ADENOSINE AND ITS ROLE IN CARDIOPLEGIA

Experimental evaluation in the isolated rat heart
and in an in-vivo primate model

Dieter H. Boehm, MD

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Department of Cardiothoracic Surgery,
University of Cape Town,
Medical School,
Cape Town,
South Africa

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CONTENTS

ABSTRACT

This study was designed to investigate the role of adenosine, an endogenous cardioprotectant agent, without high potassium and as cardioplegic additive to high potassium solutions. Adenosine cardioplegia and potassium cardioplegia supplemented by adenosine (K + ADO) were investigated in terms of hemodynamic, metabolic and ultrastructural recovery in the isolated rat heart and in the in-vivo baboon model during periods of global myocardial ischemia, simulating the clinical situation during open heart surgery. The results obtained in both models show that adenosine improved postischemic hemodynamic function when used without high potassium cardioplegia. The combination of adenosine and high potassium was less effective in both models in terms of hemodynamic recovery; however, improved rhythm stability and coronary vasodilatation were still present. In addition adenosine alone was able to induce fast electromechanical arrest in the isolated rat heart. However, failure of even high concentrations of adenosine to limit ventricular fibrillation in the baboon exclude its use as cardioplegic agent on its own without additional interventions. It appears likely that adenosine without high potassium is cardioprotective via activation of A_1 receptors and opening of ATP-sensitive potassium channels, a mechanism which is probably non-functional in a high potassium environment.

In view of the limited cardioprotection achieved with the combination of adenosine and high potassium further studies should aim for additional interventions to induce cardioplegia with adenosine and normokalemic solutions.

ORGANIZATION OF THE THESIS

This thesis deals with the role of adenosine in the field of intraoperative myocardial protection. Adenosine is a cardioprotective agent, especially during phases of induced or spontaneous regional or global ischemia, and it acts via multiple mechanisms. Additionally, adenosine might act as an alternative agent to high potassium solutions to induce cardiac arrest. This form of polarized arrest might offer certain advantages over commonly achieved cardiac arrest at depolarized membrane potentials.

Chapter 1 gives an introduction to the historical development of cardioplegia and focuses on current concepts of cardioplegia and organ preservation in general. Clinically used cardioplegic solutions are compared with the exact composition and discussion of advantages and disadvantages. The impact of cardioplegic myocardial protection on operative mortality and postoperative complications at Groote Schuur Hospital is outlined in a further paragraph. Finally the physiologic and clinical roles of adenosine are discussed.

Chapter 2 describes the models used in this thesis in detail. First, the isolated rat heart model according to Langendorff used in the pilot experiments is discussed in detail. The second model, the in-vivo baboon model developed for the studies, is a preclinical model, closely resembling the clinical setting.

Chapter 3 explains the hypothesis under test. It explains the rationale for using adenosine as cardioplegic agent or as cardioplegic additive in the context of intraoperative myocardial protection.

Chapter 4 focuses on the effect of adenosine as cardioplegic agent and its effect on cardiac arrest in the isolated rat heart, which was used as a screening model. Further studies in the isolated rat heart highlight the effect of adenosine either as cardioplegic agent in a low potassium solution or as cardioplegic additive in a high potassium solution. The effects are demonstrated in a short-term (30 minutes) global ischemia model as well as in a medium-term (90 minutes) global ischemia model.

Chapter 5 describes the studies in the primate model, used as a preclinical model. In a first set of experiments the crystalloid cardioplegic solution resulting in the best postischemic recovery amongst the various solutions used in South Africa was determined. All further studies were based on this solution, which was the St. Thomas' Hospital solution No. 2. In a second step, adenosine was added to a non-cardioplegic (low potassium) solution and compared to St. Thomas' Hospital solution No. 2. The last set of experiments performed in the in-vivo baboon model included adenosine as adjunct to St. Thomas' Hospital solution No. 2. in a dose response study.

Chapter 6 briefly describes some additional in-vitro studies comparing the activity of adenosine deaminase in various species, including man. These experiments were thought to determine the optimal adenosine concentration in cardioplegic solutions intended for clinical application.

Chapter 7 includes a discussion and summary of the thesis and the models used.

ABBREVIATIONS USED IN THE TEXT

Units of measurement:

°C	degrees Celsius	min	minute
cm	centimeter	ml	milliliter
δ	delta (change in)	mm	millimeter
g	gravitational force	mMol	millimole
gm	gram	mOsm	milliosmole
h	hour	mV	millivolt
kg	kilogram	*N*	normality
kPa	kilopascal	ng	nanogram
L	liter	p	partial pressure
M	molarity /mol/L	s	second
m	meter	U	Unit
mEq	milliequivalent	μg	microgram
mg	milligram		

Chemical compounds:

1,3 DPG	1,3-diphosphoglycerate
ADP	adenosine diphosphate
AMP	adenosine monophosphate
ATP	adenosine triphosphate
Ca^{2+}	calcium
cAMP	cyclic adenosine 3', 5'-monophosphate
cGMP	cyclic guanosine 3',5'-monophosphate
CK	creatine kinase
CK-MB	MB fraction of creatine kinase
Cl^-	chloride
CO^2	carbon dioxide

CoA	coenzyme A
CP	creatine phosphate
CPD	citrate-phosphate-dextrose
Cu^{2+}	copper
DHAP	dihydroxyacetone phosphate
ECGF	endothelial cell growth factor
EDRF	endothelium derived relaxing factor (nitric oxide)
EDTA	ethytenediamine-tetraacetic acid
F-6-P	fructose-6-phosphate
FAD	flavin adenine dinucleotide (oxidized from)
FADH	dihydroflavin adenine dinucleotide (reduced from)
FC-43	Fluosol-43 emulsion
FDP	fructose-1,6-diphosphate
FE^{2+}	irion
G-1-P	glucose-1-phosphate
G-6-P	glucose-6-phosphate
G-6-PDH	glucose-6-phosphate dehydrogenase
GIK	glucose-insulin-potassium
GI-3-P	glyceraldehyde-3-phosphate
GI-3-PDH	glyceraldehyde-3-phosphate dehydrogenase
H^+	hydrogen
H_2CO_3	carbonic acid
H_2O	water
H_2O_2	hydrogen peroxide
Hb	hemoglobin
HCl	hydrochloric acid
HCO_3	bicarbonate
Hg	mercury
HK	hexokinase
K^+	potassium
LDH	lactate dehydrogenase
Mg^{2+}	magnesium

N_2	nitrogen
Na^+	sodium
NAD	nicotinamide adenine dinucleotide (oxidized form)
NADH	dihydronicotinamide adenine dinucleotide (reduced form)
NADP	nicotinamide adenine dinucleotide phosphate (oxidized form)
NADPH	dihydronicotinamide adenine dinucleotide phosphate (reduced form)
NaOH	sodium hydroxide
α-KGI	α-ketoglutarate
O_2	oxygen
OAA	oxaloacetic acid
PDH	pyruvate dehydrogenase
PEP	phosphoenolpyruvate
PFK	phosphofructokinase
PGI_2	prostacyclin
PK	pyruvate kinase
PO_4^{3-}	phosphate
SO_4^{2-}	sulfate
SOD	superoxide dismutase
THAM	tromethamine
tPA	tissue plasminogen activator
TRIS	tris (hydroxymethyl) aminomethane hydrochloride
UW-CSS	University of Wisconsin cold storage solution

Other abbreviations:

AO	aortic flow
BSA	body surface area
CABG	coronary artery bypass graft
CI	cardiac index
CO	cardiac output
DPTI	diastolic pressure time index

e^-	electron
ECG	electrocardiogram
HR	heart rate
IABP	intra aortic balloon pump
LA	let atrium
LAP	left arterial pressure
LV	left ventricle
LV dP/dt	first derivative of LV developed pressure
LVEDP	left ventricular end diastolic pressure
MAP	mean arterial pressure
PB	Plasmalyte B electrolyte solution
PVR	pulmonary vascular resistance
SV	stroke volume
SVI	stroke volume index
SVR	systemic vascular resistance
SWI	stroke work index

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Reichenspurner H, MD PhD

Rose A.G., MD PhD

Statistical assistance

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CHAPTER 1

INTRODUCTION

1.1. Cardioplegia

History of cardioplegia

The evolution of cardiac surgery reflects a dilemma between the needs of surgeons for a still, bloodless operating field and the need of the myocardium for a continuous supply of oxygen and substrates. As a consequence of this dilemma the development of specialized solutions allowed a compromise between these two extremes.

The beginning of open heart surgery

The beginning of clinical open heart surgery dates back to 1953 when Lewis and Taufic in Chicago closed an atrial septal defect using inflow occlusion and moderate systemic hypothermia with surface cooling (Lewis,1953). But only after the development of the heart-lung machine in 1954, by Gibbon, which took about twenty years, was it possible to develop and extend open heart procedures to today's level (Gibbon, 1954). It was, however, the correction of Fallot's tetralogy by Lillehei in 1954 in Minneapolis, still using controlled cross-circulation from a donor, which initiated the explosive development of open heart surgery (Lillehei,1955). Kirklin in Rochester using a modified Gibbon screen oxygenator (Kirklin,1955) and Cooley in Houston using a bubble oxygenator, paved the way for further development (Cooley,1957).

Hypothermia and intermittent aortic cross-clamping

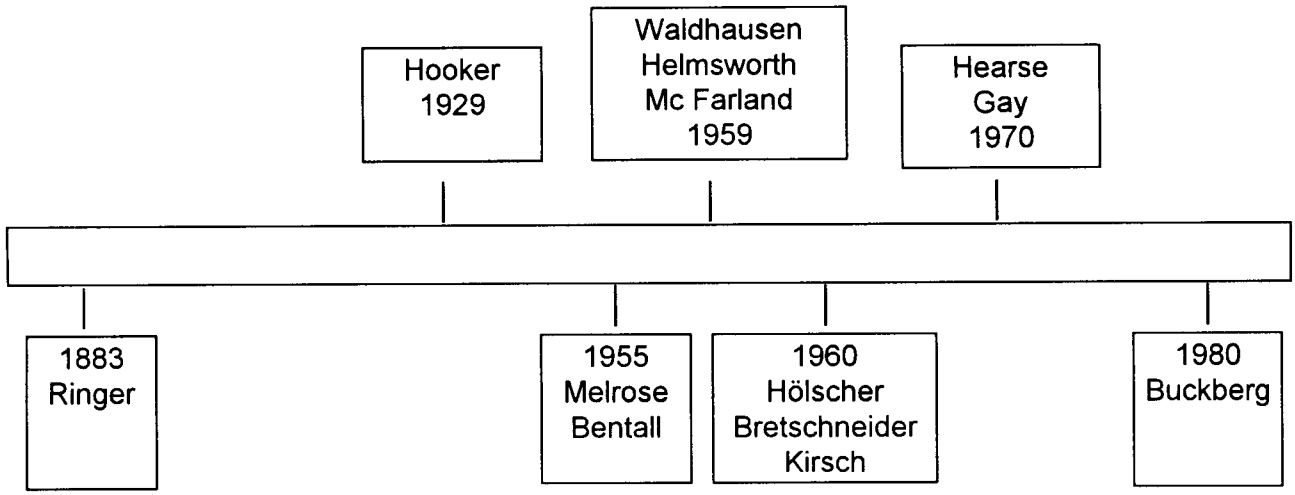
Before long the technique of hypothermia allowed only a short period of cardiac arrest, but suboptimal operating conditions became a limiting factor in extending the complexity of the surgical repair. Alternative techniques of keeping the coronary arteries perfused and the heart beating seriously obscured the operating field. Intermittent aortic cross-clamping seemed a possible alternative, but was still far from being ideal.

Introduction of cardioplegia

Cardioplegia by definition means: 'intentional reversible paralysis of the heart'. It was Melrose in 1955 who introduced the concept of 'elective cardiac arrest' based on animal and clinical studies (Melrose,1955). The observation that ions in the extracellular medium had a profound effect on cardiac action was first established in the classical studies of Ringer in 1883. Whereas Hooker in 1929 suggested that potassium chloride might be able to alter the ventricular fibrillation observed in experimental animals (Hooker,1929), it was Melrose who introduced the concept into clinical practice. It needs to be remembered, that this new concept came into existence for the purpose of improving exposure for the operation by providing the surgeon with a quiescent heart, rather than for protection of the myocardium (Figure 1.1.)

However, use of induced cardiac arrest fell into disfavor several years later after a report of some unexpected complications. They were seemingly related to the administration of potassium citrate and Helmsworth and associates (1959) described a focal area of microscopic myocardial injury believed to result from administration of a toxic substance. Swedish investigators, Björk and Fors (1961), reported that less myocardial damage resulted after a period of ischemia alone than with potassium citrate or acetylcholine induced arrest. The outcome was that elective cardiac arrest was virtually abandoned for nearly 15 years (Figure 1.1.).

FIGURE 1.1. HISTORICAL DEVELOPMENT OF CARDIOPLEGIA



Abandonment of potassium cardioplegia

With the abandonment of potassium induced cardioplegia, coronary perfusion became the technique of choice for myocardial preservation. The heart was kept beating and perfused through the aortic root. However, coronary venous return had constantly to be sucked from the beating heart during the operation. Many surgeons used electrically induced ventricular fibrillation to improve operative conditions. However, Buckberg, Hottenrot and coworkers (1975) at the University of California in Los Angeles showed that subendocardial necrosis was aggravated especially if the hypertrophic heart fibrillated spontaneously or if a normal heart was kept in ventricular fibrillation artificially. The reason being that the blood was diverted away from the subendomyocardium and even though the overall coronary flow increased in the fibrillating, compared with the empty beating heart, resistance to coronary flow rose progressively with increased period of fibrillation. Severe depression of left ventricular function associated with subendocardial necrosis was a consequence and subsequently the technique of ventricular fibrillation was largely abandoned. However, more and more surgeons turned to clamping the aorta or stopping coronary perfusion during the most difficult or critical steps of the operation so that continuous coronary perfusion became de facto intermittent. From St. Thomas' Hospital in London it was reported that for example continuous coronary perfusion of

the left coronary artery during aortic valve surgery was on the average only carried out during 70% of the overall aortic occlusion time. The usual pattern was to occlude the aorta for ten minutes and then to allow three minutes of coronary perfusion in order to correct the oxygen debt incurred during the ischemia.

The return of cardioplegia

The concept of protecting the myocardium and producing a clear operative field had been kept alive in Germany by Hölscher, who had published his work on the experimental study of various forms of cardiac arrest in the American literature (Hölscher,1961) (Table 1.1.). However, it was the work of Bretschneider and Spieckermann published in 1964 that stimulated the first cardiac surgeon to initiate the new wave of cardioplegia (Bretschneider,1964). Bretschneider published the principle of arresting the heart with a sodium poor, calcium free, procaine containing solution that became known as Bretschneider's solution (Table 1.1.). The principle of his solution was a sodium content equal to the intracellular sodium concentration to prevent generation of the action potential. The lack of calcium ions was intended to prevent excitation-contraction coupling and procaine was added to stabilize the cell membrane. Mannitol was added for osmotic reasons. Søndergaard adopted Bretschneider's cardioplegic solution routinely for myocardial protection in clinical practice, achieving a remarkably low mortality of 6% in a consecutive series of 100 aortic valve replacements (Søndergaard,1967). However, he used a 4° C cold blood/glucose mixture to perfuse the heart prior to cardioplegic perfusion.

Kirsch at the university clinic in Hamburg worked on the theory that a cardioplegic agent should contain no component that would stimulate the breakdown of energy-rich phosphates by activation of phosphorylases (Kirsch, 1970). Nor should it contain calcium, potassium, or sodium, as adenosine triphosphate (ATP) is used in membrane transport. Local anesthetics and magnesium, being membrane-stabilizing agents, were believed to slow the decay of organic phosphate in the cell (Table 1.1.). From 1969, the Kirsch magnesium-aspartate-procaine bolus solution was in

regular clinical use in Rodewald's unit in the university clinic in Hamburg (Kirsch,1972).

Stimulated by this work, Hearse tested experimentally the individual components of the Bretschneider and Kirsch solutions in the isolated rat heart at the Rayne Institute at St. Thomas' Hospital in London (Hearse,1976). In contrast to their ideas, he advocated the principle that cardioplegic solutions should retain as closely as possible extracellular, rather than intracellular concentrations of ions with only those additions that could individually be shown to be effective and then only in the optimal concentrations. He proposed the St. Thomas' solution which was based on Ringer's solution with its normal concentrations of sodium and calcium, to which was added 16 mM per liter potassium chloride to arrest the heart instantly, 16 mM per liter magnesium chloride which had in this concentration been shown to have a marked effect additive to the potassium for myocardial protection, and 1 mM per liter procaine hydrochloride (Table 1.1.). This solution was introduced into regular clinical practice by Braimbridge at St. Thomas' Hospital in 1975 (Braimbridge,1977).

There had been, during the 1960s, some interest in cardioplegia in the United States (Greenberg,1960). It was Gay and Ebert in 1973, who, quoting Kirsch and Rodewald's sizable clinical experience, used a 25 mM per liter solution of potassium chloride in 200 mM per liter of sodium with glucose and bicarbonate (Gay,1973). They were able to protect isolated dog hearts against an hour of ischemia without microscopic damage, and they made the fundamental point that this improvement on the Melrose technique was due to the fact that their solution was isotonic. Tyers and co-workers emphasized the importance of the lower concentrations of potassium (Tyers,1978; Todd, 1975).

Clinical experience of cardioplegia was reported in the United States by Roe et al. who had introduced potassium-induced cardioplegia into his practice in San Francisco in 1973 and reported his initial series in 1976 (Roe,1977). He reported an impressively low mortality at this time of 5.4 %. Tyers et al. in 1977 reported over 100 consecutive patients in whom cardioplegia had been used (Tyers,1977). The excellence of their myocardial protection was supported by the facts that over half the hearts had spontaneously defibrillated, the post-operative use of inotropic agents was infrequent, the immediate post-operative level of cardiac output was improved,

and the release of myocardial iso-enzyme levels was relatively low. He had used a 25 mM per liter of potassium-enriched solution with some calcium in Normosol® at 15° C.

TABLE 1.1. THE EVOLUTION OF CARDIOPLEGIA

1st generation:	*Melrose solution*	Melrose 1955	high K^+-citrate
		Hoelscher 1961	Mg^{2+}/Procaine
2nd generation:	*German solutions*	Bretschneider 1964	low Na^+/ Ca^{2+}-free
		Kirsch 1970	Mg^{2+}/Procaine
		Hearse 1976	high K^+/Mg^{2+}
3rd generation:	*Extracellular solutions*	Gay-Ebert 1973	high K^+
4th generation:	*Blood cardioplegia*	Buckberg 1979	high K^+/blood

By the end of 1978 coronary perfusion as a means of preserving the myocardium during a period of aortic occlusion had virtually disappeared from clinical practice throughout the world. The discussion by then was not whether but what sort of cardioplegia should be used clinically. There were initially three fairly well-defined geographical groups in this respect (Table 1.2.). The continent of Europe by and large, particularly east of the Rhine, used the sodium-poor, calcium-free magnesium and procaine containing solutions introduced by Bretschneider. Great Britain and much of western Europe used solutions based on an extracellular formula with potassium, magnesium and procaine added to Ringer's solution, such as the St. Thomas' solution. The United States used primarily potassium-enriched solutions containing little or no magnesium and no local anesthetic (Table 1.2.) A variant in American practice was the use of blood as a vehicle for infusing potassium into coronary arteries, a concept introduced by Buckberg in Los Angeles (Buckberg,1979, Folette, 1978) (Table 1.1.). Blood was preferred to purely ionic solutions because of its better oxygenating and buffering capacities.

TABLE 1.2. REGIONAL DISTRIBUTION OF PREFERRED FORM OF CARDIOPLEGIA

INTRACELLULAR COMPOSITION	EXTRACELLULAR COMPOSITION
Europe esp. Germany	North America Great Britain

The question today is not whether to use cardioplegia or not, but rather what sort of cardioplegia should be used. Today cardiac surgeons are faced with a multitude of different cardioplegic solutions and cardioplegic techniques being used clinically and experimentally. However, the question of ideal myocardial preservation during open heart surgery still remains unsolved.

In summary, it was Melrose in 1955 who initiated the clinical use of chemical cardioplegia. Because of observations of myocardial damage with this technique there was a shift in the sixties to sustained coronary perfusion in order to approximate the normal state. In the seventies there was a revival of cardioplegia with reduced potassium concentrations or principles of sodium depletion or magnesium induced arrest. Blood cardioplegia came into existence in the eighties and was succeeded in the early nineties by the technique of continuous warm blood cardioplegia.

Current concepts of cardioplegia and organ preservation

Principles and definitions

Cardioplegia is a specialized form of organ protection and is historically the first form of intraoperative organ protection. Myocardial protection during open heart surgery includes all perioperative measures to protect the structure and function of myocardial tissue, including conducting tissue and endothelial cells (Table 1.3.).

Myocardial protection during open heart surgery includes all preoperative, intraoperative and postoperative measures to reduce myocardial damage. Cardioplegia is defined as intentionally induced, chemical electromechanical arrest of the heart in diastole and was initially developed to improve operative conditions during open heart surgery and to prevent air embolism.
The emphasis on protecting the ischemic heart came only later during the historical development. However, electrical arrest of the myocardium enhances structural and functional protection, as the major energy-consuming process of electromechanical activity is stopped. The term *cardioplegia* was probably first used by Lam in 1957, when describing his acetylcholine technique (Lam,1957).

TABLE 1.3. MYOCARDIAL PROTECTION

Definition.:	all perioperative measures to protect the structure and function of myocardial tissue, conducting tissue and vasculature, including vascular smooth muscle and endothelium
Cardioplegia:	chemically induced, reversible electromechanical cardiac arrest
Cardioplegic solutions:	specifically formulated solutions to induce reversible cardiac electromechanical arrest in diastole and to protect the ischemic myocardial, conducting and vascular tissue
Cardioplegic procedures:	- high-volume cardioplegia - oxygenated crystalloid cardioplegia - blood cardioplegia (cold/warm/continuous/hot shot) - antegrade cardioplegia - retrograde cardioplegia - depending on the type of solution used
Preservation solutions:	partially modified cardioplegic solutions used for ex-vivo storage (storage via immersion or perfusion)
Adaptation for specific requirements:	- energy-depleted myocardium - neonatal myocardium - senescent myocardium

Cardioplegic solutions

The use of cardioplegic solutions is only part of the myocardial protection regimen used during cardiac surgery today. With regard to individual cardioplegic solutions it appears more appropriate to talk about myocardial protective measures as for instance high volume cardioplegia, oxygenated crystalloid cardioplegia, ante- or retrograde cardioplegia, intermittent cold blood cardioplegia or continuous warm blood cardioplegia (Table 1.3.). The protective measures used are in general

dependent on the type of solution used, but several measures can also be combined as an integral form of myocardial protection.

