheter in the coronary sinus, so as to have the soft tip of the catheter on the leading edge as it is advanced. After an initial learning curve, this maneuver becomes easier and injuries to the coronary sinus very rare.

Postoperative Neurological Deficits and Stroke Rates after WBC and Normothermic Cardiopulmonary Bypass

Concern regarding increased incidence of neurological deficits and stroke rates following CABG surgery with normothermic cardiopulmonary bypass and WBC were first raised by the Emory University group. In their randomized trial[32] of warm versus cold cardioplegia which was summarized earlier, they reported a significantly higher incidence of total neurologic events (4.5% versus 1.4%) and perioperative strokes (3.1% versus 1.0%) in the warm cardioplegia group. Since then, many additional studies have been reported looking at this problem.[42-45] In a review article, Christakis et al[46] analyzed all available controlled studies of warm versus cold and antegrade versus retrograde cardioplegia, to assess the incidence of perioperative stroke and adverse neuropsychological outcomes. Nine randomized trials and two studies with immediate historical controls were included. The pooled event rates for perioperative stroke were 1.5% for warm antegrade, 3.14% for warm retrograde, 1.7% for cold antegrade and 0-1.2% for cold retrograde. In further analysis, they determined that the differences found are unlikely to be due to temperature, but may be related to antegrade versus retrograde coronary perfusion.

Tepid Cardioplegia

Warm cardioplegia implies perfusate temperature of 37°C. Some groups have taken the middle of the road approach in order to get the maximum benefits of both warm and cold blood cardioplegia, while simultaneously reducing the disadvantages of the two extremes. They are proponents of the tepid (or lukewarm) cardioplegia.[45,47,48] Good results are reported with lukewarm (32°C) temperature of cardioplegia.

Cold Agglutinins and Warm Blood Cardioplegia

The technique of normothermic cardiopulmonary bypass with warm blood cardioplegia is an advantageous technique for performing cardiac surgical procedures in patients with cold autoimmune disease. Several case reports have documented this advantage[49-51]

Future Perspectives

As with any technique of myocardial protection, WBC received great attention as we searched for the "ideal solution" to the cardioplegic method. The initial crystalloid solutions added to blood in different mixtures (4:1 or 8:1) caused many problems such as hemodilution, hyperglycemia, hyperkalemia and others. "Miniplegia" solved these issues and, more recently, perfusion of the coronary sinus with blood seems to us to be even more advantageous. We are now performing complete revascularization on a beating heart without cardiopulmonary bypass. The role of cardiopulmonary bypass and cardioplegia in coronary artery surgery is being reassessed by our group, who has spent the last 20 years working on myocardial protection. Some of the techniques and developments in cardioplegia are already of historical value. All of them have made cardiac surgery safer but, with advancement of technology, are being replaced by innovative techniques aimed at a simpler and safer operations. There will always be the need for cardioplegia in cardiac surgery for as long as we have to perform intracardiac repair and/or complex ascending aorta and arch surgery. Simultaneous antegrade/retrograde infusion of warm blood (or WBC) comes close to being the "ideal" method of myocardial protection. Surgeons can now utilize a variety of myocardial protective strategies which compliment each other during cardiac surgery. There should be no adversarial position of one method against the other. Each technique is tailored to each patient's needs.

References

1. Gott VL, Gonalez JL, Paneth M et al. Cardiac retroperfusion with induced asystole for open heart surgery upon the aortic valve or coronary arteries. Proc Soc Exp Biol Med 1957; 94(4):689-692.
2. Gott VL, Gonzalez JL, Zuhdi MN et al. Retrograde perfusion of the coronary sinus for direct vision aortic surgery. Surg Gynecol Obstet 1957; 104:319-328.
3. Bomfim V, Kaijser L, Bendz R et al. Myocardial protection during aortic valve replacement. Cardiac metabolism and enzyme release following continuous blood cardioplegia. Scan J Thorac Cardiovasc Surg 1981; 15:141-147.
4. Salerno TA. Continuous blood cardioplegia: Option for the future or return to the past? J Mol Cell Cardiol 1990; 22(Suppl V):S49.
5. Panos A, Ashe K, El-Dalati H et al. Clinical comparison of continuous warm (37°C) versus continuous cold (10°C) blood cardioplegia in CABG surgery. Clin Invest Med 1989; 12(5 suppl):C55.
6. Panos A, Abel J, Slutsky AS et al. Warm aerobic arrest: A new approach to myocardial protection. J Mol Cell Cardiol 1990; 22(suppl V):S31.
7. Panos A, Kingsley SJ, Hong AP et al. Continuous warm blood cardioplegia. Surg Forum 1990; 41:233-235.
8. Calafiore AM, Teodori G, Mezzetti A et al. Intermittent antegrade warm blood cardioplegia. Ann Thorac Surg 1995; 59:398-402.
9. Mezzetti A, Calafiore AM, Lapenna D et al. Intermittent antegrade warm cardioplegia reduces oxidative stress and improves metabolism in the ischemic-reperfused human myocardium. J Thorac Cardiovasc Surg 1995; 109:787-795.
10. Tian GH, Xiang B Butler KW et al. Effects of intermittent warm blood cardioplegia on myocardial high energy phosphates, intracellular pH and contractile function—a ^{31}P NMR study in isolated rat and pig hearts. J Thorac Cardiovasc Surg (in press 1995; 109).
11. LeHouerou D, Pargaonkar S, Lessana A. Cardioplegia delivery systems for warm blood cardioplegia. Warm Heart Surgery 1995; 46-54.
12. Ye J, Sun J, Shen J et al. Does retrograde warm blood cardioplegia provide equal protection to both ventricles? A magnetic resonance spectroscopy study in pigs. Circ (Suppl II) 1997; 96(9):II210-II215.
13. Partington MT, Acar C, Buckberg GD et al. Advantages of antegrade/retrograde cardioplegia to optimize distribution in jeopardized myocardium. J Thorac Cardiovasc Surg 1989; 97:613-622.

14. Buckberg GD, Salerno TA. Simultaneous antegrade/retrograde cardioplegia: Experimental/clinical study of a new cardioprotective technique. Warm Heart Surgery 1995; 90-98.
15. Pleet AB, Engelman RM. Cerebral protection during warm heart surgery. Warm Heart Surgery 1995; 178-188.
16. Lichtenstein SV, El Dalati H, Panos A et al. Long cross-clamp times with warm heart surgery. Lancet 1989; 1:1443-1444.
17. Biagioli B, Borrelli E, Maccherini M et al. Reduction of oxidative stress does not affect recovery of myocardial function: Warm continuous versus cold intermittent blood cardioplegia. Heart 1997; 77(5):465-473.
18. Chello M, Mastroroberto P, De Amicis V et al. Intermittent warm blood cardioplegia preserves myocardial beta-adrenergic receptor function. Ann Thorac Surg 1997 Mar; 63(3):683-688.
19. Rainio P, Sormunen R, Lepojarvi M et al. Ultrastructural changes during continuous retrograde warm and mild hypothermic blood cardioplegia for coronary bypass operations. J Thorac Cardiovasc Surg 1995 Jul; 110(1): 81-88.
20. Tasdemir O, Katircioglu SF, Kucukaksu DS et al. Warm blood cardioplegia: Ultrastructural and hemodynamic study. Ann Thorac Surg 1993 Aug \; 56(2):305-311.
21. Menasche P, Fleury JP, Droc L et al. Metabolic and functional evidence that retrograde warm blood cardioplegia does not injure the right ventricle in human beings. Circ 1994 Nov; 90(5 Pt 2):II310-II315.
22. Hanafy HM, Allen BS, Winkelmann JW et al. Warm blood cardioplegic induction: an underused modality. Ann Thorac Surg 1994 Dec; 58(6):1589-1594.
23. Vaughn CC, Opie JC, Florendo FT et al. Warm blood cardioplegia. Ann Thorac Surg 1993 May; 55(5):1227-1232.
24. Gundry SR, Wang N, Bannon D et al. Retrograde continuous warm blood cardioplegia: Maintenance of myocardial homeostasis in humans. Ann Thorac Surg 1993 Feb 55(2): 358-361.
25. Bel A, Aznag H, Faris B et al. Warm blood cardioplegia in high risk patients. Europ J Cardiothorac Surg 1997 Jun; 11(6):1118-1123.
26. Borman JB, Arbell D, Izhar U et al. Luke warm blood cardioplegia for CAB surgery in patients with severely impaired LV function. J Cardiovasc Surg 1995 Dec; 36(6):545-550.
27. Tolis GA, Astras G, Sfyras N et al. Experience with warm blood cardioplegia in 480 patients. Cardiovasc Surg 1995 Apr; 3(2): 175-180.
28. Calafiore AM, Teodori G, Di Giammarco G et al. Intermittent antegrade cardioplegia: warm blood vs cold crystalloid. A clinical study. J Cardiovasc Surg 1994 Dec; 35(6 Suppl 1):179-184.
29. Mikaeloff P, Jegaden O, Montagna P et al. Is continuous warm retrograde blood cardioplegia completely safe for coronary artery surgery? Europ J Cardiothorac Surg 1994; 8(11):569-574.
30. Ali IM, Kinley CE. The safety of intermittent warm blood cardioplegia. Europ J Cardiothorac Surg 1994; 8(10):554-556.
31. Pelletier LC, Carrier M, Leclerc Y et al. Intermittent antegrade warm versus cold blood cardioplegia: a prospective, randomized study. Ann Thorac Surg 1994 Jul; 58(1):41-48.
32. Martin TD, Craver JM, Gott JP et al. Prospective, randomized trial of retrograde warm blood cardioplegia: Myocardial benefit and neurologic threat. Ann Thorac Surg 1994 Feb; 57(2):298-302.
33. Gerola LR, Oliveira SA, Moreira LF et al. Blood cardioplegia with warm reperfusion versus intermittent aortic crossclamping in myocardial revascularization. Randomized controlled trial. J Thorac Cardiovasc Surg 1993 Sep; 106(3):491-196.
34. Lajos TZ, Espersen CC, Lajos PS et al. Comparison of cold versus warm cardioplegia. Crystalloid antegrade or retrograde blood? Circ 1993 Nov; 88(5 Pt 2):II344-349.
35. Landymore R, Murphy JT, Hall R et al. Randomized trial comparing intermittent antegrade warm blood cardioplegia with multidose cold blood cardioplegia for coronary artery bypass. Europ J Cardiothorac Surg 1996; 10(3):179-184.
36. Rashid A, Fabri BM, Jackson M et al. A prospective randomized study of continuous warm versus intermittent cold blood cardioplegia for coronary artery surgery: Preliminary report. Europ J Cardiothorac Surg 1994; 8(5):265-269.
37. Naylor CD, Lichtenstein SV, Fremes SE et al. Randomized trial of normothermic versus hypothermic coronary bypass surgery. The warm heart investigators. Lancet 1994 Mar; 343(8897):559-563.
38. Calafiore AM, Teodori G, Bosco G et al. Intermittent antegrade warm blood cardioplegia in aortic valve replacement. J Card Surg 1996 Sep-Oct; 11(5):348-354.
39. Lichtenstein SV, Naylor CD, Feindel CM et al. Intermittent warm blood cardioplegia. Warm Heart Investigators. Circ 1995 Nov; 92(9 Suppl):II341-346.

40. Christakis GT, Buth KJ, Weisel RD et al. Randomized study of right ventricular function with intermittent warm or cold cardioplegia. Ann Thorac Surg 1996 Jan; 61(1): 128-134.
41. Panos AL, Ali IS, Birnbaum PL et al. Coronary sinus injuries during retrograde continuous normothermic blood cardioplegia. Ann Thorac Surg 1992; 54(6):1137-1138.
42. Craver JM, Bufkin BL, Weintraub WS et al. Neurologic events after coronary bypass grafting: further observations with warm cardioplegia. Ann Thorac Surg 1995 Jun; 59(6): 1429-1433.
43. Guyton RA, Mellitt RJ, Weintraub WS. A critical assessment of neurological risk during warm heart surgery. J Card Surg 1995 Jul; 10(4 Suppl):488-492.
44. Guyton RA, Gott JP, Brown WM et al. Cold and warm myocardial protection techniques. Advances in Card Surg 1996; 7:1-29.
45. Engelman RM, Pleet AB, Rousou JA et al. What is the best perfusion temperature for coronary revascularization? J Thorac Cardiovasc Surg 1996 Dec; 112(6):1622-1632.
46. Christakis GT, Abel JG, Lichtenstein SV. Neurological outcomes and cardiopulmonary temperature: A clinical review. J Card Surg 1995 Jul; 10(4 Suppl):475-480.
47. Hayashida N, Ikonomidis JS, Weisel RD et al. The optimal cardioplegic temperature. Ann Thorac Surg 1994 Oct; 58(4):961-971.
48. Hayashida N, Weisel RD, Shirai T et al. Tepid antegrade and retrograde cardioplegia. Ann Thorac Surg 1995 Mar; 59(3):723-729.
49. Muehreke DD, Torchianna DF. Warm heart surgery in patients with cold autoimmune disorders. Ann Thorac Surg 1993 Feb; 55(2):532-533.
50. Hearnsberger J, Ziomek S, Tobler G et al. Management of cold agglutinemia with warm heart surgical intervention: a case report. J Thorac Cardiovasc Surg 1993 Oct; 106(4): 756-757.
51. Donatelli F, Mariani MA, Triggiani M et al. Warm heart surgery in cold haemagglutinin disease. Cardiovasc Surg 1995 Apr; 3(2): 191-192.

Chapter 15

Interrupting Warm Blood Cardioplegia

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Warm blood cardioplegia has emerged as an alternative method of myocardial protection. Initial retrospective clinical studies using warm blood techniques showed good myocardial protection; however, prospective studies comparing warm blood cardioplegia with cold blood cardioplegia were lacking.[1,2] Furthermore, the ability of warm blood cardioplegia to adequately protect acutely ischemic myocardium was unknown. This prompted us to undertake an experimental study to compare the effectiveness of warm blood cardioplegia versus cold blood cardioplegia in protecting areas of ischemic myocardium.[3] In 40 adult pigs, the second and third diagonal coronary arteries were occluded with snares for 90 minutes, followed by 45 minutes of cardioplegic arrest and 180 minutes of reperfusion during which time the coronary snares were released. During the period of cardioplegic arrest, 10 pigs received antegrade continuous warm blood cardioplegic solution (37°C) at 100 ml/min; 10 animals received retrograde continuous warm blood cardioplegic solution at 100 ml/min; 10 received intermittent, antegrade cold blood cardioplegic solution (4°C), and 10 received intermittent, antegrade/retrograde cold blood cardioplegic solution. Our results showed that antegrade continuous warm blood cardioplegia resulted in the least optimal myocardial protection in the area at risk. Although retrograde continuous warm blood cardioplegia resulted in significantly lower areas of necrosis than in the antegrade warm blood cardioplegia group, it did not achieve any superior protection to that obtained with antegrade/retrograde cold blood cardioplegic techniques. During the period of cardioplegic arrest, both warm cardioplegic techniques had significantly lower tissue pH values in both the area at risk and in unobstructed myocardium than the cold cardioplegia groups. Upon reperfusion, pH values returned to preischemic values in the unobstructed myocardium in all cardioplegic groups. In addition, wall motion scores were not significantly different in the unobstructed myocardial segments, suggesting that temperature and the route of delivery are not as critical in myocardial tissue with normal coronary anatomy. In contrast, the method of cardioplegic delivery was an important determinant in the degree of necrosis seen in the area at risk. All retrograde cardioplegic techniques, regardless of temperature, had much lower areas of necrosis when compared with antegrade delivery. This suggested that it was the adequacy of distribution and not the temperature of the cardioplegic solution that is most critical in achieving optimal myocardial protection.

A potential disadvantage of warm blood cardioplegia techniques is that the continuous infusion of cardioplegia will obscure the operative field necessitating that the cardioplegia be interrupted. However, the effects of interrupting warm blood cardioplegia during

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the revascularization of acutely ischemic myocardium were unknown. We, therefore, added another group to our previous study of 90 minutes of coronary occlusion followed by 45 minutes of cardioplegic arrest and 180 minutes of reperfusion.[4] In 10 pigs, the retrograde infusion of warm blood cardioplegia (37°C at 100 ml/min) was interrupted for three consecutive 7 minute periods. This group of animals were compared to our previous groups of continuous retrograde warm blood cardioplegia and intermittent, antegrade/retrograde cold blood cardioplegia.[3] After 45 minutes of cardioplegic arrest, pH increased in hearts receiving antegrade/retrograde cold blood cardioplegia, remained the same in the continuous retrograde warm blood cardioplegia group, and actually decreased in the animals when the retrograde warm blood cardioplegia was interrupted. This was reflected in the wall motion scores in the area at risk which were significantly lower in the interrupted retrograde warm blood cardioplegia group. Furthermore, wall motion scores in non-ischemic areas of the myocardium were also significantly lower in the interrupted group. This resulted in an area of necrosis which was significantly higher when warm blood cardioplegia was interrupted. We concluded that interrupting warm blood cardioplegia accentuates damage in acutely ischemic myocardium and may also be detrimental to areas of myocardium without obstructed vessels.

Our experimental work raised serious questions about the safety of interrupting warm blood cardioplegia. While our results showed that regional ischemia was accentuated by interrupting warm blood cardioplegia, the effects of interrupting warm blood cardioplegia in myocardium supplied by unobstructed myocardium was still unknown. Lichtenstein, Abel, and Salerno suggested that interrupting warm blood cardioplegia actually preconditioned the heart to subsequent periods of ischemia.[1] This was based on studies of regional ischemia in the beating heart which showed that brief intermittent episodes of normothermic ischemia reduces myocardial stunning and limits necrosis after subsequent periods of prolonged regional ischemia.[5] However, interrupting warm blood cardioplegia after global ischemic arrest differs from a beating heart subjected to repeated episodes of regional ischemia. Several important issues needed to be resolved before the efficacy of interrupting warm blood cardioplegia could be established. These included determining

1. The flow rates that would be necessary to meet the metabolic needs of the myocardium in both normal and hypertrophied hearts, and in obstructed and unobstructed territories of the myocardium,
2. how long the cardioplegia could be interrupted in normal and acutely ischemic muscle,
3. what clinical methods could be used to monitor ongoing ischemia during cardioplegic interruption, and
4. what technique could be used to reverse ischemic changes following periods of intermittent warm blood cardioplegic delivery.

The aim of this chapter will be to review the current status of interrupting warm blood cardioplegia in the clinical practice of cardiac surgery. Recent experimental and clinical studies will be highlighted in an attempt to further defined the limits and role of this technique as a method of myocardial protection.

Experimental Studies

Carrier and his co-workers[6] sought to determine the effects of interrupting cold and warm blood cardioplegia on myocardial pH and the release of metabolic markers in a canine model. Following sequential arrest periods of 5, 10, 20, and 40 minutes, hearts were reperfused with either cold (12°C) or warm (37°C) blood cardioplegia at 100 ml/min for 10 minutes. Although pH decreased significantly in both groups there was no difference in the absolute pH or ΔpH between warm and cold blood cardioplegia. The amount of lactate production and creatine kinase release was also similar in both groups. The release of troponin T was higher in the warm blood cardioplegia group but this did not reach statistical significance. Their results

also showed that periods of warm ischemic arrest > 10 minutes and periods of cold ischemic arrest > 20 minutes were more likely to result in greater pH changes and more release of lactate, creatine kinase, and troponin. This suggests that the margin of safety for ischemic arrest is prolonged when hypothermic rather than warm blood techniques are used. In another canine model, Ko and his colleagues[7] arrested three groups of seven dogs with antegrade warm blood cardioplegia. The control group received continuous warm blood cardioplegia while the other two groups received multidose warm blood cardioplegia administered every 5 or 10 minutes during a 30 minute period of ischemic arrest. Systolic and diastolic recovery was impaired after arrest in animals that had received intermittent warm blood cardioplegia every 10 minutes, and mild stunning was observed when warm blood cardioplegia was administered every 5 minutes. Landymore and co-workers[8] extended the period of interruption to 15 minutes in a dog model comparing intermittent antegrade warm blood cardioplegia to intermittent antegrade cold blood cardioplegia. Hearts were arrested by giving an initial dose of 30 ml/kg delivered at a rate of 150 ml/min. Subsequent infusions were given every 15 minutes (10 ml/kg) at a rate of 150 ml/min during the 90 minute arrest period. Left ventricular systolic elastance and end-diastolic elastance was assessed using pressure-volume loops. Systolic function was well preserved whereas diastolic function decreased slightly in both groups. High energy phosphates decreased significantly in both groups during reperfusion. This study used global parameters to assess LV function in normal animals without coronary artery lesions or ventricular hypertrophy. Landymore could not demonstrate any superiority for interrupting warm blood cardioplegia over established cold blood cardioplegia techniques in this model.

Further insight into the metabolic effects of interrupting warm blood cardioplegia was provided by Tian and co-workers[9] using NMR techniques. Isolated blood perfused pig hearts were divided into three groups. One group received continuous warm blood cardioplegia (37°C) for 90 minutes. Another group received six 5 minute periods of warm blood cardioplegia infusion followed by six 20 minute periods of ischemia. The last group had no ischemia during the 150 minutes of perfusion. NMR studies showed that a 10 minute interruption of warm blood cardioplegia decreased creatine phosphate levels and intracellular pH by approximately 47% ($p < 0.01$) and 0.12 units ($p < 0.05$). Resumption of cardioplegia for 5 minutes resulted in almost complete recovery of creatine phosphate and intracellular pH values. More importantly, subsequent interruptions did not result in any cumulative changes in creatine phosphate levels or intracellular pH beyond those changes observed after the initial cardioplegic interruption. During reperfusion, there were no significant differences in ATP and creatine phosphate levels among the groups. Recovery of contractility as assessed by balloon function curves did not differ amongst the groups. Hence, in this experimental study using normal, isolated hearts, interruption of warm blood cardioplegia for up to 10 minutes appeared to be well tolerated. Similar results were reported by Tonz and co-workers[10] in an intact pig model. Antegrade warm blood cardioplegia was given for 15 minutes followed by a 5 minute interruption or for 10 minutes followed by a 10 minute period of interruption during a 180 minute period of ischemic arrest. Interrupting antegrade warm blood cardioplegia for 10 minute intervals resulted in no significant difference in systolic or diastolic function compared to 5 minutes of interruption or to those animals which received 180 minutes of continuous cardioplegic perfusion. Torracca and co-workers[11] also concluded that 10 minutes of antegrade warm blood cardioplegia interruption was safe in an isolated blood-perfused rabbit model. Antegrade warm blood cardioplegia was stopped for 10 minutes followed by 5 minutes of reperfusion. The sequence was repeated three times for a total of 45 minutes. Compared to continuous warm blood cardioplegia, the intermittent group had no significant changes in ATP content or contractility as assessed by balloon function curves. However, there was a transient but significant

release of creatine kinase and lactate after the first minute of reperfusion suggesting anaerobic glucose metabolism. This may be potentially harmful in areas of acutely ischemic myocardium or when cross-clamp times are longer than 45 minutes which is frequently seen in clinical practice.