Preservation solutions are either standard cardioplegic solutions used for intraoperative myocardial protection (St. Thomas' Hospital solution, Bretschneider HTK4 solution, Stanford solution etc.) or specially developed solutions for ex-vivo storage and subsequent transplantation (Collins solution, University of Wisconsin solution) (Table 1.4.).

Generally the concept of a single solution protecting all organs equally effectively is not feasible, owing to different organ-specific needs. However some commercially available solutions like the University of Wisconsin solution are effectively used clinically to protect intraabdominal organs as well as the heart.

However, recently concern has been raised about the intracellular composition of these preservation solutions including a high concentration of potassium (< 100mM). Pearl et al. found impaired endothelial function after prolonged storage in University of Wisconsin solution (Pearl,1994). Drinkwater et al. from the University of California, Los Angeles published a clinical study showing an increased rate of accelerated graft atherosclerosis when University of Wisconsin solution had been used for storage preservation (Drinkwater, 1995).

TABLE 1.4. CARDIAC PRESERVATION SOLUTIONS

PRESERVATION SOLUTIONS		
In-vivo protection	*Ex-vivo protection*	
Cardioplegic solutions	**Storage Solutions**	**Perfusion solutions**
St. Thomas'	Collins	Wicomb
Bretschneider	Euro-Collins	Proctor
Kirklin	Sacks	Copeland
Stanford	UW*	Watson

Legend: Cardioplegic solutions can be used for in-vivo and ex-vivo protection, whereas storage and perfusion solutions are developed purely for ex-vivo protection.

* University of Wisconsin solution

Current concepts of myocardial protection during cardiac surgery

The majority of cardiac operations performed today involve cross-clamping of the ascending aorta in order to facilitate the procedure by producing a still, bloodless operative field, but this inevitably causes myocardial ischemia. The intra-operative objective is to correct the underlying defect without causing additional damage, in other words causing no deterioration of myocardial function. Important factors in achieving this aim are pre-operative, intra-operative, and also anesthetic management (Table 1.5.). Surgical technique is important and finally the methods of intra-operative myocardial protection while the patient is on cardiopulmonary bypass. As started initially, cardioplegia is only one component of the complex term myocardial protection.

TABLE 1.5. PHASES OF PERIOPERATIVE MYOCARDIAL PROTECTION

preoperative	preoperative medication preoperative energy status of the myocardium myocardial hypertrophy premedication induction of anesthesia
intraoperative	anesthesia cardioplegic regimen reperfusion phase surgical technique
postoperative	ICU-treatment postoperative medication

Legend: The three phases of perioperative myocardial protection. Cardioplegia is only one part of the whole cardioprotective strategy.

Mechanisms of injury

Ischemia

Ischemia is defined as the total absence of blood flow. Ischemia causes irreversible injury if allowed to persist. Periods of ischemia shorter than those that produce irreversible injury may predispose the heart to reperfusion-related damage and myocardial stunning (Bolli, 1990).

The reversibility of tissue injury

The time sequence of cellular and subcellular events following myocardial ischemia, showing the progressive development, is listed in table 1.6. Jennings and colleagues (Jennings, 1961) have divided tissue Injury into a number of phases. However severe the ischemia, the cellular changes which occur during the early minutes are fully reversible. If adequate coronary flow is restored, there will be rapid and complete resumption of normal functional activity. If the duration of severity of ischemia is increased, reperfusion may not result in immediate return of normal contractile activity. However, several hours or days after the repletion of critical and limiting factors such as adenine nucleotide precursors, full recovery may be observed. Up to this stage cellular injury can be considered as reversible. However, beyond a certain point tissue injury becomes so severe that reperfusion will not result in resumption of full contractile activity. In this state, under certain conditions, reperfusion may even cause a major extension of damage (Hearse, 1973, 1977, 1990). Irreversible injury occurs progressively with increasing numbers of cells becoming destined for cell death (Jennings, 1974). The objective of effective myocardial protection is therefore either to reperfuse the tissue before the onset of significant irreversible injury or to use interventions which prevent or delay the critical transition from reversible to irreversible injury.

Effects on myocytes

The time sequence of cellular events following myocardial ischemia is listed in table 1.6.

Ischemia affects both the myocardium and the coronary vasculature as well as the conducting system. Mechanical function dwindles dramatically with the onset of ischemia. End-diastolic pressures increase and ventricular compliance decreases. These phenomena are manifestations of ATP depletion and intracellular calcium uptake.

The contractile apparatus is the major consumer of ATP in the beating heart. When substrate substrate supply is cut off, ATP stores are rapidly consumed by mechanical activity. ATP is also required to dissociate the cross-bridges between actin and myosin. In the absence of ATP and the presence of calcium, cross-bridges are formed and an irreversible contracture, called rigor, results.

Ischemia induces a change within the contractile apparatus that reduces sensitivity of the proteins to calcium and reduces the maximal rate of ATP hydrolysis (Hess,1981). The first of these effects is reversible. The sensitivity of the contractile apparatus to calcium returns to normal on reperfusion. However, the maximal activity of the myofibrillary ATPase remains depressed. The reduction in this enzyme activity explains the observed decrease in dP/dt (change in pressure with time) following ischemic injury.

Adenine nucleotides are found in coronary sinus blood following a variable period of ischemia. Loss of adenine nucleotides from the myocardium results in sustained low cellular ATP levels. Up to three days are required for ATP levels to return to normal in the dog following only 15 minutes of normothermic ischemia (De Boer, 1980)

Endogenous glycogen stores are utilized to generate ATP via glycolysis. This process continues only as long as cytosolic $NADH^+$ levels are below the threshold for inhibition of glycolysis (Rovetto, 1975). The ongoing hydrolysis of ATP and the glycolysis itself contribute to steadily increasing levels of $NADH^+$. Eventually, glycolysis ceases, and ATP production with it. ATP consumption continues until

stores are depleted. An ischemic contracture is the manifestation of severe ATP depletion. The resultant injury may be irreversible. Glycolysis results in the generation of high osmolality, acidosis, and altered redox states within the cell that can slow the generation of free radicals.

There is a substantial body of evidence linking increased glycolytic flux rates to decreased ischemic injury (Owen, 1990). Decreased glycolytic flux is associated with increased severity of ischemic injury and may contribute to lethal injury, since lack of cytosolic ATP fails to provide the energy required to move calcium across the cell membranes (Opie, 1988).

However, the regulation of glucose metabolism during ischemia remains poorly understood. Myocardial ischemia results in an increase in the rate of glycolysis and a switch from lactate uptake by the heart to lactate production and protons as metabolic end products. The glucose for glycolysis originates from both the breakdown of myocardial glycogen stores and the uptake of glucose from the blood (Lopaschuk, 1997). In a recent study by Cross et al. (1996) the authors found high endogenous glycogen to be protective during brief periods of no-flow ischemia. In contrast, during prolonged no-flow ischemia glycogen stores became fully depleted and myocardial injury occurred, which was exacerbated by the lower ischemic pH in these hearts, leading to increased Na^+ - H^+ exchange during reperfusion. The authors concluded, that the effect of high glycogen on the ischemic heart may be due to the differences in the extent of glycogen depletion during ischemia, which is linked to the extent and severity of ischemia. King et al. (1995) defined the conditions for a beneficial effect of glucose to the ischemic heart in a rat heart model investigating coronary flow and glucose delivery as determinants of contracture in the ischemic myocardium. The adequate coronary flow to deliver glucose and to remove end-product inhibition was found to be greater than 0.06 ml/g/min. In the surgical setting during cardioplegic arrest with the aorta crossclamped there is still residual non-coronary collateral flow from preexisting collaterals which may provide about 6-10% of the normal coronary flow, sufficient to remove the harmful accumulation of metabolites. Intermittent reinfusion of cardioplegia may have a similar effect. However, in conditions of prolonged total ischemia as occurs during storage for transplantation, glucose might be harmful as there is no adequate removal of end products. Tissue levels of lactate have been inversely correlated with

post-ischemia recovery (Neely, 1984). High concentrations have been found to cause mitochondrial injury (Armiger, 1974). However, more recent data from isolated cell systems suggest that lactate alone is not sufficient to cause anoxic damage or predispose to reperfusion injury (Geisbuhler, 1990). There is now recurrent interest in the use of glucose-insulin-potassium (GIK) for treatment of acute myocardial infarction (Apstein, 1997) and in a curent meta-analysis Fath-Ordoubadi (1997) found a beneficial effect on hispital mortality.

During ischemia, the mitochondria are unable to generate ATP. The activity of mitochondrial complex I (NADH: ubiquinone reductase) decreases significantly during ischemia and is further reduced on reperfusion. Changes occur within the cytochrome oxidase system that result in an univalent reduction of oxygen rather than the normal tetravalent reduction (Veitch,1990). This change results in the generation of *free oxygen radicals* upon reperfusion. Free oxygen radicals may also be formed by the mitochondria during periods of ischemia. Post-ischemic dysfunction can result from the effects of lipid peroxidation caused by these free radicals.

The other organelle affected by ischemia is the sarcoplasmic reticulum. Calcium released from the sarcoplasmic reticulum by Ca^{2+}-induced Ca^{2+} release intitiates contraction. Calcium uptake by the ATP-dependent Ca^{2+}-pump initiates relaxation. This process consumes ATP (1 mM/2 mM Ca^{2+} transported) and is stimulated by calmodulin activity. Ischemia interferes with calcium uptake by the sarcoplasmic reticulum through

1) ATP depletion,
2) reduction in calmodulin activity (Turla,1985)
3) change in intracellular pH.

Decreasing intracellular pH decreases calcium uptake (Turla,1985). The effect of pH change decreases with time but ATP depletion (Ca^{2+}-ATPase) and reversal of Na^{+}/Ca^{2+} exchanges continue to depress sarcoplasmic reticulum function during ischemia (Krause,1984).

The consequence of reduced calcium uptake via the sarcoplasmic reticulum increases cytosolic free calcium concentrations. High levels of intracellular calcium in conjunction with calmodulin can activate phospholipases (Nayler, 1981; Krause, 1984). During ischemia, calmodulin activity remains normal except in the sarcoplasmic reticulum (Turla, 1985). Calcium-calmodulin activation of phospholipase A_2 may result in membrane damage with loss of intracellular contents, including creatine kinase (van der Vusse, 1983, Das, 1986). Elevated blood levels of the MB-CK isoform are indicative of myocardial injury.

TABLE 1.6.: CELLULAR CONSEQUENCES OF MYOCARDIAL ISCHEMIA

ONSET OF SEVERE ISCHEMIA
Disturbed transmembrane ion balance
Utilization of dissolved oxygen
Reduced mitochondrial activity and oxidative metabolism
Reduced ATP production
Reduction of creatine phosphate stores
Reduction of amplitude and duration of action potential
Leakage of potassium .
ST segment changes
Accumulation of sodium and chloride ions
Catecholamine release
Stimulation of adenyl cyclase
Stimulation of glycogenolysis
Net utilization of high-energy phosphate
Accumulation of protons, carbon dioxide, and inorganic phosphate
Stimulation of phosphofructokinase activity
Increase of glycolytic flux
Development of intracellular acidosis
Reduction or blockage of mitochondrial electron transport
Suppression of fatty acid oxidation
Leakage of magnesium ions
Utilization of glycogen
Leakage of inorganic phosphate
Accumulation of NADH
Increased LDH and a-GPDH activity
Accumulation and leakage of lactate
Accumulation of fatty acyl CoA derivatives
Depletion of creatine phosphate
Leakage of adenosine, inosine, and other metabolites
Vasodilatation
Inhibition of adenine nucleotide transferase activity
Possible stimulation of triglyceride synthesis and degradation
Increasing cellular acidosis
Suppression of PFK and G-3-PDH activity
Slowing of glycolytic flux
Cell swelling
Increase in cytoplasmic ionized calcium content
Possible exhaustion of glycogen reserves
Inhibition of glycolysis
Severe reduction of ATP
Minor ultrastructural changes, for example, mitochondrial swelling
Possible onset of contracture
ONSET OF IRREVERSIBLE DAMAGE?
Lysosomal changes activation of hydrolases and lipoprotein lipases
Increasing cellular edema
Loss of mitochondrial respiratory control
Nonspecific electrocardiographic changes
Ultrastructural changes in mitochondria and myofibrils
Complete depletion of energy reserves
Loss of mitochondrial components
Leakage of enzymes to interstitial space and lymph
Severe ultrastructural damage and membrane deterioration
Disruption of mitochondria, myofibrils, and cell membranes
CELL DEATH AND TISSUE NECROSIS

(modified from Hearse, 1981)

Effects on coronary vasculature

The changes that occur within the coronary vasculature during ischemia are rather subtle but no less deleterious to the heart. The purine metabolism of the coronary endothelium and the myocytes are closely related. Much of the adenine nucleotide salvage occurs in the vascular endothelium (Gerlach,1985). Ischemic injury affects the endothelium resulting in loss of nucleotides even after ischemia of short duration.

The coronary capillary endothelium is the only site of uric acid production within the myocardium (Jarasch,1981). Uric acid is produced from hypoxanthine and xanthine by the enzyme xanthine dehydrogenase. During ischemia, xanthine dehydrogenase is converted to xanthine oxidase by the action of a calcium-dependent protease (Jarasch, 1986).

Xanthine oxidase converts xanthine and hypoxanthine to uric acid, but it also catalyses the formation of toxic oxygen radicals. These substances are formed on reperfusion and are capable of mediating reperfusion injury. This view is not universally accepted. Data acquired from ischemic rat hearts indicate that there is only minimal conversion of xanthine dehydrogenase xanthine oxidase (Kehrer, 1987). Whether this observation is unique to the rat remains to be determined.

Toxic oxygen radicals are formed in low concentrations in the normal heart. They are detoxified by endogenous superoxide dismutase, catalase and peroxidases.

The activity of these free radical scavengers is dramatically reduced by ischemia (Otani, 1985). Ischemia renders them ineffective during the reperfusion period; consequently, free radical mediated damage ensues. The activity of lytic enzymes is increased during ischemia.

The vascular endothelium is a source of endothelium-dependent relaxing factor (EDRF), which is probably nitric oxide (NO), and prostacyclin. These substances are critical for the maintenance of normal coronary flow. Ischemia and possibly cardioplegic solutions themselves alter the responsiveness of the coronary vasculature to 5-hydroxytryptamine, which causes release of EDRF (Saldanha, 1989, Pearl, 1994). Loss of normal endothelial function leads to pathologic modes of constriction by the coronary vessels and response to various biochemical

metabolites. Ischemic injury may also leave the endothelium vulnerable to activation of platelets and neutrophils and their adhesion upon reperfusion, resulting in further injury (Olafsson, 1987).

Specialized conduction cell damage

The specialized conduction cells become non-functional early in the course of global myocardial ischemia in humans, and it may be speculated that their recovery takes longer than does recovery of myocytes. Some support for this is that five or so minutes after initially hyperkalemic reperfusion, the ventricular myocardium in some patients responds well and strongly to direct ventricular pacing, although it remains silent with atrial pacing or without pacing. Then, after a couple or more minutes, a sinus rhythm may appear. Also, when blood cardioplegia and uncontrolled normokalemic reperfusion are used, about 50 per cent of patients have transient atrioventricular conduction defects when the cardiopulmonary bypass is discontinued (Baermann, 1987). This appears to represent a formal specialized conduction cell stunning rather then necrosis, since the conduction defects have disappeared by the time of hospital discharge in half of the patients in whom it developed. Even third degree AV block persisting as long as two months have been observed to give way to sinus rhythm (Baermann, 1987). This speculation remains to be validated, however.

Reperfusion

At the cellular level, reperfusion is potentially the most destructive phase during the operative repair of a heart defect. Generation of oxygen radicals and calcium influx both occur during reperfusion of the myocardium. Both of these phenomena may injure the heart although the myocardium must be reperfused after regional or global ischemia if total necrosis is to be avoided. Several studies have demonstrated that injury may be accelerated or extended by interventions at the time of reperfusion (Simpson, 1987; Opie, 1989; Hearse, 1990). However, reperfusion, though in some

ways harmful, does improve many deleterious changes. The reason is that with the resumption of mitochondrial function ATP resynthesis restarts and reversible ultrastructural changes improve over time.

The existence of this so-called reperfusion injury has been debated. However, descriptions of its characteristics, time course and mechanisms are convincing. Furthermore, evidence is mounting that reperfusion injury is indeed a process amenable to modification (either extension or regression) by intervention initiated at the time of reperfusion (Vinten-Johansen, 1985; Vinten-Johansen, 1986; Opie, 1989; Nakanishi, 1991; Nakanishi,1995) .

Cardiac surgeons have spoken extensively about reperfusion injury, possibly because they, unlike their cardiology colleagues, can modify the conditions under which reperfusion is conducted or the composition of the reperfusate with relative ease by means of extracorporeal technology, chemical cardioplegia and modified reperfusates. We can define reperfusion injury as pathology that is extended, accelerated or expressed de novo from the profile observed during ischemia, resulting from events occurring after reperfusion has been initiated. This definition is rather broad in that it takes into consideration that the *rate* of injury as well as the final long-term *extent* of injury may be altered by reperfusion or by reperfusion therapy. Deceleration of the rate of reperfusion injury in the early post-operative period may be important in counteracting myocardial stunning, which is defined as post-ischemic contractile dysfunction in the absence of morphologic injury or necrosis (Jennings, 1991; Appleyard, 1993; Mangano,1993).

The concept of reperfusion injury had its genesis in the 1960s and 1970s. Jennings et al. (Jennings, 1960) reported development of ultrastructural abnormalities after reperfusion, while Hearse and colleagues (Hearse, 1973; Hearse, 1977) demonstrated that the reintroduction of oxygen and the initiation of reperfusion were associated with abrupt myocardial injury. In the following decades, a series of experiments were performed to describe the pathologic characteristics of reperfusion injury or reoxygenation injury and researchers have striven to determine the mechanisms involved.

There is a burst of free radical production that peaks 2 - 4 minutes after reperfusion and continues for 3 hours or more after reflow. Much of the damage produced by toxic oxygen radicals is mediated by the hydroxyl radical $OH^{\cdot}$ (Burton, 1984). This entity is formed by the interaction of the superoxide radical, $O_2^{\cdot}$ and hydrogen peroxide via the Haber-Weiss reaction (Fridovich, 1978). The reactant $O_2^{\cdot}$ is formed by the conversion of hypoxanthine into xanthine by xanthine oxidase in the presence of oxygen. An alternative pathway for $O_2^{\cdot}$ generation is via one-electron reduction of O_2 catalyzed by the enzyme NADPH-oxidase that is present in neutrophils and is activated by the complement C5a. Complement activation is a frequent event during bypass. The hydroxyl radical is especially toxic because it has been shown to react with polyunsaturated fatty acids (a common membrane constituent) to form lipid hydroperoxides (Flamm, 1978). Lipid hydroperoxides are capable of causing sustained chain reactions resulting in extensive membrane damage and possible disruption of cellular integrity.

Ultrastructural damage produced by the hydroxyl radical ($OH^{\cdot}$) includes vacuolization and edema of the vascular endothelium, severe swelling of the myocardial mitochondria and myocyte basement membrane blebbing (Krause, 1984). Membranous cellular debris was found in the vessels of septal preparations exposed to $O_2^{\cdot}$ and $OH^{\cdot}$. The primary damage caused by free radicals is not the generation of holes in the cell membrane but rather inactivation of proteins critical to maintenance of cellular homeostasis. Free radical damage to the glycolytic pathway has been postulated to cause increased intracellular calcium by impaired sodium/hydrogen exchange with consequent calcium/sodium exchange. Functional changes induced by $OH^{\cdot}$ include significant decreases of developed tension and diminished ability of the sarcoplasmic reticulum to take up calcium.

The diminished calcium uptake is a consequence of the uncoupling of calcium transport from ATP hydrolysis (Rowe,1983); this effect is exacerbated by acidosis. Both radicals and excess calcium have been implicated in the decrease in NADH ubiqinone oxidase (complex I) activity in mitochondria on reperfusion with O_2 containing perfusate. Loss of complex I activity uncouples oxidative phosphorylation. Another effect of the generation of oxygen radicals is the production of arachidonic acid metabolites capable of mediating constriction within the coronary vasculature.

This effect, combined with direct damage to the vascular endothelium could, theoretically lead to microinfarcts in the areas of myocardium involved.

Calcium influx

Events during the ischemic period can expose the myocyte to a massive influx of calcium on reperfusion. The SR is unable to take up calcium normally because of depressed ATP stores and OH. - mediated injury. Five to 10 μM or greater intracellular calcium concentrations activate lytic enzymes and uncouple substrate oxidation from phosphorylation (Swan, 1953). The combined effects of mitrochondrial respiratory chain damage and calcium entry can be significant. Stunned, reperfused hearts have a fourfold greater than normal oxygen consumption at a given work load (Bavaria, 1991). It has been suggested that while a prolonged accumulation of total cell calcium may be indicative of irreversible cell damage (Shen and Jennings, 1972), an early transient increase in intracellular calcium levels may cause and be indicative of reversible reperfusion injury (Opie, 1991). Opie proposed first, that reperfusion after a brief period of ischemia induces a transient calcium overload which contributes to reperfusion stunning and arrhythmias and secondly, that elevated intracellular calcium levels during ischemia and early reperfusion may promote abnormal intracellular calcium transients which could exacerbate stunning and/or initiate and perpetuate arrhythmias (Opie, 1989, Opie 1991).

Other mechanisms

In addition to oxygen free radicals and calcium influx, a number of mechanisms have been implicated in reperfusion injury, including activation of neutrophils and platelets, and development of microvascular injury with impaired blood flow (Olafsson, 1987). However, it must be remembered that the stage for reperfusion injury is set by the severity of the preceding ischemia (Veitch, 1990; Lucchesi,1990): the more severe the ischemia (i.e. regional ischemia in evolving myocardial

infarction, poor myocardial protection stemming from failure to deliver cardioplegia beyond obstructions or failure to formulate a solution to meet specific needs), the more severe will be the ensuing reperfusion injury.

Methods of myocardial protection

Non-cardioplegic methods of myocardial protection

During the past years a variety of different methods of protecting the myocardium during cardiac surgery have evolved (Hearse, 1981; Kirklin, 1993). Non-cardioplegic methods of myocardial protection are used infrequently today but are still occasionally applicable. More recently, techniques for performing coronary artery bypass surgery without cardiopulmonary bypass and cardioplegia have begun to emerge (Benetti, 1991; Borst, 1996).

1. Continuous cardiac perfusion

The myocardium is continuously perfused with oxygenated blood via the aortic root or during aortic valve surgery by direct cannulation of the coronary arteries. This can be performed at normothermia or moderate hypothermia (25° C - 32° C) and with or without electrical ventricular fibrillation. This method, however, does not produce ideal operative conditions. Although an empty beating heart has lower myocardial oxygen demands (Hottenrott, 1974), altered compressive forces in ventricular geometry impede the distribution of intra-myocardial bloodflow resulting in sub-endocardial ischemia (Miyamoto, 1978).

2. Intermittent cardiac ischemia

The aorta is intermittently cross-clamped for short periods (less than 15 minutes) with subsequent 5 - 15 minutes' periods of reperfusion of the beating heart. This method can be used at normothermia (Moran, 1986; Van der Vusse, 1987) or at moderate cardiac hypothermia, with or without electrical fibrillation in order to obtain a still operative field (Bonchek, 1987). Interestingly, a low morbidity and mortality has been reported using this method for coronary revascularization procedures and it has been speculated that intermittent aorta cross-clamping might cause ischemic preconditioning and thereby make the heart more tolerant of ischemia (Abd-Elfattah, 1995). There are clinical studies comparing intermittent aortic cross-clamping and hypothermic cardioplegic arrest which have shown equivalent outcomes (Baur, 1986; Moran, 1986). However, high-energy phosphates and cellular ultrastructure are better preserved in the cardioplegia group, although the use of cardioplegic solutions is also associated with an increased incidence of temporary post-operative rhythm disturbances (Flameng, 1984). Furthermore, a large number of experimental studies have shown cumulative damage as a result of intermittent aortic cross-clamping when compared to hypothermic cardioplegic arrest (Follete, 1978; Hearse, 1981; Levitsky, 1977, Wright, 1978).

3. Prolonged ischemic cardiac arrest

The aorta is simply cross-clamped for prolonged periods (15 - 60 minutes) at profound myocardial hypothermia (22° C). After 20 - 30 minutes the myocardium becomes electromechanically silent and still because of exhaustion of intramyocardial energy supplies. This method was pioneered successfully by Norman Shumway (Shumway, 1959), but it is now no longer used routinely. Aortic cross-clamp periods of up to 96 minutes were tolerated with acceptable clinical results, but prolonged cardiovascular support was necessary after these periods of anoxic rest and high-energy phosphate depletion (Griepp, 1973).

Hypothermic ischemic arrest may still have a role today in pediatric cardiac surgery when combined with deep hypothermic circulatory arrest, as neonatal hearts react

differently to cardioplegic solutions compared to adult hearts (Anderson, 1994; Baker, 1988; Baker,1990; Bove, 1986; Bove, 1988; Bull, 1984, Mangovern, 1988).

Cardioplegic methods of myocardial protection

The principles of cardioplegic myocardial protection are summarized in table 1.7.

Hypothermia

Hypothermia reduces the metabolic rate of the heart: Enzymatic systems obey van' t Hoff's law: there is an exponential decrease in reaction rate with decreasing temperature. This relationship applies to oxygen consumption. Oxygen consumption decreases by a factor of 2.8 for each 10-degree fall in temperature. Hypothermia has a stabilizing effect on membranes and alters membrane fluidity (Frank, 1982). Low temperatures also protect the heart from the "calcium paradox" (Rich, 1982). Although preventing the development of the calcium paradox, hypothermia increases the sensitivity of the myocardium to exogenous calcium. However, the ability of hypothermia alone to prevent myocardial ischemic injury is limited. Additionally, the effects of temperature on the myocardium appear to be age dependent. In the neonatal heart, hypothermia alone may offer better protection than hypothermia plus cardioplegic solutions (Mangovern, 1988; Karck, 1995).

TABLE 1.7.: PRINCIPLES OF CARDIOPLEGIC PROTECTION

Maximize energy conservation

1. Stop electromechanical activity

Chemical arrest of the heart in diastole

Produce immediate arrest

Must be easily maintained and reversed

Must not cause myocardial damage

2. Reduce metabolic rate

Hypothermia

Maintain intracellular homeostasis without energy-consuming membrane pumps

Prevent unfavorable ischemia-induced changes

1. Modify extracellular ionic environment

Prevent calcium influx

2. Maintain energy production

Supply substrates

Oxygen

Glucose?

Amino acids?

High energy phosphates?

Remove end products of metabolism

3. Counteract acidosis

Modify pH

Provide buffers

4. Prevent edema

Hyperosmolarity, Impermeants

Colloid oncotic pressure

(Modified from Buckberg, 1987)

Several strategies have been developed to reduce myocardial temperatures safely. They include surface cooling, core cooling, topical cooling of the myocardium and perfusion cooling of the myocardium.

Myocardial cooling is most effectively achieved by perfusing the coronary vasculature with iced (2-4°C) solution. Myocardial temperatures below 20°C are attained within minutes of the initiation of cold perfusion of the coronary arteries (Reitz, 1981). This rapid cooling can produce myocardial standstill, besides protecting the myocardium from ischemic damage during the period of aortic cross-clamping.

This technique may, however, predispose to later injury (Rebeyka, 1990) Temperatures of 6°C or below produce cardiac arrest (Hearse, 1975) but can result in tissue damage (Speicher, 1962). Cardioplegic solutions are used to induce cardiac arrest at higher temperatures. Other advantages of the use of cardioplegia include a more rapid and reliable onset of electromechanical silence.

Since the introduction (Melrose,1955) and development of cardioplegic solutions, there are now almost as many cardioplegic solutions as there are centers using them, but there is a more recent trend to greater uniformity and an increasing number of centers throughout the world are using properly formulated solutions which have been adequately tested under experimental conditions close to the clinical setting. Despite the variations in formulation, two broad types of cardioplegic solutions can be defined: blood cardioplegia and crystalloid cardioplegia (with or without the addition of a colloid substance). At present, there is no overwhelming evidence in favor of one type of cardioplegic solution for myocardial protection during intraoperative cardiac repair, but there is a general trend towards the use of blood cardioplegia in most countries, despite the more complex technical procedure and the higher costs.

Crystalloid cardioplegia

Crystalloid cardioplegic solutions are formulated to resemble either intracellular (prototype: Bretschneider's solution) or extracellular (prototype: St. Thomas' Hospital

solution) fluid. The degree of myocardial protection afforded by a solution resembling intracellular fluid is affected by the rate and volume of infusion. Decreased protection was previously noted if high rates of infusion or large volumes were used (Jynge, 1978). However, more recent studies find better protection if high volumes are used (Preusse, 1987, Human, 1995). This is explained by the longer period required by intracellular solutions to equilibrate the interstitial space. For Bretschneider's solution (HTK 4) an infusion period of 7-8 minutes with a total volume of 3-4 liters per patient is suggested in order to use the full preservation potential of the solution. If a solution based on extracellular fluid is used, the degree of protection does not generally vary with the volume or rate of infusion of the cardioplegic solution (Jynge, 1978) although better protection was found in one study (Engelman, 1983) with higher infusion volumes for St. Thomas' Hospital solution. The authors concluded, however, that improved protection by the higher volumes was probably related to the lower temperatures achieved with larger volumes. However, not all solutions based on extracellular fluid afford the same protection. Only properly formulated crystalloid solutions are as effective as blood cardioplegia in preserving high-energy phosphate stores and preventing ischemic injury (Hearse, 1981).

Blood cardioplegia

The use of blood cardioplegia was initially based upon the assumption that the oxygen carried by the hemoglobin would provide extra protection against ischemic injury. This assumption has seemingly been both supported and refuted by experimental evidence (Magovern,1982; Bing, 1982). The reasons for the discrepancy relate to the temperature of the cardioplegic solution infused (Magovern, 1982). The ability of hemoglobin to deliver oxygen to tissue decreases at lower temperatures. At 5°C, more oxygen is available to tissue from oxygenated crystalloid solution than from oxygenated blood cardioplegic solution. However, if the cardioplegic solution is delivered at higher temperatures, such as 20-27°C, then blood cardioplegic solutions will be able to deliver more oxygen to tissues than crystalloid cardioplegic solutions.

Blood contains many other components besides hemoglobin, including calcium, magnesium, protein, hormones, and cellular elements. Some studies claiming that blood cardioplegic solutions provided better myocardial protection than crystalloid cardioplegic solutions failed to add divalent cations to the crystalloid solutions used in the comparative studies. The omission of these cations from the crystalloid cardioplegic solutions may have played a significant role in the observed superiority of blood cardioplegia over the nonsanguineous cardioplegic solutions. If blood and crystalloid cardioplegic solutions have the same ionic constituents added (not that the concentrations of the ionic species are the same as in blood), there is little or no advantage in the use of blood cardioplegic solution in cases for which low infusion temperatures are used (Magovern, 1982).

The rheologic properties of blood cardioplegia are worthy of note. The viscosity of blood increases as temperature falls. Because of higher viscosity, cold blood solutions require longer infusion times than does an nonsanguineous cardioplegic solution. Prolonged infusions may have a beneficial effect on distribution in non-hypertrophied hearts.

Distribution of the cardioplegic solution in a non-hypertrophied heart after multiple doses (Heitmiller, 1985) was found to be more uniform with blood than with crystalloid. This improvement in perfusion is observed even if the red blood cells have been rendered incapable of O_2 transport. The presence of red blood cells or other microparticles allows perfusion of the entire capillary bed. No flow is seen in the true capillaries if they are perfused with nonparticulate solutions (colloid or crystalloid) (Zweifach, 1940). Despite this theoretical advantage, blood and calcium-containing crystalloid cardioplegic solutions were found to have identical intracardiac distribution after three doses (40-minute aortic cross-clamp time).

The use of blood cardioplegic solutions requires a delivery system. This is usually part of the cardiopulmonary bypass unit and includes a pump, heat exchanger and reservoir. A quantity of blood is removed from the bypass circuit and stored in the reservoir. Potassium is added to achieve the desired K^+ concentration or alternatively a crystalloid concentrate is mixed with blood at a 4:1 ratio (Buckberg, 1989). The solution is infused by a pump into the aortic root using pressures ranging from 50 to 100 mmHg. Care must be taken to avoid excessive intravascular pressure during infusion of the solution. Flows of 400-500 mL/min/m^2 produce rapid arrest of

electromechanical activity and give effective myocardial cooling. The pressures needed to yield these flows depend on hematocrit, delivery temperature, coronary vessel patency, and vascular tone. Technical factors such as conduit, coronary or aortic cannula diameters also affect the required infusion pressure. Blood cardioplegic solution should be infused frequently during the cross-clamp period. It is most often used at delivery temperatures of 20°C or greater and consequently does not achieve the same degree of myocardial cooling. Excessive rewarming of the heart can occur if the solution is not reinfused at regular intervals (approximately every 20 minutes). Factors that increase the likelihood of excessive myocardial rewarming are a high rate of bronchial return and systemic perfusion temperatures of 27°C or greater (Rousou, 1988).

Components of cardioplegic solutions

The formulation of cardioplegic solutions remains a subject of lively debate. However, potassium, magnesium, calcium, sodium and buffers appear to be beneficial in preserving myocardial function. The osmolarity of cardioplegic solutions also merits comment.

Potassium

Melrose's original cardioplegic solution contained potassium citrate. The excessive potassium concentration, the chelating properties of citrate (magnesium and calcium) and the increased osmolarity contributed to the myocardial damage caused by clinical use of the Melrose solution. Excessively high potassium (K^+) concentrations (40mM/L or greater) are potentially injurious to the myocardium and endothelium. Most clinically used solutions today employ K^+ concentrations of 15 - 40 mM/L.

A 1970 study found that under conditions of hypothermia and ischemia, ATP and creatine phosphate levels are better maintained if electromechanical arrest is induced than in the absence of electromechanical arrest (Kubler, 1970).

In 1975, Hearse and co-workers (Hearse, 1975) demonstrated that hypothermia and hyperkalemia had additive effects on the preservation of myocardial ATP stores during ischemia. This finding was subsequently confirmed by other investigators.

The major effect of potassium is the production of electromechanical arrest that results from depolarization of the sarcolemmal membrane. A state of diastole is maintained as long as there is increased extracellular potassium. The potassium concentration needed to produce diastolic arrest is a function of temperature; it decreases as temperature is decreased, (e.g., at 37°C, 20 mM/L of K^+ is required to produce arrest, and at 24°C , only 13 mM/L is needed) (Hearse,1975). The benefit derived from electromechanical arrest is a function of temperature and potassium concentration.

Potassium concentrations greater than 40 mM/L appear to alter the permeability of the myocyte to calcium (Rich,1974). Extracellular calcium may enter the cell and thereby increase energy consumption and calcium overload. The measured oxygen consumption of hearts exposed to very high potassium concentrations (170mM/L) is twice that measured in hearts exposed to lower concentrations (80 mM/L) of potassium (Bretschneider, 1975). The left ventricular diastolic pressure of isolated hypothermic (17°C) perfused neonatal rabbit hearts increases with the onset of potassium-induced arrest. The magnitude and duration of the increase in end-diastolic pressure increased dramatically as potassium concentration exceeded 60 mM/L. Rich and Brady (1974) found that myocardial contracture, or "stoneheart," could be induced in hearts exposed to solutions containing more than 100 mM/L of potassium.

There is not unanimous acceptance of hyperkalemic cardioplegic solutions. Laboratory and clinical studies (Del Nido, 1985; Ellis, 1978) found no improvement in postischemic recovery between groups of hearts treated with either high K^+(20-30 mM/L) or low K^+ (4-10 mM/L) cardioplegic solutions. Data from one of the laboratory studies suggest that in the presence of a strong buffer, high K^+ concentrations (30 mM/L or greater) are detrimental. Myocardial function was better preserved during the reperfusion period with a 10 mM/L K^+ buffered cold cardioplegic solution (Del Nido, 1985). The cardioplegic solutions used in this study had no calcium, low sodium (27 mM/L), and a high pH (7.8). The addition of buffer (histidine) and 30 mM/L of K^+ resulted in extensive damage on reperfusion. The pattern of injury was

similar to that produced by massive Ca^{2+} influx. This suggests that there are complex interactions between ionic species that must be investigated before new cardioplegic formulations are used clinically.

Magnesium and calcium

Divalent cations have profound effects on membrane fluidity and stability (Trauble, 1974). Perfusion of isolated interventricular septum with calcium-free medium results in membrane damage that is not entirely prevented by adding cadmium (a divalent cation with an ionic radius closer in size to Ca^{2+} than to Mg^{2+}. Hypothermia (18°C) prevents the membrane damage seen with calcium depletion (Frank, 1982). Tolerance to calcium depletion diminishes with increasing temperature. At 18°C, nearly complete functional recovery is seen in hearts exposed to Ca^{2+} -free medium for 30 minutes and then reperfused with Ca^{2+} -containing medium. Recovery is only 70% at 22°C and less than 20% at 28°C for the same duration of exposure to Ca^{2+} -free medium prior to reexposure to Ca^{2+}. The injury responsible for the decreased function is termed calcium paradox. Ca^{2+} concentrations as low as 50 μM/L prevent the development of the calcium paradox (Rich, 1982). In a recent study of cardioplegic solutions containing one of three Mg^{2+} concentrations (0, 1.2 and 15 mM) in combination with one of three Ca^{2+} concentrations (0.05, 1.5 and 4.5 mM), Brown and co-workers (Brown, 1991) determined that optimal recovery from ischemia occurred at 0.05 mM Ca^{2+} and 15 mM Mg^{2+}. Functional and metabolic recovery was reduced by decreasing [Mg^{2+}] or by increasing [Ca^{2+}]. The decrement in recovery with increasing Ca^{2+} was more pronounced as the temperature decreased.

The St. Thomas' Hospital solution has a [Mg^{2+}] of 15 mM/L and a [Ca^{2+}] of 1.2 mM/L. This solution is optimal in adult hearts as demonstrated by multiple experiments. There is general agreement that inclusion of Mg^{2+} in cardioplegic solutions is beneficial. Magnesium is lost from the myocardium during ischemic arrest. This loss may result in impairment of cardiac recovery because Mg^{2+} is known to reduce the trans-sarcolemmal flux of Ca^{2+} and inhibit Na^{+} influx into the cell, among other effects

that are related to its function as a cofactor for many enzymatic reactions. Magnesium can produce cardioplegia in high concentrations. This observation was the basis for the "Kirsch" solution (Kirsch, 1970). Most clinically used solutions have a lower magnesium concentration than the Kirsch solution. Hearse and coworkers (Hearse,1978) found a 15 mM/L concentration to be optimal.

Nuclear magnetic resonance (NMR) spectroscopic studies have found that the addition of magnesium to cardioplegic solutions improved maintenance of high-energy phosphate stores under conditions of hypothermic arrest (Pernot, 1983), yet these same investigators found that the addition of K^+ to a magnesium - based cardioplegic solution failed to protect ATP stores. This is at variance with the study of Hearse and coworkers (Hearse,1974), who found that additional protection was conferred by the addition of K^+ to a Mg^{2+} solution. As in the case of comparisons between blood and crystalloid solutions, the temperature involved may be the important factor in the different conclusions.

Sodium

Intracellular sodium and calcium concentrations are interrelated. These two ions can be exchanged via a common channel termed the Na^+-Ca^{2+} exchanger. Interventions that increase intracellular sodium activity may cause a dramatic increase in intracellular Ca^{2+} activity. In the absence of Ca^{2+} and Mg^{2+} , sodium may enter the cell through Ca^{2+} channels.

The effects of changes in extracellular sodium concentrations are more complex than would be predicted by consideration of transcellular Na^+ - Ca^{2+} exchange alone. Perfusion of isolated hearts with low sodium perfusate causes calcium-dependent increases in diastolic pressure, energy consumption, and dissociation of oxygen consumption from work. The effect is postulated to be mediated by alterations in SR calcium cycling (Renlund,1985).

Despite the theoretical considerations, a cardioplegic solution with decreased Na^+, Bretschneider's "HTK" solution, is used clinically with success. This solution has essentially no Ca^{2+} (15-20 μM) and a high buffering capacity. Low Na^+ concentrations can arrest the heart by inhibition of the "fast" inward current required for depolarization. If K^+ is added, Na^+ -pump activity is stimulated and intracellular

Na^+ decreases in response. This effect reduces cell swelling and Ca^{2+} influx on reperfusion (Jynge, 1980).

Hypertonic saline should be avoided because it increases intracellular sodium concentration with a subsequent increase in intracellular Ca^{2+} concentration. The "ideal" sodium concentration for standard cardioplegia is not firmly established, but is probably between 90 and 120 mM/L (Pernot, 1983; Stinner, 1989). The St. Thomas' solution contains 120 mM Na^{2+} (110 mM as NaCL and 10 mM as NaHC03).

The action of Na^+ ion is complex. Entry into the cell during ischemia is mediated via Na^+-H^+ exchange. Thus, accumulation of H^+ within the cell can increase Na^+ during the early phases of reperfusion with a consequent rise in Ca^{2+}. If the Na^+-H^+ exchanger is blocked by amiloride, the increases in cell Na^+ and Ca^{2+} seen on reperfusion are blocked (Meng, 1991).

Buffer

ATP consumption continues during ischemia. Hydrogen ion is generated as a consequence of ATP hydrolysis causing intracellular acidosis unless some provision is made for buffering. The cell has some limited buffering capacity. An important buffer is the amino acid histidine. Histidine has an imidazole group that can exist in a protonated or deprotonated state. The latter can be a proton acceptor and can thereby buffer intracellular pH changes: The cost of using histidine residues as buffer is high because the activity of enzymes is reduced or destroyed if histidine residues in active sites undergo protonation.

If ATP stores decrease during ischemia, endogenous glycogen is converted to glucose for metabolism to ATP and lactate (Del Nido, 1985). If intracellular levels of ATP are maintained, exogenous glucose is used in preference to glycogen. The problem then is the maintenance of ATP levels during ischemia.

These problems can be mitigated by the use of a buffer in the cardioplegic solution. The choice of buffer may be important. The bicarbonate ion is an inefficient buffer of intracellular pH change and has even been found to cause intracellular acidosis, whereas other species such as phosphate can buffer intracellular pH changes.

Adequate intracellular buffering is associated with better functional recovery (Hearse, 1976).

Lange and colleagues (Lange, 1984) studied the effects of alkalinity and temperature on the effectiveness of multidose bicarbonate-buffered cardioplegic solutions. The use of 37°C, pH 8.2, bicarbonate-buffered cardioplegic solution failed to alter intracellular pH. The same solution when cooled to l0°C before infusion produced transient increases in intracellular pH. The increases in pH paralleled decreases in myocardial temperature. The magnitude of the intracellular pH change was equal to that pH change which could be predicted on the basis of the temperature change alone. There was no need to invoke buffering of intracellular H^+ by the bicarbonate in the cardioplegic solution to explain the pH change.

Tromethamine (THAM) and histidine are both effective in increasing intracellular pH if supplied in the perfusate (Preusse, 1982). Removal of reducing equivalents from the cytosol by a buffer prevents the inhibition of glycolysis by nicotine adenine dinucleotide hydrogen (NADH). However, THAM is toxic to certain tissues.

Histidine has the desirable property of shifting its pKa into the alkaline range with decreasing temperature. The change is in the same direction and of the same magnitude as the change in pH of pure water with temperature (Reeves, 1976). It has been postulated that the pKa shift of the imidazole group of histidine residues of intracellular proteins accounts for the increase in intracellular pH with hypothermia. This pH change with temperature protects the activity of intracellular enzymes and maintains chemical neutrality. Histidine buffering maintains ATP levels during ischemia and prevents glycogenolysis by promoting utilization of exogenous glucose (Del Nido, 1985). Histidine appears theoretically to be the ideal buffer for use in cardioplegic solutions; however, in clinical practice, phosphate is very effective.

Osmolarity

Melrose's original cardioplegic solution had an osmolarity of greater than 500 mOsm/L, which contributed to the myocardial damage it produced in clinical use. Severe myocardial damage is caused by solutions with osmolarities greater than 400 mOsm/L. Hyperosmolar solutions cause myocardial injury by several mechanisms, one of which is intracellular water loss, which produces conformation changes in

protein structures within the cell. Another mechanism by which hypertonic cardioplegic solutions may cause damage is by raising intracellular sodium ion activity. Lado and co-workers (Lado, 1984) studied the effects of solutions of high osmolarity on intracellular sodium and calcium ion activity. The solutions were made hyperosmolar by the addition of sucrose while ion concentrations were kept constant. Segments of heart exposed to hypertonic (448 and 610 mOsm/L) solutions developed rapid increases in resting tension and dramatic decreases in contracted size.