In summary, experimental studies seem to suggest that antegrade warm blood cardioplegia can be interrupted for as long as 10 minutes without significant changes in high energy phosphate levels or contractility after a period of reperfusion. However, it should be noted that these studies were performed in normal hearts without coronary lesions or periods of ongoing ischemia, or ventricular hypertrophy. Based on these experimental results, clinical studies were conducted to further define the efficacy of the intermittent warm blood cardioplegia technique in clinical practice.

Clinical Studies

Yau and co-workers[12] performed a randomized trial comparing continuous antegrade warm blood cardioplegia with intermittent cold blood cardioplegia in patients undergoing elective coronary artery bypass graft surgery performed by a single surgeon. The cases were of low risk; over 80% of patients had an ejection fraction ≥ 40%. Proximal and distal anastomoses were performed under a single cross-clamp period. After an initial arresting dose of 500 ml; antegrade, warm blood cardioplegia was infused at 80 ml/min and interrupted "whenever necessary" to allow adequate visualization of each distal anastomosis. After the interruption, a "catch-up" infusion was given through the aortic root and in each completed vein graft. The volume of this infusion was calculated by the perfusionist to precisely compensate for the period of cessation of flow and to maintain the preset cardioplegic flow rate. These patients were not actively cooled; systemic temperatures were allowed to drift toward 30-32°C. In the cold blood group, after an arresting dose of 500 ml; 100 ml of low K+ (10 mEq/L) cardioplegia was infused intermittently into each vein graft after completion of each distal anastomosis, and a 400 ml infusion was delivered into the aortic root. Myocardial temperatures were maintained between 12 and 18°C. Patients were systemically cooled to 25-28°C. Warm blood cardioplegia was delivered during 60 ± 3% of the arrest period; while cold blood cardioplegia was delivered only 28 ± 5% of the cross-clamp time ($p = 0.009$). There were no significant differences between operative mortality, sternal infections, perioperative myocardial infarctions, strokes, and the need for inotropic or intra-aortic balloon support between the groups. Immediately after removal of the cross-clamp, warm hearts showed more washout of accumulated lactate ($p < 0.05$ versus cold). This lactate production was thought to be related to the inadequate delivery of cardioplegic solution in the LAD distribution which was grafted with an internal mammary artery and was the last territory to be grafted. Unfortunately, regional function in the LAD distribution was not assessed in this study but end-systolic elastance and preload recruitable stroke work index were not depressed in the warm blood group. Furthermore, the MB fraction of creatine kinase was lower in the warm hearts. Since the incidence of infarcts was similar in both groups, this suggests that the increased release of creatine kinase in the cold hearts may have been due to reversible disruption of sacrolemnal integrity, allowing transient transmembrane loss of these high-molecular weight proteins. In summary, interrupting antegrade warm blood cardioplegia appeared to have been well tolerated in these low risk patients. Delivering cardioplegia through the completed vein grafts undoubtedly decreased ischemic damage in some areas of the heart with critical coronary stenoses. Nevertheless, concern is raised about the increased lactate washout in the warm blood group; especially in the LAD distribution. This potentially could have resulted in regional dysfunction in more acutely ischemic myocardium.

A similar prospective, randomized study was performed by Pelletier and co-workers[13] comparing intermittent, warm blood cardioplegia and intermittent cold blood cardioplegia in 200 low risk CABG patients. After an initial arresting dose of 300 ml of high K^+

cardioplegia, a low K^+ antegrade, warm blood cardioplegic solution was administered (200-300 ml) after each distal anastomosis. The maximal single time of interruption was 12-15 minutes; cardioplegia was delivered 30-40% of the cross-clamp period. In 13% of the intermittent warm blood group, a sustained electromechanical arrest could not be achieved and these patients crossed over to the cold blood group. The cold blood group had similar rates of infusion through the aortic root. The mortality (1% vs 1%) and infarct rates (2% warm, 4% cold) were similar in each group. There was no difference in the need for pharmacologic or mechanical support between the two groups. The serum levels of postoperative creatine kinase were again significantly lower in the warm blood group suggesting that hypothermia may result in temporary membrane dysfunction resulting in a higher leak of creatine kinase which is not indicative of permanent injury or myocardial necrosis. These results appear to confirm Yau's[12] observation that warm blood cardioplegia can be safely interrupted in low risk surgical patients. However, it should be noted that Pelletier and his co-workers had to alter their cardioplegia technique in 13% of patients because a sustained electromechanical arrest could not be achieved.

In a further attempt to define the safe period that warm blood cardioplegia can be interrupted, Lichtenstein and co-workers[14] conducted a randomized study of 720 patients comparing cold blood cardioplegia with warm blood cardioplegia. Following an initial arresting dose of high K^+ cardioplegia, a low K^+ solution was delivered at 50-150 ml/min. After periods of interruption, warm blood cardioplegia was delivered at 200-300 ml/min after which the rate was reduced to 50-150 ml/min. Cardioplegia was also administered through completed vein grafts. No retrograde infusions were given. Seventeen percent of patients in the warm group crossed over to the cold blood group because of excessive coronary "flooding" or difficulty achieving or maintaining a cardiac arrest. In this study, warm blood cardioplegia was interrupted for approximately 50% of the cross-clamp time. The longest single ischemic interval averaged 11.4 ± 4.0 minutes but extended from 6.8 ± 5.4 to 15.3 ± 5.0 minutes according to the individual surgeon. In this study, repeated interruptions of antegrade warm blood cardioplegia were less likely to lead to adverse clinical results if single interruptions were ≤ 13 minutes. Their results were similar to earlier experimental studies which suggested that the longest ischemic interval was more important than the cumulative ischemic time.[9,10] Nevertheless, their results also showed that the chance of an adverse outcome increased when the period of interruption exceeded 13 minutes; especially in patients with left main disease, decreased LV function, and those requiring more urgent surgery.

Chritakis and his co-workers[15] studied the effects of intermittent antegrade, warm blood cardioplegia on postoperative right ventricular (RV) function in 52 isolated CABG patients. Patients were randomized to receive either intermittent antegrade, warm blood cardioplegia (37°C) with systemic pump flow at 35-37°C or intermittent antegrade, cold blood cardioplegia (5-8°C) with systemic temperatures ranging from 25-30°C. All hearts received an arresting high K^+ dose of 200-300 ml/min followed by a continuous low K^+ infusion of 100-150 ml/min. Cardioplegia was interrupted only during the construction of the distal anastomoses. After the completion of each distal, a 500 ml bolus of cardioplegia was given at 200-300 ml/min after which the rate was decreased to 100-150 ml/min. Cardioplegia was also given through each completed vein graft and both groups received a terminal infusion of 350 ml of warm (37°C) cardioplegia prior to removing the cross-clamp. Total ischemic time was longer in the cold group (42 ± 8 vs 31 ± 14 min; $p < 0.001$). Right ventricular ejection fraction, RV end-diastolic volume index, and RV end-systolic volume index, as derived from a thermodilution REF-1 catheter, were found to be significantly higher in the warm blood group at 6 and 8 hours after bypass. However, these measurements are affected by changes in RV afterload, and their significant improvement over the cold group most likely reflect the decreased pulmonary vascular resistance and

warmer systemic temperatures in the warm blood group. This may be a beneficial effect of normothermic cardiopulmonary bypass and not warm blood cardioplegia. Furthermore, at 6 and 8 hours postop, nearly one-third to one-quarter of the patients in both groups were excluded because they required either inotropes or afterload reducing agents, or were shivering and had a tachycardia. Christakis noted in his discussion that ischemic times were longer in the cold group "because all surgeons felt more confident with the luxury of hypothermia" and that "surgeons were more expeditious when constructing anastomoses in the warm group." Their data actually showed that the increased ischemic time taken to perform the distal anastomoses in the cold group had no detrimental effects on right ventricular or left ventricular function.

Our earlier experimental studies showed that the presence of an LAD occlusion limits cardioplegia delivery when given in an antegrade fashion and that this damage may be accentuated when warm antegrade cardioplegia is used for myocardial protection.[3,4] Although continuous retrograde cardioplegia results in better delivery of cardioplegia beyond a coronary occlusion,[3] it may still provide limited perfusion to the posterior septum and right ventricle.[16] Hayashida and his colleagues[17] therefore postulated that a combination of antegrade and retrograde delivery of cardioplegia might be required to provide better perfusion of the myocardium when warm blood cardioplegia techniques are used. They undertook a prospective, randomized clinical study in low risk patients undergoing CABG comparing intermittent, warm antegrade blood cardioplegia, intermittent, retrograde warm blood cardioplegia, and a combination of antegrade/retrograde warm blood cardioplegia. Both antegrade and retrograde groups received an initial arresting antegrade dose of 500 ml of high K^+ cardioplegia. Cardioplegia was then administered either retrograde or antegrade at 200 ml/min. The cardioplegia was interrupted when necessary for visualization of the anastomosis. A "catch-up" infusion was then given, averaging 150-200 ml/min to compensate for the period of cessation of cardioplegia. Cardioplegia was also administered directly into each completed vein graft in both groups. In the patients receiving antegrade/retrograde cardioplegia, a continuous infusion of retrograde cardioplegia was interrupted only for visualization of the anastomosis. Intermittent infusions (250 ml) of antegrade cardioplegia were given after completion of each proximal anastomosis. Infusion of cardioplegia was never done simultaneously in an antegrade and retrograde manner. Cardioplegia was least interrupted in the combined group (17 ± 3% combined vs 28 ± 4% retrograde vs 36 ± 4% antegrade $p < 0.05$). The combined group also received significantly more cardioplegic solution (6.3 ± 0.4 L combined vs 4.8 ± 0.3 L retrograde; vs 4.6 ± 0.2 L antegrade; $p < 0.001$). No differences in clinical outcomes were found among the groups. Myocardial lactate concentrations were highest in the LAD territory during antegrade cardioplegia delivery. Retrograde cardioplegia had greater lactate production in the right and left ventricles and decreased ATP concentrations. The combination of antegrade and retrograde cardioplegia resulted in the best preservation of ATP, least lactate production and accumulation, and the best global LV and RV function. Hence, the technique which resulted in the most cardioplegic delivery and least interruptions gave the best myocardial protection.

Summary and Conclusions

This chapter has reviewed the experimental and clinical studies in the development of techniques used to interrupt warm blood cardioplegia. Most experimental studies have been performed in animals without coronary artery disease or ventricular hypertrophy. Many involve isolated preparations which may not be analogous to clinical practice. The majority of clinical studies have been performed on low risk patients with normal ejection fractions, no previous infarcts, and without any on-going ischemia. Assessment of left ventricular performance has generally been global with load dependent indices. Since many of the cold blood cardioplegia patients were cooled to 25-28°C, their systemic vascular resistance

tended to be higher in the earlier postop period, thus resulting in lower stroke work indices compared to patients treated with warm blood cardioplegia. Despite these limitations, certain conclusions can be drawn regarding the technique of interrupting warm blood cardioplegia.

How long can warm blood cardioplegia be safely interrupted? It appears that interrupting warm blood cardioplegia for up to 10 minutes in low risk patients without active ischemia is well tolerated and results in no increased mortality or infarct rate and that global left ventricular function appears to be preserved.[6,9-11,14] The length of the period of interruption of cardioplegia and not the total ischemic time is the most important parameter in determining myocardial performance and patient outcome.[9,14] The safe period for interrupting warm blood cardioplegia in actively ischemic patients is still undetermined. It appears however, that when cardioplegia is interrupted for longer than 13 minutes, there is a greater likelihood of an adverse outcome in patients with left main disease, decreased left ventricular function, and those requiring urgent surgery.[14]

What are the optimal flow rates to meet the needs of the myocardium when warm blood cardioplegia is interrupted? The exact flow rates for normal, ischemic, and hypertrophied myocardium have yet to be determined. Nevertheless, experimental and clinical studies using antegrade techniques suggest that an arresting dose of high K^+ cardioplegia (25-30 mEq) at 100-150 ml/min should be adequate for the nonischemic, nonhypertrophied myocardium. Following the interruption of cardioplegia, a "pay-back" dose of 200 ml/min is usually given for 2-3 minutes followed by the baseline flow rate (100-150 ml/min).[15,17]

What clinical methods can be used to monitor ongoing ischemia during the period of cardioplegic interruption? NMR is impractical in the operating room and may not be able to localize abnormalities in regional myocardial metabolism. Lactate washout is also non-specific and impractical in clinical cardiac surgery. ATP levels may not always be correlated with ongoing ischemia in the arrested heart and could not be used as an on-line measurement of active ischemia. Myocardial pH has been used to reliably detect regional ischemia during cardiac surgery and can give on-line data.[18] Surgeons using warm blood cardioplegia in patients where the solution must be interrupted for greater than 10 minutes may wish to consider monitoring pH values.

What techniques could be used to reverse ischemic changes following periods of intermittent warm blood cardioplegic delivery? It is the adequacy of distribution of cardioplegia and not the temperature which is the most important factor in limiting ischemia during aortic cross-clamping. In the presence of significant coronary stenoses, antegrade cardioplegia alone may not provide adequate myocardial protection.[3,19] This may result in further ischemic injury when warm blood cardioplegia is interrupted.[4] Infusing cardioplegia through completed vein grafts is one method by which cardioplegic distribution may be improved. Furthermore, continuous infusion of cardioplegia through completed vein grafts during the time that antegrade flow is interrupted may limit problems with visualization in the operative field while still adequately perfusing other areas of the myocardium. Another technique to maximize cardioplegic distribution is the combination of antegrade/retrograde delivery. Hayashida and his colleagues[17] demonstrated that intermittent antegrade infusions of warm cardioplegic solution during continuous warm retrograde cardioplegic delivery provided more homogenous distribution and prevented ischemic myocardial injury during warm heart operations. It would seem that the combination of antegrade/retrograde cardioplegia plus infusion of cardioplegia through the completed vein grafts would provide the most optimal myocardial protection in those instances when warm blood cardioplegia must be interrupted.

What is the role of interrupting warm blood cardioplegia in the current practice of cardiac surgery? Randomized clinical studies have yet to show a clear-cut superiority of warm blood cardioplegia over cold blood cardioplegic techniques. It is important to

remember that cardioplegia is merely an adjunct to the surgeons' main goal which is to perform a technically perfect operation. This is even more important in the current practice of cardiac surgery where optimal visualization is needed to bypass vessels which are smaller with more diffuse disease, to reconstruct valves, and to replace valves with advanced deterioration. Surgeons must rely on cardioplegic techniques which provide an unobscured field to construct a perfect technical anastomosis as well as afford excellent myocardial protection for acutely ischemic hearts with lower ejection fractions. Surgeons employing intermittent warm blood cardioplegia must understand the limitations of this technique and be able to alter their methodology when necessary in order to safely achieve both these goals.

References

1. Lichtenstein SV, Abel JG, Salterno TA. Warm heart surgery and results of operation for recent myocardial infarction. Ann Thor Surg 1991; 52:455-460.
2. Salerno TA, Houck JP, Barrozo CAM et al. Retrograde continuous warm blood cardioplegia: a new concept in myocardial protection. Ann Thorac Surg 1991; 51:245-247.
3. Matsuura H, Lazar HL, Yang X et al. Warm versus cold blood cardioplegia—Is there a difference? J Thorac Cardiovasc Surg 1993; 105:45-51.
4. Matsuura H, Lazar HL, Yang XM et al. Detrimental effects of interrupting warm blood cardioplegia during coronary revascularization. J Thorac Cardiovasc Surg 1993; 106:357-361.
5. Cohen MV, Liu GS, Downey JM. Preconditioning by a brief coronary occlusion preserves wall motion in ischemia/reperfusion. Circ 1990; 82:271-275.
6. Carrier M, Tourigny A, Thoribe N et al. Effects of cold and warm blood cardioplegia assessed by myocardial pH and release of metabolic markers. Ann Thorac Surg 1994; 58:764-767.
7. Ko W, Zelano J, Fahey L et al. Warm ischemic tolerance of the arrested heart. European J Cardiothorac Surg 1993; 7:295-299.
8. Landymore RW, Marble AE, Fris J. Effect of intermittent delivery of warm blood cardioplegia on myocardial recovery. Ann Thorac Surg 1994; 57:1267-1272.
9. Tian G, Xiang B, Butler KW et al. A ^{31}P-nuclear magnetic resonance study of intermittent warm blood cardioplegia. J Thorac Cardiovasc Surg 1995; 109:1155-1163.
10. Tonz M, Krogmann ON, Hess OM et al. Effect of intermittent warm blood cardioplegia on functional recovery after prolonged cardiac arrest. Ann Thorac Surg 1996; 62:1146-1151.
11. Torracca L, Pasini E, Curello S et al. Continuous versus intermittent warm blood cardioplegia: Functional and energetic changes. Ann Thorac Surg 1996; 62:1172-1179.
12. Yau TM, Ikonomidis JS, Weisel RD et al. Ventricular function after normothermic versus hypothermic cardioplegia. J Thorac Cardiovasc Surg 1993; 105:833-844.
13. Pelletier LC, Carrier M, Leclerc Y et al. Intermittent antegrade warm versus cold blood cardioplegia: A prospective, randomized study. Ann Thorac Surg 1994; 58:41-49.
14. Lichtenstein SV, Naylor CD, Feindel CM et al. Intermittent warm blood cardioplegia. Circ 1995; 92:341-346.
15. Christakis GT, Buth KJ, Weisel RD et al. Randomized study of right ventricular function with intermittent warm or cold cardioplegia. Ann Thorac Surg 1996; 61: 128-135.
16. Caldarone CA, Krukenkamp IB, Misare BD et al. Perfusion deficits with retrograde warm blood cardioplegia. Ann Thorac Surg 1994; 57:403-406.
17. Hayashida N, Ikonomidis JS, Weisel RD et al. Adequate distribution of warm cardioplegic solution. J Thorac Cardiovasc Surg 1995; 110:800-812.
18. Khuri SF, Josa M, Martson W et al. First report of intramyocardial pH in man. II. Assessment of adequacy of myocardial preservation. J Thorac Cardiovasc Surg 1983; 86:667-678.
19. Haan C, Lazar HL, Bernard S et al. Superiority of retrograde coronary sinus cardioplegia over antegrade cardioplegia following revascularization of an acute coronary occlusion. Ann Thorac Surg 1991; 51:408-412.

Section IV:
Surgical Techniques
B. Protection Strategies

CHAPTER 16

Myocardial Protection Strategies in Routine Coronary and Valve Operations

Kiyozo Morita and Michio Yoshitake

Integrated Myocardial Management

The goal of every cardiac operation must be a technically perfect anatomic result contributing to functional improvement that requires adequate visualization in a quiet, bloodless operative field without postoperative deterioration in cardiac function. The essential prerequisite for a successful cardiac operation is the use of simple and safe cardioprotective techniques to avoid myocardial damage, while maintaining a quiet, bloodless field.

A variety of myocardial protection strategies have been developed, and the clinical effectiveness of each strategy has been confirmed.[1-4]

This chapter describes an integrated technique of myocardial protection with warm and cold blood cardioplegia in conjunction with the combined use of antegrade and retrograde delivery for routine adult cardiac operations. This approach of integrated myocardial management, developed by Buckberg and associates,[5] combines the advantages of various cardioprotective strategies to compensate for their individual shortcomings and incorporates the strategies of warm/cold blood cardioplegia, antegrade/retrograde delivery, continuous/intermittent infusion, and blood/blood cardioplegic perfusion during a single period of aortic clamping.

This method is based on the following principles:

1. Adequate visualization for surgical precision is optimized by a still, bloodless field, accomplished by intermittent administration of cold potassium blood cardioplegia.
2. Uniform myocardial distribution of cardioplegic solution is ensured by the combination of antegrade and retrograde cardioplegic delivery.
3. Warm blood cardioplegia is used for active resuscitation of preoperatively impaired myocardium and/or avoidance of reperfusion injury, in addition to cold multidose blood cardioplegia.
4. Ischemia is unnecessary under the circumstances of the procedure when visualization is nonproblematic and continuous coronary perfusion is possible in an arrested myocardium.
5. Continuous blood perfusion of the cold arrested heart does not require cardioplegic solution. Noncardioplegic normal cold blood can be used to maintain arrest.

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Background and Logic of Integrated Myocardial Management

Combined Cold and Warm Blood Cardioplegia

The primary advantage of cold blood cardioplegia is that it couples the myocardial nourishment by intermittent oxygenation and substrates supply with the capacity to lower myocardial oxygen demands and the rate and development of ischemic damage in the hypothermic arrested heart.[1]

Despite the theoretical advantage of continuous warm blood perfusion to prevent ischemia,[2,3] the use of cold blood cardioplegia and subsequent myocardial hypothermia may be essential to prevent myocardial damage during intentional ischemia when blood supply must be interrupted for surgical precision. The capacity of cold intermittent blood cardioplegia to prevent ischemic damage in the normal myocardium is adequaet for up to 4 hours of the aortic clamping, as shown experimentally.[6] Intermittent cold blood cardioplegia techniques, however, can only prevent further deterioration but cannot correct metabolic defects that may compound the underlying mechanical cardiac disorder requiring correction. The method may be insufficient in the energy- and substrate-depleted heart, and impaired function may persist, as demonstrated experimentally.[7]

Warm blood cardioplegia was initially introduced as "warm blood cardioplegic reperfusion" to limit reperfusion injury by a brief period of administration of warm blood cardioplegia at the initial phase of reperfusion (before aortic unclamping).[8-10] Subsequently, the concept of "warm cardioplegic induction", delivery of warm blood cardioplegia to induce cardiac arrest after aortic clamping, was introduced to actively resuscitate the ischemically damaged, energy-depleted heart and improve its tolerance to subsequent intervals of cold ischemia.[7] Administration of warm blood cardioplegic solution restores aerobic ATP production by maximizing myocardial metabolic activity by normothermic blood perfusion and supplementation of depleted substrates, while keeping the heart arrested to lower its oxygen demands.[8] Thus, ATP is channeled to reparative processes in myocardial metabolism. The formulation of warm cardioplegic solution must be modified (hypocalcemic, alkalotic and enriched with amino acids, glutamate and aspartate) to limit calcium influx during reflow, buffer acidosis[9] and replenish key Krebs cycle intermediates depleted during ischemia.[10]

The clinical study by Teoh and associates[10] demonstrated that warm cardioplegic reperfusion (terminal warm blood cardioplegia) accelerates myocardial metabolic recovery after reperfusion. It is now generally accepted that warm, controlled reperfusion provides a powerful tool to limit reperfusion injury and nullify the adverse effects of prolonged aortic clamping.[12] Additionally, the clinical benefits of warm induction in energy-depleted hearts were demonstrated in coronary patients with cardiogenic shock[13,14] and advanced valvular disease.[15] In the integrated management strategy, multidose cold blood cardioplegia to allow interrupting coronary perfusion for surgical precision is incorporated with warm blood cardioplegic induction and reperfusion in order to combine the individual benefits of warm and cold cardioplegia to maximize the cardioprotective effects of blood cardioplegia.