Intracellular calcium ion activity was increased beyond that expected on the basis of osmolar concentration effects due to osmotic water loss. The increase in calcium activity was thought to be a consequence of Na^+ -Ca^{2+} exchange. The hyperosmolar solutions first induced a rise in intracellular sodium ion activity that resulted in a change in the Na^+ electrochemical gradients across the cell membrane. Na^+ -Ca^{2+} exchange reduces the intracellular Na^+ load at the cost of increasing myoplasmic Ca^{2+}.

The effects of hypo-osmolar solutions on intracellular ion activities are opposite to those seen with hyperosmolarity. Intracellular sodium ion activity is reduced to a level predicted on the basis of osmotic dilution. Calcium activity decreases much more than predicted on the same basis. This decrease in intracellular calcium activity is offset by increased cell swelling secondary to water gain. Experimentally, the optimum osmolarity for solutions designed for either cardioplegia or donor organ preservation is between 300 and 320 mOsm/L.

Miscellaneous constituents

Substrates

At one time, glucose was thought to be a desirable component of cardioplegic solutions. In theory, glycolysis might be maintained and energy stores preserved (Opie, 1991). Von Oppell (1991) was able to demonstrate a beneficial effect from glucose added to St. Thomas' Hospital cardioplegia in the isolated rat heart, but only in a concentration range between 7-11mM, whereas higher concentrations

decreased recovery. However, this could not be confirmed by in vivo studies in a baboon model (Boehm, unpublished data). The possibilities that interspecies differences might be responsible for this discrepancy and that on the other hand the washout of end products of metabolism might have been relatively greater in the isolated rat heart model.

However, because of the detrimental effects of ongoing glycolysis during hypothermic ischemic arrest, few clinically used cardioplegic solutions contain glucose. Provision of other substrates, i. e., those involved in purine synthesis, may provide better recovery of both myocardial energy stores and function.

In studies of isolated hearts, continuous provision of adenosine in the pre- and postischemic periods improved post-ischemic function (Ely, 1985). Hypoxanthine and ribose provided independently also enhanced postischemic function (Lasley, 1988; Pasque, 1982). All three substrates were included in a standard hyperkalemic (16 mM K^+) crystalloid cardioplegic solution and administered prior to 1 hour of normothermic (37°C) global ischemia. The animals receiving the adenosine, hypoxanthine, and ribose-supplemented cardioplegic solution (AHR) exhibited significantly better postischemic recovery of both ATP stores and function as compared with a group receiving only the standard cardioplegic solution (Wyatt, 1989).

The mechanisms by which these substrates improve recovery are complex but include utilization of the salvage pathways in which adenosine is phosphorylated to adenosine monophosphate (AMP) and eventually to ATP. Hypoxanthine is phosphorylated in the presence of phosphoribosyl pyrophosphate (in the presence of ribose) to form inosine mono-phosphate and subsequently AMP. Additionally, provision of these compounds may retard loss of purine metabolites during ischemia and reperfusion.

Steroids

Although not universally accepted as being effective in improving myocardial preservation, steroids are another frequently added ingredient of cardioplegic solutions. However, an earlier study suggests that dexamethasone prevents

myocardial injury mediated by peroxidation stimulated by calcium load (Saxon, 1985). Similar protection was provided by α-tocopherol (vitamin E) (Erin,1983).

Calcium entry blockers

Calcium channel blockers have been tested as additives to cardioplegic solutions. Initial studies demonstrated that verapamil, diltiazem, and nifedipine protected the normothermic, globally ischemic heart (Nayler, 1980; Weishaar, 1980; Jolly, 1981). The mechanism of protection was not directly related to preservation of ATP stores but to limitation of mitochondrial Ca^{2+} uptake. The observed protective effect could be masked if the myocardial temperature was reduced to 25°C. (Nayler, 1981; Hearse, 1984). Following 60 minutes of ischemia at 25°C, there was no significant difference in postischemic recovery between hearts protected with hypothermia alone and those protected with hypotherrnia plus nifedipine. Nifedipine did, however confer additional protection if ischemia time was extended to 3 hours. Nifedipine also appears to protect the myocardium from the deleterious effects of profound cooling; i.e., temperatures of 5°C. The mechanism of the protective effect is unknown. Diltiazem added to cardioplegic solution (150 mg/kg) improved recovery after 30 minutes of cold 15-20°C) ischemia compared with standard cardioplegia; segmental shortening and dye indicators were used as measures of function and injury respectively (Rebeyka, 1990).

In a clinical trial, patients receiving diltiazem cardioplegia remained asystolic during the reperfusion period and required pacing to be weaned from bypass (Christakis, 1986). Nimodipine, one of the dihydropyridine calcium channel blockers, has been studied for its ability to reduce central nervous system (CNS) damage following ischemic injury. However, a clinical trial could not reveal a neuroprotective effect in the Nimodipine treated patient group. has also been found to improve post-reperfusion cardiac function in patients aged 6 months to 20 years undergoing cardiac surgery when added to a cardioplegic solution in a concentration of 0.25 mg/L (Mori, 1990). There were no differences between the nicardipine group and control group in age, bypass or cross-clamp time, temperature (23°C), or rate of spontaneous defibrillation (54-75%). Both groups had a 35-38% use of inotropes, but the nicardipine group had better cardiac indices and left ventricular stroke work

index (LVSWI) at lower wedge pressures than did the control group. The experimental group also had less evidence of myocardial injury as judged by MB-CK levels. Nicardipine is less likely than diltiazem or verapamil to cause decreased inotropy or conduction defects. It may be a useful adjunct to standard cardioplegic solutions.

Reperfusion solutions

Reperfusion solutions are useful for the purpose of replenishing lost intracellular substrates. Key metabolic intermediates lost during ischemia and reperfusion include purine nucleotides, succinate, and pyruvate (Taegtmeyer, 1978). NAD^+ is converted to NADH during anaerobic glycolysis. The cell has a limited ability to reconvert NADH to NAD^+ via cytosolic malate dehydrogenase. The net reaction is NADH + malate. In an anaerobic environment, malate cannot be reconverted to oxaloacetate (OAA) by mitochondrial malate dehydrogenase. The result is the depletion of cytosolic OAA and accumulation of NADH.

The provision of glutamate during reperfusion accelerates restoration of normal intracellular levels of NAD^+ and α-ketoglutarate. Glutamate and mitochondrial OAA can undergo transamination to yield ketoglutarate and aspartate. The mitochondrial membrane is freely permeable to aspartate. Aspartate can then be deaminated in the cytosol to yield OAA. Provision of glutamate allows $NADH^+$ to be converted to NAD^+, with repletion of cytosolic OAA levels and regeneration of Krebs cycle intermediates.

The five-carbon sugar ribose is an essential intermediate for both the de novo and the salvage pathway synthesis of purine nucleotides (Goldthwait, 1957). Ribose can also serve as a source of pyruvate for oxidative phosphorylation if the glycolytic pathway is blocked by metabolites produced during ischemia (Metzler, 1977). Provision of ribose during reperfusion can result in improved function by virtue of its roles in various biochemical pathways. The combination of ribose and glutamate in a potassium blood cardioplegic reperfusion solution is superior to unmodified pump blood, and other solutions, in restoring postischemic cardiac function in dogs (Haas, 1984).

Depleted intracellular stores of ATP can be replenished providing ATP and magnesium chloride during the reperfusion period (McDonagh, 1984). The degree of functional recovery of the heart is sensitive to the amount of ATP provided. Hearts reperfused with excessive amounts of ATP have worse postischemic function than those reperfused with control solution. If ATP and magnesium chloride was provided at a low rate (0.13 mg/kg/min) for the first 30 minutes of reperfusion, nearly 100% recovery of left ventricular ejection function was attained. However, myocardial compliance remained depressed, as did oxygen extraction. The depressed oxygen extraction probably reflected the diminished availability of mitochondrial substrate for oxidative phosphorylation. The alternative explanation is mitochondrial injury.

A nonsanguineous reperfusion solution has been used clinically with apparent benefit (Menasche, 1984). The solution was designed to maintain electrical silence, reintroduce Ca^{2+} in physiologic concentration, and provide glutamate, buffering, and moderate hyperosmolarity (by adding mannitol). The addition of mannitol protects against free radical damage and reduces cellular edema. This reperfusate was infused via the aortic root just before removal of the cross-clamp. A total of 1000 mL was given at a rate of 200-300 mL/min. Patients receiving this solution had significantly better indices of myocardial function than did those not receiving it. Of patients undergoing mitral valve replacement, those receiving the nonsanguineous reperfusion required less inotropic support than the control group. The same phenomenon was not observed in the group of patients undergoing aortic valve surgery.

Much research is being directed toward prevention of reperfusion injury. Recognition of the involvement of blood elements in this process has led to new areas of investigation (Lucchesi, 1989). A very interesting area of research is the use of neutrophil antibodies as a means of reducing postischemic injury. Endothelial injury, which is postulated as a result of ischemia and reperfusion, promotes neutrophil adhesion and subsequent inflammatory damage. The endothelial injury can be prevented experimentally using antibodies against the neutrophil surface glycoprotein CD18. In a dog model of regional ischemia (at 37°C), the use of the neutrophil antibody anti-Mol reduced the infarcted area (as a percentage of at-risk area) by 46% after 6 hours of reperfusion. If the anti-Mol treatment is continued for 48 hours postischemia, a reduction in infarcted area is noted at 72 hours of

reperfusion. (Takahashi, 1990). Inhibition of neutrophil adhesion to the vascular endothelium in at-risk areas represents a new and potentially promising area in the field of myocardial preservation.

Donor heart preservation

The routine repair of cardiac defects does not test the limits of myocardial preservation as does organ preservation for transplantation. The donor heart must be stored for transport in such a way that it will not be damaged by preservation techniques and will function effectively after 4 hours of storage. There are, however, major differences between in-vivo cardioplegic protection and ex-vivo storage preservation, which are summarized in Table 1.8. Recent studies have found that hearts protected with an "intracellular" (University of Wisconsin, UW)-type solution were more likely to regain full function after transplant than hearts protected with an "extracellular" solution (Stanford vs St. Thomas II) (Wicomb, 1989; Ledingham, 1990). For a given solution, the infusion conditions are extremely important. For the St. Thomas' solution, if initial arrest is produced at 37°C, followed by infusion of cold (7,5°C) solution, postischemic function is better than if initial cold infusion is used (Takahashi, 1990). The opposite is true for the UW solution. If initially infused at 20°C with subsequent cooling of the heart to 4°C, function decreases as compared with hearts receiving the initial infusion of UW-solution at 4°C (Ledingham, 1990).
The UW solution used in these studies did not contain Ca^{2+}. Recent studies by the University of Wisconsin group indicate that the addition of 0.5 mM Ca^{2+} greatly enhances the posttransplant survival of kidneys (up to 5-day preservation with continuous perfusion) (McAnultry, 1989).

TABLE 1.8.: DIFFERENCES BETWEEN CARDIOPLEGIC AND STORAGE PRESERVATION

	Cardioplegia	Storage
Site	**in-vivo**	**ex-vivo**
Frequency	**multiple doses**	**single dose**
Temperature	**15° C - 20° C**	**4° C**
Flow	**collateral flow**	**no flow**
Time	**short ischemia**	**long ischemia**
Precondition	**predamaged heart**	**healthy heart**
Na^+ - K^+ pump	**partial inactivation**	**complete inactivation**
K^+-concentration	**excludes excessive K^+**	**high K^+ used**
Membrane potential	**polarized or depolarized**	**depolarized**
Tissue edema	**importance of colloids**	**importance of impermeants**

Legend: Major differences exist between in-vivo cardioplegic protection and storage preservation for transplantation.

As in the case of cardioplegic solutions, the optimum solution, technique, and mode of organ preservation for transplantation is yet to be developed. However, the current UW solution has been found to protect rabbit hearts for up to 8 hours at 4°C with no decrease in function (Takahashi, 1990). The period during which the heart is removed from the cold environment and placed in the body and prior to reperfusion must of necessity be minimized to reduce the likelihood of loss of myocardial function. It is during this period that technical factors can either help or hinder the effectiveness of the preservation technique employed at organ harvest.

Failure of myocardial preservation

The following concepts should guide both present and future myocardial preservation techniques:

1. Preservation of ATP levels does not ensure adequate functional recovery, much less optimum functional recovery.

2. The optimum Ca^{2+} concentration in cardioplegic solutions varies with age of the myocardium.

3. The interactions between Mg^{2+}, Ca^{2+}, and Na^{+} are complex and temperature dependent.

4. Extracellular buffering alone is not enough; buffers that protect against intracellular acidosis (which $HC0_3^-$ does not) are preferred.

5. Prevention of reperfusion injury by means of antioxidants, free radical scavengers, or other means improves functional recovery. Preservation of vascular endothelial function must be optimized.

Even the most well-formulated cardioplegic solution may fail to provide protection from myocardial injury.

TABLE.1.9.: REASONS FOR FAILURE OF MYOCARDIAL PRESERVATION*

Preexisting myocardial injury
Technical factors
Premature cardiac rewarming
Myocardial distention
Prolonged fibrillation
Premature return of electromechanical activity
Infrequent use of cardioplegic solution
Coronary air emboli
Free radical damage
Myocardial substrate depletion
Calcium influx

Legend * As manifested by poor myocardial performance on completion of rewarming, necessitating use of inotropes or continued support on bypass.

There are multiple reasons for this failure during open heart surgery and after storage preservation, and the majority of them are technical (Table 1.9.). Cunningham and co-workers (1979) found that technical errors during aortic cross-clamping led to inadequate myocardial protection. These errors were failure to re-inject the cardioplegic solution frequently enough, allowing electrocardiographic activity to return and myocardial temperatures to exceed 28°C. The surgeon and anesthesiologist must be aware of the time intervals between injections of cardioplegic solution. Ideally, the solution should be reinfused every 20 minutes and generally should not be allowed to exceed 30 minutes unless deep hypothermic arrest is used. If cardiac electrical activity is noted, immediate reinfusion of cardioplegic solution is necessary. Despite all precautions, there appears to be a limit to the ischemia time during the surgical repair of lesions. This time limit was 85 minutes in work by Bull and colleagues (Bull, 1984). After longer times, postoperative mortality increased.

Myocardial rewarming can be a difficult problem. There are multiple contributing factors such as room temperature, heat generated by operating room lights, systemic perfusion temperature, and noncoronary collateral flow. The right atrium appears most susceptible to rapid rewarming to the systemic perfusion temperature. This phenomenon has been blamed for atrial arrhythmias during the postischemic period (Chen, 1985). Noncoronary collateral flow can defeat the best myocardial preservation efforts. It contributes to myocardial distention, rewarming, and washout of cardioplegic solution. Washout is helpful in eliminating poorly formulated cardioplegic solutions but more often prematurely terminates the beneficial effects of well-designed solutions (Buckberg, 1981). High rates of noncoronary collateral flow are seen in patients with well-developed bronchial vessels or high grade coronary stenoses. Noncoronary collateral flow is related to the systemic perfusion index. Low indices reduce the return to the left heart through these channels, thus reducing myocardial distention and rewarming. Adequate left ventricular venting is necessary to prevent cavity distention and premature subendocardial rewarming. The rate of left ventricular subendocardial rewarming can be slowed by the use of low (20°C) systemic perfusion temperature. Even the most carefully conducted procedure cannot prevent all the biochemical events that lead to ischemic and reperfusion injury.

Comparison of cardioplegic solutions used clinically

Large numbers of different cardioplegic solutions have been formulated and are used clinically today. However, the formulation of cardioplegic solutions remains a subject of lively debate. Many conclusions regarding cardioplegic solutions are based on studies carried out in isolated heart preparations. The experience with solutions found to be beneficial in the laboratory may be disappointing in the clinical area because of poorly controlled factors such as non-collateral coronary flow. Conversely, solutions that are less than optimal in the isolated heart may perform well in a clinical setting. Therefore, cardioplegic solutions must be evaluated in models comparable to the clinical settings in which they are used before clinical use. Three different types of cardioplegic solutions are in use today (Table 1.10):

1. Crystalloid cardioplegic solutions

2. Colloid cardioplegic solutions

3. Blood cardioplegia.

The cardioplegic techniques used today are summarized in Table 1.11.

TABLE 1.10.: COMPOSITION OF CARDIOPLEGIC SOLUTIONS

COMPOSITION OF CARDIOPLEGIC SOLUTIONS			
CRYSTALLOID COMPOSITION		COLLOID COMPOSITION	
EXTRACELLULAR	INTRACELLULAR	ALBUMIN	BLOOD
St. Thomas' No.1+2	Bretschneider HTK	Hamburg	Buckberg
Kirklin No.1	Stanford	Kirklin No. 2	
Sabax			

Legend: Cardioplegic solutions can be divided into crystalloid and colloid solutions. Crystalloid solutions resemble either extracellular or intracellular ionic composition. Colloid solutions are either albumin-containing crystalloid solutions, or blood-based solutions with additional oxygen carrying capacity.

TABLE 1.11.: CARDIOPLEGIC TECHNIQUES

1. Composition of the cardioplegic solution

   crystalloid: extracellular/ intracellular electrolyte content
   colloid: addition of oncotic agents to crystalloid solution
   blood: mixed 1:4 with crystalloid concentrate

2. Temperature
   - a) of the myocardium:
     - normothermic
     - mild hypothermia
     - deep hypothermia
   - b) of the body
     - normothermic
     - mild hypothermia
     - deep hypothermia

3. Application
   - a) site
     - antegrade (aortic root, ostia)
     - retrograde (coronary sinus)
     - combined (alternating or simultaneously)
   - b) volume:
     - normal volume (15 ml/kg/BW*)
     - high volume (50 ml/kg/BW*)
   - c) pressure
     - low pressure
     - high pressure
   - d) time
     - induction
     - intermittent
     - continuous
     - terminal
     - reperfusion

Legend: Different cardioplegic techniques in clinical use according to composition, temperature, and application.
*BW: body weight

1. Crystalloid cardioplegic solutions

These solutions have frequently a normal or slightly supranormal osmolarity but no oncotic or colloid osmotic active substances. This means that the forces binding water are produced only by the low-molecular electrolytes and not via membrane impermeable high-molecular colloid substances. That implies that pressure control during perfusion is extremely important. Amongst many solutions, the most important crystalloid solutions used today are Bretschneider's solution (Table 1.12.), St. Thomas' Hospital solution No. 1 and No. 2 (Table 1.13.), Stanford cardioplegia (Table 1.14.), and Kirklin cardioplegia (Table 1.15). The common principle of all these solutions is to reduce myocardial metabolism mainly via inactivation of the contractile apparatus.

TABLE 1.12.: BRETSCHNEIDER'S CARDIOPLEGIC SOLUTION (HTK4)

NaCl	15 mM/L
KCl	9 mM/L
MgCl2	4 mM/L
Histidine	180 mM/L
Tryptophan	18 mM/L
Mannitol	2 mM/L
α-Ketoglutarate	20 mM/L
pH	7.1
osmolarity	280-300 mosm/l

TABLE 1.13.: ST. THOMAS HOSPITAL CARDIOPLEGIA NO.1 AND 2

	No. 1 (MacCarthy)	**No. 2 (Plegisol)**
NaCl	144 mM/L	110 mM/L
KCl	20 mM/L	16 mM/L
MgCl2	16 mM/L	16 mM/L
CaCl2	2.4 mM/L	1.2 mM/L
NaHCO3	---	10 mM/L
Procaine-HCL	1.0 mM/L	---
pH	5.5-7.0	7.8
osmolarity	300-320 mosm/l	285-300 mosm/l

TABLE 1.14.: STANFORD CARDIOPLEGIA

Na^+	25 mM/L
Cl^-	30 mM/L
K^+	30 mM/L
HCO_3^-	25 mM/L
Mannitol	1.25%
Dextrose	5 %
pH	8.5-8.7
osmolarity	450 mosm/l

TABLE 1.15.: KIRKLIN CARDIOPLEGIA (UAB = UNIVERSITY OF ALABAMA/BIRMINGHAM)

	with Albumin	**without Albumin**
Na+	100 mM/L	110 mM/L
K+	30 mM/L	30 mM/L
Cl-	84 mM/L	85 mM/L
HCO3-	28 mM/L	27 mM/L
Ca2+	1.4 mM/L	1.0 mM/L
Glucose	5 g/L	5 g/L
Mannitol	5 g/L	9.9 g/L
Albumin	50 g/L	---

2. Colloidal cardioplegic solutions

These solutions contain water-binding oncotic or colloid-osmotic substances. These substances may be albumin, mannitol or high molecular weight polyglycoses such as hydroxyethyl starch. Important colloid cardioplegic solutions are the Hamburg cardioplegia (Table 1.16.) or the modified Kirklin cardioplegia containing albumin (Table 1.15.).

TABLE 1.16.: HAMBURG CARDIOPLEGIA

KCl	5 mM/L
$CaCl_2$	0.5 mM/L
$NaHCO_3$	25 mM/L
Mg-aspartate	2 mM/L
Procaine-HCl	4 mM/L
Glucose	10 mM/L
Mannitol	200 mM/L
Hydroxyethyl starch	6%
Methylprednisolon	250 mg/L
total Na^+	50 mM/L
osmolarity	320 mosm/L
colloid osmotic pressure	46 cmH_2O
pH	7.4
pO_2	600mmHg

3. Blood cardioplegia

In the years 1978 and 1979, Buckberg in Los Angeles and Barner in St. Louis introduced the procedure of blood potassium cardioplegia into clinical practice. The blood from the oxygenator of the heart-lung machine was used not only as the vehicle for potassium cardioplegia, but also for oxygen transport and as substrate carrier. However, as indicated in the previous chapter, oxygen delivery to the myocardium is limited at low temperatures. Buckberg additionally advocated application of a modified blood cardioplegic solution as reperfusion solution (Table 1.17.b) before opening the aortic cross-clamp. Other advantages of blood cardioplegia are improved buffering capacity via endogenous buffering substances. Furthermore, blood contains endogenous oxygen-free radical scavengers which potentially can protect the myocardium from reperfusion-related damage (Table 1.17.).

TABLE 1.17a.: CONCENTRATE FOR 4:1 BLOOD CARDIOPLEGIA (ACCORDING TO BUCKBERG, 1989) WARM INDUCTION

Additive	Volume (ml)	Delivered concentration	
THAM (300 mM/L)	225	pH	± 7.6
CPD	225	Ca^{2+}	± 0.15 mM/L
50% D/W	40	Osm	±400 mOsm/kgH2O
5% D/W	220	Glucose	± 70 mM/L
KCl (2 mM/L)	40	K^+	20-25 mM/L
Glutamate (7.32%)	125	Glutamate	13 mM/L
Aspartate (7.32%)	125	Aspartate	13 mM/L

Legend: Infused at 37°C at a rate of 250 - 350 ml/min till arrest, then at 150 ml/min for a total of 5 min. Thereafter standard cold (4°C - 8°C) cardioplegia is given for approximately 3 min. CPD: Citrate phosphate dextrose, D/W: Dextrose/Water; 4:1 ratio for blood: crystalloid concentrate.

TABLE 1.17b.: CONCENTRATE FOR 4:1 BLOOD CARDIOPLEGIA (ACCORDING TO BUCKBERG, 1989) REPERFUSION SOLUTION

Additive	Volume (ml)	Delivered concentration	
THAM (300 mM/L)	225	pH	± 7.6
CPD	225	Ca^{2+}	± 0.15 mM/L
50% D/W	40	Osm	±400 mOsm/kgH2O
5% D/W	220	Glucose	± 70 mM/L
KCl (2 mM/L)	**15**	K^+	**8-10** mM/L
Glutamate (7.32%)	125	Glutamate	13 mM/L
Aspartate (7.32%)	125	Aspartate	13 mM/L

Legend: Infused at 37°C, at a rate of 150 ml/min antegrade for 1.5min, retrograde for 1.5 min. Systemic rewarming commenced 3-4 min before delivery. CPD: Citrate phosphate dextrose, D/W: Dextrose/Water; 4:1 ratio for blood: crystalloid concentrate.

Effect of cardioplegia on operative mortality

The fact that myocardial protection and cardioplegia can influence operative outcome has been demonstrated in large studies (see also chapter 1.2. : Cardioplegia at Groote Schuur Hospital). The CASS-Study comprising 6652 CABG-patients between 1975 and 1978 in 15 US hospitals reported an operative mortality varying between 1.4 and 4.5% depending on the subgroup of patients studied, with an average risk of operative death of 2.9% (Table 1.18), (Kennedy, 1980).

Amongst other factors, hospital mortality was related to the myocardial protection regimen which was used. Berger, also from the CASS-study, had a high operative mortality of 6.8% if normothermia was used (Berger,1981) (Table 1.19.). Hypothermia and topical cooling lowered operative mortality to 5.3%, whereas if cold cardioplegia was used operative mortality was only 1.9% (Table 1.19.). The rate of perioperative myocardial infarction correlated with the decrease in mortality (Table 1.19.), showing a rate of perioperative myocardial infarction of 7.5% for the normothermic group and of 8.9% for the hypothermic group, where only topical cooling was applied, whereas the subgroup of patients treated with cold cardioplegia had the lowest rate of perioperative myocardial infarction with only 3.8%.

TABLE 1.18.: OPERATIVE MORTALITY IN THE CASS STUDY

Number of patients	Description	Mortality %
6652	all patients	2.9
6176	only CABG patients	2.3
4913	elective CABG-patients	1.7
1263	emergency CABG-patients	4.4
4303	CABG-patients<60y	1.4
1873	CABG-patients>60y	4.2
5197	male CABG-patients	1.8
979	female CABG-patients	4.5

Legend: Operative mortality of 6652 CABG-patients who were operated upon in US hospitals between August 1975 and December 1978 (modified from Kennedy,1980)

TABLE 1.19.: INFLUENCE OF MYOCARDIAL PROTECTION

	Operative mortality		Rate of perioperative MI	
	n	%	*n*	%
Normothermia	44	6.8	40	7.5
Hypothermia	150	5.3	135	8.9
Cold cardioplegia	745	1.9	677	3.8

Legend: Myocardial protection regimen in relation to operative mortality and rate of perioperative myocardial infarction (modified from Berger, 1981)

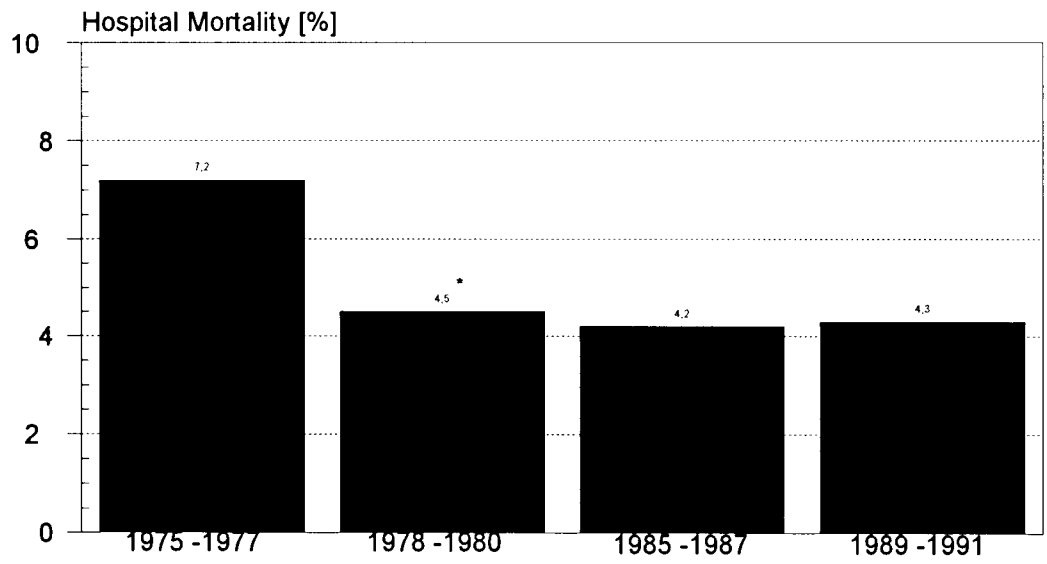
1.2. Cardioplegia at Groote Schuur Hospital

Hypothermic cardioplegic cardiac arrest was used routinely at Groote Schuur Hospital from approximately 1978; initially a modified Bretschneider intracellular type solution was used. However, major modifications especially in calcium content and other important components made the composition quite different from Bretschneider's original formulation; therefore it will be called Groote Schuur Hospital solution from now on. Hospital mortality decreased from 7.2% in the precardioplegic period (1975 - 1977) to 4.5% in the period from 1978 -1980 ($p \leq 0.01$) (Figure 1.2). After our initial experiments, which are part of this thesis, we were able to show better myocardial protection with St. Thomas' Hospital cardioplegia No. 2. This solution was subsequently introduced into clinical practice at Groote Schuur Hospital and in most cardiac surgery units throughout South Africa. Although overall mortality did not decrease, being 4.2% in the period from 1985 to 1987 compared to 4.3% in the period from 1989 to 1991 (Figure 1.2), there was a decrease in mortality from 16.7% (1985 - 1987) to 5,6% (1989 - 1991) ($p \leq 0.01$), in the higher risk procedures (CABG + valve replacement) (Figure 1.3). Furthermore, post cardiopulmonary bypass usage of the intraaortic balloon pump decreased from 3.0% (1985 -1986) to 1.0% (1990 - 1991) ($p \leq 0,01$) (Figure 1.4).

These data reflect the fact that improved cardioplegic myocardial preservation is at least one factor that has helped to increase the safety of open heart surgery. Naturally, also improved surgical technique and further improvements in cardiopulmonary bypass technique have probably contributed to this decreased

mortality and morbidity, but on the other hand the complexity and extent of cardiac surgical repair has increased considerably during the same time period.

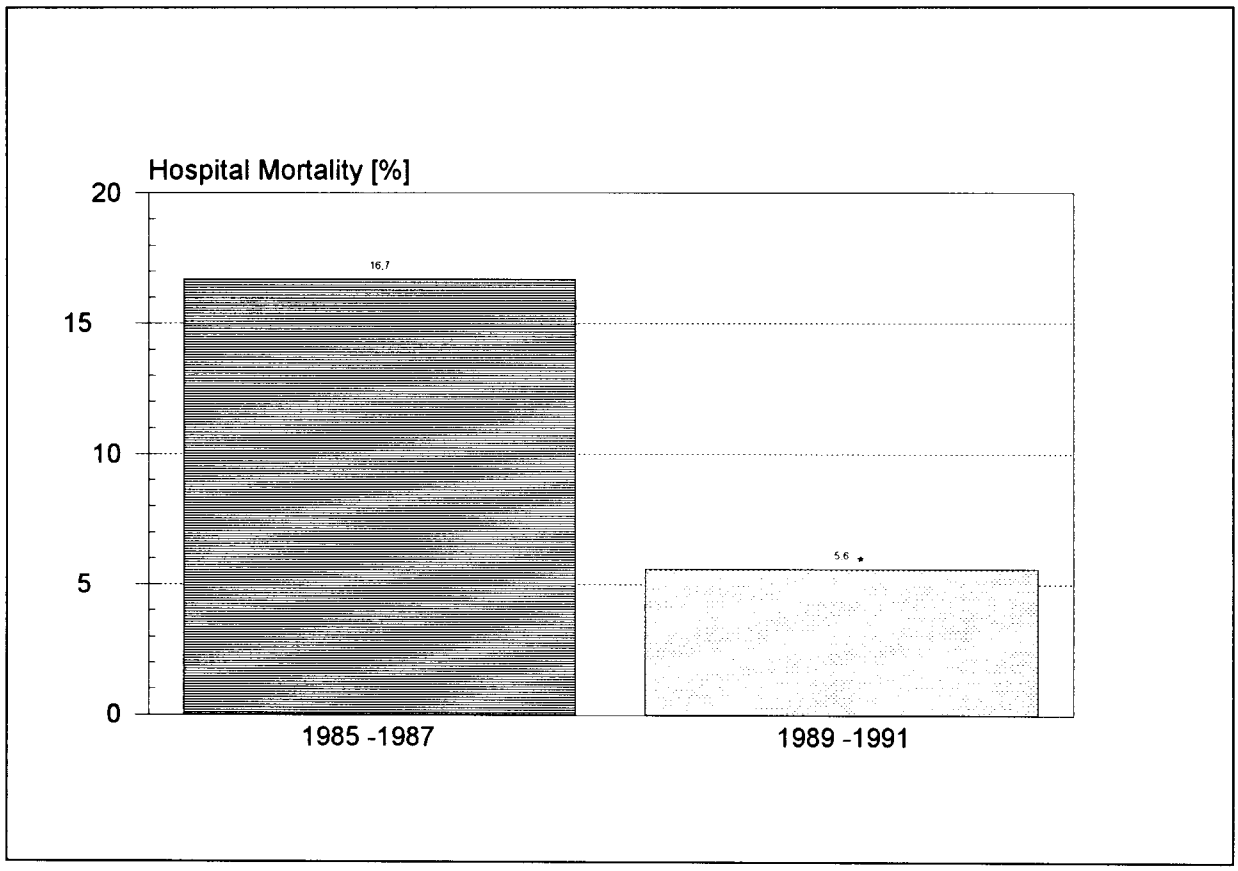
FIGURE 1.2.: HOSPITAL MORTALITY FOR ADULT CARDIAC SURGERY AT GROOTE SCHUUR HOSPITAL DURING FOUR DIFFERENT PERIODS



Legend: 1975 - 77: precardioplegic period
1978 - 80: Groote Schuur Hospital cardioplegia
1985 - 87: Modified Bretschneider solution
1989 - 91: St. Thomas' Hospital solution No. 2

* = $p < 0.01$ vs 1975 - 1977

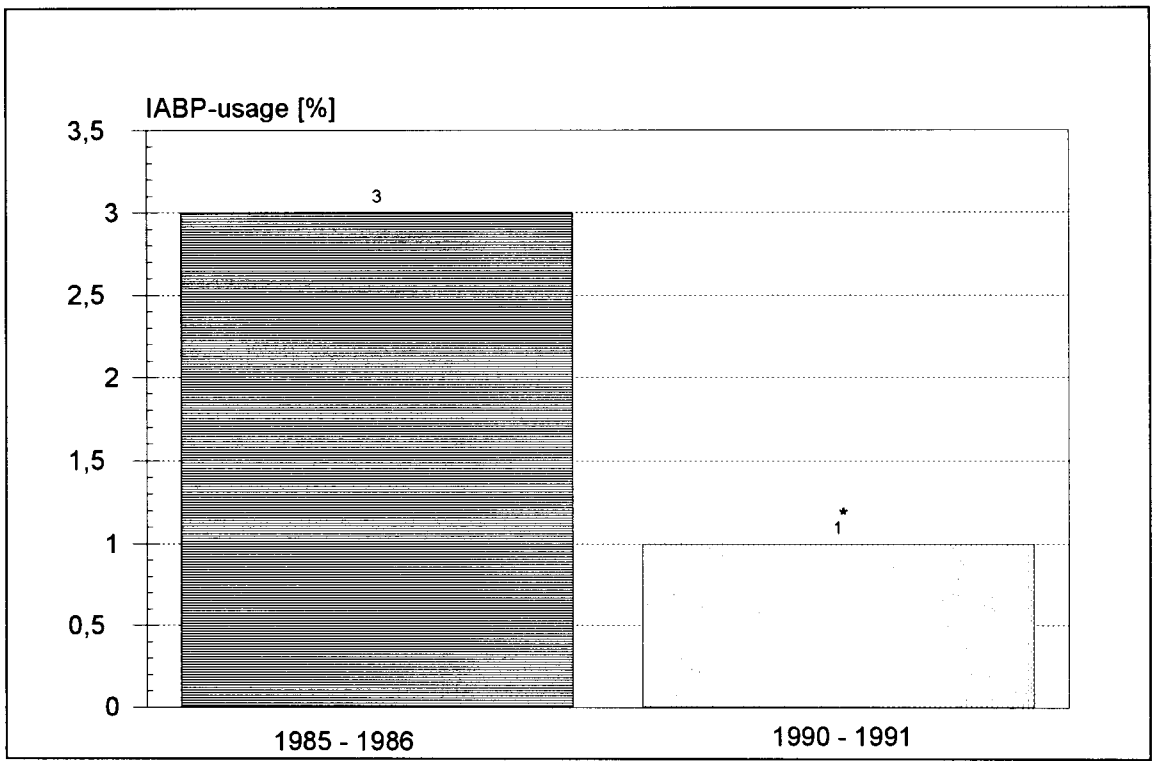
FIGURE 1.3.: HOSPITAL MORTALITY FOR HIGH RISK PROCEDURES (CABG + VALVE REPLACEMENT) AT GROOTE SCHUUR HOSPITAL



Legend: 1985-87: Modified Bretschneider's solution
1989-91: St. Thomas' Hospital solution No. 2

* = $p < 0.01$

FIGURE 1.4.: POSTOPERATIVE USAGE OF THE INTRAAORTIC BALLOON PUMP AT GROOTE SCHUUR HOSPITAL



Legend: 1985-87: Modified Bretschneider's solution
1989-91: St. Thomas' Hospital solution No. 2
* = $p < 0.01$

1.3. Adenosine

Adenosine, a physiologic purine compound nucleoside, has stimulated renewed interest in the context of induced or spontaneous myocardial ischemia. It has been called the natural cardioprotector (Van Belle, 1990) owing to its ability to attenuate ischemia induced damage. It has further been called the 'signal of life' for intercellular responses by indicating cell viability to inflammatory cells (Engler, 1991) and it has been associated with the attribute autacoid, meaning anti-injury hormone, that links ATP catabolism to inhibition of granulocyte adherence, microvascular obstruction and superoxide anion formation (Gruber, 1989). There is in fact a whole new class of pharmacological agents that augment adenosine release from ATP-catabolism, called adenosine promoter agents.

Physiological roles

Precursor of high energy phosphates and regulator of coronary flow

The potential physiologic roles of adenosine are summarized in Table 1.20.
Adenosine is a purine nucleoside compound of fundamental importance for the structure of nucleic acids and as a backbone for energy storage and use by living cells (ATP-system). It is also a regulator of biological functions as it controls coronary flow especially under ischemic conditions (Berne and Rubio hypothesis) (Rubio, 1969):

TABLE 1.20.: POTENTIAL ROLES OF ADENOSINE

Potential roles of Adenosine

Cardioprotection

>supply	<demand
coronary vascular resistance	adrenergic stimulation (pre-postsynaptic)
renin release	O_2-consumption
endothelin release	antilipolytic effects
platelet activation	membrane stabilization
leucocyte activation	Ca^{++} / K^{+} homeostasis
no reflow phenomenon	
glucose utilization	
high energy phosphates	

mediator of ischemic preconditioning
reduction of myocardial stunning

Neuroprotection

Analgesic properties

side effects: SVR*>
GFR**>
Bronchospasm
AV-Block

Legend: Potential roles of adenosine in improving the supply/demand ratio of the myocardium
* systemic vascular resistance
** glomerular filtration rate
<: increase
>: decrease

Periods of increased metabolic needs of the myocardium result in increased ATP catabolism and subsequent adenosine release, which in turn acts via purinergic A_2 receptors (P_1-receptor subtype) on the small coronary resistance arterioles thereby self-regulating coronary blood flow and metabolic needs. Although the adenosine hypothesis is widely accepted in coronary vasodilatation arising during hypoxia or ischemia, there is evidence that it plays no major role during physiologic exercise (Bache, 1988) and coronary vasodilatation in response to acute hypoxia is not uniquely dependent on adenosine (Gerwitz, 1987). Normal serum levels (in man) of adenosine range between 0.1 and 0,3µmol/L (Sollevi A, 1987) and any excess adenosine is rapidly broken down by adenosine deaminase.

Reduction of superoxide anion production from neutrophils

Cronstein (Cronstein, 1983) described adenosine as a physiologic modulator of superoxide anion generation by human neutrophils, but found no effect on either degranulation or aggregation. In a second study in 1986 (Cronstein, 1986) he confirmed the role of adenosine as an endogenous inhibitor of neutrophil- mediated injury to endothelial cells in an in vitro model. He concluded that the engagement of adenosine receptors prevents both the adhesion of neutrophils and the injury they cause to endothelial cells. More recently investigators from the University of Munich (Thiel, 1992) showed that the inhibition of oxygen radical production via adenosine results from receptor-mediated inhibition of stimulus-induced increases in intracellular calcium.

In an attempt to reduce ischemic injury in a coronary microembolization model with three antioxidant agents (superoxide dismutase, allopurinol, CV3611), Hori found less edema, increased numbers of microspheres required for complete stoppage of flow, less dysfunction during progressive embolization and increased adenosine release when these antioxidants were used (Hori, 1991). When the adenosine receptor antagonist 8-phenyltheophylline was used the beneficial effects of superoxide dismutase were blocked, indicating that augmented adenosine was a mechanism of this free radical scavenger.

This raises the possibility that increased local adenosine levels are the common pathway of protection from reactive oxygen species during ischemia and reperfusion (Engler, 1991). Is there an additional deleterious effect by which free radicals lower effective adenosine levels and thereby defeat a microvascular protective mechanism (Engler, 1991)? Originally it was thought that the principal source of superoxide production was the metabolism of hypoxanthine to uric acid via endothelial xanthine oxidase (McCord, 1985). Recent studies have confirmed that xanthine oxidase inhibition by allopurinol or oxypurinol may be protective, but the conclusions are controversial (Werns, 1986; Lasley, 1988). Even more recently a non-purine based agent that also inhibits xanthine oxidase failed to limit reperfusion injury, whereas oxypurinol reduced injury under identical circumstances. This raises the question of the role of xanthine oxidase inhibition in the mechanism of protection; perhaps oxypurinol and allopurinol have additional mechanisms of protection. The release of adenosine as the mechanism by which viable cells are recognized by cell-mediated inflammation has prompted Engler to call adenosine the signal of life (Engler, 1987), a marker that indicates cell viability to inflammatory cells. Cells that are no longer capable of ATP catabolism no longer excrete adenosine and can be recognized as necrotic debris for disposal by phagocytic cells.

Inhibition of platelet aggregation

Endogenous adenosine has been shown to inhibit platelet aggregation during myocardial ischemia (Kitakaze, 1991). During low flow ischemia in open-chest dogs, treatment with 8-phenyltheophylline, an adenosine receptor antagonist, reduced coronary flow and led to thromboembolization in the small coronary arteries. The authors demonstrated that this thromboembolization during lack of adenosine activity is triggered by stimulation of A_2-adrenoreceptors by release of norepinephrine during ischemia.

Sollevi (Sollevi, 1985) demonstrated in 1985 in a clinical study that adenosine spared platelets during cardiopulmonary bypass without causing systemic vasodilatation. However, the study failed to show any reduction in postoperative blood loss when adenosine was infused.

Inhibition of adrenergic stimulation and calcium antagonistic properties

Adenosine inhibits the myocardial effects of catecholamine stimulation (Schrader, 1977). Adenosine inhibited the contractile response to catecholamine stimulation by inhibiting isoproterenol-induced rise in cyclic 3`5`-AMP levels. Summarizing the electrophysiological effects of adenosine on cardiac tissues, Rubio pointed out that the negative inotropic effect of adenosine on atrial muscle, associated with a shortened action potential, the decrease of AV-conduction and a negative inotropic effect, are probably caused by a depression of calcium influx (Rubio, 1979). Kato confirmed in 1990 that adenosine decreases Ca^{2+} current under ß-adrenergic stimulation mainly by reducing channel availability (Kato, 1990). Since adenosine also opens the K_{ATP} channel, it can also reduce net calcium influx as has been demonstrated for K_{ATP} channel openers (Behling, 1995).

Stimulation of glycolytic flux

Wyatt published a study in 1989 in which he could demonstrated increased glycolytic flux via exogenous adenosine in the isolated rat heart (Wyatt, 1989). Adenosine stimulated glycolytic flux in the normoxic as well as in the hypoxic myocardium and the effect was mediated by interaction with A_1- adenosine receptors. However Dale conducted a study on isolated rat myocytes in which adenosine failed to alter glycolytic rates (Dale, 1991). Rather it was found that PIA (N6-(L-2-phenylisopropyl) adenosine, a nonmetabolizable adenosine analogue, inhibited glucose utilization with glucose as the sole exogenous substrate, as it inhibited glucose transport by binding to the glucose transport molecule. These discrepancies are difficult to explain by differences in methodology in estimating glycolysis or different perfusion systems alone and further studies will be required to clarify this issue.

Clinical roles

Diagnosis and treatment of supraventricular arrhythmias

Adenosine has a depressant effect on the SA node and impairs impulse conduction through the AV node, but has little effect on impulse generation and conduction in ventricular myocardium. These effects are mediated at the cellular level by an increase in potassium and a decrease in calcium conductance and are not antagonized by atropine. The effects of adenosine are rapid in onset (5-30 sec) and transient because the half life is 10-30 seconds only, because of a rapid uptake and breakdown by adenosine deaminase in erythrocytes and endothelial cells. These differing regional cardiac effects and short half life have led to the use of adenosine in the treatment of cardiac arrhythmias (Belardinelli, 1990)

Adenosine can terminate supraventricular arrhythmias in 86-100% of cases if the AV node is involved and forms one of the limbs of the re-entrant circuit, such as atrio-ventricular reciprocating tachycardia and atrioventricular nodal re-entry. Adenosine effectively terminates these types of supraventricular arrythmias by transiently interrupting impulse propagation through the AV node. However, the arrhythmias may reoccur quickly in up to one third of cases after the effects of adenosine dissipate. Adenosine will nearly always terminate tachyarrhythmias of atrial origin. Adenosine may be of diagnostic value because it slows the ventricular rate by causing block at the AV node level and reveals the unaffected atrial arrhythmia, as in automatic atrial tachycardia, atrial flutter and atrial fibrillation. Although adenosine is not effective in terminating ventricular tachycardia it has been safely and effectively used to help differentiate ventricular tachycardia from supraventricular tachycardia with a wide QRS complex. It seems to be safer for this procedure than verapamil which often has deleterious hemodynamic effects in patients with ventricular tachycardia.