Antegrade/Retrograde Cardioplegic Delivery

The benefits of cardioplegia are provided only if the solutions are distributed to all myocardial regions in sufficient amounts. Antegrade cardioplegia is used by most surgeon and produces prompt cardiac arrest, but is distributed poorly in coronary patients with severely stenotic or occluded coronary arteries unless it is delivered through the vein graft.[4,16,17] These strategies are ineffective in patients who receive arterial grafts or who have diffuse coronary artery disease. Further limitation of antegrade cardioplegia include: poor distribution in patients with aortic regurgitation unless the aorta is opened and the coronary ostia are perfused directly; risk of potential ostial injury during aortic valve procedure from direct perfusion; and the need

to interrupt the continuity of mitral valve operations to remove the retractors and avoid aortic distortion during cardioplegic replenishment. These limitations of antegrade cardioplegia can be overcome by the use of retrograde cardioplegic delivery.[4,16] In the presence of complete coronary artery occlusion, this method would appear to result in a better perfusion of the ischemic myocardial area.[4]

The development of transatrial coronary sinus cannulation technique has made simple, safe, and rapid access to the coronary sinus feasible,[18] and retrograde cardioplegia is now generally accepted as a routine method for intraoperative myocardial protection. Nevertheless, experimental[17] and clinical[19-21] studies have shown that the right ventricle and posterior septum are not adequately perfused with retrograde cardioplegia. Inconsistent right ventricular protection may be problematic in patients with marked right ventricular hypertrophy or pulmonary hypertension. This recognized limitation of right ventricular hypoperfusion can be counteracted partially by cold cardioplegic perfusion, inasmuch as coronary sinus retroperfusion provides right ventricular hypothermia as the effluent traverses conductance vessels, and therefore confers hypothermic lowering of oxygen demands to counteract this limited nutritive oxygen supply due to veno-veno shunts. This benefit is achieved only if hypothermia is used in conjunction with retrograde cardioplegic delivery.

Aforementioned limitations of antegrade and retrograde perfusion have stimulated the use of combined antegrade/retrograde infusion of cardioplegia (alternating between antegrade and retrograde[20-24] or simultaneous antegrade/retrograde perfusion[25]). In an editorial comment, Menasche[4] suggested that a combined antegrade and retrograde approach may be safer and more effective in obtaining a more uniform myocardial distribution of blood cardioplegia.

Intermittent Blood Cardioplegia and Continuous Perfusion of Noncardioplegic Cold Blood

A dry, quiet operative field is a prerequisite for a technically precise cardiac procedure. Most surgeons arrest the heart with high dose potassium blood cardioplegia (20 mEq/L) and use multidose low dose potassium (8-10 mEq/L) as interrupting coronary perfusion for the remainder of the operation. In a routine adult cardiac operation, however, ischemia is unnecessary under the circumstances, when visualization is nonproblematic, and therefore continuous coronary perfusion is feasible if the hearts can be kept arrested.

For that purpose, continuous blood perfusion of the cold arrested heart does not require cardioplegic solution (noncardioplegic normal cold blood is possible).

Our recent experimental study[26] showed that cold arrested hearts remain quiescent and both ventricles recovered completely when perfused with cold 4-10c retrograde noncardioplegic blood. Consequently, the advantages of continuous perfusion and nourishment to shorten real ischemic interval can be achieved without the drawbacks of excessive hemodilution and cardioplegic overdose. These benefits are possible only with "cold" noncardioplegic blood since electromechanical activity (i.e., beating or fibrillation) returns and myocardial oxygen demands rise when warm blood is delivered.

Method of Integrated Myocardial Management and Surgical Techniques

The Cardioplegic Solution

The blood cardioplegic formulations for the standard 4:1 ratio (Blood:Cardioplegia) is listed in Table 16.1. The formulation is modified for the other ratio (i.e., 8:1 Blood:Cardioplegia), as reported previously.[5]

For standard protocol, four cardioplegic solutions are prepared: a high and low K^+ amino acid enriched solution for warm induction and reperfusion, and a high and low K^+ nonamino acid enriched solution for cold

Table 16.1. The blood cardioplegic formulations for the standard 4:1 ratio (Blood:Cardioplegia) in the integrated myocardial management. The formulation is modified for the other ratio (i.e., 8:1 Blood:Cardioplegia), as reported previously.[5]

Cardioplegic Additive	Volume Added(mL)	Component Modified	Concentration Delivered
		Cold Induction	
Tham (0.3M)	200	pH	7.7-7.8
CPD	50	Ca++	0.5-0.6 mM/L
5% dextrose & H_2O	550	Osm	340-360 mOsm
KCL (2mEq/mL)	30	K+	18-20 mEq
		Warm induction	
Tham (0.3M)	225	pH	7.5-7.6
CPD	225	Ca++	0.15 mM/L
50%dextrose	40	Glucose	> 400mg
KCL (2mEq/mL)	40	K+	20-25 mEq
Glutamate			13 mM/L
	250	substrate	
Aspartate			13 mM/L
5% dextrose & H_2O	220	%Osm	380-400 mOsm
		Cold Maintenance	
Tham (0.3M)	200	pH	7.7-7.8
CPD	50	Ca++	0.5-0.6 mM/L
5% dextrose & H_2O	550	Osm	340-360 mOsm
KCL (2mEq/mL)	10	K+	8-10 mEq
		Warm Reperfusion	
Tham (0.3M)	225	pH	7.5-7.6
CPD	225	Ca++	0.15-0.25 mM/L
50%dextrose	40	Glucose	> 400mg %
KCL (2mEq/mL)	15	K+	8-10 mEq
Glutamate			13 mM/L
	250	substrate	
Aspartate			13 mM/L
5% dextrose & H_2O	200	Osm	380-400 mOsm

induction and maintenance doses. Currently, only two cardioplegic solutions: a high K^+ amino acid enriched solution and a low K^+ nonamino acid enriched solution are made up for each procedure, as the solution for warm induction is used also for cold induction and warm reperfusion in a routine procedure to simplify the preparation. Glutamate and aspartate are not added to the maintenance solution, as peripheral vasodilatation may occur when large volumes of amino acids are used.

Cardioplegic Delivery System

Blood cardioplegia delivery system used for the integrated myocardial management contains a single roller pump, heat exchanger for altering cardioplegic temperature, and coronary perfusion- monitoring set for alternating between antegrade and retrograde routes (Fig. 16.1). The blood component of cardio-

plegic solution is withdrawn from the coronary port of the oxygenator. The blood is mixed in a ratio of 4:1 (blood:cardioplegia) by fixing the calibers of the tubing coming from the cardioplegic solution and coronary port of the oxygenator. The tubes are then routed through a single occlusive roller pump, with an insert to accommodate the different sizes of the tubing and into a heat exchanger with the capacity of rapid alteration of blood temperature so that the cardioplegic solution can be delivered either warm (37°C) or cold (8-10°C) as required under different circumstances.

As a heat exchanger, standard Advance BCD™ or more recently developed Buckberg BCD Vanguard™ (Sorin Biochemical Inc., Irvine, CA) is exclusively utilized.

Two heater/coolers (one for the cardioplegic solution, and the other for systemic perfusion) are required in order to control temperature of the heart and body independently.

A heat exchanger, Buckberg BCD Vanguard™ (Fig. 16.2) has efficient heat exchange performance, rapid priming capacity and low priming volume (35 mL device only), integral filter, bubble trap and pressure monitoring port.

The pressure port is essential so the perfusionist can determine if there is obstruction in the system during cardioplegic administration, and make adjustments to avoid disruption of the heat exchanger by excessive pressure.

The outlet arm of the heat exchanger is connected to a cardioplegic infusion line. For alternating between antegrade and retrograde delivery, we use antegrade/retrograde infusion and pressure monitoring set (MIS-001-CP) (Research Medical Inc., Midvale, Utah) containing two arms of each infusion and pressure monitoring line specially designed to allow rapid switching from antegrade to retrograde cardioplegia and simultaneous monitoring of aortic and coronary sinus pressure. The side arm attached to the retrograde cardioplegic delivery arm permits perfusion via vein graft or coronary ostial cannula (Fig. 16.3).

Antegrade Cannula

An aortic cannula containing ports of monitoring perfusion pressure and aortic venting is required for delivery of antegrade cardioplegia, when used in combination with retrograde cardioplegia. The aortic vent line is outfitted with a one-way pop-off valve which is kept on low suction throughout the procedure. Alternatively, the vent line can be connected to a Y-connector in the venous line to provide gravity drainage.

Aortic pressure monitoring is crucial to ensure cardioplegic delivery and it allows: A) detection of aortic regurgitation; B) inadvertent failure to close the aortic vent line; C) determination of optimal flow rate in hypertrophied ventricle; and D) regulation of the pressure during controlled reperfusion.

Clinical cardioplegic perfusion pressure of 80-100 mmHg are safe during cardioplegic induction. Conversely, keeping perfusion pressure at or below 50 mmHg during reinfusion and reperfusion will be desired to protect edema formation and endothelial integrity.

Retrograde Cannula

The cannula for retrograde delivery of choice is used routinely to deliver retrograde cardioplegia via coronary sinus and simultaneously monitoring coronary sinus pressure. We exclusively use a Retroplegia™ cannula with the self-inflated textured balloon (RC-014-T, Research Medical, Inc., Midvale, Utah) containing a specially designed stylet with a low pressure, self inflating and deflating balloon and an integral second lumen to permit pressure monitoring. The flow lumen is designed to have the small holes distal to the balloon and larger holes present within the balloon. This design allows the balloon to become self inflated as the cardioplegic solution flows through the cannula, and therefore occludes back flow from the coronary sinus as the remainder of the solution flows through the smaller distal holes. This also allows the balloon to be self deflated spontaneously when the infusion is completed. The inflated balloon is only 1.8 cm in diameter and contains allow intraluminal pressure, so that it cannot

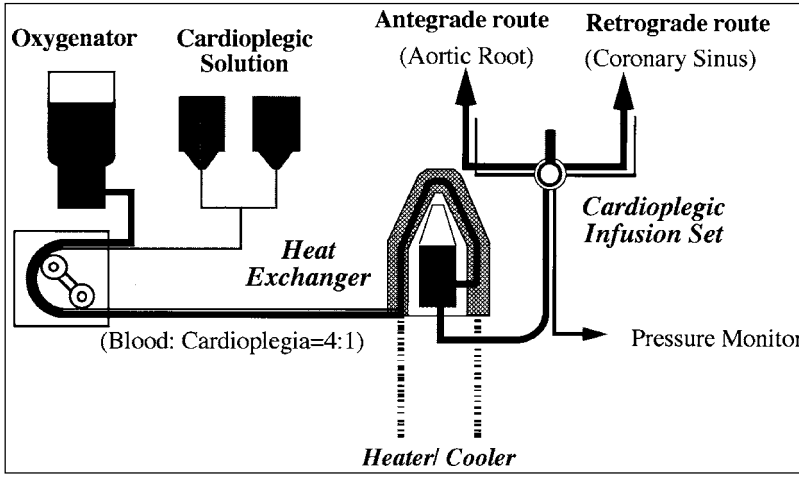
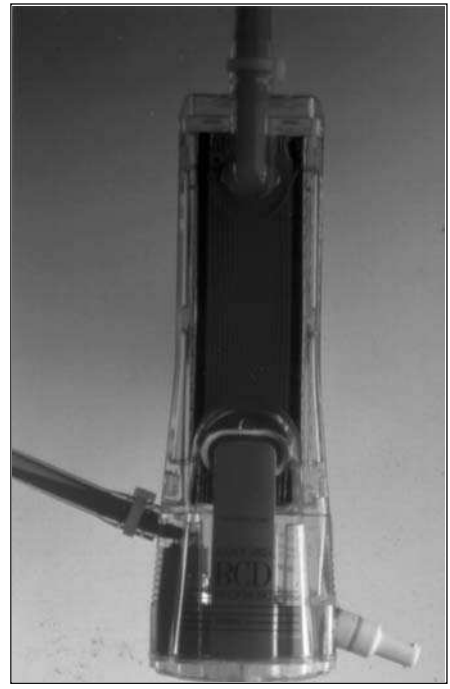


Fig. 16.1. Cardioplegic delivery system containing a single occlusive roller pump, tubing system, heat exchanger with the capacity of rapid alteration of blood cardioplegic temperature and mixture of blood and cardioplegic solution, heater/cooler, and the specially designed cardioplegic infusion line for alternating between antegrade and retrograde delivery. As a heat exchanger, standard Advance BCD ™ or Buckberg BCD Vanguard ™ (Sorin Biochemical Inc., Irvine, CA) is routinely utilized.

Fig. 16.2. Buckberg BCD Vanguard™ (Sorin Biomedical Inc. Irvine, CA) used for warm/cold blood cardioplegic delivery. This heat exchanger has efficient heat exchange performance, rapid priming capacity and low priming volume (35 mL device only), integral filter, bubble trap and pressure monitoring port.



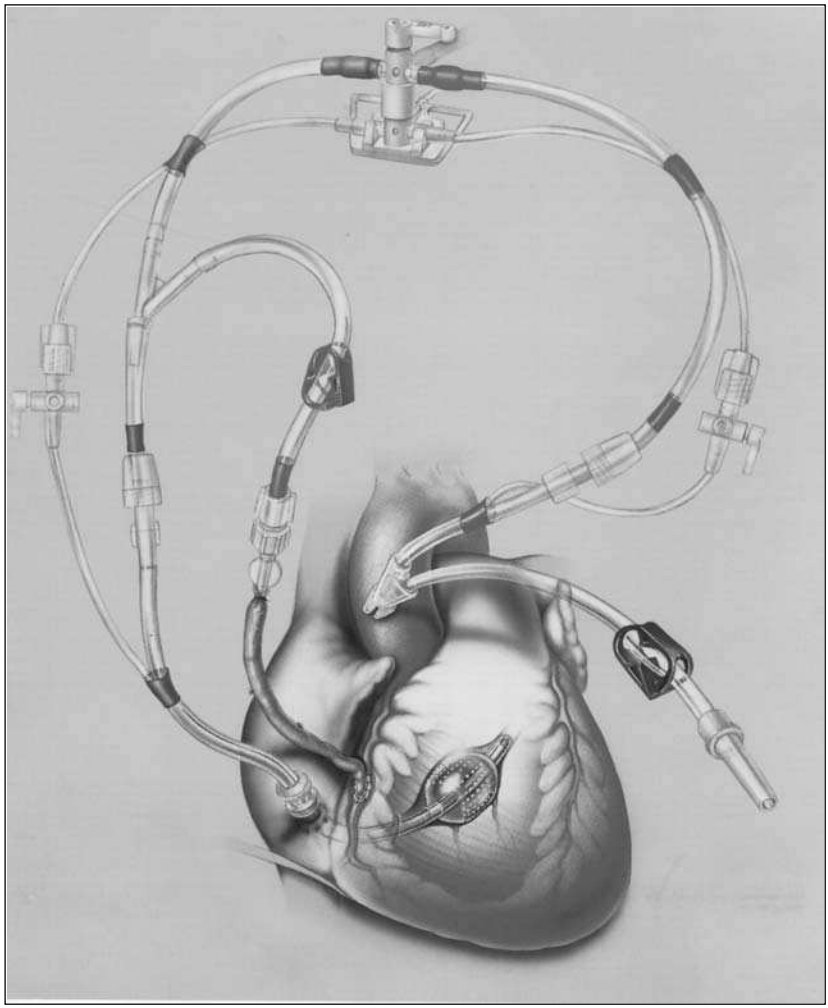


Fig. 16.3. Schematic for delivering antegrade/retrograde blood cardioplegia and pressure monitoring. Antegrade/retrograde infusion and pressure monitoring set (MIS-001-CP) (Research Medical Inc., Midvale, Utah) is utilized for cardioplegic infusion, and Retroplegia ™ with self-inflated textured balloon (RC-014-T, Research Medical, Inc., Midvale, Utah) is used for retroperfusion

damage the coronary sinus by over inflation and produce barotrauma during infusion.

The coronary sinus pressure must be displayed so that the surgeon and perfusionist can be aware of it during infusion.

Placement of Retroplegia

The Retroplegia cannula is always placed after venous cannulation to avoid inadvertent dislodgment when the IVC cannula is inserted. Retroplegia cannulation is accomplished most readily before starting extracorporeal circulation or can be done on partial bypass. A 3-0 prolene suture is placed in the low right atrium, near the IVC junction, and is withdrawn through a rubber tourniquet. This low position for placement is selected to avoid dislodgment of the retroplegia cannula during retroperfusion or retraction of the heart for

exposure of the circumflex and distal branches of the right coronary artery.

The stylet and cannula are introduced into the right atrium through a small puncture anterior to the two-stage cannula, and inserted far enough to advance the balloon into an intra-atrial position. The suture and tourniquet are snagged to prevent bleeding. Placement of the index finger at the IVC-right atrial junction will allow palpation of the tip of the cannula, guide its entrance into the coronary sinus and verify its correct placement position. By this maneuver, advancement of cannula into coronary sinus can be accomplished readily. Normally coronary sinus course is in a direction that extends at a approximate 45° angle directly toward the left shoulder from its orifice to its position beneath the left atrial appendage. Verification of the correct placement of the stylet is made by palpating the course of the coronary sinus behind the heart. When the proper positioning is confirmed, the cannula is advanced little further over the stylet until it meets any resistance, while holding the stylet in place. This maneuver will place cannula at the coronary sinus great coronary vein junction beneath the left atrial appendage. The stylet is then withdrawn while cannula is held in place. The pressure port is connected to ensure coronary sinus pressure. A high coronary sinus pressure (i.e., > 20 mmHg) indicates the catheter is wedged.

Pressure Monitoring During Retrograde Cardioplegia

The coronary sinus pressure ranges usually 30-50mmHg when retrograde cardioplegia is delivered at 200-250 mL/min. The surgeon and perfusionist must be aware of the coronary sinus pressure during infusion so that the appropriate correction can be made if the coronary sinus pressure is either too high or too low. A coronary sinus pressure > 50 mmHg means the catheter is lodged too far into the coronary sinus or is in the posterior descending coronary vein and this can be corrected by reducing the flow rate immediately, withdrawing the catheter and restarting retrograde flow. Conversely, a coronary sinus pressure < 20 mmHg during infusion indicates that the self-inflating balloon is not occluding the coronary sinus completely, or that the catheter is dislodged.

Under these circumstances, the position of the catheter tip must be tested by palpation of the coronary sinus. The adequacy of retroperfusion can be assumed when the catheter has been retracted, but remains in the coronary sinus, by simply compressing the coronary sinus-right atrial junction against the IVC cannula. This always results in a prompt rise in coronary sinus pressure and avoids the need for repositioning the cannula. Sometimes the cannula is retracted and slipped away from the coronary sinus due to the back pressure in the coronary sinus at the initiation of retrograde infusion. A gradual increase of the flow rate over 12-15 seconds will minimize catheter retraction during infusion. Failure to ensure adequate coronary sinus pressure (20-50 mmHg) during cardioplegic infusions will result in inadequate retrograde myocardial protection and therefore retrograde cardioplegia must be abandoned.

Standard Protocol for Integrated Blood Cardioplegia

The cardioplegic dose is generally divided by alternating between antegrade and retrograde delivery in a standard protocol for a routine adult open heart surgery. Flow guidelines used for alternative administration of antegrade and retrograde blood cardioplegia for induction, maintenance, reperfusion and continuous noncardioplegic cold blood perfusion are listed in Table 16.2.

Cardioplegic Induction

Antegrade cardioplegia is always given first to produce prompt arrest, followed by retroperfusion of the remainder of the cardioplegic dose, except aortic valve insufficiency patients. Failure to achieve prompt arrest (i.e., 1-2 minutes) suggests that either there is incomplete aortic clamping, aortic regurgitation, or inadequate venous drainage with admixture of cardioplegia with blood that escapes the venous return cannula. Reapplication of the aortic clamp will ensure that the aorta is occluded completely. The presence of

Table 16.2. Flow guidelines used for alternative administration of antegrade and retrograde blood cardioplegia for induction, maintenance, reperfusion and continuous noncardioplegic cold blood perfusion. The flow rates are increased by 50-100mL/min for hypertrophied hearts.

Cardioplegia	Antegrade	Retrograde
Cold Induction	300 mL/min (2 min)	200mL/min (2min)
Warm induction	300 mL/min until arrest then 150 mL/min (2.5 min)	150 mL/min (2.5 min)
Maintenance	200 mL/min (1 min)	200 mL/min (1 min)
Noncardioplegic Blood		150 mL/min
Warm reperfusion	150 mL/min (2 min)	150 mL/min (2 min)

aortic regurgitation will be noted in case of a low aortic perfusion pressure during cardioplegic induction, and the most common cause of this is distortion of the noncoronary cusp by venous cannula. This can be corrected readily by retracting the venous cannula downward and to the right. Clinically unsuspected mild aortic regurgitation can be handled by increasing the flow rate of the antegrade cardioplegia to 500 mL/min, and by compressing the right ventricular outflow with a sponge stick to push the septum against the posterior left ventricular wall beneath the aortic valve. Failure of these maneuvers to restore satisfactory aortic perfusion pressure at the increased flow rate immediately should lead to abandonment of the antegrade cardioplegic infusion and to complete reliance upon retrograde cardioplegia as the only method of myocardial protection.

Cold Induction

Cold cardioplegic induction with 8-10°C blood cardioplegia is used during all elective operations where preoperative cardiac function is reasonable (i.e., LVEF > 40%). The antegrade dose is given at 300-350 mL/min for 2 minutes to stop the heart and the remainder of the induction dose is delivered retrograde at 200-250 mL/min for 2 minutes. The cardioplegic volume and infusion rate are increased in hypertrophied hearts (2-minute antegrade at 350 mL/min, and 2-minute retrograde at 250 mL/min). After the completion of retrograde cardioplegic perfusion, coronary perfusion is interrupted when there is a need to ensure a bloodless operative field, otherwise retrograde perfusion of noncardioplegic cold blood is continued. The conversion from antegrade to retrograde cardioplegia is made by turning the stopcock and activating the aortic vent line.

The aortic vent line is kept open until the next antegrade cardioplegia to provide cardiac decompression and to evacuate air from the aorta when the next antegrade infusion is started.

Warm Induction

The heart is stopped with a warm (37°C) substrate enriched (glutamate/aspartate) high potassium (20-25 mEq/L) cardioplegic solution (Table 16.1) when there is clinically significant preoperative impairment of cardiac function (i.e., cardiogenic shock, advanced valve disease, extending myocardial infarction). The purpose of warm induction is to "resuscitate" the heart and improve its tolerance to subsequent ischemia. Warm induction is achieved by circulating warm water from the heater/cooler into a heat exchanger. Warm induction is delivered antegradely first. The initial infusion is started at 300-350 mL/min until the heart stops, and the infusion rate is then slowed to 150-200 mL/min. The total dose is divided relatively equal between antegrade and retrograde delivery. The total period of warm infusion is 5 minutes (2.5 minutes antegrade and 2.5 minutes retrograde)

Warm induction is followed always by a 3-4 minute infusion of cold cardioplegia (8-10°C) at 200-250 mL/min (1/2 antegrade and 1/2 retrograde) with the low K^+ cardioplegic solution to ensure cardioplegic distribution and cooling. For this purpose, one to two minutes before the completion of initial warm induction, the heater/cooler is switched to the maximum cooling mode to lower the temperature of cardioplegic solution rapidly down to 10°C.