Measurement of maximal coronary vasodilator reserve

More recently adenosine has been used successfully to measure maximal coronary vasodilatation (Wilson, 1990) in humans. Intracoronary bolus injection (16 μg), intracoronary infusion (>80 μg/min) and intravenous infusion (140 μg/kg/min) caused coronary hyperemia similar to that caused by papaverine. The duration of coronary vasodilatation was much shorter after adenosine bolus injection than after papaverine administration; no side effects were recorded apart from a brief decrease in arterial pressure similar to that caused by papaverine. In particular, there was no alteration in heart rate and cardiac conduction in the dosage used. One clear advantage over papaverine is the short half life of adenosine, whereas the total dose of papaverine that can be given is limited by its relatively slow systemic excretion (half life 3-6 hours). Consequently intravenous or prolonged intracoronary infusion lead to systemic hypotension. In addition intracoronary papaverine prolongs the QT interval and can cause polymorphous ventricular tachycardia. Intravenous dipyridamole, another drug which has been used and which acts via inhibition of adenosine deaminase, has the disadvantage of prolonged action (>30 minutes) and therefore precludes repeated measurements in the same study. Adenosine was therefore found to be a safer, more practical agent for measuring maximal coronary vasodilator reserve.(Wilson, 1990)

Induced hypotension

Recently adenosine has also been introduced as an agent to induce controlled hypotension during anesthesia in patients undergoing cerebral aneurysm surgery and in patients with peripheral vascular disease (Sollevi, 1984; Oewall 1987). Intravenous infusion at a rate of 90 μg/kg/min lowered mean arterial blood pressure by approximately 20%. Whereas systemic and pulmonary vascular resistance indices decreased by 36 and 32% respectively, cardiac index increased by 18%. Heart rate, ventricular filling pressures and whole body oxygen consumption were not affected (Oewall, 1988). Coronary sinus flow increased by 128% in parallel with a 96% increase in coronary sinus oxygen content. Signs of myocardial ischemia

were demonstrated in one patient while myocardial lactate uptake was unchanged in all subjects. However, more recently Zaell reported a significant depression of the ST segment in six out of fourteen patients after coronary bypass surgery with intravenous infusion rates of 30-120 µg/kg/min (Zaell, 1991). The authors therefore concluded that adenosine, while inducing systemic, pulmonary and coronary vasodilatation, increased the intrapulmonary shunt fraction and may cause or aggravate myocardial ischemia via coronary steal phenomena. Unfavorable coronary redistribution is therefore an undesirable side effect which needs further elucidation in controlled clinical trials before adenosine can be recommended for routine use to induce controlled hypotension.

Experimental roles

Mediation of ischemic preconditioning:

It is now well established that the extent of any myocardial infarction depends on the duration and the severity of the ischemic insult, the presence and degree of any collateral circulation, the rate of oxygen consumption, and the area of myocardium at risk (Walker, 1992). However the exact biochemical mechanism of cell death remains unclear. At present most theories relate to either the loss of high energy metabolites such as ATP and phosphocreatinine or the accumulation of catabolites such as lactate, H^+-ions and inorganic phosphate.

In experiments to separate the effects of high energy phosphate depletion from those of catabolite accumulation, Reimer et al. found that brief periods of ischemia and reperfusion were able to protect the heart through a longer sustained ischemic insult (Reimer, 1986). Induction of ischemic tolerance in this way has been termed ischemic preconditioning.

Global ischemic preconditioning as a protective mechanism in the clinical setting, especially in the field of cardiac surgery, has been extensively studied by Yellon et al. (Jenkins, 1995; Alkhulaifi, 1994; Speechly-Dick, 1995; Yellon, 1993). According to a recent survey 28% of British cardiac surgeons still use intermittent aortic cross clamping and ventricular fibrillation instead of cardioplegic arrest and yet have

acceptable clinical results (Izzat, 1994). Yellon (Yellon,1994) found significant preservation of high energy phosphate content in preconditioned hearts of patients undergoing CABG surgery. Walker and Yellon (Walker, 1993) had already postulated in 1993 that global ischemic preconditioning might account for the successful use of cross-clamp fibrillation during cardiac surgery. Abd-Elfattah and Wechsler (Abd-Elfattah, 1995) confirmed that intermittent aortic cross clamping prevents cumulative adenosine triphosphate depletion, postischemic ventricular fibrillation and ventricular dysfunction, and represents a form of preconditioning.

Adenosine has subsequently evolved as the most likely candidate as an endogenous mediator of this phenomenon (Walker, 1995). Being released from ischemic myocytes, adenosine is thought to act as a local regulator of the cell via a feedback mechanism. Probably the adenosine which accumulates during ischemia acts on myocardial A_1 receptors which in turn trigger biochemical changes in the heart that confer lasting protection against a subsequent ischemic insult. Most likely, these protective changes could be stimulation of glycolysis and opening of ATP regulated K^+ channels (Walker, 1992). Kloner and Yellon (Kloner, 1994) conclude that in the future preconditioning or "preconditioning mimetic" agents have the potential to be applied to a wide array of cardiovascular disorders and might result in better preservation of the heart in the settings of cardiopulmonary bypass, heart transplantation, angina and myocardial infarction'.

However, no consensus has been reached about the exact mechanism of preconditioning and although the role of adenosine has been well studied it does not seem to play a role in the rat (Cave, 1993) and can therefore not be the universal mediator. Several other mechanisms have been proposed including limitation of glycolysis, maintenance of high energy compounds and increased activity of the inhibitory G protein and increased activity of protein kinase C activity (King, 1996; Lawson, 1993).

Amelioration of myocardial stunning

Myocardial stunning is defined as transient postischemic contractile dysfunction of myocytes that have not undergone irreversible cell injury (Braunwald, 1985, Bolli, 1988). The most likely mechanisms for the development of stunning are free radical formation and intracellular calcium overload. Depletion of high energy phosphate stores and impairment of regional blood flow may play an additional role. Forman speculated that adenosine could accelerate the recovery of contractile function through a number of mechanisms (Forman, 1991). Adenosine may enhance intracellular ATP levels via the salvage pathway while conserving utilization through its negative inotropic effect in the presence of catecholamine stimulation. Adenosine could also reduce free radical mediated injury by inhibiting superoxide anion release from neutrophils (Cronstein, 1986) and decreasing catecholamine release and lipid peroxidation. Adenosine also blocks calcium dependent channels and therefore may restore intracellular calcium homeostasis. Finally adenosine may enhance oxygen delivery and washout of "toxic" metabolites through arteriolar vasodilatation and antiplatelet and antineutrophil effects (Forman, 1991).

Adenosine during ischemia and reperfusion

In view of the cardioprotective properties of adenosine during ischemia and reperfusion numerous experimental models have been used to investigate the potential benefits of adenosine during these periods.

Reibel and Rovetto showed 1979 that postischemic perfusion of the isolated rat heart with adenosine for 5 hours could restore ATP values to control levels (Reibel, 1979). At this stage the main effect of adenosine was thought to be restoration of high energy phosphates via precursor availability. These initial findings were emphasized by Sollevi in 1987 (Sollevi, 1987) in a clinical study, who showed that considerable amounts of purines are washed out of the myocardium during reperfusion, impeding restoration of the normal energy state and return of myocardial function.

Starting from the theory that catabolism of high energy phosphate precursors is the limiting factor for their regeneration during reperfusion, Foker found in 1980 that pretreatment with EHNA, an adenosine deaminase inhibitor, together with adenosine infusion during ischemia increased myocardial blood flow and ATP content at reperfusion in dogs (Foker, 1980). However, adenosine infusion alone, while altering myocardial blood flow similarly, did not alter subsequent ATP levels.
Using a similar approach Humphrey showed in 1982 (Humphrey 1982) that suppression of adenosine catabolism by adenosine deaminase inhibitors (EHNA) improved the functional recovery of ischemic myocardium. EHNA alone or EHNA together with adenosine prior to ischemia resulted in 100% recovery of aortic output after 30 minutes of global ischemia in the isolated rat heart. However, as in the previous study, only the combination of adenosine and EHNA was able to improve postischemic ATP levels significantly.

Ely from Virginia, USA demonstrated in 1985 improved functional recovery in an isolated rat heart model of global ischemia with adenosine infusion prior to ischemia and at reperfusion (Ely, 1985). In these experiments, in contradiction to the previously mentioned studies, adenosine treatment alone enhanced metabolic recovery as evidenced by better preserved ATP levels. Furthermore, adenosine (100 μmol) increased the time to onset of ischemic contracture by 50%. Ely concluded from his study that adenosine enhanced myocardial preservation by reducing the net degradation of ATP during ischemia and facilitated repletion of ATP levels during reperfusion.

Starting in 1989, Bolling published several studies showing improved postischemic recovery when adenosine was added during ischemia in the isolated rat heart (Bolling, 1989). He found improvement of ventricular function which was maximal with adenosine 400 μM including better preservation of diastolic function. He noted no differences in postischemic coronary flow and attributed the improved recovery to an enhanced repletion of high energy phosphates with adenosine, although his study did not include measurements of high energy phosphates. The same author published a second study where he found improved recovery with adenosine or 2-deoxycoformycin, a non-competitive inhibitor of adenosine deaminase

(Bolling,1990). Combination of these two drugs enhanced recovery even further. This time he measured ATP levels and found elevated levels at reperfusion in the drug treated hearts, although ATP levels declined equally during ischemia in the control and treatment groups. Confirming his results he found in 1991 inferior recovery in a third study in animals treated with adenosine deaminase (Bolling 1991). Postischemic functional recovery closely paralleled the availability of myocardial adenosine and he concluded that myocardial adenosine levels at the end of ischemia and at early reperfusion are important determinants of functional recovery after global ischemia.

Adenosine releasing agents

A relatively new approach with an old drug is the enhancement of endogenous adenosine release via adenosine-releasing agents. AICA riboside (5-amino-4-imidazole carboxamide riboside), described as long ago as 1952 (Greenberg, 1952), augments adenosine release only from energy deprived cells with net ATP catabolism (Gruber, 1989). Gruber found AICA riboside pretreatment of myocardium reduced granulocyte accumulation and improved collateral flow in the ischemic area. In addition the episodes of ventricular arrhythmias during coronary artery ligation were significantly reduced in AICA riboside pretreated hearts. The study also confirmed that adenosine inhibits granulocyte superoxide anion production in vitro, as already demonstrated by Cronstein (Cronstein 1983,1986) .

Conclusion

A review of all these experimental investigations shows numerous potential cardioprotective qualities of adenosine. However, it is difficult to attribute these protective qualities to one single mechanism. It seems more likely that these properties act together via synergistic mechanisms. However, it would be interesting to separate the vascular effects (A_2 receptor-mediated) from the myocardial effects (A_1 receptor-mediated). This would be possible with currently available selective

agonists for one receptor subtype. Whereas isolated heart models allow rapid screening tests in large numbers and control of pre- and afterload conditions, they often lack clinical relevance and the species tested is often distant from man. In-vivo models, while more closely simulating the clinical situation, allow less control of loading conditions for assessment of myocardial function (Bretschneider 1980). It also seems that the initially postulated ATP repletion via adenosine as precursor is not the main mechanism involved. Especially during ischemia there seems to be no significant regeneration of ATP even when high concentrations of adenosine are present. However, during reperfusion, the presence of adenosine probably enhances fast restoration of high energy phosphates. On the other hand there seems to be no close correlation between high energy phosphate content and mechanical recovery and Hohlfeld and Hearse (Hohlfeld 1989) have questioned whether elevation of high energy phosphate levels postischemia is important. They concluded that total tissue ATP is not necessarily a good indicator of functional capabilities under conditions of normothermic ischemia and reperfusion in the isolated rat heart, but they did not question the uncontradicted value of ATP as a parameter of ischemia related tissue damage. Probably different adenine nucleotide compartments characterized by different specific activities with varying contributions during normoxia and hypoxia preclude total tissue ATP as a reliable marker of mechanical function postischemia (Schrader, 1976).

Two other questions remain: Is the presence of adenosine essential before or during ischemia or during the reperfusion phase? The answer is not yet established, but most probably adenosine will be beneficial in all three phases.

The other question is whether supplementation via exogenous adenosine, inhibition of adenosine breakdown via adenosine deaminase or adenosine transport inhibition via nucleoside transport inhibitors is the most successful way to ensure high local myocardial levels of adenosine. Again the answer is not yet established, but the combination of those three approaches seems likely to offer most promise. There is sufficient experimental evidence to support the effectiveness of either method. Different experimental setups and animal species have to be taken into consideration.

More recently adenosine releasing substances (ARA) stimulating endogenous adenosine release have become available. This is perhaps the most effective

approach especially as the undesirable side effects of high and prolonged adenosine administration such as generalized receptor activation (Van Belle, 1990), renal vasoconstriction (Osswald, 1978; Spielman, 1987) and bronchospasm (Holgate, 1987) can be avoided.

Adenosine has been called the natural cardioprotector (Van Belle, 1990) and has been termed autacoid, meaning anti-injury hormone, that links ATP catabolism to inhibition of granulocyte adherence, microvascular obstruction and superoxide anion formation (Gruber, 1989). In view of the experimental evidence discussed above it appears that adenosine is in fact an anti-injury hormone with multiple sites of action. Apart from its role of enhancing coronary flow during ischemic stress associated with ATP catabolism in order to meet metabolic needs, it also slows heart rate and AV-conduction resulting in preservation of energy and decreased O_2 consumption. The antiadrenergic effects act additionally to counteract the systemic hormonal stimulation during these periods. Whereas the potential of stimulating glycolytic flux has been disputed it could, once proven, act to ensure the functioning of metabolic pathways during ischemic periods. The Ca^{2+}- antagonistic properties could prevent Ca^{2+} accumulation during ischemia and reperfusion with all its adverse consequences. Inhibition of leukocyte adherence and superoxide anion generation together with diminishing platelet aggregation act together to maintain adequate flow during reperfusion and to reduce the deleterious consequences of the reintroduction of oxygen. All these multiple sites of action aim in the same direction of ameliorating ischemia and reperfusion induced damage.

While further experimental results elucidating the various protective mechanisms are necessary the time has come to start clinical trials with this promising substance. To my knowledge the only commercially available solution employing adenosine is UW-solution for heart storage prior to transplantation, although it was originally designed for storage of intraabdominal organs, such as liver and kidney. Cardioplegic and reperfusion solutions for local and global myocardial ischemia could be substantially enhanced by addition of adenosine. For blood-based solutions a combination with adenosine deaminase inhibitors or nucleotide transport inhibitors would be necessary to prevent rapid degradation.

CHAPTER 2

MODELS USED TO STUDY MYOCARDIAL PROTECTION

Cardioplegic solutions need to be developed and evaluated in animal models prior to human use. Thereafter, multivariate clinical trials of experimentally investigated solutions are valuable and necessary. However, the multitude of uncontrolled variables, as a result of the varied diseases necessitating cardiac surgery, makes evaluation of cardioplegic solutions difficult in the clinical situation.

In animal studies, it is important to undertake evaluations in more than one species because of interspecies differences. Hearse D J, Jynge P and co-workers evaluated the St. Thomas' Hospital No 2 cardioplegic solution in both rats and dogs (Jynge,1981). However, the ideal species for evaluating solutions intended for human use is possibly the baboon, as it is one of the closest species to man. In this thesis two models and species were used to assess the efficacy of cardioplegic solutions used in South Africa, and experimental modifications of the St. Thomas' Hospital cardioplegic solution.

All experimental animals used in our laboratory received care in accordance with the „Principles of laboratory animal care" of the National Society for Medical Research and the „Guide for the care and use of laboratory animals" prepared by the National Academy of Sciences (NIH Publication No 80 - 23, revised 1978). In addition, experimental protocols were reviewed and authorized by the Animal Research Review Committee of the University of Cape Town.

2.1. ISOLATED RAT HEART MODEL

The isolated perfused rat heart was used as the experimental model for the majority of studies in this thesis. This model has two components; the Langendorff preparation and the working heart model.

Langendorff preparation

The Langendorff model was originally described in 1895 (Langendorff, 1895) and since then has been used extensively to study cardiac metabolism in a number of different animals (Hearse, 1981). Male rats of the Sprague-Dawley strain maintained on a standard diet were used for our experiments.

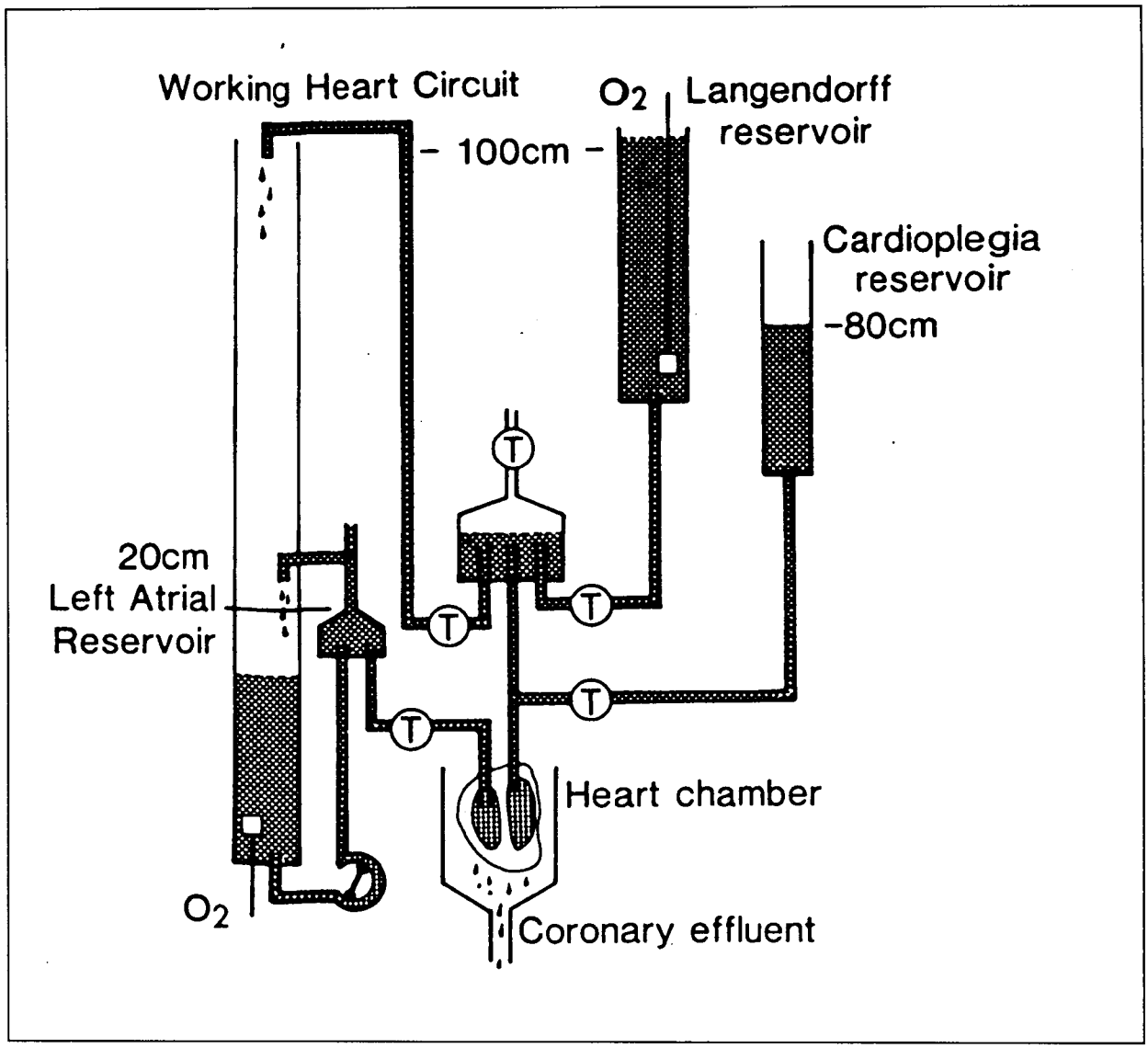
The rat was anesthetized with diethyl ether, heparinized with 200 U heparin injected into the exposed femoral vein, and the heart was then carefully but rapidly excised and placed into cold (4 °C - 10 °C) Krebs-Henseleit buffer (Table 2.1). The low temperature arrests the heart and thus provides some protection to the myocardium during this initial ischemic period. The aorta was then cannulated and the heart mounted on the perfusion apparatus (Fig. 2.1), within one minute of excision. Normothermic (37 °C), Krebs-Henseleit buffer oxygenated with 95 % O_2 and 5 % CO_2 (pO_2 greater than 600 mm Hg) was filtered through a cellulose filter (0.8 µm size pores; Millipore Corporation, Bedford, Massachusetts, USA) and then allowed to flow retrograde into the aorta at a constant pressure of 100 cm H_2O (75 mm Hg), thus establishing coronary perfusion. Normal sinus rhythm recommenced immediately. The pulmonary artery was then incised to ensure adequate drainage of coronary effluent, and the heart was maintained at 37 °C in a water-jacketed chamber. This model thus allows the perfused heart to continue beating in a nonworking mode, and was used to stabilize the experimental hearts prior to conversion to the working model. In addition, this mode of perfusion was also used for the initial reperfusion of the postischemic heart.

Working heart model

In order to allow the heart to perform external work so that mechanical performance could be monitored, the Langendorff model was then converted to the working model originally described by Neely and colleagues (Neely, 1967). The left atrium was cannulated through the pulmonary veins, and filled with oxygenated Krebs-Henseleit buffer at a constant pressure of 20 cm H_2O (15 mm Hg). The left ventricle then spontaneously ejects the perfusate against a hydrostatic pressure of 100 cm H_2O (75 mm Hg), and the coronary flow was collected and recirculated. Aortic pressure, aortic flow, coronary flow, and heart rate were then recorded in the control working period, prior to the ischemic period, and also in the postischemic recovery period.