Multidose Administration of Cold Blood Cardioplegia (Cold Maintenance Cardioplegia)

Multidose administration of cold low K^+ blood cardioplegia are repeated to replenish the solution washed away by noncoronary collateral flow at approximately 20-25 minutes intervals. Reinfusion are delivered at 200-250mL/min for 2 minutes, with 1-minute antegrade and 1-minute retrograde, whether or not electromechanical activity returns for the following reasons: maintains arrest; restores hypothermia; buffers acidosis; washes away acid metabolites; and replenishes substrates.

In coronary operations, all reinfusions are divided equally between antegrade and retrograde routes of delivery (1 minute antegrade and 1 minute retrograde). Conversely, all reinfusions during mitral and aortic valve operations are delivered retrograde for 2 minutes so that the procedure can proceed uninterrupted, unless the right ventricle starts to contract. If this occurs, 1 minute infusion is delivered at 20-25 minutes intervals via the antegrade route in mitral operations or the right coronary ostia in aortic valve procedures in order to ensure right ventricular protection.

Noncardioplegic Cold Blood Retroperfusion

After the completion of antegrade and retrograde reinfusion of cold blood cardioplegia, retrograde cold noncardioplegic blood is continuously perfused usually at 150 mL/min, when it does not compromise visualization during the aspects of the procedure (i.e., proximal anastomosis of coronary graft, placing sutures into the prosthetic valve or annuloplasty ring and closure of the atriotomy or aortotomy).

A one-minute infusion of the cardioplegic maintenance solution is delivered every 10-20 minutes during prolonged cold noncardioplegic blood perfusion or if electromechanical activity returns spontaneously. This ensures maintenance of arrest and avoids cold noncardioplegic blood perfusion of the heart with electromechanical activity.

Perfusion is always stopped if blood obscures vision, as visualization is never compromised to maximize surgical precision.

Warm Reperfusion

The objective of warm reperfusion is to limit reperfusion injury by lowering energy demands. This is done by keeping the heart arrested during the initial period of reflow, and optimizing metabolic rate of repair by raising the temperature to normothermia, while adding substrates, buffering acidosis, and limiting the calcium load.[27] Warm reperfusate (Table 16.1) is delivered before aortic unclamping in all patients at 150 mL/min for 3-5 minutes, while monitoring perfusion pressure and is divided equally by alternating between antegrade and retrograde delivery. During application of warm reperfusion, it is crucial to maintain persistent cardiac arrest without any electromechanical activity and to avoid distention of the left ventricle. Furthermore keeping perfusion pressure at or below 50 mmHg during antegrade warm reperfusion by controlling flow rate will be desired to protect edema formation and endothelial integrity. Rewarming of the blood cardioplegic solution is started approximately 2-3 minutes before starting the warm reperfusate, and is accomplished by switching the heater-cooler to warming mode. The duration of warm cardioplegic reperfusion is prolonged to 20 minutes when the operation is for acute evolving myocardial infarction, since prolonged controlled reperfusion enhances early functional recovery after regional ischemia, as experimentally shown in a report by Allen and associates.[27]

Special Consideration for the Type of Surgical Procedure

Coronary Operation

In general, the aforementioned standard protocol for integrated management is applied to all coronary patients. In coronary operations, all infusions of cardioplegia are divided equally between antegrade and retrograde routes of delivery, inasmuch as the adequate distribution of cardioplegic solution is of crucial importance in patients with coronary disease. The current strategies for coronary bypass grafting include constructing all anastomoses during a single period of aortic clamping, delivering the cardioplegic solution through either aortic root or coronary sinus, and perfusing cardioplegic solution through the graft after each distal anastomosis if needed. Graft perfusion is particularly important if the right coronary artery is revascularized with the vein graft, since the right ventricle may not be sufficiently perfused and protected with either antegrade aortic root perfusion or retroperfusion. The side arm of cardioplegic infusion set is used for graft perfusion. Alternatively, cardioplegic delivery is ensured by antegrade perfusion provided that each proximal anastomosis is accomplished immediately after each distal anastomosis.

After antegrade and retrograde cardioplegic induction, cold ischemia is often required during distal anastomosis, but continuous retroperfusion of noncardioplegic cold blood is used when it does not compromise visualization (i.e., dissection of coronary arteries, preparation of arterial grafts, or proximal anastomosis of vein grafts). The obligatory prolongation of aortic clamping is counterbalanced by the improved cardioplegic distribution.

Anterograde warm reperfusion is started retrogradely prior to the completion of proximal anastomosis and followed by antegrade warm reperfusion. During antegrade warm reperfusion, the clamps of vein grafts are released so that antegrade perfusion is delivered to the segments that were revascularized.

Valve Operations

Aortic Valve Operation

In aortic stenosis, an antegrade cardioplegic induction is started antegradely, followed by retrograde delivery as the aorta is opened. With aortic regurgitation, cardiopulmonary bypass is initiated normothermically to prevent ventricular distention and ventricular fibrillation; cardioplegia is administered retrograde at a higher flow rate than the standard protocol, while the aorta is opened. The coronary perfusion cannula connected to the side arm of cardioplegic infusion set is available for selective coronary ostial perfusion. The left and right coronary ostia is cannulated promptly in patients with aortic regurgitation and cardioplegia is delivered until the standard dose for antegrade induction is completed and electromechanical activity completely disappears. A coronary perfusion cannula must be introduced into the right coronary ostia to ensure right ventricular protection.

Retrograde cold cardioplegia is given for 2 minutes each 20-25 minutes exclusively at 200 mL/min to avoid ostial perfusion for valve excision, placement of sutures in the annulus, and securing the prosthesis.

Cold noncardioplegic blood is infused continuously at 150 mL/min while sutures are placed from the annulus to the valve ring, and while the aortotomy is closed. Particularly in complicated aortic and mitral operation (i.e., double valve replacement with annular enlargement procedure), a relatively extended period of continuous perfusion is technically feasible to shorten ischemic interval.

The color of the effluent blood from the coronary ostia is inspected during retroperfusion. A well oxygenated effluent is interpreted to reflect adequate ventricular perfusion (sufficient flow to meet the low demands of arrest during hypothermia) and operation is performed exclusively with retroperfusion via the coronary sinus with blood cardioplegia and noncardioplegic blood. Conversely, intermittent right coronary ostial perfusion is mandatory if the right coronary effluent is very desaturated, as this is considered to indicate

that right ventricular perfusion is potentially deficient.

The retrograde warm blood cardioplegic reperfusate is initiated approximately 4-5 minutes before it is anticipated that the aortotomy incision will be closed and is followed by antegrade perfusion after the aorta is de-aired completely.

The clamp is removed and replaced immediately 1/3-1/2 way across the site of clamping to create a dome to purge retained air.

Mitral Valve Operation

After antegrade and retrograde cardioplegic induction (1/2 antegrade and 1/2 retrograde), cold cardioplegic or blood perfusion is exclusively retrograde for the remainder of the operation with the exception of the period of warm reperfusion. Primary reliance is placed upon retrograde perfusion, since mitral retractors may make the aortic valve incompetent and make antegrade perfusion ineffective.

Cold cardioplegia is given for 2 minutes at 20-25 minute interval exclusively via retrograde route at 200 mL/min for valve repair, valve excision, placement of sutures in the annulus, and securing the prosthesis. During mitral procedures, however, the coronary effluent from the retroperfusion drainage which comes from the coronary ostia is successfully harvested from suction placed into the left ventricle, and continuous perfusion of noncardioplegic cold blood is maintained without compromising visualization in most of the aspects of mitral procedure. Perfusion is always stopped if blood obscures vision, as visualization is never compromised to maximize surgical precision.

Intermittent doses of the cardioplegic maintenance solution are administered for one minute every 10-20 minutes of prolonged cold noncardioplegic blood retroperfusion or whenever electromechanical activity returns.

The retrograde warm blood cardioplegic reperfusate is initiated as the atriotomy closure is begun. The antegrade portion of the warm reperfusate is then delivered

Combined Valve and Coronary Procedures

The distal coronary anastomoses are always constructed first in all combined valve and coronary procedures using cold intermittent doses of antegrade and retrograde blood cardioplegia as described previously. This allows all subsequent cardioplegic infusions to be delivered from the graft (if needed).

The vein introducers in the coronary graft are connected to the cardioplegic infusion set containing one or more side arms so that the cardioplegic solution can be delivered via the grafts during the valve procedure. Myocardial management during aortic and mitral procedures are as described previously. Retroperfusion of cold noncardioplegic blood is continued as the proximal anastomoses are started. During antegrade warm reperfusion, the clamps of vein grafts is released so that antegrade perfusion is delivered to the segments that had antegrade revascularization.

References

1. Follette DM, Mulder DG, Maloney JVJ et al. Advantages of blood cardioplegia over continuous coronary perfusion and intermittent ischemia. J Thorac Cardiovasc Surg 1978; 76:604-619.
2. Lichtenstein SV, Ash KA, El-Dalati H et al. Warm heart surgery. J Thorac Cardiovasc Surg 1991; 101:269-274.
3. Salerno TA, Houck JP, Barrozo CAM et al. Retrograde continuous warm blood cardioplegia: a new concept in myocardial protection. Ann Thorac Surg 1991; 51:245-247.
4. Menasche' P, Subayi JB, Veyssie' L et al. Efficacy of coronary sinus cardioplegia in patients with complete coronary artery occlusions. Ann Thorac Surg 1991; 51:423-428.
5. Buckberg GD, Beyersdorf F, Allen BS. Integrated myocardial management in valvular heart disease. J Heart Valve Disease 1995; 4 (suppl. II):S 198-213.
6. Robertson JM, Vinten-Johansen J, Buckberg GD et al. Safety of prolonged aortic clamping with blood cardioplegia. I Glutamate enrichment in normal hearts. J Thorac Cardiovasc Surg 1984; 88:395-401.

7. Rosenkranz ER, Okamoto F, Buckberg GD et al. Safety of prolonged aortic clamping with blood cardioplegia. III Aspartate enrichment of glutamate-blood cardioplegia in energy-depleted hearts after ischemic and reperfusion injury. J Thorac Cardiovasc Surg 1986; 91:428-435.
8. Follette DM, Steed DL, Foglia RP et al. Reducing postischemic myocardial damage by maintaining arrest during initial reperfusion. Surg Forum 1977; 28:281-283.
9. Follette D, Fey K, Buckberg GD et al. Reducing postischemic damage by temporary modification of reperfusate calcium, potassium, pH, and osmolarity. J Thorac Cardiovasc Surg 1981; 82:221-238.
10. Lazar HL, Buckberg GD, Manganaro AM et al. Myocardial energy replenishment and reversal of ischemic damage by substrate enhancement of secondary blood cardioplegia with amino acids during reperfusion. J Thorac Cardiovasc Surg 1980; 80:350-359.
11. Teoh KH, Christakis GT, Weisel RD et al. Accelerated myocardial metabolic recovery with terminal warm blood cardioplegia. J Thorac Cardiovasc Surg 1986; 91:888-895.
12. Kirklin JW. Technical and scientific advances in cardiac surgery over the past 25 years. Ann Thorac Surg 1990; 49:26-31.
13. Rosenkranz ER, Buckberg GD, Mulder DG et al. Warm induction of cardioplegia with glutamate-enriched blood in coronary patients with cardiogenic shock who are dependent on inotropic drugs and intra-aortic balloon support: Initial experience and operative strategy. J Thorac Cardiovasc Surg 1983; 86:507-518.
14. Beyersdorf F, Kirsh MM, Buckberg GD et al. Warm glutamate/aspartate-enriched blood cardioplegic solution for perioperative sudden death. J Thorac Cardiovasc Surg 1992; 104:1141-1147.
15. Cunningham J, Rosenkranz ER, Okamoto F et al. Benefits of normothermic induction of blood cardioplegia in energy-depleted hearts with maintenance of arrest by multidose cold blood cardioplegia. J Thorac Cardiovasc Surg 1982; 84:676-683.
16. Noyez L, van Son JAM, van der Werf T et al. Retrograde versus antegrade delivery of cardioplegic solution in myocardial revascularization. J Thorac Cardiovasc Surg 1993; 105:854-863.
17. Partington MT, Acar C, Buckberg GD et al. Studies of retrograde cardioplegia. II. Nutritive blood flow distribution in normal and jeopardized myocardium. J Thorac Cardiovasc Surg 1989; 97:613-.
18. Buckberg GD, Drinkwater DC, Laks H. A new technique for delivering antegrade/retrograde blood cardioplegia without right heart isolation. Eur J Cardiothorac Surg 1990; 4:163-168.
19. Allen BS, Winkelmann JW, Hanafy H et al. Retrograde cardioplegia does not adequately perfuse the right ventricle. J Thorac Cardiovasc Surg 1995; 109:1116-1126.
20. Ardehali A, Gates RN, Laks H et al. The regional capillary distribution of retrograde blood cardioplegia in explanted human hearts. J Thorac Cardiovasc Surg 1995; 109:935-940.
21. Carrier M, Gre'goire J, Khalil A et al. Myocardial distribution of retrograde cardioplegic solution assessed by myocardial thallium-210 uptake. J Thorac Cardiovasc Surg 1994; 108:1115-1118.
22. Buckberg GD. Antegrade/ retrograde blood cardioplegia to ensure cardioplegic distribution: operative techniques and objectives. J Card Surg 1989; 4:216-238.
23. Jegaden O, Eker A, Montagma P et al. Antegrade/retrograde cardioplegia in arterial grafting: metabolic randomized clinical trial. Ann Thorac Surg 1995; 59:456-461.
24. Aldea GS, Hou D, Fonger JD et al. Inhomogeneous and complementary antegrade and retrograde delivery of cardioplegic solution in the absence of coronary artery obstruction. J Thorac Cardiovasc Surg 1994; 107:499-504.
25. Ihnken K, Morita K, Buckberg GD et al. The safety of simultaneous arterial and coronary sinus perfusion: experimental background and initial clinical results. J Cardiac Surg 1994; 9:15-25.
26. Ihnken K, Morita K, Buckberg GD. New approaches to blood cardioplegic delivery to reduce hemodilution and cardioplegic overdose. J Card Surg 1994; 9:26-36.
27. Allen BS, Okamoto F, Buckberg GD et al. Studies of controlled reperfusion after ischemia. XV. Immediate functional recovery after 6 hours of regional ischemia by careful control of conditions of reperfusion and composition of reperfusate. J Thorac Cardiovasc Surg 1986; 92:621-635.

CHAPTER 17

Acute Myocardial Infarction and Cardiogenic Shock

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The surgical approach to myocardial infarction during the acute evolving phase has been controversial and its role has changed during the last decades. In fact emergency coronary bypass surgery has been adopted in some centers since the 1970s.[1-3] Results were acceptable, but far from satisfactory. However, this applied to medical therapy as well. Thus, research was directed towards improvement of both, surgical and nonsurgical, strategies. What is certainly well documented is that revascularization procedures should preferably be accomplished within approximately 6 hours from the onset of myocardial ischemia. This fact rises logistic problems in the standardization of in-hospital treatment of such a widely diffused and life-threatening disease.

Initial reports of surgically treated patients with acute evolving myocardial infarction documented mortality rates as low as 2%,[1,2] but selection certainly played an important role: factors such as three-vessel disease or transmural infarction, as well as cardiogenic shock, adversely affected outcome raising overall mortality to about 25%. The advent of thrombolytic therapy for acute coronary occlusion has added knowledge to the effect of early reperfusion on myocardial function and survival, and has gradually become the most widely adopted treatment for acute evolving infarction, due to its simplicity. Many trials have shown improvement in left ventricular function and survival if reperfusion is achieved within 4-6 hours from the onset of symptoms.[4]

In parallel, the other technique that has been developed for the treatment of acute evolving myocardial infarction is percutaneous transluminal angioplasty (PTCA) of the diseased vessel. Reperfusion of the ischemic area can be achieved in about 90% of cases, in-hospital mortality is around 10% and systolic left ventricular function improves in more than 50%. On the other hand, an early reocclusion rate up to 30% has been documented and results are not satisfactory in case of elderly patients, women, multivessel coronary artery disease, poor left ventricular function and acute infarction associated with cardiogenic shock.[5,6] PTCA as an adjunctive procedure associated with successful thrombolysis does not appear to provide clear benefits compared with isolated thrombolysis and is correlated with higher morbidity.[7,8]

Most data concerning surgical revascularization in acute myocardial infarction are nonrandomized and selection of patients is likely to be linked to encouraging results. A randomized trial has demonstrated low mortality rates (2.9%) when bypass surgery was accomplished at a mean of 4.3 hours from the onset of ischemia.[9] On the contrary, mortality was 8.8% in the medically treated patients. This trial, however, excluded patients over 65 years of age, or with a history of cardiogenic shock or prior myocardial infarction, but it is interesting to notice that mortality, at an 18

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months follow-up, raised to 20% in the medical series, while all surgical survivors where alive.

It appears that some form of revascularization, simple thrombolysis of the acutely occluded coronary vessel, PTCA, or surgical bypass, during the acute phase of evolving myocardial infarction, i.e., during the first 6 hours from the onset of ischemia, is beneficial in reducing the necrotic area of myocardium and hospital mortality.[10] In particular, surgical revascularization offers some advantages to the alternative and less invasive procedures:

1. it allows complete revascularization in virtually all patients (in 100% of patients that could benefit from PTCA or thrombolysis) reducing the incidence of recurrent infarction and angina pectoris, and reducing mortality at 1 year after infarction from 10% to about 2-3%;[11]
2. specific protocols of myocardial protective measures can be aggressively instituted to allow recovery of optimal metabolic conditions of the acutely ischemic and energy-depleted myocardium: this obviously applies to the nonperfused segment depending from the acutely occluded coronary artery, but also, and very importantly, to the viable, and hypercontractile, remaining remote myocardium;
3. despite suboptimal mortality rates, around 15% for elderly patients with multivessel disease or left ventricular dysfunction and often well over 20-25% for patients presenting in cardiogenic shock,[10,12-15] it gives the possibility to improve results in high-risk situations compared to conventional therapy. In this respect, it is worth remembering that mortality in medically treated patients in cardiogenic shock due to acute evolving myocardial infarction approaches 100% and that pump failure is the most common cause of death in this population.

Despite the considerations cited above, coronary artery bypass grafting for acute evolving myocardial infarction is seldom performed, in the current era, in the absence of complications and additional risk factors. Patients are generally selected for surgery when they present with diffuse coronary artery disease, a history of prior myocardial necrosis often with left ventricular dysfunction of varying severity, and especially when the hemodynamic state is unstable or cardiogenic shock is already present. Surgical treatment for acute myocardial infarction is also widely considered the treatment of choice in case of failed PTCA procedures or unsuccessful thrombolytic therapy. In the latter case coronary bypass surgery has been performed with mortality rates of about 15%, but often bleeding complications represent a major concern.[11,16,17] Obviously, patients with mechanical complications of myocardial infarction are surgical candidates, almost invariably on an urgent basis or as true emergencies. Encouraged by the good results obtained with the myocardial protective strategies suggested by Buckberg and associates, and taking into account that the high mortality and morbidity regarding the surgical treatment of patients with acute evolving myocardial infarction reported in the literature are possibly determined by selection of poor candidates for revascularization procedures in general and not to coronary bypass surgery itself, we started since 1994 to perform coronary operations in selected patients with acute myocardial infarction.[18,19]

Indications for Operation, Preoperative Management and Surgical Technique

Indications for Operation and Preoperative Management

Patients are selected for coronary artery bypass grafting during acute evolving myocardial infarction not only in emergency conditions or after failed thrombolysis or PTCA, but also in case of hemodynamically stable patients with ongoing ischemia and extensive areas of jeopardized myocardium. In other words, surgery is performed not only in the case no other therapeutic option is available, but is primarily selected in some patients. The

extension of the myocardial area at risk of necrosis is the critical issue in the indication for immediate operation: when the infarct results in a loss of approximately 40% or more of the left ventricular mass, cardiogenic shock results. In this respect, the extension of the critically ischemic area is dependent also on other important factors such as the presence of prior myocardial infarction or left ventricular dysfunction (not necessarily on an ischemic basis) and the gradual dysfunction of the hypercontractile remote ischemic muscle, the severity of which is generally well correlated with the presence of multivessel coronary artery disease.

Summarizing, we select for surgery patients in the following conditions:

Acute Evolving Myocardial Infarction (Within 6 Hours from the Onset of Myocardial Ischemia)

1. anterior infarction with ST segment elevation in at least 4 leads;
2. infarction with concomitant inferior, posterior and lateral extension;
3. infarction with inferior, but biventricular involvement;

Cardiogenic Shock Secondary to Acute Myocardial Infarction (Within 3 Hours from the Onset of Shock)

It is to be noted, however, that indication for operation may still be present despite an interval longer than 6 hours from the onset of *ischemia* because the rationale for surgery in this setting is primarily based on the principle that survival is dependent on reperfusion of the reversibly ischemic peri-infarct segments and remote areas of hypercontractile myocardium supplied by critically stenotic coronaries. Cardiogenic shock is clinically defined as the presence of hypotension (mean arterial pressure < 60 mmHg), anuria (urine output < 0.5 ml/Kg/hr) and metabolic acidosis. As outlined previously, mortality of medically treated cardiogenic shock approaches 100%. Although intra-aortic balloon counterpulsation can reverse this state in about 80% of cases, the majority of these patients become balloon-dependent and eventually die in the hospital: the one-year mortality rate is around 95%.[15]

On admission, primary diagnosis is established by electrocardiography and echocardiography. If the patient's clinical picture corresponds to one of the conditions listed above, no attempt to perform successful thrombolysis is made and the coronary anatomy is immediately outlined by angiography. The patient is then transferred to the operating room as quickly as possible. Preoperative intra-aortic balloon counterpulsation is instituted when necessary, namely, in case of hemodynamic instability or cardiogenic shock. A widespread use of aortic counterpulsation in this setting would be reasonable from a theoretical and pathophysiologic standpoint, but may often necessitate additional time to insert the balloon (especially if percutaneous techniques are unsuccessful, e.g., in the presence of severe peripheral vascular disease); therefore we do not recommend its routine use.

Not all patients that present in the aforementioned clinical conditions are surgical candidates. Although absolute and relative contraindications for operation may be present (as for any other surgical candidate), they are *not* related to the severity of the hemodynamic status, except for moribund patients. Patients over 75 years of age have not yet been considered for immediate coronary surgery, but this limit is probably arguable.

What must finally be emphasized is the importance of logistic and organizational aspects of such a therapeutic strategy. Coordination between cardiologists, cardiac surgeons, anesthesiologists, the emergency department and, eventually, physicians or cardiologists from other hospitals in the surrounding area, is crucial in avoiding time loss and is greatly responsible in determining a successful outcome: more in particular, it is essential to perform coronary angiography as soon as possible.

Surgical Technique

The general planning of the operation is similar to standard coronary artery bypass

grafting. However, some details in surgical technique and especially in myocardial protective strategies are, in our opinion, important and should be emphasized. In fact, we started this surgical protocol encouraged by the good results obtained adopting the method of myocardial management suggested by Buckberg and associates. In our experience, this consideration applies, in particular, to coronary artery surgery in patients with unstable angina or severe left ventricular dysfunction, and to energy-depleted hearts in general.

Cardiopulmonary bypass is maintained at moderate levels of systemic hypothermia (32°C). In patients with acute myocardial infarction, as in patients with left ventricular dysfunction, the left ventricle is vented through the right superior pulmonary vein. An aortic root vent is also placed as it is part of the cardioplegia circuitry. As already stated above we follow the Buckberg protocol for all cardiac operations. More in particular, in this population the strategy for energy-depleted hearts[20-22] is applied in all cases. This consists of the infusion of antegrade and retrograde (coronary sinus) normothermic blood (blood:cardioplegia ratio 4:1) substrate-enriched cardioplegic induction (KCl 20-25 mEq/L, glutamate and aspartate 13 mM/L, 37°C, 2.5 minutes antegradely and retrogradely respectively), followed by hypothermic induction (KCl 8-10 mEq/L, 4-8°C, 1.5 minutes antegradely and retrogradely), maintaining infusions (KCl 8-10 mEq/L, nonsubstrate-enriched, 4-8°C, 1 minute antegrade and retrograde) and controlled antegrade reperfusion (500 ml, KCl 8-10 mEq/L, 37°C, substrate-enriched; then unmodified blood for 2 minutes to wash-out the cardioplegic solution prior to aortic unclamping). When surgery is performed for acute myocardial infarction, the protocol includes 20 minutes of additional controlled reperfusion of the infarcted area through a saphenous vein graft after aortic unclamping, with the heart beating and unloaded. It is to be remarked that, if the indication for operation is acute evolving myocardial infarction, grafts should be performed to reperfuse the acutely ischemic region first in order to provide maximal cardioplegic protection in the most vulnerable area. In contrast, in the presence of cardiogenic shock secondary to myocardial infarction, emergency surgery is indicated primarily to rescue the stunned remote viable muscle as this situation often ensues *after* 6 hours from the onset of ischemia: consequently, the bypass graft to be constructed last should be the one supplying the acutely occluded vessel.

In the clinical practice, we have slightly modified this operative protocol. This relates in particular to a wider use of retrograde cardioplegia. In fact (not only in this subset of patients) we frequently infuse maintaining doses for 2 minutes retrograde rather than 1 minute antegrade and 1 minute retrograde, as originally suggested, because this does not interrupt the flow of the operation (it is undoubtedly an ideal route for continuous cardioplegia). In addition to several technically convenient situations (aortic valve surgery and regurgitation; severely calcified or porcelain ascending aorta; mitral valve operations after exposure of the left atrium with a distorted aortic valve; aortic dissection), retrograde infusion, and occasionally induction, may actually provide superior protection in a number of patients with diffusely stenotic or occluded coronary arteries, and in case of left main disease.[23-28]

In this respect we perform the 20-minute-period of controlled reperfusion in a retrograde fashion at the end of all distal anastomoses. This technique has, in our opinion, several advantages:

1. it provides controlled and homogeneous controlled reperfusion to all myocardial segments. Some degree of stunning in myocardial regions other than the infarct (remote myocardium) is likely to occur in the presence of multivessel disease or prior myocardial infarction;
2. even if this technique necessitates aortic clamping, the heart is perfused and, theoretically, optimally protected. Thus, the aortic cross-clamp time, traditionally correlated with myocardial damage,

relates to a somewhat different condition if compared to standard cardioplegic arrest;

3. this reperfusion period is ideal (also from a technical point of view) to construct the proximal anastomoses of the vein grafts with the heart in a flaccid and empty state, and avoiding side-biting clamps on the ascending aorta. In fact we now perform such anastomoses during a single aortic cross-clamp period for all coronary operations;
4. finally, the internal mammary artery may more easily be used to revascularize the left anterior descending coronary artery (LAD) in selected cases. This option avoids the necessity to place a vein graft on the LAD to assure controlled reperfusion. The construction of two separate bypass grafts on the LAD has been advocated,[29] but it is probably less recommendable being time-consuming. In this regard, the choice to use the internal mammary artery must obviously be carefully evaluated and the pedicle should be dissected as expeditiously as possible, eventually after the institution of cardiopulmonary bypass, preferably by a senior surgeon. In our experience, a judicious use of the internal thoracic artery has been possible in about 50% of patients with acute myocardial infarction. It has not been used in case of cardiogenic shock because long-term prognosis is not the critical issue in this subset of patients.

Personal Experience and Conclusive Considerations

As we have reported,[19] results can be divided into two groups of patients reflecting the two different indications for operation. In particular, results are much more disappointing in the presence of cardiogenic shock, as it is with conventional medical therapy. In our preliminary experience on 23 patients, in-hospital mortality was very low in patients who underwent operation for acute evolving myocardial infarction (1/15 due to cerebral hemorrhage 5 days after surgery) and these data compare favorably with surgical series previously reported in the literature,[2,17] especially because, in the latter, surgical candidates were not primarily selected on the basis of the extension of the ischemic area.

On the contrary, mortality was 50% (4/8) in patients with cardiogenic shock. This outcome is strongly affected by preoperative conditions and confirms the dramatic advantages of surgical treatment compared with conventional measures. All patients (2/8) requiring cardiopulmonary resuscitation before operation died.

Intraaortic balloon counterpulsation is very useful in this subset of patients. We have placed a balloon after operation in 20% of patients with acute myocardial infarction and in all patients with cardiogenic shock. Preoperative balloon counterpulsation has been necessary in 75% of cases with cardiogenic shock. Moreover, 25% of patients with cardiogenic shock required preoperative cardiopulmonary resuscitation. Similarly, inotropic support before operation was needed in 20% of patients with acute myocardial infarction and in all patients with cardiogenic shock. Postoperatively, most cases required intravenous catecholamines (80% of patients with acute myocardial infarction and in all patients with cardiogenic shock). Occasionally we performed a delayed sternal closure in patients showing hemodynamic deterioration while attempting to close the sternal edges.[30]

Peak CK-MB enzyme serum levels in patients operated for acute evolving infarction averaged about 70 mg/L and were recorded at arrival in the intensive care unit in the majority of cases. Left ventricular function appears to improve after coronary bypass. Although our data are not statistically significant, mean left ventricular ejection fraction markedly increased in patients with cardiogenic shock, especially during the first months following the operation (22% preoperatively; 25% at discharge from the hospital; 35% at 6-months follow-up), while only mild variations were encountered, in case surgery was performed for extensive acute myocardial infarction (40%

preoperatively; 45% at hospital discharge and follow-up).

Finally, from an economic standpoint, this technique may appear expensive. It is certainly a subset of critically ill patients compared to elective coronary surgical candidates, but it must be emphasized that the acute ischemic event and the underlying coronary artery disease are treated during a single hospitalization, thus reducing costs. In addition, this particular population is more likely to require a prolonged hospitalization and a higher probability to develop congestive heart failure on the medium-to-long term, if patients are treated in a more conventional manner.

In conclusion, we are convinced that emergency surgical therapy can play a substantial role in improving results in patients with acute evolving myocardial infarction and cardiogenic shock. If coronary bypass is performed within 6 hours from the onset of ischemia, we feel that the majority of patients with extensive ongoing infarction, i.e., patients at risk for secondary cardiogenic shock or, less dramatically, for the development of congestive heart failure on the medium-long term, have a better prognosis. Myocardial protective strategies are a crucial issue in performing such surgical procedures and, in fact, emergency coronary bypass grafting in this setting may well be considered a "myocardial protective operation."

References

1. Berg R, Kendall RW, Duvoisin GE et al. Acute myocardial infarction. A surgical emergency. J Thorac Cardiovasc Surg 1975; 70:432-439.
2. De Wood MA, Spores J, Notske RN et al. Medical and surgical management of myocardial infarction. Am J Cardiol 1979; 44:1356-1364.
3. Berg R Jr, Selinger SL, Leonard JJ et al. Immediate coronary artery bypass for acute evolving myocardial infarction. J Thorac Cardiovasc Surg 1981; 81:493.
4. Yusuf S, Sleight P, Held P et al. Routine medical management of acute myocardial infarction. Lessons from overviews of recent randomized controlled trials. Circulation 1990; 82(suppl. II):II-117-II-134.
5. O'Keefe JH, Rutherford BD, McConahay DR et al. Early and late results of coronary angioplasty without antecedent thrombolytic therapy for acute myocardial infarction. Am J Cardiol 1989; 64:1221-1230.
6. Stack RS, Califf RM, Hinohara T et al. Survival and cardiac event rates in the first year after emergency coronary angioplasty for acute myocardial infarction. J Am Coll Cardiol 1988; 11:1141-1149.
7. Salem DN, Desnoyers MR, Berman AD et al. Coronary angioplasty and thrombolysis for acute myocardial infarction: is two a crowd? Am J Med 1989; 86:259-261.
8. Ryan TJ. Revascularization for acute myocardial infarction. Strategies in need of revision. Circulation 1990; 82(suppl. II):II-110-II-116.
9. Koshal A, Beanlands DS, Davies RA et al. Urgent surgical reperfusion in acute evolving myocardial infarction. Circulation 1988; 78(suppl. I):I-171-I-178.
10. Flameng W, Sergeant P, Vanhaecke J et al. Emergency coronary bypass grafting for evolving myocardial infarction. Effects on infarct size and left ventricular function. J Thorac Cardiovasc Surg 1987; 94:124-131.
11. Barner HB, Lea JW IV, Naunheim KS et al. Emergency coronary bypass not associated with preoperative cardiogenic shock in failed angioplasty, after thrombolysis, and for acute myocardial infarction. Circulation 1989; 79(suppl. I):I-152-I-159.
12. Goldman BS, Weisel RD. Surgical reperfusion of acute myocardial ischemia: a clinical review. J Cardiac Surg 1986; 1:167-199.
13. Jones EL. Surgical revascularization during acute evolving myocardial infarction. Circulation 1987; 76(suppl. III):III-146-III-148.
14. Athanasuleas CL, Geer DA, Arciniegas JG et al. A reappraisal of surgical intervention for acute myocardial infarction. J Thorac Cardiovasc Surg 1987; 93:405-414.
15. Laks H, Rosenkranz E, Buckberg G. Surgical treatment of cardiogenic shock after myocardial infarction. Circulation 1986; 74(suppl. III):III-11-III 16.
16. Bolooki H. Emergency cardiac procedures in patients in cardiogenic shock due to complications of coronary artery disease. Circulation 1989; 79(suppl.I):I-1137-I-148.
17. Phillips SJ, Kongtahworn C, Skinner JR et al. Emergency coronary artery reperfusion: A choice therapy for evolving myocardial infarction. Results in 339 patients. J Thorac Cardiovasc Surg 1983; 86:679-688.
18. Donatelli F, Benussi S, Triggiani M et al. Emergency surgical revascularization in extensive acute myocardial infarction: a prospective protocol. Circulation 1996; 94(suppl. I):I-476.

19. Donatelli F, Benussi S, Triggiani M et al. Surgical treatment for life-threatening acute myocardial infarction: a prospective protocol. Eur J Cardio-Thorac Surg 1997; 11:228-233.
20. Rosenkranz ER, Buckberg GD. Myocardial protection during surgical coronary reperfusion. J Am Coll Cardiol 1983; 1:1235-1246.
21. Rosenkranz ER, Okamoto F, Buckberg GD et al. Safety of prolonged aortic clamping with blood cardioplegia. III. Aspartate enrichment of glutamate-blood cardioplegian energy depleted hearts after ischemic and reperfusion injury. J Thorac Cardiovasc Surg 1986; 91:428-435.
22. Allen BS, Rosenkranz E, Buckberg G et al. Studies on prolonged acute regional ischemia. Myocardial infarction with left ventricular power failure: a medical/surgical emergency requiring urgent revascularization with maximal protection of remote muscle. J Thorac Cardiovasc Surg 1989; 98:691-703.
23. Gundry GR, Kirsh MM. A comparison of retrograde cardioplegia versus antegrade cardioplegia in the presence of coronary artery obstruction. Ann Thorac Surg 1984; 38: 124-127.
24. Guiraudon GM, Campbell CS, McLellan DG et al. Retrograde coronary sinus versus aortic root perfusion with cold cardioplegia: Randomized study of enzyme levels in 40 patients. Circulation 1986; 74(suppl. III):III-105-III-115.
25. Diehl JT, Eichhorn EJ, Konstam MA et al. Efficacy of retrograde coronary sinus cardioplegia in patients undergoing myocardial revascularization: A prospective randomized trial. Ann Thorac Surg 1988; 45:595-602.
26. Eichhorn EJ, Diehl JT, Konstam MA et al. Protective effects of retrograde compared with antegrade cardioplegia on right ventricular systolic and diastolic function during coronary bypass surgery. Circulation 1989; 79: 1271-1281.
27. Fiore AC, Naunheim KS, Kaiser GC et al. Coronary sinus versus aortic root perfusion with blood cardioplegia in elective myocardial revascularization. Ann Thorac Surg 1989; 47:684-688.
28. Haan C, Lazar HL, Bernard S et al. Superiority of retrograde cardioplegia after acute coronary occlusion. Ann Thorac Surg 1991; 51:408-412.
29. Myocardial management during cardiac surgery with cardiopulmonary bypass. In: Kirklin JW, Barratt-Boyes BG, eds. Cardiac surgery, 2nd edition, New York: Churchill Livingstone 1993:155-156.
30. Donatelli F, Triggiani M, Benussi S et al. Advantages of delayed sternal closure in cardiac-compromised adult patients. J Cardiac Surg 1995; 10:632-636.

CHAPTER 18

Protection Strategies in Reoperations

Fumiyuki Okamoto and Keisuke Sakai

As the life-expectancy in patients undergoing cardiac surgery has increased, the frequency of cardiac reoperations for acquired heart disease has correspndingly increased. Reoperation is accompanied by difficult problems deriving from substernal and intrapericardial tissue adhesions, severely compromised heart function with advanced cardiac lesions, and older and sicker patients with other organ dysfunctions.[1,2] These patients often require prolonged cardiopulmonary bypass and aortic cross clamping. In such circumstances, we must maximally preserve cardiac function by means of myocardial protection.

The majority of adult cardiac reoperations involve coronary bypass; for example, in our hospital over the past several years 70.1-77.0% are coronary cases. Valve surgery and thoracic aortic aneurysms are the second and third most common indications for reoperation, respectively. Myocardial protection techniques differ more or less based upon the problems inherent in the underlying pathology, e.g., the embolization of atherosclerotic debris from previously placed vein grafts, annular defects caused by infectious endocarditis after prosthetic valve replacement, and severe aortic regurgitation secondary to proximal extension of aortic dissection.[1-5] In this chapter, myoprotective strategies will be described in accordance with these three major categories.

Coronary Bypass Reoperations

The median sternotomy is the standard access in coronary reoperation as with primary surgery. There may be, however, danger in sternal re-entry, e.g., injury to substernally adherent structures such as the free wall of the right ventricle, anteriorly extending aorta, patent vein grafts overlying heart, in situ internal thoracic artery (ITA) grafts crossing the aorta or pulmonary artery, and so forth.[1,4] Often it is helpful to assess the substernal anatomy by preoperative CT scan. In addition, preoperative coronary angiograms—including vein, ITA and other arterial grafts—are a prerequisite regardless of whether patent or occluded in order not only to plan the newly constructed coronary bypass, but to avoid unnecessary injury against these conduits.[1,4] If a graft is occluded, for instance, there is no need to make special efforts to avoid injuring it. But if patent, compression trauma or direct injury during dissection may cause intraoperative myocardial infarction before cardiopulmonary bypass is established either by interruption of blood supply or embolization of easily detachable atheromatous debris.[1-4] Therefore, extensive intrapericardial dissection, especially around bypass conduits. should be delayed until cardiopulmonary bypass is established or until cardioplegic arrest occurs.[3-5]

Sometimes arterial and venous access for cardiopulmonary bypass should be attained prior to sternal re-entry, usually by femoral exposure (artery and vein)[1,2,5] or axillary arterial approach.[4] Once sternal re-entry with subsequent prebypass dissection confined to aorta and right atrium is safely established, the conventional cannulation technique can be undertaken and femoral or axillary access can be

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discontinued. If sternal re-entry is not easily accomplished, cardiopulmonary bypass via femoro-femoral access or axillary approach should be established to decompress the heart, thereby keeping cardiac structures, including patent graft conduits, away from the sternum.[2,4,5] This substantially enhances the safety of sternal re-entry with an oscillating saw and the subsequent intrapericardial dissection. In addition, hemodynamic stability can be maintained by cardiopulmonary bypass, even if serious injury to substernal cardiac structures occurs.[4] On some occasions, however, dissection around heart should be limited to minmize the risk of intravascular detachment of atheromatous debrises in diseased, but still patent vein grafts, even though cardiopulmonary bypass is established. Moreover cardiac quiescence induced by cardioplegia facilitates intrapericardial dissection.[1-5] The technical aspects of sternal re-entry and a more detailed description of cannulation strategies for cardiopulmonary bypass are described elsewhere.[4]

Systemic hypothermia below 25°C used to be an indispensable adjunct in cardiac surgery, but current advancements in myocardial protection have rendered it redundant.[3,7,21] In routine cardiac operations, we keep the temperature at subnormothermic levels, around 34°C; this can be applied to coronary reoperations as well. However, whenever a prolonged aortic cross clamp time is expected, the systemic temperature should be lowered to increase the margin of safety. For the purpose of placing ice slush, complete isolation of the heart from pericardial adhesions is ideal. Yet more and more institutions, including ours, have abandoned topical hypothermia as an adjunct to myoprotection.[6,7]

Cardioplegic delivery, either antegrade via the ascending aorta or retrograde via the right atrium, or both, should be accomplished after cardiopulmonary bypass. Recently combined antegrade and retrograde cardioplegia has become the method of choice,[2,3,5,7] but is not always indicated in re-do coronary surgery since antegrade cardioplegia sometimes causes embolization of atheromatous debris from severely diseased vein grafts and possibly from advanced native coronary lesions.[4] If embolization is expected, functioning grafts should be divided[1-3,5] and new anastomoses constructed. Once functioning, but severely diseased vein grafts are transected, the combined antegrade and retrograde cardioplegic infusion may be indicated as in routine cardiac surgery without risk of embolization. Another option is continuous retrograde cold blood perfusion following aortic cross clamping, which gives rise to cardiac quiescence adequate to ensure accurate intrapericardial dissection.[1-5]

Functioning arterial grafts, such as ITA or gastroepiploic artery (GEA), are problematic[2,3] since they tend to reduce the myoprotective effect and to hamper the operative procedure by continuously draining noncardioplegic blood into the myocardium, delaying cardioplegic arrest and early return of electromechanical activity. The careful dissection of pedicled arterial grafts and temporary placement of occlusion clamps to interrupt noncardioplegic blood flow into the myocardium overcomes these problems.[1,3] In such cases, retrograde infusion is extremely useful to provide relatively uniform distribution of cardioplegic solution and to optimize myocardial protection, particularly when these arterial conduits supply a considerable amount of blood flow to the myocardium.[3,4] Coronary anastomoses are usually constructed under induced cardioplegic arrest followed by proximal anastomoses performed while cold retrograde noncardioplegic blood is continuously infused.[3,24]

If pedicled arterial grafts are difficult to dissect, aortic cross-clamping and cardioplegia often are effective so the pedicles can be freed-up under relative cardiac quiescence, even though electromechanical activity may resume. With functioning arterial grafts, hypothermic systemic circulatory arrest[8] that does not require clamping arterial pedicles may be indiccated, but this considerably prolongs cardiopulmonary bypass and increases the risk of cerebral ischemia. The distal coronary anastomoses also can be constructed under induced ventricular fibrillation as conducted in the past,[9] but this tends to underperfuse the subendocardium with subsequent myocardial

necrosis.[7,10] Intermittent aortic cross-clamping for each distal anastomosis, with ischemic arrest or antegrade cardioplegic infusion via patent native coronary arteries, is another option although the pedicle has to be clamped temporarily and the proximal anastomoses constructed under tangential aortic clamping.[3] Neither technique, however, fulfills the myoprotection obtained in primary surgery.

Right or left thoracotomy[11] thoracotomy[4,12] are sometimes safer approaches for re-entering the mediastinum in coronary reoperations, but they are suited only to the right coronary system or mainly to posterolateral portions of the left ventricle (sometimes to left anterior descending coronary artery-LAD). In these cases, either ventricular fibrillation, topical cardiac hypothermia or intermittent interruption of coronary blood flow by occlusion snare is needed while the anastomoses are constructed. Nonetheless, these techniques are not as effective as conventional myocardial protection. urgical benefits have to be compromised considerably by technical feasibility. On the other hand, minimally invasive cardiac surgery likely can be applied to coronary reoperations, for example, a left ITA to LAD anastomosis through a limited anterior thoracotomy or a right GEA to distal right coronary artery through a divided xyphoid and small upper abdominal incision.[13,14,32]

Valve Reoperations

Although recent advances in artificial valve design have led to excellent long-term clinical results, there are still reasons for reoperation: structural defects of mechanical valves, degenerative alterations of bioprosthetic valves (porcine aortic or bovine pericardium) or hemodynamic impairments of arising from perivalvular thrombi or fibrotic tissue proliferation.[2,15,16,20] In these circumstances, the patient may have adapted with left and/or right ventricular distention secondary to pulmonary hypertension which can interfere with sternal re-entry.[2] In patients who require reoperative valve surgery repair is sometimes deferred until the hemodynamic status alread is deteriorated; accordingly the incidence of reoperative mortality and morbidity is elevated.[2,17,20] If the functional class of patients is NYHA-IV, the risk of reoperation is higher than in NYHA-I to -III patints in whom operative mortality and morbidity are comparable to that seen in primary surgery.[2] Therefore, it is important that reoperative valve surgery be undertaken before the patient's hemodynamic status deteriorates.

Infectious endocarditis is another serious problem in re-do valve operations[18] since it produces impaired left ventricular function, e.g., perivalvular leakages cause aortic or mitral regurgitation, and destroys periannular anatomy with the potential of cerebrovascular embolism.[19,20] In these cases, the meticulous surgical repair of periannular defects along with the careful re-reimplantation of a new valve are needed, often requiring long cardiopulmonary bypass and aortic cross-clamp times. For this reason, innovative myoprotection is required.

Whenever difficulties in sternal re-entry are expected, prebypass exposures of femoral artery and vein are recommended to readily initiate cardiopulmonary bypass and to decompress the heart by inferior vena caval drainage; this facilitates meticulous intrapericardial dissection.[2] The substernally adherent failed right ventricle may be easily injured because it tends to dilate and its wall thins secondary to left ventricular failure, often necessitating complicated repair. Therefore, the preoperative chest x-ray[2] and CT are helpful in avoiding these dangers. Even if sternal re-entry is accomplished without installing femorofemoral bypass, dissection to establish cardiopulmonary bypass should sometimes be confined to limited areas of the ascending aorta and right atrium—just enough to permit cannulation. Further intrapericardial dissection can be ndertaken after the heart is decompressed completely during cardiopulmonary bypass. Because topical hypothermia has decreased in popularity in recent years,[6,7] isolation of the heart from the pericardium is not necessarily needed to optimize myoprotection. In any case, this disection is associated with persistent intrapericardial bleeding, particularly when the patients are anticoagulated preoperatively. Nevertheless,

care must be taken during deairing when the aorta and/or left atrium are closed after valve replacement. The intraoperative transesophageal echocardiogram is useful to detect residual air frequently existing in the left atrium, which otherwise often enters the right coronary orifice, leading to postoperative right ventricular dysfunction and resultant low cardiac output despite appropriate myocardial protection.[2]

The combined use of antegrade and retrograde cardioplegia is the method of choice to protect the heart in re-do valve surgery because of the uniform distribution of cardioplegic solution.[2,21] Although cardiac quiescence can be more readily achieved by antegrade than retrograde cardioplegia, aortic regurgitation delays cardioplegic arrest considerably, and antegrade cardioplegia should be used only at the outset with subsequent immediate retrograde cardioplegic perfusion.[2,3,21] The direct intracoronary infusion of cardioplegia following aortotomy, either as cardioplegic induction or subsequent to initial infusion of cold retrograde cardioplegia, may be another alternative to induce immediate cardioplegic arrest in aortic valve reoperations with severe aortic regurgitation.[3,21] But care must be taken not to inadvertently injure the coronary orifices, particularly when the aorta is severely calcified.