Cardioplegic solutions were infused into the aorta at a pressure of 80 cm H_2O (60 mm Hg) prior to the ischemic period, by simultaneously closing the aortic and atrial cannulae (Fig. 2.1), and infusing the cardioplegic solution through a side arm of the aortic cannula. Constant hypothermia (10 °C) was maintained throughout the ischemic period by another separate cold (10 °C) water-jacketed chamber.

FIGURE 2.1. ISOLATED WORKING RAT HEART



Legend: Three way taps (T) allow easy conversion to either Langendorff mode, the working heart circuit or infusion of cardioplegic solutions. Reprinted from J Thorac Cardiothorac Surg 1991; 102; 405-412 (with permission Mosby Year Book Inc.)

TABLE 2.1.: KREBS-HENSELEIT BUFFER

Compound	mM/L	gm/L	Electrolyte	mM/L
NaCl	118	6.9	Na^+	143
$NaHCO_3$	25	2.1	K^+	6
KCl	4.8	0.36	Ca^{2+}	1.25
KH_2PO_4	1.2	0.16	Mg^{2+}	1.2
$CaCl_2 \cdot 2H_2O$	1.25	0.18	Cl^-	125.3
$MgSO_4 \cdot 7H_2O$	1.2	0.3	PO_4^{3-}	1.2
D-Glucose • H_2O	11.1	2.2	Glucose	11.1

Legend: Modified Krebs-Henseleit buffer used in our laboratory

Experimental protocols

All hearts were stabilized for 10 minutes in the Langendorff mode, prior to changing to the working model. Pre-ischemic control hemodynamic parameters were measured at 2,5 and 10 minutes during this working period. Any heart that was unstable during this period was rejected. The 10-minute values were then recorded as the preischemic controls for future comparisons.

Cardioplegic arrest was induced by infusing 10 ml of cold (10 °C) cardioplegic solution into the aorta.

At the end of the ischemic period each heart was reperfused in the Langendorff mode with standard oxygenated Krebs-Henseleit buffer (37 °C) for 10 - 20 min at a pressure of 80 - 100 cm H_20 (60 - 75 mm Hg) according to individual experimental protocols. The hearts were then converted to the working model for a further 10 minutes, at which time the postischemic hemodynamic parameters were recorded. The coronary sinus effluent was collected during the ischemic period when multidose

cardioplegia was administered, as well as during the postischemic Langendorff reperfusion period. At the end of the experiments the hearts were freeze-clamped with Wollenburg tongs to determine their high-energy phosphate content.

Exclusion criteria:

In the preischemic control period the following values were used to discard hearts:
Aortic output < 30 ml/min
Coronary flow > 22 ml/min
Heart rate < 200 beats/min, or irregular rhythm
In the postischemic period any heart whose coronary flow increased significantly by more than 50 % - suggesting a left atrial leak - was excluded.

Parameters of recovery

To evaluate the efficacy of myocardial protection both mechanical, metabolic and morphological parameters may be measured (Hearse, 1981). However, ultimately the most important parameter is mechanical recovery, which was measured under controlled conditions (both preload and afterload), in this isolated rat heart model.

Hemodynamic parameters

Cardiac output (CO) in this preparation is the sum of aortic flow (AO) and coronary flow. Stroke volume (SV) was then calculated using the following formula:

$$SV = CO / HR \text{ (ml/beat)}$$

Values obtained during the postischemic working period were expressed as a percentage of their individual preischemic control values. Results were then presented as percentage means and standard errors of percentage means.

Metabolic parameters

Loss of myocardial high energy phosphate stores is an integral component of ischemia, and Bretschneider and co-workers predicted poor recovery if ATP levels fell below 4 µmol/gm wet weight (Bretschneider, 1985). ATP levels have been used in clinical studies and correlate with postoperative outcome (Hammon, 1987). However, other studies have demonstrated that postischemic levels of ATP of less than 2 µmol/gm wet weight neither predict recovery of contractile function nor irreversible damage (Vary, 1979). Thus, although newer 'on line' nuclear magnetic resonance techniques for measuring ATP may provide valuable information, one cannot predict functional recovery from these values (Rosenkranz, 1986; Saks, 1989). High energy phosphates were measured in the studies in this thesis by freeze-clamping the hearts with Wollenberg tongs and freeze drying the samples. The levels were then determined by the method of Lamprecht and Trautschold, expressed as µmol/gm wet weight (Lamprecht, 1974). Preischemic control values for ATP and CP were derived from hearts subjected to the same initial experimental protocol, i.e. 10 min Langendorff and 10 min working heart modes, but thereafter immediately freeze clamped. Tissue ATP and CP levels were then also measured at the end of the ischemic period in some of the studies, by freeze clamping a second set of hearts prior to reperfusion. In addition, high energy phosphate levels were also measured in most of the studies after reperfusion, at the end of the experimental protocol.

Analysis of data

Extreme observations or „outliers" were tested for by Dixon's criteria (Snedecor, 1980), in order to exclude major experimental errors, and if found those hearts were then excluded from further analyses.

The statistical tests used to compare differences between groups were the Anova one-way, two-way or three-way analyses of variance. However, the Anova depends upon an assumption of common variance amongst observations within each

treatment group. Therefore, if variance was not consistent necessary adjustments for the violation of common variance were made. If statistical significance was obtained with ANOVA then pairwise comparisons of means were performed using either t-statistics with a nominal level of significance set at 5 %, or by the f-test. Appropriate tables were then used to determine p-values for comparisons of interest. Statistical significance was assumed when the p-value was less than 0.05. Bonferroni correction was performed for multiple comparisons.

2.2 In vivo primate model

Juvenile and adult Chacma baboons (*Papio ursinus*) were used in the primate experiments in this thesis. An in vivo model using cardiopulmonary bypass to simulate the clinical situation as close as possible was used.

Animal preparation

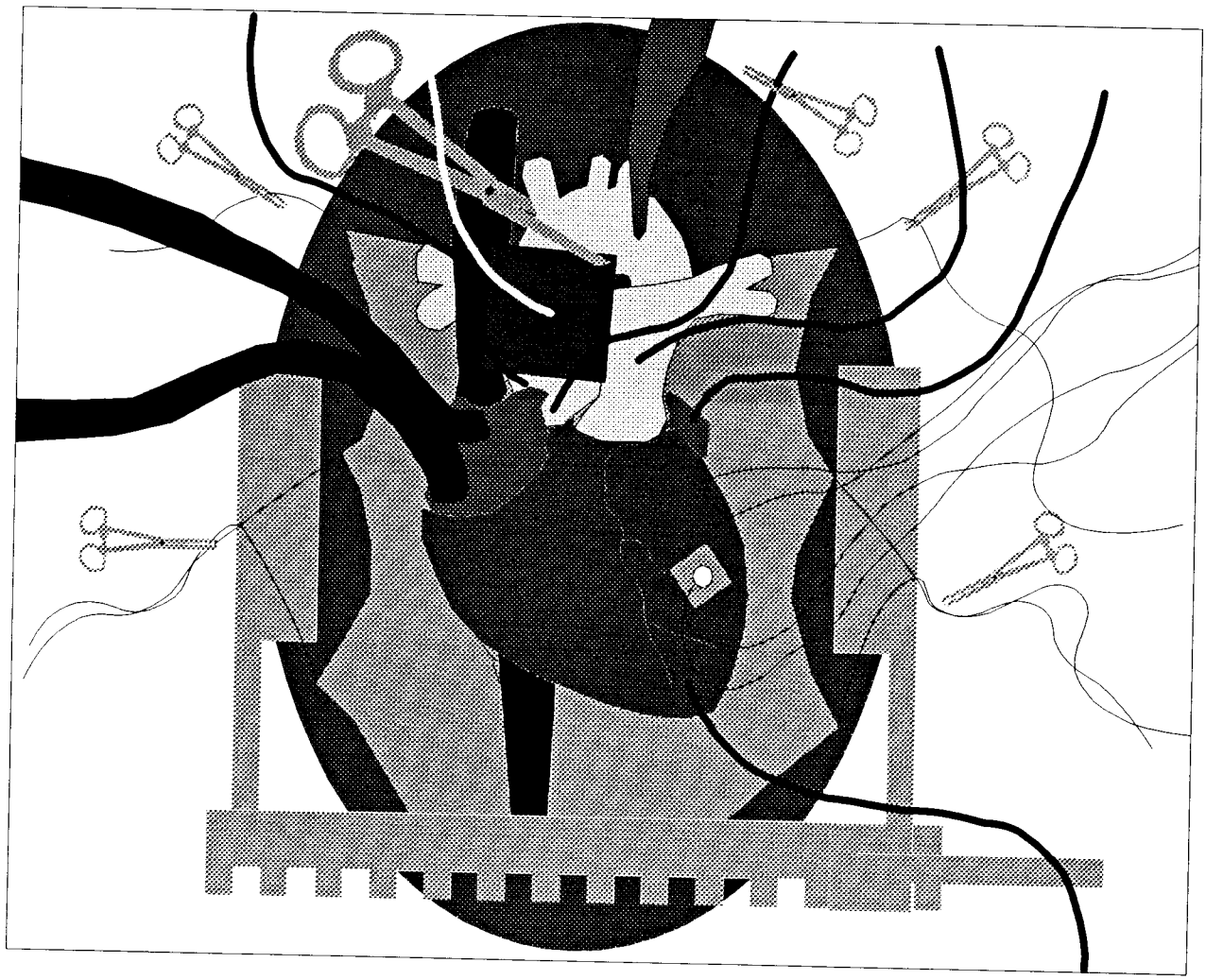
The baboons were fed on a standard diet which was withheld the day prior to surgery.

The animals were premedicated with intramuscular ketamine hydrochloride (10 mg/kg body weight). An endotracheal tube was then inserted, which was stabilized and secured with a cardboard ring and Elastoplast. A narcotic analgesic morphine sulfate (0.25 mg/kg), an antisialogogue, atropine sulfate (0.5 mg) and a neuromuscular blocker, pancuronium bromide (0.1 mg/kg) were administered intravenously. Anesthesia was maintained with a mixture of nitrous oxide (7 L/min), oxygen (3 L/min), and 0.5 % halothane, administered via a Bird Mark 8 respirator (Bird Corporation, Palm Springs, California, USA) at a respiratory rate of 10 - 20 breaths/min. Tidal volume was adjusted according to animal size, ventilator pressures were kept at 15 - 20 cm H_2O, and blood gases were monitored throughout the experiment and ventilation adjusted accordingly. The animals were also monitored for signs of insufficient anesthesia (hypertension, bradycardia,

tachycardia, pupillary dilatation, lacrimation, salivation and muscular tension), and the level of anesthesia was appropriately adjusted. Intra-arterial and central venous lines were inserted into the femoral vessels.

Immediately prior to performing the median sternotomy a second dose of morphine sulfate was given. After full heparinization (500 U/kg) the heart was cannulated in preparation for cardiopulmonary bypass. Venous blood was drained via a single venous cannula inserted into the right atrium, oxygenated by a bubble oxygenator (Polystan Venotherm Oxygenator, low prime adult / pediatric Cat 012500, or infant Cat 011200; Polystan A/S, Vaerlose, Denmark), temperature controlled with a heat exchanger incorporated in the oxygenator, and the blood was then returned by the cardiopulmonary bypass machine (Polystan A/S; Vaerlose, Denmark), via a cannula inserted into the ascending aorta. A Swan-Ganz thermodilution catheter (American Edwards 7F; American Edwards Laboratory, American Hospital Supply corporation, Santa Ana, California, USA) was inserted through the superior vena cava and positioned in the pulmonary artery for the measurement of cardiac output. Cannulae were also inserted into the left atrium and left ventricle and a thermistor probe (Microprobe thermometer Model BAT-12; Physitemp, Clifton, New Jersey, USA) was positioned in the ventricular septum.

FIGURE 2. 2.: SITUS OF THE ANIMAL PREPARATION INTHE PRIIMATE MODEL



Legend: Situs of the animal preparation in the baboon. CPB is connected via cannulae in the right atrium and ascending aorta. A Swan-Ganz thermodilution catheter is inserted via the SVC. Additional monitoring lines are inserted in the left atrium and left ventricle. A thermistor probe is inserted in the interventricular septum.

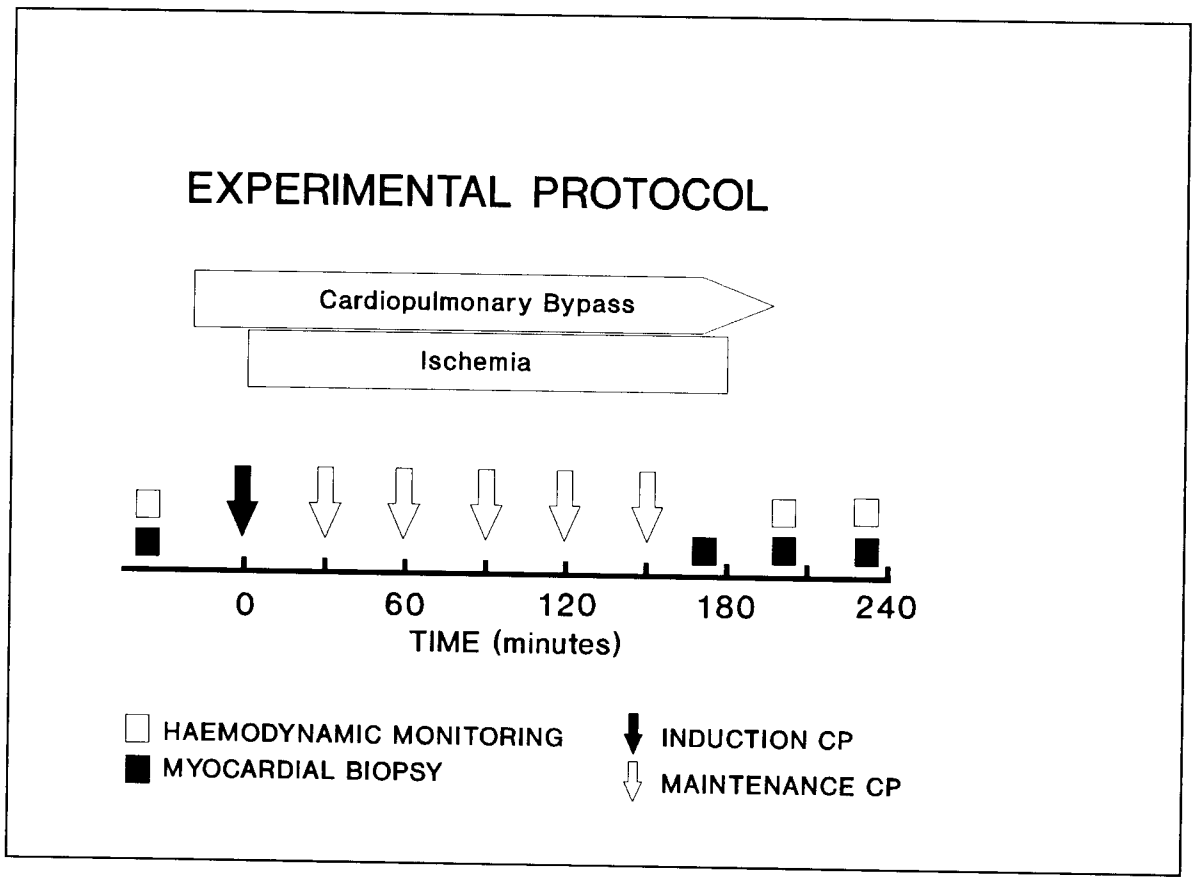
Experimental protocol

Systemic temperature was lowered and maintained at 26 °C after commencing cardiopulmonary bypass. The aorta was then cross-clamped and cardioplegic solution (15 ml/kg) infused via a separate cannula inserted into the ascending aorta, at a constant pressure of 80 mm Hg. In addition, the pericardial cavity was irrigated with iced saline during each infusion of cardioplegic solution. Multidose maintenance cardioplegia (100 ml/dose) was reinfused every 30 minutes throughout the 180-minute cross-clamp period. Systemic rewarming was commenced 30 minutes before the release of the aortic cross-clamp, and continued throughout the 15-minute

reperfusion period until normothermia was attained. The left ventricle was vented during the cross-clamp period and initial reperfusion period to ensure left ventricular decompression, by a cannula inserted via the right superior pulmonary vein. The animal was then weaned from cardiopulmonary bypass without the use of any inotropes.

The animals were killed while still under full anesthesia by the intravenous infusion of a strong solution of potassium chloride, thereby effecting immediate cardiac arrest at the completion of each experiment.

FIGURE 2.3.: EXPERIMENTAL PROTOCOL OF THE IN-VIVO BABOON MODEL



Legend: CPB was commenced after positioning of all cannulae and after prior hemodynamic measurements and obtaining a left ventricular biopsy specimen with a Truecut needle. Subsequently the aorta was crossclamped and the heart was perfused with cardioplegic solution at 80 mmHg controlled via a pressure regulating feedback device. During 180 minutes of global hypothermic ischemia the hearts were perfused in 30 minutes intervals with maintenance cardioplegia and additional cooling with topical cold saline was provided. Prior to release of the aortic cross clamp at the end of the ischemic interval a further myocardial biopsy was taken and subsequently the animals were weaned from CPB. After 5 and 30 minutes after termination of CPB hemodynamic measurements were performed and a myocardial biopsy specimen was obtained.

Parameters of recovery

Myocardial temperature and hemodynamic parameters - heart rate, arterial blood pressure, right and left atrial pressure, first derivative of left ventricular developed pressure (LV dP/dt), and cardiac output (by thermodilution) were recorded with a Honeywell AR-6 Simultrace Recorder (Electronics for Medicine, Honeywell Inc, Pleasantville, New York, USA). Preischemic hemodynamic parameters were recorded after the positioning of all cannulae before starting cardiopulmonary bypass. A left ventricular function curve was plotted by recording these parameters at increasing left atrial filling pressures (5, 10, 15, 20 mm Hg), obtained by infusing cardiopulmonary bypass prime into the animal. In addition, two left ventricular transmural needle biopsy specimen were taken from the apex of the left ventricle for determination of high-energy phosphates and transmission electron microscopic assessment in some experiments.

Postischemic hemodynamic and biochemical parameters were again taken at 5 and 30 minutes after termination of cardiopulmonary bypass, (a postischemic LV function curve was only obtained at the 30 minute postischemic period).

Analysis of data

Values were expressed as an index, to exclude the influence of animal size. The following formulae were used to derive cardiac index (CI), stroke volume index (SVI), and left ventricular stroke work index (SWI).

BSA (cm^2) = Weight $^{0.425}$ (kg) X Height $^{0.725}$ (cm) X 71.84 (Du Bois, 1916)
CI ($L/min/m^2$) = CO (L/min) / (BSA X 10^{-4} m^2/cm^2)
SVI ($ml/beat/m^2$) = (CI X 1000 ml/L) / HR (beats/min)
SWI ($gm \cdot m/beat/m^2$) = SVI X (LV systolic pressure (mm Hg) - LVEDP (mm Hg) X 0.014348 (Saksena, 1983)

Postischemic values were then expressed as a percentage of each individual preischemic control, and the same statistical tests as previously described in this section were used.

For statistical comparisons of SWI left ventricular function curves, the SWI at each left atrial filling pressure was expressed as a percentage of the stroke work achieved in the preischemic control curve for each individual animal. Mean percentages and standard errors of mean percentages were then compared by ANOVA. However, for display purposes an average preischemic control left ventricular function curve was derived by pooling the data from all control function curves. Postischemic function curves for each group were then normalized to the average preischemic curve; by multiplying the mean percentage recoveries and their standard errors for each group, at each left atrial filling pressure from 0 - 20 cm H_2O, by the average control SWI at the corresponding pressure.

The volume of cardioplegic solution administered to each animal (15 ml/kg) was according to the recommended dose for the St. Thomas' Hospital solution (Hearse, 1981).

CHAPTER 3

HYPOTHESIS UNDER TEST

3.1. Adenosine in intraoperative myocardial protection

Because of its ability to attenuate ischemia induced damage, adenosine - a physiologic purine compound nucleoside - has stimulated renewed interest in the context of induced or spontaneous myocardial ischemia.

The initial idea was to use adenosine as an alternative cardioplegic agent or as an adjunct to high potassium cardioplegic solutions in order to achieve fast arrest and improved postischemic myocardial function. During pilot studies we had found that adenosine in high concentration was able to achieve fast electromechanical cardiac arrest in the isolated rat heart. At that time there were only a few studies using adenosine as adjunct to high potassium, showing an improvement in postischemic recovery. However, no study had used adenosine for induction of cardioplegic arrest.

3.2. Aim of the study

The objective of the present study was to test the anti-ischemic properties of adenosine in intraoperative myocardial protection. Two animal models were developed:

1. The isolated rat heart model as a preclinical screening model to test the hypothesis, and

2. A large animal model in the in-vivo primate as a clinically orientated model to verify the findings in the expectation of clinical application.

In both models the two potentially important factors for intraoperative myocardial preservation were to be investigated.

1. Is adenosine a cardioplegic agent on its own without high potassium, by virtue of its inhibition of the SA and AV nodes?

2. Can adenosine on its own or in conjunction with high potassium improve myocardial preservation during global, hypothermic ischemia as measured by hemodynamic, biochemical and ultrastructural recovery?

A further aim was to elucidate at least some of the mechanisms by which adenosine would achieve this aim, although this was not the primary intention of the study. In order to achieve this aim adenosine was tested as a cardioplegic agent and as an adjunct to high potassium in the isolated rat heart during 30 and 90 minutes of global ischemia.

The in vivo-baboon model was used to first compare different crystalloid cardioplegic solutions used clinically in South Africa. The solution giving the best recovery should be used as control solution to test the effects of an intervention with adenosine.

End points of the study were the hemodynamic recovery of the postischemic hearts, the biochemical status as measured with ATP and CP levels of the myocardium and in some experiments the ultrastructural changes during ischemia and reperfusion.

CHAPTER 4

STUDIES IN THE ISOLATED RAT HEART

4.1. Adenosine vs high potassium to induce cardiac arrest: Langendorff and working heart mode

Rationale

Adenosine, an endogenous nucleoside, formed mainly as a degradation product of ATP, is an intermediate metabolite in many important biochemical pathways and has been shown to play an important role in the regulation of a number of physiologic processes (Arch,1987; Drury,1929; Sparks,1986). Among its various effects adenosine was found to produce bradycardia and atrioventricular block (Bellardinelli,1983; Dobson,1987). These electrophysiologic properties of adenosine have led to its use as potent antiarrhythmic agent for the management of paroxysmal supraventricular tachycardias (Belhassen,1984; Di Marco,1985; Pelleg,1986).