Once cardiac quiescence is achieved in aortic valve re-do surgeries, we prefer to utilize simultaneous selective antegrade to right coronary artery and retrograde cardioplegic perfusion to ensure the uniform distribution of cardioplegia solution. Since one of the biggest disadvantages of retrograde perfusion is inadequate nutritional flow to the right ventricle with poor myoprotection despite internal cooling through the venovenous shunt,[22-24] the additive perfusion of retrograde cardioplegia simultaneously directed to the right coronary artery can yield better distribution of cardioplegic solution. This can be achieved easily by connecting the intracoronary cannula to the side-arm of the retrograde perfusion line as advocated by Buckberg et al.[3,24] Unless extraordinary surgical precision is required as in repair of periannular defects, removal of a previously replaced malfunctioning valve, annular suture placements et cetera, the retrograde perfusion of cold plain blood can be continuous, thereby virtually offsetting the ischemic insult despite prolonged aortic cross-clamping. Most surgical procedures (e.g., suture placement to a prosthetic valve ring, refixation of a valve by securing sutures, aortorrhaphy) can be accomplished without creating a further ischemic burden in functionally impaired hearts. As a consequence, in most aortic valve reoperations, the strict use of combined antegrade and retrograde cardioplegic perfusion may no longer be absolutely necessary, and the simultaneous antegrade to right coronary combined with retrograde cardioplegia would be a better choice to reduce technical complexity.

In mitral re-do operations, minimal intrapericardial dissections mainly around the right atrium (i.e., trans-septal approach) or right-sided left atrium allow suffcient access to the mitral valve, unless a more complicated procedure like "Cox-maze operation" is required or visualization of mitral valve is insufficient.[25,26] As performed in other cardiac re-dos, the initial cardiac quiescence can be provided by antegrade cardioplegic perfusion whenever possible, and then followed by retrograde perfusion.[2,3,24] If the operative procedure requires a brief period of aortic cross-clamping—as in a single mitral valve re-replacement—, repeated retrograde cardioplegia may be adequate to protect the heart. If reoperation requires a long period of aortic cross-clamping, the combined use of antegrade and retrograde cardioplegia is recommended, particularly in functionally-compromised hearts associated with left ventricular dysfunction. In this case, however, air must not be pushed inadvertently into coronary arteries since an open left atrium and empty left ventricle usually accompanies an air-filled aorta. Regardless of the method of cardioplegia, it may be useful to perfuse the myocardium retrogradely with cold noncardioplegic blood throughout surgery where technical precision is not strictly required, e.g., in valve fixation with securing sutures, closure of an atrial septum or left atriorrhaphy.[3,21,24] This may also be helpful in flushing back any intracoronary

air or emboli toward the coronary orifices in the aorta; this prevents postoperative impairment of cardiac function.[2,3,21,24,33] In exceptional cases of re-do mitral valve operations, as with multiple previous surgeries, a right anterior thoracotomy with femoro-femoral bypass can be employed for access to the mitral valve in order to avoid massive bleeding from incision by means of a median sternotomy.[2,27] Since the conventional myoprotective strategy of combined antegrade and retrograde cardioplegia is difficult to conduct in this approach, systemic hypothermia to 20°C is concomitantly utilized to protect the heart.

Thoracic Aortic Aneurysm Reoperations

Re-do thoracic aortic aneurysm surgery involving the aortic root is the most difficult type of reoperative cardiac surgery because large, adherent ascending aortic aneurysms sometimes make sternal re-entry extremely dangerous and distort adjacent cardiac structures including aortic valves and coronary arteries in unexpected directions.[2,28] In order to ensure safer sternal re-entry, exposure of the femoral artery and vein for initiation of retrograde cardiopulmonary bypass is routine although there is the risk of fatal cerebral or even myocardial embolizations from debris dislodged from the descending aorta and/or proximal aortic dissection.[29] Therefore, careful monitoring by a transesophageal echo is extremely useful to detect abnormalities in the descending aorta and if such devastating complications are likely to happen, antegrade cardiopulmonary bypass via the ascending aorta or arch should be taken into account. Deep hypothermic systemic arrest is almost always required to protect multiple organs, particularly brain as well as heart. The various modalities for brain protection have inherent advantages and disadvantages, and the technical aspects, along with cannulation strategies, are described in detail elsewhere.[30,31]

Often ascending aortic aneurysms are associated with aortic regurgitation because of annular dilatation or direct involvement of dissection,[2] which then leads to functional impairment of the left ventricle making it less tolerant to ischemic insult. Although antegrade cardioplegic induction via the aortic root produces prompt cardiac arrest despite aortic regurgitation, the left ventricle has to be vented immediately to avoid overdistension, and retrograde cardioplegia should be given to confer more efficient myocardial protection. Alternatively, the direct intracoronary infusion of cardioplegia is a delivery option after aortotomy to ensure reliable myoprotection. In these cases, where left ventricular hypertrophy exists, the total amount of cardioplegia should be increased[3,21] as much as 1.5 times with anticipation of more complete distribution of cardioplegia.

Reoperations of thoracic aortic aneurysms require extremely difficult and time-consuming reconstruction which should be performed in a relatively bloodless field. We, therefore, preferentially utilize retrograde, continuous noncardioplegic blood perfusions to reduce ischemia duration whenever. Absolutely bloodless operative fields are mandatory only in aortic root reconstructions which include coronary orifices. As with aortic valve reoperations, repeated cardioplegic infusions subsequent to initial induction can be substituted by simultaneous selective antegrade (to right coronary artery) and retrograde perfusion even while noncardioplegic blood is being given.[3,24] This avoids the cumbersome placement of intracoronary cannulae at each cardioplegic interval and prevents traumatic injury to the coronary ostia. Additionally, any debris and/or air bubbles can be evacuated from coronary arteries retrogradely.[2,3,24,33] Almost all forms of myoprotection used in re-do coronary and valve surgery are applicable in reoperations of thoracic aortic aneurysms.

Summary

The current protection strategies for reoperations have been reviewed with regard to the three major indications for adult cardiac surgery. Although the fundamental precepts of myocardial protection in redo surgery are almost identical to those in primary surgery, there are numerous factors which complicate re-do operations. The complexities in

any individual case cannot be overcome by any single, universal method. Comprehensive integration of myoprotective techniques is required in various re-do surgical situations.

References

1. Loop FD. The value and conduct of reoperations for coronary atherosclerosis. Semin Thorac Cardiovasc Surg 1994; 6(2): 116-119.
2. Cohn LH. Myocardial protection for reoperative cardiac surgery in acquired heart disease. Semin Thorac Cardiovasc Surg 1993; 5(2):162-167.
3. Buckberg GD, Allen BS. Myocardial protection management during adult cardiac operations. In: Baue A, Geha A, Hammond G et al. eds. Glenn's Thoracic and Cardiovascular Surgery. Stamford, CT: Appleton & Lange 1995:1653-1687.
4. Lytle BW. Coronary reoperations. In: Edmunds LH Jr., ed. Cardiac Surgery in the Adult. New York: McGraw-Hill Health Professions Division, 1997:573-596.
5. Savage EB, Cohn LH. 'No touch' dissection, antegrade-retrograde blood cardioplegia, and single aortic cross-clamp significantly reduce operative mortality of reoperative CABG. Circulation 1994; 90(Part 2):II-140-II-143.
6. Allen BS, Buckberg GD, Rozenkranz ER et al. Topical cardiac hypothermia in patients with coronary disease: An unnecessary adjunct to cardioplegic protection and cause of pulmonary morbidity. J Thorac Cardiovasc Surg 1992; 104:626-631.
7. Buckberg GD. Myocardial Protection: An overview. Semin Thorac Cardiovasc Surg 1993; 5(2):98-106.
8. Michielon G, Doty DB. Hypothermic circulatory arrest for cardiac valve replacement reoperations. Ann Thorac Surg 1994; 57: 1281-1283.
9. Senning A. Ventricular fibrillation during extracorporeal circulation. Acta Chir Scand 1952; 171(Suppl):1-79.
10. Najafi H, Henson D, Dye WS. Left ventricular hemorrhagic necrosis. Ann Thorac Surg 1969; 7:550-561.
11. Uppal R, Wolfe WG, Lowe JE et al. Right thoracotomy for reoperative right coronary artery bypass procedures. Ann Thorac Surg 1994; 57:123-125.
12. Ungerleider RM, Mills NL, Wechsler AS. Left thoracotomy for reoperative coronary bypass procedures. Ann Thorac Surg 1985; 40: 11-15.
13. Benetti FJ, Ballester C, Sani G et al. Video assisted coronary bypass surgery. J Card Surg 1995; 10:620-625.
14. Benetti F, Mariani MA, Sani G et al. Video-assisted minimally invasive coronary operations without cardiopulmonary bypass: A multicenter study. J Thorac Cardiovasc Surg 1996; 112(6):1478-1484.
15. Cohn LH, Koster JK Jr, Van de Vanter S et al. The in-hospital risk of rereplacement of dysfunctional mitral and aortic valves. Circulation 1982; 66(Suppl I):I-153-I-156.
16. Bortolotti U, Milano A, Mossuto E et al. Early and late outcome after reoperation for prosthetic valve dysfunction: Analysis of 549 patients during a 26-year period. J Heart Valve Dis 1994; 3(1):81-87.
17. Blackstone EH, Kirklin JW. Death and other time-related events after valve replacement. Circulation 1985; 72:753-767.
18. Larbalestier RI, Kinchla NM, Aranki SF et al. Acute Bacterial endocarditis. Optimizing surgical results. Circulation 1992; 86(Suppl II):II-68-II-74.
19. Baumgartner WA, Miller DC, Reiz BA et al. Surgical treatment of prosthetic valve endocarditis. Ann Thorac Surg 1983; 35: 87-104.
20. Oakley CM. Investigations before reoperation for acquired heart disease. In: Stark J, Pacifico AD, eds. Reoperations in Cardiac Surgery. Berlin Heidelberg New York: Springer-Verlag, 1989:17-37.
21. Allen BS, Murcia-Evans D, Hartz RS. Integrated cardioplegia allows complex valve repairs in all patients. Ann Thorac Surg 1996; 62:23-30.
22. Gates R, Laks H, Drinkwater D et al. Gross and microvascular distribution of retrograde cardioplegia in explanted human hearts. Ann Thorac Surg 1993; 56:410-417.
23. Allen B, Winkelmann J, Hanafy H et al. Retrograde cardioplegia does not perfuse the right ventricle. J Thorac Cardiovasc Surg 1995; 109:1116-1126.
24. Buckberg GD, Beyersdorf F, Allen BS et al. Integrated myocardial management: Background and initial application. J Card Surg 1995; 10:68-89.
25. Cox JL, Schuessler RB, D'agostino HJ et al. The surgical treatment of atrial fibrillation. III. Development of a definitive surgical procedure. J Thorac Cardiovasc Surg1991; 101(4):569-583.
26. Duran CG. Reoperations on the mitral and tricuspid valves. In: Stark J, Pacifico AD, eds. Reoperations in Cardiac Surgery. Berlin Heidelberg New York: Springer-Verlag, 1989:325-349.
27. Cohn LH, Peigh PS, Sell J et al. Right thoracotomy, femoro-femoral bypass, and deep hypothermia for re-replacement of the mitral valve. Ann Thorac Surg 1989; 39:53-55.

28. Crawford ES, Crawford JL, Safi HJ et al. Redo operations for recurrent aneurysmal disease of the ascending aorta and transverse aortic arch. Ann Thorac Surg 1985; 40: 439-455.
29. Wareing TH, Davila-Roman VG, Barzilai B et al. Management of the severely atherosclerotic ascending aorta during cardiac operations. J Thorac Cardiovasc Surg 1992; 103(3):453-462.
30. Crawford ES, Saleh SA. Transverse aortic arch aneurysm: Improved results of treatment employing new modifications of aortic reconstruction and hypothermic cerebral circulatory arrest. Ann Surg 1981; 194:180-188.
31. Svensson LG, Crawford ES, Hess KR et al. Deep hypothermia with circulatory arrest: Determinants of stroke and early mortality in 656 patients. J Thorac Cardiovasc Surg 1993; 106(1):19-31.
32. Watanabe G, Misaki T, Kotoh K et al. Bilateral minimally invasive direct artery bypass grafting with the use of two arterial grafts. J Thorac Cardiovasc Surg 1997; 113(5): 949-951.
33. Sandhu AA, Spotnitz HM, Dickstein ML et al. Cardiopulmonary bypass, myocardial management, and support techniques. Retrograde cardioplegia preserves myocardial function after induced coronary air embolism. J Thorac Cardiovasc Surg 1997; 113(5):917-922.

CHAPTER 19

Protection Strategies for Heart Transplantation

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Safe procurement and effective preservation are fundamental features in heart transplantation. The preservation technique might influence the rate of early graft failure as well as the incidence of graft vasculopathy.[1,2]

Data from the Registry of the International Society for Heart and Lung Transplantation demonstrate, that the early mortality rate after heart transplantation remains 8-10% and 40% of these deaths are caused by nonspecific graft failure.[3]

One important fact related to early graft failure may be that brain death is associated with hemodynamic deterioration, so that donor hearts often require inotropic support and are of questionable value.[4-6] These injuries are greater in the right ventricle and may contribute to early right ventricular failure after transplantation.[7]

Furthermore, heart excision and preservation techniques are thought to play a role in myocardial damage.[8] Research efforts are in progress to develop preservation methods that would decrease myocardial injury both during the period of preservation and during the period of reperfusion.

There are some special topics of donor heart preservation in comparison with protection techniques in other open heart procedures. One topic is that the donor heart is a completely isolated organ without any collateral blood flow. Therefore, the preservation solution can not be washed out and periodic replenishment of cardioplegia is not necessary.

During storage the heart is surrounded by ice-cold solution at a constant myocardial temperature of 0-4°C. This allows extended periods of storage without extracorporal circulation.

During heart transplantation 4 Phases of myocardial protection can be distinguished:[9]

Phase 1: Preharvesting phase
Phase 2: Cardioplegic induction phase
Phase 3: Organ storage
Phase 4: Reperfusion phase

Preharvesting Phase

Brain death causes myocardial energy and substrate depletion with a metabolic profile similar to ischemically damaged hearts.[10] Data confirm depletion of ATP, CP, glycogen as well as of tissue glutamate, a precursor of the Krebs cycle intermediate alpha-ketoglutarate.[11] Furthermore, dysfunction of the hypothalamic-pituitary axis results in acute hypothyroidism, adrenal insufficiency, and diabetes insipidus. These factors may contribute to early nonspecific graft failure.

Due to the universal shortage of donor organs, optimal management of the brain dead patient is essential before organ harvest.

Ischemia-Reperfusion Injury in Cardiac Surgery, edited by Friedhelm Beyersdorf.

Pretreatment with Substances that Limit Ischemic and Reperfusion Damage

Allopurinol

Controversial results exist concerning the effect of the xanthine oxidase inhibitor allopurinol. Some experiments have shown a reduction in irreversible myocardial injury following pretreatment with allopurinol, but others have failed to show an effect.[12-14] A possible explanation for this discrepancy could be that the concentration of xanthine oxidase varies widely between species and has been reported as being undetectable in both the porcine and human hearts.[15, 16]

Calcium Channel Blockers

Damage of cardiac membranes after ischemia and reperfusion may result in a transient intracellular calcium overload, what may be responsible for the prolonged postischemic dysfunction. Calcium channel blockers can attenuate stunning of the myocardium and enhance the recovery of wall motion during reperfusion.[17]

Furthermore, calcium channel blockers inhibit superoxide production in human neutrophils.[18]

Ischemic Preconditioning and Adenosine Pretreatment

Ischemic preconditioning describes the phenomenon whereby a significant reduction of cell necrosis, contractile malfunction or arrhythmias induced by ischemia and reperfusion can be achieved if the injurious period of ischemia is preceded by one or more brief (2-5 min) periods of ischemia and reperfusion. Studies in the rat[19] and the rabbit[20] have shown that ischemic preconditioning can improve functional recovery after a prolonged period of global ischemia.

The mechanism underlying preconditioning has not yet been elucidated but one suggestion is that the protective effect is mediated by adenosine that is released during the short episode of preconditioning ischemia. Liu et al[21] have shown that an intracoronary infusion of adenosine can substitute for the 5 min of ischemia that are required to precondition the isolated blood perfused rabbit heart against necrosis. The effects of extracellular adenosine are mediated predominantly via membrane receptors (A_1 and A_2 receptors) in the myocytes and coronary vasculature. The mediated effects of these receptors include: the stimulation of glycolysis,[22] delaying of ischemic contracture[23] and, reduction of neutrophil activation.[24]

Support for preconditioning in humans comes from several sources: studies showing increased tolerance to repetitive balloon inflations during angioplasty, some studies suggesting that preinfarction angina confers an early beneficial effect and studies showing that patients can develop sudden tolerance to repetitive exercise- or pacing-induced ischemia.[25]

Alkhulaifi (1994) and co-workers performed examinations on patients during aortocoronary bypass-surgery.[26] Ischemic preconditioning was achieved by 2 periods of cross-clamping for 3 minutes. Following 10 minutes of global ischemia they found better preservation of myocardial ATP-content when preconditioning was performed.

Karck et al[27] demonstrated in an isolated rat heart model that ischemic preconditioning can improve contractile function after a 10-hr period of hypothermic ischemia. This endogenous mechanism of cardioprotection was effective regardless of whether preservation was accomplished using different cardioplegic solutions or topical hypothermia alone. This may have clinical implications in myocardial preservation for heart transplantation.

Rat experiments in a Langendorff model indicated the benefit of adenosine pretreatment for prolonged cardiac storage.[28] In contrast, investigations in an isolated ejecting rat heart preparation showed no evidence to support the involvement of adenosine to any major extent in preconditioning induced protection of postischemic contractile function.[29]

Acadesine (5-amino-4-imidazole carboxamide reboside) and its derivatives protect against myocardial injury during ischemia and reperfusion. This effect is probably mediated by augmented extracellular adenosine.[30]

Pretreatment with Thyroid Hormone

Myocardial deterioration after brain death is related to metabolic and hormonal changes.[31] Recent studies have shown that thyroid hormone and vasopressin administration in the brain dead donor may be beneficial to long-term graft survival.[32] T_3 replacement can reverse the increased anaerobic to aerobic metabolism and improve hemodynamics in brain dead animals.[11]

In contrast, Meyers and co-workers[33] found no significant change in cardiac function of brain dead pigs after T_3 treatment.

Vasopressin

Hemodynamic instability commonly observed in brain dead patients has been explained by diabetes insipidus secondary to vasopressin depletion as the primary mechanism. Hemodynamic support of the brain dead patient has been based on vasopressin and catecholamine therapy. Unfortunately, vasopressin, a potent vasoconstrictor, may improve hemodynamics at the expense of decreasing splanchnic and myocardial perfusion.[34]

Cardioplegic Induction Phase

The aim of myocardial protection in this phase should be to prevent that severely compromised hearts enter the storage phase with substrate depletion.

Metabolic depletion can be reversed by inducing cardiac arrest with a warm substrate-enriched blood cardioplegic solution during harvesting.[9,35] Brain dead dogs received a 10 minute infusion of warm blood cardioplegia containing glutamate and aspartate. Metabolic investigations of the myocardium showed a:

1. return of creatine phosphate levels to normal
2. replenishment of glutamate
3. reduction in myocardial lactate content.

The authors conclude that warm substrate-enriched blood cardioplegic solution may increase the tolerance to subsequent ischemia during organ storage, provide potential expansion of the donor pool, or improve the potential function of hearts harvested form hemodynamically impaired donors.

Usually, cold crystalloid cardioplegic solutions are used for induction of cardiac arrest of donor hearts. But the initial use of profound hypothermic solutions may have detrimental effects.[36-38]

In the isolated working rat heart model cardiac arrest was induced with St. Thomas cardioplegia before the hearts were stored for 6 hours in cardioplegic solution. Thereafter cardiac output and creatine kinase leakage were measured. Induction was started with cardioplegic solution at 7.5, 22, or 37°C for 1 minute and was followed by a secondary infusion (2 minutes) with cold (7.5°C) cardioplegia. Primary infusion with warm solutions resulted in decreased coronary vascular resistance during infusion and greater postischemic cardiac function.

Organ Storage

The period of cold organ storage is one that "prevents" further injury of hearts but "does not make a poor donor heart a good one.[35]

The main principle of organ storage is deep hypothermia because it

1. reduces metabolic rate
2. avoids cumbersome continuous organ perfusion
3. prevents swelling and oxygen free radical injury

It is generally accepted that a temperature of 4°C should be used for the cardioplegic solution. This temperature is believed to be achieved when the harvested heart is placed on ice and transported in a cooler. Experimental assessments in dogs showed that after 4 hours of cooling with ice and saline solution in a cooler temperature was below 0°C throughout the myocardium. Examination with an electron microscope revealed serial changes, including moderate-to-severe cytoplasmic and nuclear swelling and mitochondrial calcium deposits. Cell membranes remained intact, which suggests that the damage was not irreversible.[39] Nevertheless, unacceptably low temperatures during organ storage (< 4°C) should be avoided.

In contrast, investigations on isolated Wistar rat hearts after 6 hours of preservation with University of Wisconsin solution showed a better recovery of cardiac function, myocardial adenine nucleotides content, and prevention of myocardial edema when subzero nonfreezing storage at –1°C as compared with 4°C was used.[40]

An increase in early mortality with an increase in donor organ ischemic times beyond 4 hours has been reported.[41,42]

To avoid edema and swelling, slightly hyperosmolar cardioplegic solutions are used for heart storage. The optimal osmolality is 280-290 mOsm. Moderate or severe hyperosmolality can be detrimental to cardiac function.[43]

To prevent edema formation and improve postischemically coronary flow hyaluronidase has been added to preservation solutions.[44] Hyaluronidase acts by specifically reducing the hyaluronate content of tissues, which is the principal glycosaminoglycan of the interstitium, has a high water-binding capacity and exerts a strong osmotic force. Application of hyaluronidase decreases the interstitial volume of edematous tissues and prevents ischemically induced increase in vascular resistance, but does not change the microvascular permeability. Thus, hyaluronidase is very effective to diminish the no reflow phenomenon.

Myocardial contracture seems to play a major role in the reduced vasodilatory capacity of coronary vessels after an extended period of cold storage. This hypothesis is illustrated by the finding, that rat hearts treated with 2,3-butanedione monoxime (BDM) showed a reduced degree of left ventricular stiffness and an increase in coronary flow after 10 hours of hypothermic storage.[45] BDM is a reversible inhibitor of cross-bridge formation in the weak binding state and actively cycling cross bridges.[46]

Ischemic and reperfusion injury is associated with vascular dysfunction. Vasodilation in the heart is mediated through the release of nitric oxide by the endothelium.[47,48] After begin of reperfusion nitric oxide release from the endothelium is impaired.[49] Therefore, the supplementation of cardioplegic solutions with L-arginine, a nitric oxide precursor can reduce vascular dysfunction after heart transplantation.[45, 50]

Reperfusion Phase

Reperfusion injury occurs when ischemically stored heart transplants are abruptly reoxygenated by the recipient's blood. Damage to the myocardium is generated through a variety of mechanisms including free radical generation, calcium overload, and abnormalities of energetics.

Analysis of coronary sinus and arterial blood samples in 11 transplant recipients showed a lactate release and pyruvate uptake in the first minute of reperfusion. A massive efflux of nucleotide catabolites was observed for more than 30 minutes of reperfusion. There was nearly no oxygen uptake after onset of reperfusion. The oxygen saturation of hemoglobin in coronary sinus blood decreased gradually over the first 45 minutes, indicating gradual restoration of myocardial oxygen uptake.[51]

Postischemic reperfusion damage can be minimized by a brief (i.e., 3-5 minutes) controlled reperfusion with normothermic, substrate-enriched blood cardioplegia ("hot shot") during the initial phase of reoxygenation.[52,53] The factors which minimize reperfusion injury include precise control of the conditions and composition of the reperfusate. This involves keeping the heart vented, delivering reperfusate normothermically to optimize metabolism, providing a gentle reperfusion pressure (< 50 mmHg), keeping the heart arrested during the initial phase of reperfusion to minimize oxygen demands, lowering reperfusate calcium, providing a buffer to reverse acidosis, replenishing substrates (i.e., amino acid precursors of Krebs cycle intermediates which have been depleted during ischemia), providing hyperosmolarity to limit edema formation, and providing extra glucose as metabolic substrate. Controlled reperfusion has been shown excellent results after revascularization of severely ischemically damaged hearts,[54] but only few clinical studies have assessed this method after heart transplantation.[55-57]

There is increasing evidence that damage from acute myocardial ischemia is not solely due to the interruption of nutrient blood flow nor to the failure to remove metabolic products. Rather, reperfusion initiates a cascade of reactions, including oxygen-radical formation, mechanical capillary obstruction by granulocytes, and inflammatory events that markedly increase the injury. Numerous mechanism have been proposed, including interactions between reactive oxygen species, neutrophils, and the capillary endothelium.

Free radicals cause peroxidation of membrane lipids and denaturation of proteins. Measurements on isolated rat hearts after graded ischemia showed a peak of hydroxyl radical generation 30-90 seconds after the onset of reperfusion.[58]

Normally, protective mechanisms are present in the cell to prevent damage by free radicals. Superoxide dismutase catalyzes the dismutation of O_2^- to H_2O_2 and O_2. The cytoplasmic enzymes glutathione peroxidase and catalase provide the final detoxification steps with the reduction of H_2O_2 to H_2O. Glutathione peroxidase seems to be a more active enzyme than catalase in protecting myocardial cells from H_2O_2 mediated damage.[59]

There is increased evidence that oxygen free radical scavengers i.e., superoxide dismutase (SOD), catalase (CAT), and mercaptopropionyl-glycine (MPG) inactivate free radicals and thus ameliorate reperfusion injury.[60-62] Sodium lactobionate has been shown to reduce free hydroxyl radical generation. The compound can complex large amounts of metal ions. Especially iron ions participate in the Fenton reaction and Haber-Weiss cycle, the most important source of hydroxyl radicals.[63] Lactobionate is contained in Henry Mondor and Celsior cardioplegic solution.

The best time for administration of scavengers is just before and just after the onset of reperfusion.[61,64]

Formerly, the source of the oxygen-radical superoxide was thought to be xanthine oxidase in the endothelial cell.[65] Recent studies indicated that granulocytes are the major source of superoxide radicals.[66]

Granulocytes contain the enzyme NADPH oxidase, can produce large quantities of superoxide and are an important origin of oxygen-derived free radicals.[67]

Experimental[68] and clinical[69] investigations showed an accumulation of polymorphonuclear leukocytes in myocardial capillaries during ischemia and reperfusion. This phenomenon has been termed leukocyte plugging and is the main mechanism of the capillary no-reflow phenomenon occurring during the first 5 hours of reperfusion after an initial period of reactive hyperemia. Accumulation of leukocytes results from the rheologic properties of the granulocyte such that marked deformation is necessary for capillary transit, and because of natural adherence, there is a small safety margin for granulocyte transport through capillaries. Following 1 hour of ischemia and brief reperfusion in dogs, 24% of myocardial capillaries remained obstructed by granulocytes.[68] The phenomenon of leukocyte plugging can not be observed by routine histologic methods, probably because single granulocytes obstruct individual capillaries in a stochastic fashion, which is quite different from leukocyte infiltration.

An additional effect of granulocyte activation during myocardial ischemia and reperfusion is degranulation. The effect of enzymes contained within these granules in ischemic myocardium is not known. The serine proteinase elastase has been implicated most consistently in polymorphonuclear leukocyte-mediated tissue damage. Elastase may hydrolyze a host of proteins in the extracellular matrix (i.e., elastin, fibronectin, and collagen) as well as complement proteins and clotting factors. In addition, elastase may inhibit platelet function by proteolysis of platelet membrane glycoproteins.[70]

A means to halt the inflammation might allow reperfusion with granulocyte-depleted blood and prevent granulocyte-mediated injury. Furthermore, adenosine and perfluorochemicals have been shown to reduce neutrophil adherence and cytotoxicity to endothelial cell cultures.[71]

Adenosine may counteract the potassium chloride-induced vasoconstriction that occurs

during hyperkalemic reperfusion and may thus improve coronary flow and myocardial function.[72]

Future Directions

The established concept for heart allograft preservation involves single-dose cardioplegia and static hypothermia. But intensive research activities exist to develop effective methods for long term storage and to improve long term results after transplantation.

24-Hour Storage

The safe period for clinical heart preservation remains approximately 4 hours, whereas clinical liver or kidney preservation times routinely exceed 12 hours, and exceptionally 24 hours.[73]

Successful prolonged storage in clinical practice from 4-8 hours has been reported only in a few single cases.[74,75] Recently, Obadia et al[76] reported 14 heart transplantations performed in exceptional circumstances after a long preservation period (10-13 hours). The hearts were perfused with St. Thomas cardioplegia number 2 + 100 mg procaine and stored in the same solution at 4°C. Immediately before aortic unclamping cardiopulmonary bypass (CPB) was stopped and CPB flow rate was increased by 500 ml/min every 30 seconds to achieve low reperfusion pressure of the heart. A glucose-insulin-potassium (GIK) infusion was started at the unclamping of the aorta and maintained for the first hour after operation. The survival rate was 75% at 1 year and 71% at 5 years.

The problems resulting from prolonged heart storage are mainly contracture and reduced coronary flow which inhibit sufficient recovery of cardiac function in reasonable time to guarantee the survival of the recipient. Many attempts have been made in animal experiments to improve the outcome of prolonged-stored hearts including continuous perfusion systems, modifications of storage solutions, and development of new preservation solutions.

Rabbit hearts that were arrested, cooled, preserved for 24 hours in an extracellular cardioplegic solution and reperfused in a Langendorff model recovered 84% of control contractile function.[77]

Piglet hearts were arrested with crystalloid cardioplegia, excised, and stored for 12 hours in saline solution at 0°C. Initial reperfusion (10 min) revealed a left ventricular stroke work index of 3.8 ± 2.3, 14.6 ± 1.3, and $19.8 \pm 1.6 \times 10^3$ erg/g when whole blood, unmodified perfluorochemical, or aspartate/glutamate-enriched perfluorochemical cardioplegia was used.[78]

Kojima et al[79] tested a hexanol cardioplegic solution containing pyruvate in an isolated rat heart model after 18-hours preservation. The percent recovery of left ventricular developed pressure and rate-pressure product were significantly better with the hexanol cardioplegic solution compared with St. Thomas and Stanford solution.

Wicomb[80] tested rabbit hearts in an in vitro model after 24-hour storage. For perfusion and storage a simplified UW solution (Cardiosol) with polyethylene glycol instead of hydroxyethyl starch was used. The mean cardiac output of the hearts reached 80% of controls.

In an in vitro human right atrial muscle preparation recovery of function after a 24 hour storage period at 4°C or 12°C was assessed. The developed forces (DF) in the 4°C group were 59, 77, and 61% of the control for Euro-Collins, University of Wisconsin, and Bretschneider solution. For those cooled to 12°C, DF were 10, 30, and 96%, respectively.[81]

Intermittent Perfusion

In an isolated rat heart model Zhu and co-workers[82] investigated poststorage function after 24 hour storage at 0°C and intermitted perfusion with oxygenated crystalloid cardioplegic solution (CP-11EB) for 3 minutes at 10 and 17 hours of storage. Poststorage aortic flow was 65%, coronary flow 44%, cardiac output 58% and work 53% of the unstored controls.

Continuous Perfusion

The concept of continuous normothermic perfusion with blood was discussed by Lower

et al[83] and was advocated by Robicsek and co-workers[84] in 1969. But logistically problems and cumbersome methods have prevented it from being accepted in clinical practice.

In an isolated rat heart model the effects of glucose, insulin, and aspartate as components of oxygenated St. Thomas' solution on recovery of cardiac function after 24-hour preservation were assessed.[85] When used for continuous perfusion the best recovery of cardiac output was achieved at a temperature of 20°C. Recovery at 4°C was significantly lower than at 20°C.

The efficacy of oxygenated UWS containing endothelin-A receptor antagonist FR139317 for 24-hour preservation was tested in continuously perfused isolated rabbit hearts.[86] Percent recovery rates of cardiac output and coronary flow were 93.3% and 94.3%, respectively.

In a swine model of heart transplantation simple hypothermic storage and continuous hypothermic perfusion with crystalloid solution were compared. For the hypothermic perfusion group there was significant improvement of myocardial function after orthotopic transplantation.[87]

Ferrera et al[88] compared isolated pig hearts were preserved in cold St. Thomas solution for 24 hours either by simple storage or continuous microperfusion. After reperfusion the microperfusion group showed higher levels of tissue adenosine triphosphate and higher mean left ventricular developed pressure.

Non-Heart-Beating Donors

The use of donor hearts harvested 30 minutes or later after circulatory arrest could expand the donor pool. A number of potential donors die, before the procurement of organs can be performed. After cardiac arrest the organs of these "non-heart-beating donors" (NHBD) are exposed to unprotected normothermic ischemia.

Experiments in dogs[89] and primates[90] showed that hearts from NHBD could be successfully transplanted if the donors received cardioprotective drugs before cardiac arrest, i.e., prostaglandins, calcium antagonists and radical scavengers.

But this is not in conformity with the ethical premise formulated by the First International Workshop on NHBD that the therapy of a patient should not be compromised on the grounds that he is a potential donor and that a therapy just for the purpose of organ procurement can also not be accepted.[91]

Using the in-vivo pig model we harvested hearts 30 minutes after circulatory arrest. The animals were not pre-treated with cardioprotective drugs. We performed a perfusion with cold leukocyte-depleted blood cardioplegia (BCP) containing the Na^+-H^+-exchange (NHE) inhibitor HOE 642. NHE inhibitors have been shown excellent protective efficiency to reduce ischemia and reperfusion injury in different in vitro and in vivo models.[92,93] After orthotopic transplantation a second controlled reperfusion with BCP and HOE 642 was performed.

The contractility of these hearts expressed as maximal left and right ventricular stroke work index was not significantly different as compared to the control group (transplantation without normothermic ischemia). In contrast, the contractility was reduced significantly when the reperfusions were performed without HOE 642.[94]

Preservation Solutions

Currently, the majority of transplant programs use crystalloid-based cardioplegic solutions for myocardial preservation. The hypothermic, hyperkalemic crystalloid cardioplegia techniques have enabled safe myocardial preservation for up to 4 hours.

Bretschneider's HTK Solution

In 1979 Bretschneider introduced his Histidine-Tryptophane-Ketoglutarate (HTK) solution into clinical practice (Table 19.1). Cardioplegia for open heart surgery has been the first aim of this preservation solution. German heart surgeons have used Bretschneider solution in heart transplantation since 1985.[95]

Bretschneider solution contains the amino acid histidine, a biologically compatible,

administrable buffer system with a high buffer capacity. HTK solution is a flush solution like Euro-Collins or University of Wisconsin solution. To achieve optimal protective effects, the vascular and extracellular space have to be equilibrated with the fluid. This is nearly accomplished after 8-10 minutes of perfusion with a hydrostatic pressure of 80 mmHg. Therefore approximately 3000-4000 mL of cardioplegic solution are required.

In a retrospective study in cooperation with Eurotransplant and five heart transplant centers 591 heart transplant patients were included.[42] Immediate postoperative graft failure was observed in 4.2%. Within the first 30 days 11.8% of organs failed. Acute graft failure and early mortality correlated with the length of ischemic time. In addition, a higher incidence of graft failure was observed when the perfusion volume was less than 1500 ml. The 1- and 5-year survival rates were 72 and 63%, respectively. The authors advocate to avoid ischemic time of more than 4 h.

The efficacy of Bretschneider vs. University of Wisconsin solution (UWS) for long-term preservation was tested in a primate allotransplantation model.[96] Bretschneider's HTK solution allowed storage of hearts for periods of up to 10 hours. UWS provided superior results with regard to clinical outcome and hemodynamic recovery of hearts after ischemic periods of up to 16 hours.

In contrast, examinations in an in vitro human atrial muscle preparation showed a greatly improved recovery of contractility after 24-hour storage in HTK-solution compared with both Euro-Collins and UW solutions.[81] Recovery of developed force was affected by temperature (0°C vs. 12°C) for Euro-Collins and UW solutions but not for Bretschneider's solution.

University of Wisconsin Solution

The University of Wisconsin organ preservation solution (UWS, Table 19.3) was initially developed by Belzer and co-workers for pancreas transplantation.[97] It is an intracellular type solution, with a high potassium concentration. Components include the impermeables lactobionate, raffinose, and hydroxyethylstarch to prevent edema during hypothermic storage. The benefit of an intracellular potassium solution is an early and more complete arrest along with prevention of membrane flux during hypothermia. Glutathione is added at the time of administration and acts as an antioxidant. The solution also contains allopurinol to prevent xanthine oxidase-mediated production of free radicals. Adenosine is a metabolic precursor to the formation of ATP and high-energy phosphates.

In a large number of articles originating from the University of Wisconsin, but also from many other groups, UW solution was claimed to be superior of all other preservation fluids.[98, 99]

A clinical trial comparing UWS and Stanford solution revealed better preservation of ATP and creatine phosphate levels, decreased defibrillations, decreased intraoperative pacing, and trend toward decreased requirement for inotropic support in the UW group.[100]

Even for heart preservation the possibility of prolonged preservation with UW solution was reported.[80,101,102] As shown by Astier and Paul[103] the glutathione component of the UW changes from the reduced into the oxidized form with a half-life of about 4 days. UW solution containing oxidized instead of reduced glutathione is significantly less effective for preservation.[104,105]

The so-called simplified UWS (Cardiosol) contains polyethylene glycol instead of hydroxyethyl starch.

The high potassium concentration of UWS is accused to be responsible for endothelial injury of the coronary arteries. Investigations in neonatal pig hearts showed a loss of endothelium-dependent vasodilatation after UWS caused by the inability of the endothelium to release nitric oxide. In contrast, blood cardioplegia did not result in impaired endothelial function.[106]

To avoid the deleterious effect of hyperkalemic cardioplegic solution on coronary endothelium a new extracellular UWS formulation was created.[107] This solution contains 25 mEq/L of potassium and 129 mEq of sodium. The solution was tested in an isolated

Table 19.1. Composition of Bretschneider's HTK solution

Histidine	180 mmol/L
Tryptophane	18 mmol/L
Mannit	2 mmol/L
K-Ketoglutarate	20 mmol/l
Magnesium chloride	9 mmol/L
Potassium chloride	9 mmol/L
Sodium chloride	15 mmol/L
Total osmolarity	20 mOsm
pH	7.1

Table 19.2. Composition of UWS[109]

Pentafraction	0.45 mmol/L
Lactobionic acid	100 mmol/L
Magnesium sulfate	5 mmol/L
Raffinose	30 mmol/L
Adenosine	5 mmol/L
Allopurinol	1 mmol/L
Glutathione	3 mmol/L
Dexamethasone	16mg/L
Potassium	140 mEq/L
Sodium	20 mEq/L
Total osmolarity	320 mOsm
pH	7.4

Table 19.3. Composition of Stanford cardioplegic solution[109]

HCO_3	28mEq/L
Mannitol	25gm/L
Glucose	50gm/L
Potassium	30mEq/L
Sodium	28mEq/L
pH	8.0
Osmolarity	450mOsm

piglet heart model. After 24-hours storage there was no significant difference of stroke work index, high-energy phosphate stores and myocardial water content between the two UWS groups. Experiments in neonatal duroc piglets suggest that the low-potassium UWS solution provides superior protection of the endothelium by preserving the endothelium-dependent vasodilatory response to nitric oxide release.[108]

Stanford Solution

Stanford cardioplegic solution (Table 19.4) is typical of the class of extracellular type solutions. The solution contains potassium as the cardioplegic agent, bicarbonate as buffer, and glucose and mannitol for their osmotic properties. Furthermore, mannitol acts as a free radical scavenger. Most centers using this solution tend to limit the ischemic period to 4 hours.[109]

Table 19.4. Composition of St. Thomas' cardioplegic solution[112]

Sodium chloride	10mmol/L
Potassium chloride	16 mmol/L
Magnesium chloride	16 mmol/L
Calcium chloride	1.2 mmol/L
Sodium bicarbonate	10.0 mmol/L
pH	7.8
Osmolarity	285-300 mOsm

In a retrospective analysis 195 patients were reviewed for the development of cardiac allograft vasculopathy with a mean follow-up of 24 months.[75,110] These patients were treated in identical fashion, except for the type of cardioplegia used, Stanford solution or UWS. The data supported the conclusion that UWS is associated with an enhanced incidence of vasculopathy (14% versus 22%, $p < 0.03$). It is suggested that the high potassium concentration of intracellular solutions cause myocyte and endothelial cell injury resulting in a higher incidence of graft atherosclerosis.

St. Thomas Solution No. 2

In 1975 the St. Thomas' Hospital cardioplegic solution No. 1 was introduced into clinical practice. [111] The formulation of this solution was based on the desire to deviate as little as possible from the normal extracellular ionic composition and to minimize the amount of potassium that had traditionally been used to ensure rapid and complete arrest. This solution had widespread clinical use throughout the world. As a result of numerous experimental studies the St. Thomas Hospital cardioplegic solution No. 2 was introduced in 1981. The main features of this solution, as compared to the earlier solution, are a reduction in the sodium and potassium content, a reduction in calcium by 50%, the omission of procaine, the incorporation of a small amount of bicarbonate, and the adjustment of pH to 7.8. In an isolated rat heart model St. Thomas' Hospital solution No. 2 provided substantially superior protection compared with No.1.[112]

In a prospective, randomized study comparing preservation with UWS versus St. Thomas' Hospital solution (STS, Table 19.4) 39 heart transplant patients were enrolled. Hemodynamic, electron microscopic, and biochemical evaluation did not reveal any significant differences in postoperative myocardial performance. Only the number of intraoperative defibrillations and the rhythm stability after reperfusion indicated superiority of UWS.[113]

Celsior

Celsior, a new crystalloid cardioplegic solution (Table 19.5), was developed by Menasche.[114] The development of Celsior has been conducted according to the following guidelines:

1. Prevention of calcium overload by the low calcium concentration of the solution (100mM), by magnesium at a concentration high enough (13mM) to antagonize calcium fluxes and by maintaining a slight degree of acidosis (pH 7.30).
2. Prevention of myocardial edema by mannitol and lactobionate.
3. Prevention of severe intracellular acidosis by buffering with histidine. The concentration of histidine in Celsior is similar to that of blood (30mM).
4. Prevention of oxidative damage by reduced glutathione.

Furthermore, Celsior contains glutamate to restore the ischemia-induced loss of high energy phosphates, in particular ATP. Hitherto, no controlled prospective clinical trials with this solution have been published.

Table 19.5. Composition of Celsior cardioplegic solution[82]

Lactobionate	80 μmol/L
Mannitol	60 μmol/L
Reduced glutathione	3 μmol/L
Glutamate	20 μmol/L
Histidine	30 μmol/L
Potassium	15 μmol/L
Sodium	100 μmol/L
Magnesium	13 μmol/L
Calcium	0.26 μmol/L
Chloride	41.5 μmol/L
Total osmolarity	360 mOsm
pH	7.3

Blood Cardioplegia

Blood cardioplegia and warm reperfusion (Table 19.6) have demonstrated enhanced myocardial protection in standard open heart operations. But the use of blood cardioplegia for the procurement of donor hearts in clinical practice might bear problems because of the cumbersome technique.

Nataf and co-workers[55] used blood cardioplegia for the preservation of donor hearts. Harvesting of the donor graft began with a perfusion of cold crystalloid solution (Plegisol). Perfusion of a first dose of blood cardioplegia (8-10°C) for 3 minutes was immediately started on the arrival of the graft in the operating room. Reinfusions were performed each 20 minutes. At the end of the aortic anastomosis myocardial warm reperfusion with glutamate-containing blood cardioplegia was started. Retrospective comparison of two matched cohorts of 50 patients receiving either crystalloid or blood cardioplegia revealed a significantly better recovery of cardiac function in the blood cardioplegia group.

Briganti and co-workers[56] reported successful long-term outcome after transplantation of hearts with prolonged ischemic time of more than 300 minutes. Cardioplegia was achieved with oxygenated St. Thomas' Hospital No. 2 solution supplemented with aspartate. Allografts received a reinfusion of aspartate-supplemented cold blood cardioplegic solution at 20-minute intervals from time of arrival in the recipient theater to the time of implantation. In addition the hearts were reperfused with warm blood cardioplegic solution containing 20 mmol/L aspartate ("hot shot") just before aortic cross-clamp removal. No difference was found in allograft function, functional capacity, the development of transplant-associated coronary disease or actuarial survival in the groups with an ischemic time of less than 241 minutes, 241-300 minutes and more than 300 minutes.

Conclusions

The majority of available hypothermic, hyperkalemic crystalloid cardioplegia techniques in heart transplantation have enabled safe myocardial preservation for up to 4 hours. Research efforts are in progress to develop preservation methods that would decrease myocardial injury both during the period of preservation and during the period of reperfusion.

Attention has to be focused on the best "myocardial preservation" as well as on the "best endothelial cell preservation" to avoid an initial injury may result in early graft failure, chronic rejection, or the development of cardiac allograft vasculopathy.

One of the major challenges in transplantation remains the extension of the preservation period.

Benefit of blood cardioplegic solutions for the preservation and reperfusion of hearts has been shown in experimental and clinical

Table 19.6. Composition of blood cardioplegia for reperfusion[116]

THAM (Tromethamine)	8-10mEq/L
Citrate Phosphate Dextrose	Ca^{2+} 0.15-0.20 mmol/L
Glutamate	13 mmol/L
Aspartate	13 mmol/L
Glucose	40 mmol/L
Calcium	0.15-0.25 mmol/L
Potassium Chloride	8-10mEq/L
Total osmolarity	380-400 mOsm
pH	7.5-7.6

investigations but it's use for the procurement of donor hearts is limited by the cumbersome technique.

References

1. Drinkwater DC, Rudis E, Laks H et al. University of Wisconsin Solution versus Stanford Cardioplegic Solution and the development of cardiac allograft vasculopathy. J Heart Lung Transplant 1995; 14:891-6.
2. Day JD, Rayburn BK, Gaudin PB et al. Cardiac allograft vasculopathy: The central pathogenic role of ischemia-induced endothelial cell injury. J Heart Lung Transplant 1995; 14:142-9.
3. Hosenpud JD, Bennett LE, Berkeley M et al. The registry of the International Society of Heart and Lung Transplantation, 14th official report 1996. J Heart Lung Transplant 1997; 16:691-712.
4. Darby JM, Stein K, Grenvik A et al. Approach to management of the heart-beating "brain dead" organ donor. JAMA 1989; 261:2222-8.
5. Mertes PM, Burtin P, Carteaux JP et al. Changes in hemodynamic performance and oxygen consumption during brain death in the pig. Transplant Proc 1994; 26:229-30.
6. Sebening C, Hagl C, Szabo G et al. Cardiocirculatory effects of acutely increased intracranial pressure and subsequent brain death. Eur J Cardiothorac Surg 1995; 9:360-72.
7. Bittner HB, Kendall SWH, Chen EP et al. The combined effects of brain death and cardiac graft preservation on cardiopulmonary hemodynamics and function before and after subsequent heart transplantation. J Heart Lung Transplant 1996; 15:764-77.
8. Emery RW, Cosk RC, Levinson MM et al. The cardiac donor: A six-year experience. Ann Thorac Surg 1986; 41:356-62.
9. Buckberg GD. Invited letter concerning: Phases of myocardial protection during heart transplantation. J Thorac Cardiovasc Surg 1990; 100:461-2.
10. Burtin P, Mertes PM, Pinelli G et al. Myocardial ischemia during experimental brain death. Transplant Proc 1993; 25:3107-8.
11. Novitzky D, Cooper DKC, Morrell D et al. Change from aerobic to anaerobic metabolism after brain death and reversal following triiodothyronine. Transplantation 1988; 45:32-6.
12. Shatney CHO, MacCarter DJ, Lillehei RC. Effects of allopurinol, propanolol and methylprednisone on infarct size in experimental myocardial infarction. Am J Cardiol 1976; 37:572-80.
13. Arnold SL, DeWall RA, Kezdi P et al. The effect of allopurinol on the degree of early myocardial ischemia. Am Heart J 1980; 99:616-624.
14. Reimer KA, Jennings RB. Failure of the xanthine oxidase inhibitor allopurinol to limit infarct size after ischemia and reperfusion in dogs. Circulation 1985; 71:1069-75.
15. Eddy LJ, Stewart JR, Jones HP et al. Free radical-producing enzyme, xanthine oxidase, is undetectable in human hearts. Am J Physiol 1987; 253:H709-11.
16. Podzuweit T, Braun W, Muller A et al. Arrhythmias and infarction in the ischemic pig heart are not mediated by xanthine oxidase-derived free oxygen radicals (Abstract). Circulation 1986; 74(Pt2):II346.
17. Heusch G. Myocardial stunning, a role for calcium antagonists during ischaemia? Cardiovasc Res 1992; 26:14-9.
18. Irita K, Fujita I, Takshige K et al. Calcium channel antagonists induced inhibition of superoxide production in human neutrophils: Mechanism independent of antagonizing calcium influx. Biochem Pharmacol 1986; 35:3465-71.

19. Cave AC, Downey JM, Hearse DJ. Adenosine fails to substitute for preconditioning in the globally ischemic isolated rat heart (Abstract) J Mol Cell Cardiol 1991; 23(SIII):S76
20. Omar B, Hanson A, Bose S et al. Reperfusion with pyruvate eliminates ischemic preconditioning in the isolated rabbit heart: An apparent role for enhanced glycolysis. Coronary Heart Dis 1991; 2:799-804.
21. Liu GS, Thornton J, Van Winkle DM, Protection against infarction afforded by preconditioning is mediated by A_1 receptors in rabbit heart. Circulation 1991; 84:350-6.
22. Wyatt DA, Edmunds MC, Berne RM et al. Adenosine stimulates glycolytic flux in isolated perfused rat hearts by A_1 adenosine receptors. Am J Physiol 1989; 257:H1952-7.
23. Lasley R, Rhee J, VanWylen D et al. Adenosine A_1 receptor mediated protection of the globally ischemic isolated rat heart. J Mol Cell Cardiol 1990; 22:39-47.
24. Cronstein BN, Kramer SB, Weissman G et al. Adenosine: A physiological modulator of superoxide anion generation by human neutrophils. J Exp Med 1983; 158:1160-77.
25. Kloner RA, Yellon D. Does ischemic preconditioning occur in patients? J Am Coll Cardiol 1994; 24:1133-42.
26. Alkhulaifi AM, Yellon DM, Pugsley WB. Preconditioning the human heart during aortocoronary bypass-surgery. Eur J Cardiothorac Surg 1994; 8:270-6.
27. Karck M, Rahmanian P, Haverich A. Ischemic preconditioning enhances donor heart preservation. Transplantation 1996; 62:17-22.
28. Fremes SE, Zhang J, Furukawa RD et al. Adenosine pretreatment for prolonged cardiac storage. An evaluation with St. Thomas' Hospital and University of Wisconsin solutions. J Thorac Cardiovasc Surg 1995; 110:293-301.
29. Cave AC, Clifford SC, Downey JM et al. Improved functional recovery by ischemic preconditioning is not mediated by adenosine in the globally ischemic isolated rat heart. Cardiovasc Res 1993; 27:663-8.
30. Galinanes M, Bullough D, Mullane KM et al. Sustained protection by acadesine against ischemia- and reperfusion-induced injury: Studies in the transplanted rat heart. Circulation 1992; 86:589-97.
31. Finkelstein I, Toledo-Pregra L, Castellanos J. Physiologic and hormonal changes in experimentally induced brain dead dogs. Transplant Proc 1987; 19:4156-8.
32. Orlowski JP, Spees EK. Improved cardiac transplant survival with thyroxin treatment of hemodynamic unstable donors: 95.2% graft survival at 6 and 30 months. Transplant Proc 1993; 25:1535
33. Meyers CH, D'Amico TA, Peterseim DS et al. Effects of triiodothyronine and vasopressin on cardiac function and myocardial blood flow after brain death. J Heart Lung Transplant 1993; 12:68-80.
34. Gaskill HV, Sirinek KR, Levine BA. Hemodynamic effects of vasopressin. Arch Surg 1983; 118:434-7.
35. Tixier D, Matheis G, Buckberg GD et al. Donor hearts with impaired hemodynamics. Benefit of warm substrate-enriched blood cardioplegic solution for induction of cardioplegia during cardiac harvesting. J Thorac Cardiovasc Surg 1991; 102:207-14.
36. Magovern GJ Jr, Flaherty JT, Gott VL et al. Failure of blood cardioplegia to protect myocardium at lower temperatures. Circulation 1982; 66:I60-7.
37. Heitmiller RF, DeBoer LSV, Geffin GA et al. Myocardial recovery after hypothermic arrest: A comparison of oxygenated crystalloid to blood cardioplegia. The role of calcium. Circulation 1985;72:II241-53.
38. Takahashi A, Hearse DJ, Braimbridge MV et al. Harvesting hearts from long-term preservation. Detrimental effects of initial hypothermic infusion of cardioplegic solutions. J Thorac Cardiovasc Surg 1990; 100:371-8.
39. Hendry PJ, Walley VM, Koshal A et al. Are temperatures attained by donor hearts during transport too cold? J Thorac Cardiovasc Surg 1989; 98:517-22.
40. Sakaguchi H, Kitamura S, Kawachi K et al. Preservation of myocardial function and metabolism at subzero nonfreezing temperature storage of the heart. J Heart Lung Transplant 1996; 15:1101-7.
41. Young JB, Naftel DC, Bourge RC et al. Matching the heart donor and heart transplant recipient. Clues for successful expansion of the donor pool: A multivariable, multi-institutional report. J Heart Lung Transplant 1994; 13:353-65.
42. Reichenspurner H, Russ C, Überfuhr P et al. Myocardial preservation using HTK solution for heart transplantation. A multicenter study. Eur J Cardiothorac Surg 1993; 7:414-9.
43. Batty PR, Hicks GL, DeWeese JA et al. Optimal osmolality for cold storage of the cardiac explant. J Surg Res 1990; 48:601-5.
44. Fischer JH, Jeschkeit S. Minimal amounts of hyaluranidase in HTK or UW solution substantially improve the recovery of preserved hearts. Transpl Int 1996; 9 (Suppl1):442-6.
45. Kevelaitis E, Mouas C, Menasche, P. Poststorage diastolic abnormalities of heart transplants: Is vascular dysfunction or myocardial contracture the culprit? J Heart Lung Transplant 1996; 15:461-9.

46. Hajjar RJ, Ingwall JS, Gwathmey JK. Mechanism of action of 2,3-butanedione monoxime on contracture during metabolic inhibition. Am J Physiol 1994; 267:H100-8.
47. Benyo Z, Kiss G, Szabo C et al. Importance of basal nitric oxide synthesis in regulation of myocardial blood flow. Cardiovasc Res 1991; 25:700-3.
48. Mankad PS, Chester AH, Yacoub MH. 5-Hydroxytryptamine mediates endothelial dependent coronary vasodilation in the isolated rat heart by the release of nitric oxide. Cardiovasc Res 1991; 25:244-8.
49. Seccombe JF, Pearson PJ, Schaff HV. Oxygen radical-mediated vascular injury selectively inhibits receptor-dependent release of nitric oxide from canine coronary arteries. J Thorac Cardiovasc Surg 1994; 107:505-9.
50. Weyrich AS, Ma XL, Lefer AM. The role of L-arginine in ameliorating reperfusion injury after myocardial ischemia in the cat. Circulation 1992; 86:279-88.
51. Smolenski RT, Seymour AM, Yacoub MH. Dynamics of energy metabolism in the transplanted human heart during reperfusion. J Thorac Cardiovasc Surg 1994; 108:938-45.
52. Buckberg GD. Myocardial protection: An overview. Sem Thorac Cardiovasc Surg 1993; 5:98-106.
53. Buckberg GD. Update of current techniques of myocardial protection. Ann Thorac Surg 1995; 60:805-14.
54. Beyersdorf F, Mitrev Z, Sarai K et al. Changing patterns of patients undergoing emergency surgical revascularization for acute coronary occlusion. J Thorac Cardiovasc Surg 1993; 106:137-48.
55. Nataf P, Pavie A, Bracamontes L et al. Myocardial protection by blood cardioplegia and warm reperfusion in heart transplantation. Ann Thorac Surg 1992; 53:525-6.
56. Briganti EM, Bergin PJ, Rosenfeldt FL et al. Successful long-term outcome with prolonged ischemic time cardiac allografts. J Heart Lung Transplant 1995; 14:840-5.
57. Pradas G, Cuenca J, Juffe A. Continuous warm reperfusion during heart transplantation. J Thorac Cardiovasc Surg 1996; 111: 784-90.
58. Takemura G, Onodera T, Ashraf M. Quantification of hydroxyl radical and its lack of relevance to myocardial injury deriving early reperfusion after graded ischemia in rat hearts. Circ Res 1992; 71:96-105.
59. Fantone JC, Ward PA. Role of oxygen-derived free radicals and metabolites in leukocyte- dependent inflammatory reactions. Am J Pathol 1982; 107:395-418.
60. Ambrosio G, Weisfeldt MYL, Jacobus W et al. Evidence for a reversible oxygen radical-mediated component of reperfusion injury: Reduction by recombinant human superoxide dismutase administered at the time of reflow. Circulation 1987; 75:282-91.
61. Bolli R, Jeroudi MO, Bharat SP et al. Marked reduction of free radical generation and contractile dysfunction by antioxidant therapy begun at the time of reperfusion. Evidence that myocardial "stunning" is a manifestation of reperfusion injury. Circ Res 1989; 65: 607-22.
62. Sun SC, Appleyard R, Masetti P et al. Improved recovery of heart transplants by combined use of oxygen-derived free radical scavengers and energy enhancement. J Thorac Cardiovasc Surg 1992; 104:830-7
63. Charloux C, Ishii K, Paul M et al. Reduced production of the deleterious hydroxyl free radical during the final reperfusion of isolated rabbit heart with the use of an improved sodium lactobionate-based cardioplegic medium. J Heart Lung Transplant 1994; 13: 481-8.
64. Bando K, Teramoto S, Tago M et al. Oxygenated perfluorocarbon, recombinant human superoxide dismutase, and catalase ameliorate free radical induced myocardial injury during heart preservation and transplantation. J Thorac Cardiovasc Surg 1988; 96:930-8.
65. McCord JM. Oxygen-derived free radicals in postischemic injury. N Engl J Med 1985; 312:159-63.
66. Engler R, Covell W. Granulocytes cause reperfusion ventricular dysfunction after 15-minute ischemia in the dog. Circulation Research 1987; 61:20-28.
67. Babior BM. Oxygen dependent microbial killing by phagocytes. N Engl J Med 1978; 659-68.
68. Engler RL, Dahlgren MD, Morris D et al. Role of leukocytes in response to acute myocardial ischemia and reflow in dogs. Am J Physiol 1986; 252:H314-322.
69. Dhôte-Burger P, Vuillemenot A, Lecompte T et al. Is myocardial function influenced by neutrophil activation during ischemia and reperfusion in coronary artery surgery? Transplant Proc 1995; 27:2786-8.
70. Hansen PR. Role of neutrophils in myocardial ischemia and reperfusion. Circulation 1995; 91:1872-85.
71. Forman MB, Virmani R, Puett DW. Mechanisms and therapy of myocardial reperfusion injury. Circulation 1990; 81(Suppl.IV):69-78.
72. Habazettl H, Palmisano BW, Graf BH et al. Improvement in functional recovery of the isolated guinea pig heart after hyperkalemic reperfusion with adenosine. J Thorac Cardiovasc Surg 1996; 111:74-84.

73. Todo S, Nery J, Yanaga K et al. Extended preservation of human liver grafts with UW solution. JAMA 1989; 261:711-4.
74. Jeevanandam V, Auteri JS, Sanchez JA et al. Improved heart preservation with University of Wisconsin solution-experimental and preliminary human experience. Circulation 1991; 84;324-8.
75. Drinkwater DC, Stein DG, Permut LC et al. Clinical trial of University of Wisconsin Solution for cardiac transplantation-preliminary results. J Thorac Cardiovasc Surg 1991; 102:798-9.
76. Obadia JF, Girard C, Ferrara R et al. Long conservation organs in heart transplantation: Postoperative results and long-term follow-up in fourteen patients. J Heart Lung Transplant 1997; 16:256-9.
77. Burt JM, Copeland JG. Myocardial function after preservation for 25 hours. J Thorac Cardiovasc Surg 1986; 92:238-246.
78. Martin SM, Laks H, Drinkwater DC et al. Perfluorochemical reperfusion yields improved myocardial recovery after global ischemia. Ann Thorac Surg 1993; 55:954-60.
79. Kojima S, Wu ST, Wikman-Coffelt J et al. Eighteen-hour preservation of rat hearts with hexanol and pyruvate cardioplegia. J Am Coll Cardiol 1993; 21:1238-44.
80. Wicomb WN, Hill DJ, Collins GM. Twenty-four-hour ice storage of rabbit heart. J Heart Lung Transplant 1994; 13:891-4.
81. Hendry PJ, Labow RS, Keon WJ. Comparison of intracellular solutions for donor heart preservation. J Thorac Cardiovasc Surg 1993; 105:667-73.
82. Zhu Q, Yang X, Claydon MA et al. Twenty-four-hour intermittent perfusion storage of the isolated rat heart: The effect of perfusion intervals on functional preservation. J Heart Lung Transplant 1994; 13:882-90.
83. Lower RR, Stofer RC, Hurley EJ et al. Successful homotransplantation for the canine heart after anoxic preservation for seven hours. Am J Surg 1962;104:302-5.
84. Robicsek F, Tam W, Daugherty HK. Survival of heart grafts. Arch Surg 1969; 99: 750-2.
85. Shimada Y, Yamamoto F, Yamamoto H et al. Temperature-dependent cardioprotection of exogenous substrates in long-term heart preservation with continous perfusion: Twenty-four-hour preservation of isolated rat heart with St. Thomas' hospital solution containing glucose, insulin, and aspartate. J Heart Lung Transplant 1996; 15:485-95.
86. Okada K, Yamashita C, Okada M. Efficacy of oxygenated University of Wisconsin solution containing endothelin-A receptor antagonist in twenty-four-hour heart preservation. J Heart Lung Transplant 1996; 15:475-84.
87. Qayumi AK, Jamieson WR, Rosado LJ et al. Preservation techniques for heart transplantation: comparison of hypothermic storage and hypothermic perfusion. J Heart Lung Transplant 1991; 10:518-26.
88. Ferrera R, Marcsek P, Larese A et al. Comparison of continuous mircoperfusion and cold storage for pig heart preservation. J Heart Lung Transplant 1993; 12:463-9.
89. Takagaki M, Hisamochi K, Morimoto T et al. Successful transplantation of cadaver hearts harvested one hour after hypoxic cardiac arrest. J Heart Lung Transplant 1996; 15:527-31.
90. Gundry SR, Fukushima N, Eke CC et al. Successful survival of primates receiving transplantation with "dead", nonbeating donor hearts. J Thorac Cardiovasc Surg 1995; 109:1097-102.
91. Obermann K, Nagel E, Pichlmayr R. Ethical considerations in procuring organs from non-heart-beating donors after sudden cardiac death. Transplantation Proceedings 1995; 5:2924-5.
92. Hendrikx M, Mubagwa K, Verdonck F et al. New Na^+-H^+ exchange inhibitor HOE 694 improves postischemic function and high-energy phosphate resynthesis and reduces Ca^{2+}-overload in isolated perfused rabbit heart. Circulation 1994; 89:2787-98.
93. Scholz W, Albus U, Counillon L et al. Protective effects of HOE 642, a selective sodium-hydrogen exchange subtype 1 inhibitor, on cardiac ischaemia and reperfusion. Cardiovasc Res 1995; 29:260-268.
94. Martin J, Sarai K, Yoshitake M et al. Successful orthotopic pig heart transplantation from non-heart-beating donors. J Heart Lung Transplant 1999; 18:597-606.
95. Hölscher M, Groenewoud AF. Current status of the HTK solution of Bretschneider in organ preservation. Transplant Proc 1991; 23:2334-7.
96. Human PA, Holl J, Vosloo S et al. Extended cardiopulmonary preservation: University of Wisconsin solution versus Bretschneider's cardioplegic solution. Ann Thorac Surg 1993; 55:1123-30.
97. Belzer FO, Southard JH. Principles of solid-organ preservation by cold storage. Transplantation 1988; 45:673-6.
98. Breda MA, Drinkwater DC, Laks H et al. Successful long-term preservation of the neonatal heart with a modified intracellular solution. J Thorac Cardiovasc Surg 1992; 104:139-50.

99. Jeevanandam V, Barr ML, Auteri JS et al. University of Wisconsin solution versus crystalloid cardioplegia for human donor heart preservation. A randomized blinded prospective clinical trial. J Thorac Cardiovasc Surg 1992; 103:194-9.
100. Stein DG, Drinkwater DC, Laks H et al. Cardiac preservation in patients undergoing transplantation. A clinical trial comparing University of Wisconsin solution and Stanford solution. J Thorac Cardiovasc Surg 1991; 102:657-65.
101. Swanson DK, Pasaoglu I, Berkoff HA et al. Improved heart preservation with UW preservation solution. J Heart Lung Transplant 1988; 7:456-61.
102. Stein DG, Permut LC, Drinkwater DC et al. Complete functional recovery after 24-hour heart preservation with University of Wisconsin solution and modified reperfusion. Circulation 1991; 84(5Suppl):III 316-23.
103. Astier A, Paul M. Instability of reduced glutathione in commercial Belzer cold storage solution. Lancet 1989; 2:556.
104. Boudjema K, Vangulik TM, Lindell SL et al. Effect of oxidized and reduced glutathione in liver preservation. Transplantation 1990; 50:948-51.
105. Fischer JH, Jeschkeit S. Effectivity of freshly prepared or refreshed solutions for heart preservation versus commercial Eurocollins, Bretschneiders HTK, and University of Wisconsin solution. Transplantation 1995; 59: 1259-62.
106. Pearl JM, Laks H, Drikwater DC et al. Loss of endothelium-dependent vasodilatation and nitric oxide release after myocardial protection with University of Wisconsin solution. J Thorac Cardiovasc Surg 1994; 107:257-64.
107. Drinkwater DC, Ziv ET, Laks H et al. Extracellular and standard University of Wisconsin solutions provide equivalent preservation of myocardial function. J Thorac Cardiovasc Surg 1995; 110:738-45.
108. Lee J, Drinkwater DC Jr, Laks H et al. Preservation of endothelium-dependent vasodilation with low-potassium University of Wisconsin solution. J Thorac Cardiovasc Surg 1996; 112:103-10.
109. Aziz S, Tada Y, Jaffery S et al. University of Wisconsin solution provides superior myocardial preservation compared with Stanford cardioplegic solution. J Heart Lung Transplant 1994; 1099-108.
110. Drinkwater DC, Rudis E, Laks H et al. University of Wisconsin solution versus Stanford cardioplegic solution and the development of cardiac allograft vasculopathy. J Heart Lung Transplant 1995; 14:891-6.
111. Braimbridge MV, Chayen J, Bitensky L et al. Cold cardioplegia or continuous coronary perfusion? Report on preliminary clinical experience as assessed cytochemically. J Thorac Cardiovasc Surg 1977; 74:900-6.
112. Ledingham SJM, Braimbridge MV, Hearse DJ. The St. Thomas' Hospital cardioplegic solution. A comparison of the efficacy of two formulations. J Thorac Cardiovasc Surg 1987; 93:240-6.
113. Demertzis S, Wippermann J, Schaper J et al. University of Wisconsin versus St. Thomas' Hospital solution for human donor heart preservation. Ann Thorac Surg 1993; 55: 1131-7.
114. Menasche P, Termignon JL, Pradier R et al. Experimental evaluation of Celsior, a new heart preservation solution. Eur J Cardiothorac Surg 1994; 8:207-13.
115. Buckberg GD. Blood cardioplegia. Il Cuore 1988; 3:271-81.

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