Recent studies showed the beneficial effects of calcium channel blockers on the functional recovery of ischemic myocardium (Christakis,1986; Nayler 1980). As the effects of adenosine involve the depression of sinus node automaticity and of calcium-mediated slow channel conduction, we investigated the feasibility of preserving myocardial function during induced global ischemia by a high dose adenosine cardioplegia. The adenosine cardioplegia was compared with potassium as well as with a mixture of potassium and adenosine cardioplegia.

Experimental procedures, materials and methods

The experimental procedures, materials and methods used are described in detail in chapter 2.1.

Perfusion technique

All experiments were performed on male Sprague-Dawley rats, weighing 250 to 350 g. The techniques used are described in detail in chapter 2.1.

Perfusion time sequence

For hemodynamic stabilization all hearts were perfused by the Langendorff mode for 10 minutes at first. Afterwards the hearts were converted to the working mode for another 10 minutes. Coronary and aortic flow rates were measured by collecting the perfusate draining from the hearts. Aortic pressure and ECG were monitored continuously by the mean readings from a two channel recorder (Mx 2P-148).
In the initial pilot study, after infusion of 10 ml of cold (4°C) cardioplegic solution at a rate of 2 ml/min, infused over 5 minutes, time for complete electromechanical arrest was recorded as well as the number of arrest- and escape beats via the two channel recorder. Two sets of experiments were performed: the first set was performed in the Langendorff mode, the second in the working heart mode.

Perfusion solutions

Six perfusion solutions were used: [1] standard Krebs-Henseleit buffer (KHB), [2] KHB supplemented with 1 mM/L of adenosine (Boehringer Ingelheim), [3] KHB supplemented with 10 mM/L of adenosine, [4] KHB containing 20 mM/L potassium, [5] KHB supplemented with 20 mM/L potassium and 1 mM/L of adenosine, [6] KHB supplemented with 20 mM/L potassium and 10 mM/L of adenosine.

Tissue analysis

At the end of the experiments all hearts were freeze-clamped and stored in liquid nitrogen. LDH was controlled to exclude myocardial damage prior to ischemia. Myocardial contents of adenosintriphosphate (ATP) and phosphocreatine (CP) were quantified by high pressure liquid chromatography, using the technique described elsewhere (Sollevold, 1986). One further set of hearts was freeze-clamped just prior to the ischemic period to obtain normoxic values for the phosphonucleoside pool.

Results

Cardiac arrest

Langendorff perfusion

Compared with potassium cardioplegia, adenosine was found to arrest the hearts more rapidly. While potassium cardioplegia arrested the hearts within 52 seconds, a high dose of adenosine caused arrest within 2.1 (adenosine 1 mM/L) or 1.6 (adenosine 10 mM/L) seconds ($p < 0,001$). The number of arrest beats were 6 and 7 for the adenosine versus 168 for the potassium cardioplegia. Escape beats, i.e. ventricular contractions after complete cardiac arrest, were not noted with potassium cardioplegia but occurred during adenosine arrest. With 10 mM/L adenosine escape

beats were fewer compared with 1 mM/L in both groups, with and without potassium (Table 4.1.).

Working heart mode

In the working heart mode the time of arrest and the number of arrest beats were still significantly different between high potassium and adenosine (10 mM/L) cardioplegia. Using 10 mM/L of adenosine contractile myocardial function stopped within 7 seconds, while in experiments using potassium cardioplegia 63 seconds elapsed before complete cardiac arrest. The number of arrest beats was 15 in the adenosine versus 125 in the potassium trials (Table 4.2). In the control group cold Krebs-Henseleit-Buffer was used as control cardioplegia by virtue of hypothermia only.

TABLE 4.1.: CARDIAC ARREST IN LANGENDORFF PERFUSION

Cardioplegia (n = 7)	**Time to arrest (seconds)**	**Arrest beats**	**Escape beats**
Adenosine 1 mM	2.1 ± 0.6*	6 ± 1*	11 ± 2*
Adenosine 10 mM	1.6 ± 0.3*	7 ± 1*	3 ± 1*
K^+ 20 mM	52.0 ± 2.0	168 ± 9	0
K^+ 20 mM + Ado 1 mM	9.0 ± 5.0*	16 ± 8*	15 ± 7*
K^+ 20 mM + Ado 10 mM	1.9 ± 0.2*	9 ± 2*	4 ± 1*

Legend: Time to cardiac arrest, arrest and escape beats recorded in the different treatment groups in the Langendorff mode

* $p < 0.001$, modified t-test, compared to K^+ 20 mM

TABLE 4.2. CARDIAC ARREST IN WORKING HEART PERFUSION

Cardioplegia (n = 7)	Time to arrest (seconds)	Arrest beats	Escape beats
Krebs (control)	446 ± 39	640 ± 107	38 ± 9
Adenosine 10 mM	7 ± 1*	15 ± 2*	31 ± 7*
K^+ 20 mM	63 ± 12	125 ± 27	37 ± 24

Legend: Time to cardiac arrest, arrest and escape beats recorded in three different treatment groups in the working heart mode

* $p < 0.05$, t-test, compared to K^+ 20 mM

4.2. Short-term global ischemia

Adenosine as cardioplegic agent and as cardioplegic additive

Rationale

As the first study showed significantly shorter times for cardiac arrest induction in the adenosine treated hearts, we wanted to test the effect of this accelerated electromechanical arrest on hemodynamic recovery after short term global ischemia.

Experimental procedures, materials and methods

The experimental procedure used is already described in chapter 2.1 and 4.1. Additionally, following infusion of cold (10°C) cardioplegia at 2 ml/min for 3 minutes, the cardioplegic solution was washed out with Krebs-Henseleit solution at 2 ml/min for 3 minutes, in the groups indicated. Subsequently, a period of 30 min of global ischemia at 10°C was started. Reperfusion was initiated with the Langendorff-perfusion for a 10 minutes equilibration period followed by 10 minutes in the working

heart mode. Functional recovery was assessed by the recovery of aortic flow and the heart rate-systolic (mean) pressure-product expressed as a percentage of the preischemic value.

Recovery

30 min of ischemia at 10 °C

During the initial experiments adenosine cardioplegic solution (10 mM) was infused prior to ischemia in the usual way. These hearts did not improve their recovery significantly. Therefore, the adenosine was washed out immediately after infusion during the following experiments with an equal amount of Krebs-Henseleit buffer. Using this technique two out of three parameters determined for cardiac recovery after ischemia showed a maximum in the adenosine group (10 mM). The aortic output was 91.1 % while the heart rate-systolic pressure-product exceeded 100 %. Recovery in potassium treated hearts was always less compared to adenosine, but the differences did not reach significance. The mixture of potassium and adenosine showed no constant relation to either group. While the aortic output showed a paramount value (93.4 %), the heart rate-systolic pressure-product was only 86.6 % (Table 4.3.).

TABLE 4.3.: RECOVERY AFTER 30 MIN OF ISCHEMIA AT 10 °C

Group (n = 7)	Aortic output %	HR x syst. P. %	HR x mean P. %
Krebs	49.4 ± 14.2	72.6 ± 18.8	45.0 ± 11.2
Adenosine 10 mM	64.6 ± 15.9	72.7 ± 17.1	71.4 ± 16.3
Krebs◆	84.6 ± 3.0 *	95.7 ± 3.2*	87,6 ± 5.4
Ado 10 mM◆	91.1 ± 4.6*	103.0 ± 4.4*	98.0 ± 8.4
K^+ 20 mM◆	89.0 ± 5.8	97.1 ± 6.8	93.5 ± 15.5
Ado 10 + K^+ 20◆	93.4 ± 2.7	86.6 ± 4.3	96.1 ± 9.2

Legend: Hemodynamic recovery in percent of preischemic values after 30 min of global hypothermic ischemia in the different treatment groups with and without washout of adenosine.

◆ Cardioplegia wash-out right after infusion with Krebs Henseleit buffer
* $p < 0.01$, t-test, compared non-washout to washout

Energy status

After cardioplegia, before ischemia

The comparison of ATP stores showed no significant difference. All mean values ranged within the interval of 3.8 and 4.5 µmol/g wet weight, which is nearly identical with the pre-ischemic ATP energy storage of 4.1 ± 0.2 µmol/g wet weight. Phosphocreatine levels showed higher nucleoside contents for 1 and 10 mM adenosine (7.5 ± 0.7/7.1 ± 0.2 µmol/g wet weight) compared with the pre-ischemic energy status of 6.6 ± 0.3 µmol/g wet weight, whereas energy stores after potassium cardioplegia were depressed to 6.0 ± 0.3 µmol/g wet weight (Table 4.4.).

TABLE 4.4. ENERGY STATUS IMMEDIATELY AFTER CARDIOPLEGIA INFUSION IN LANGENDORFF EXPERIMENTS PRIOR TO ISCHEMIA

Group (n = 7)	ATP	PCr
Pre-cardioplegia	4.1 ± 0.2	6.6 ± 0.3
Adenosine 10 mM	4.0 ± 0.1	7.1 ± 0.2*
Adenosine 1 mM	4.5 ± 0.2	7.5 ± 0.7*
K^+ 20 mM	4.2 ± 0.1	6.0 ± 0.3
Ado. 10 mM + K^+ 20 mM	3.8 ± 0.4	6.5 ± 0.2

Legend: High energy phosphate content of the myocardium at control stage (pre-cardioplegia) and immediately after infusion of cardioplegia. Units: μmol/g wet weight

* $p < 0.05$, mod. t-test, compared to K^+ 20 mM

30 minutes of ischemia and 20 minutes of reperfusion

Out of all cardioplegic groups adenosine (10 mM) cardioplegia showed maximal high energy phosphates. ATP stores were 4.2 ± 0.1 μmol/g wet weight for adenosine; this is better than for potassium (3.8 ± 0.2 μmol/g wet weight). The potassium cardioplegia supplemented with adenosine showed even less ATP compared with potassium alone (3.3 ± 0.2 μmol/g wet weight) (Table 4.5.).

TABLE 4.5.: ENERGY STATUS AFTER 30 MINUTES OF ISCHEMIA AND 20 MINUTES OF REPERFUSION

Group (n = 7)	ATP	PCr
Krebs (KHB)	3.1 ± 0.2	4.1 ± 0.4
Adenosine 10 mM	3.3 ± 0.2	5.6 ± 0.5
Krebs◆	4.2 ± 0.1*	5.0 ± 0.4*
Ado 10 mM◆	4.2 ± 0.1*	5.4 ± 0.2
K^+ 20 mM◆	3.8 ± 0.2	4.6 ± 0.7**
Ado 10 + K^+ 20 mM◆	3.3 ± 0.2	4.0 ± 0.3**

Legend: High energy phosphate content after 30 min of global hypothermic ischemia and 20 min of reperfusion in the different treatment groups.
◆ Cardioplegia wash-out immediately after infusion with 10 ml of Krebs buffer
Units: µmol/g wet weight
* $p < 0.05$, mod. t-test, compared to KHB
** $p < 0.05$, mod. t-test, compared to KHB + washout

The same findings were observed for phosphocreatine levels. Maximal stores were identified for the adenosine group (5.4 ± 0.2 µmol/g wet weight), while potassium cardioplegia (4.6 ± 0.7 µmol/g wet weight) was superior to the admixture of adenosine and potassium (4.0 ± 0.3 µmol/g wet weight).

4.3. Medium-term global ischemia

Adenosine as cardioplegic agent and as cardioplegic additive

Rationale

Since we could only show a trend for improved recovery in the adenosine treated hearts after 30 minutes of global ischemia, we extended the time period of ischemia to 90 minutes in order to worsen the ischemic insult.

Experimental procedures, materials and methods

The experimental procedure has been described in the previous chapter. The only modification was the extension of the global ischemic period up to 90 minutes in this set of experiments.

Results

90 min of ischemia at 10 °C

As in the experiments comprising 30 minutes of ischemia, adenosine (10 mM) cardioplegia showed superior recovery. The heart rate-systolic pressure product was calculated as 92.7 % for the adenosine group while the same parameters for the potassium and the potassium-adenosine mixture were 88.0 % and 88.7 % respectively. Aortic output was evaluated as 83.9 % for the adenosine cardioplegia while all other groups hardly reached the 75 % recovery level (Table 4.6.).

TABLE 4.6.: RECOVERY AFTER 90 MIN OF ISCHEMIA AT 10 °C

Group (n = 7)	Aortic output %	HR x syst. P. %	HR x mean P. %
Krebs◆	48.0 ± 1.4 *	78.3 ± 4.2*	68.8 ± 7.2
Ado 10 mM◆	83.9 ± 1.3*	92.7 ± 2.5**	85.5 ± 4.1**
K^+ 20 mM◆	75.1 ± 2.5	88.0 ± 4.4	84.2 ± 6.1
Ado 10 + K^+ 20 mM◆	70.6 ± 3.9	88.7 ± 3.0	79.0 ± 4.7

Legend: Hemodynamic recovery in % of preischemic values after 90 min of global hypothermic ischemia in the different treatment groups.

◆ Cardioplegia wash-out immediately after infusion with of Krebs Henseleit buffer
* $p < 0.05$, mod. t-test, compared to K^+ 20 mM
** $p < 0.05$, mod. t-test, compared to KHB

Summary of findings

Adenosine is an intermediate metabolite in many important biochemical pathways. This study was designed to investigate the feasibility of preserving myocardial function by the cardioprotective properties of adenosine.

Male Sprague-Dawley rats, weighing 250 to 350 g, were isolated and attached to a Langendorff perfusion system. After conversion to the working heart mode various cardioplegic agents (adenosine 1 and 10 mM, potassium 20 mM, combination of both) were infused. Following 30/90 min of hypothermic (10°C) global ischemia, Langendorff and working heart perfusion were started again and hemodynamic recovery as well as tissue analyses of ATP and phosphocreatine (PCr) were determined.

Compared with potassium cardioplegia, adenosine was found to arrest the hearts more rapidly, i.e. ventricular function stopped within a few seconds. Hemodynamic parameters after ischemia (30/90 min) at 10 °C showed superior recovery for

adenosine 10 mM. The mixture of potassium (20 mM) and adenosine (10 mM) demonstrated no improvement in hemodynamic recovery. Tissue analysis prior to ischemia showed no increase of ATP contents, but of PCr levels in the adenosine groups (1 and 10 mM). After 30 min of ischemia at 10°C ATP and PCr were best preserved in the adenosine group, compared with potassium cardioplegia, but these values did not reach statistical significance.

We conclude that adenosine cardioplegia (10 mM) is suitable and safe for preserving myocardial function during induced global ischemia. However, wash out of adenosine was necessary to demonstrate this effect. The combination of potassium and adenosine however, is less effective than expected.

CHAPTER 5

STUDIES IN THE IN-VIVO BABOON MODEL

5.1. Comparison of cardioplegic solutions used in South Africa

Rationale

The primary function of a cardioplegic solution is to halt all myocardial electromechanical activity and thereby conserve energy (Hearse, 1983; Lell, 1983). This is achieved by manipulating the extracellular concentrations of the major cations (sodium, potassium, magnesium, calcium). Myocardial hypothermia, attained by infusing the solution into the coronary arteries at a temperature of 4°C, lowers energy demands still further (Lell, 1983). Since the use of a hyperkalemic cardioplegic solution by Melrose in 1955 (Melrose, 1955), ever increasing numbers of formulations are being used experimentally and clinically, thus complicating the objective comparison of solutions and reportedly beneficial additives. A vast amount of basic research has, however, shown the complex interrelationship of all components in a cardioplegic solution and highlighted the need for careful experimental assessment before any alterations are made to existing solutions.

In 1988 a questionnaire was sent to all perfusion technologists registered with the South African Society of Cardiovascular Perfusion Technology regarding the cardioplegic solution used by their unit. Eighty percent of the questionnaires were returned, and this revealed that three hospitals performing cardiac surgery in South Africa were using a form of blood cardioplegia whilst a further seven employed various formulations of crystalloid cardioplegia. The solution prepared by SABAX (SABAX Ltd., Samuel Evans Road, Aeraton, Johannesburg) was used by three units, a basic solution of Plasmalyte B plus 30mmol potassium chloride with various additives was employed by a further three, and only Groote Schuur Hospital was at

that time using a modified Bretschneider solution made up by the hospital dispensary (GSH). The composition of each of these solutions is tabulated in Table 5.I. In principle the St. Thomas' Hospital solution No. 2 relies on a high potassium, high magnesium system; the GSH solution on a high potassium, low sodium system and the SABAX solution on a high potassium composition.

Most of these clinically used cardioplegic solutions have, however, never been adequately or objectively evaluated, either experimentally or clinically. This study was therefore undertaken to compare the efficacy of two crystalloid cardioplegic solutions used in this country with an internationally accepted and extensively investigated solution, viz. St. Thomas' Hospital cardioplegic solution No. 2 (ST2) (Jynge, 1980; Jynge, 1981; Robinson, 1984; Ledingham, 1987). Additionally, a solution containing normal extracellular concentrations of the major cations, and therefore unable to induce chemical cardiac arrest, was employed as a control in order to highlight the effects of cardioplegic solutions as opposed to the beneficial effects of hypothermia. The physiologic saline solution, Krebs-Henseleit buffer (KHB), was selected as the control non-cardioplegic solution (Table 5.I).

The ST2 cardioplegic solution used in this study is the improved St. Thomas' Hospital solution No. 2 (Plegisol: Abbott Laboratories, North Chicago, Illinois) and not the original St. Thomas' Hospital solution No. 1 (Mac Carthy) (Ledingham,1987). This solution has previously been shown in the rat heart to be superior to other solutions such as Ringer-lactate solution with added potassium and balanced saline solution with glucose and potassium (Robinson,1984).

In contrast, SABAX cardioplegia, although used clinically in South Africa, has neither been reported to have been experimentally evaluated nor clinically compared to other attested solutions.

The GSH cardioplegic solution was originally based on the initial Bretschneider's solution which was formulated with an "intracellular" concentration of electrolytes, specifically sodium (Jynge, 1978). Over the years our GSH solution was altered without any reported experimental or clinical trials, resulting in the composition

indicated in Table 5.1. The Bretschneider's HTK-4 solution in clinical use in Europe today differs greatly from both our GSH solution and the original Bretschneider solution.

Experimental procedures, materials and methods

Materials and methods used are described in detail in chapter 2.2.

Crystalloid cardioplegic solutions tested

The composition of the tested solutions is given in table 5.1. The ST2 cardioplegic solution and the control non-cardioplegic solution KHB were prepared in the laboratory and filtered through a 0,8 µm filter to remove any bacteria and particulate matter. The SABAX solution was kindly donated by SABAX Ltd., South Africa. The GSH solution was the standard solution made by the hospital pharmacy at Groote Schuur Hospital.

Groups : Four different solutions were tested : Group ST2: St. Thomas' Hospital solution No.2 (n=8) , SBX: Sabax cardioplegic solution (n=7), GSH: Groote Schuur Hospital cardioplegic solution (n=7), KHB: control group treated with Krebs-Henseleit-buffer solution (n=7). Plasmalyte B was not evaluated in this study.

TABLE 5.1: CRYSTALLOID CARDIOPLEGIC SOLUTIONS USED IN SOUTH AFRICA (mM/L)

	Na	K	Ca	Mg	HCO_3	Glucose	Mannitol	Osmolarity
ST2	120	16	1.2	16	10	---	---	±300
SABAX	141	24/12**	0.9	1.5	38	51	68	±450/432**
GSH	15	20	1.0	12	15	240	14	±350
PI B(1)	140	34	---	1.5	37	56	---	±400
PI B(2)	130	34/14*	±0.4*	1.5	27	±4*	---	±330
PI B(3)	130	34	---	1.5	27	---	---	±370
KHB-buffer	143	5.9	1.25	1.2	25	11	---	±300

Legend: ST2 - St. Thomas' Hospital cardioplegic solution No. 2
SABAX - Cardioplegic solution available from SABAX Limited, Aeroton, Johannesburg
GSH - Cardioplegic solution previously used at Groote Schuur Hospital, Cape Town
PI B - Cardioplegic solution made up by the addition of 30 mM KCl, and additional sodium bicarbonate to 1 liter of Plasmalyte B solution in PI B(1)
KHB - Krebs-Henseleit physiological buffer solution used as a non-cardioplegic control solution

* - The maintenance cardioplegia has only 10 mM KCl added in addition to 200 ml pump blood per 800 ml crystalloid solution. This would thus change the solution from a calcium-free solution to one with an approximate calcium content of 0.4 mM/L and approximate glucose concentration of 4 mM/L.

** - In the maintenance cardioplegia the potassium concentration is diminished to 12 mM/L.

Results

The myocardial hypothermia produced by the above experimental protocol was uniform in all animals. Ventricular septal temperature consistently fell to 10°C with each infusion of cardioplegic solution. The myocardium then rewarmed to approximately 20°C in the 30 minutes between each reinfusion.

Hemodynamic recovery

Since there were no differences between hemodynamic recovery at 5 minutes and at 30 minutes, as determined by two-way analysis of variances, only the functional recovery at 30 minutes is presented in Table 5.2.

Functional recovery as assessed by SVI in the ST2 group was 113.9% $\pm$ 8.3, which was superior to all the other solutions: SABAX 81.4% $\pm$ 7.8 ($p \leq 0.05$); GSH 80.2% $\pm$ 6.0 ($p \leq 0.05$) and KHB buffer 55.6% $\pm$ 10.0 ($p \leq 0.01$). The recovery of CI and SWI of the ST2 treated animals, 95% $\pm$ 5.8 and 97.1% $\pm$ 12.7 respectively, was significantly improved when compared to GSH (73.6% $\pm$ 3.0, 51.9% $\pm$ 2.5) ($p < 0.05$) and KHB (59.9% $\pm$ 9.9 ($p < 0.05$), 34.9% $\pm$ 9.2, ($p < 0.01$)). However, CI and SWI for ST2, although higher than SABAX treated animals (84.3% $\pm$ 5.8, 71.7% $\pm$ 14.0), was not statistically different.

However, when comparing the functional recovery (SVI, CI, SWI) of SABAX to GSH cardioplegia or KHB buffer, hearts treated with SABAX cardioplegia were not superior to either the KHB control solution or the hearts perfused with the GSH cardioplegic solution.

TABLE 5.2: HEMODYNAMIC RECOVERY (PERCENTAGE OF PRE-ISCHEMIC CONTROL) OF BABOON HEARTS AT 30 MINUTES AFTER RELEASE OF AORTIC CROSS-CLAMP

n		CI	SVI	SWI	LVdP/Dt
ST2	8	94.75 5.81	113.95 ± 8.30	97.14 ±12.68	87.00 ±12.39
SBX	7	84.30 ± 5.79	81.35 ± 7.84*	71.67 ±14.03	83.96 ±10.22
GSH	7	73.58 ± 3.01*	80.21 ± 6.02*	51.91 ± 2.52*	71.43 ± 6.49
KHB	7	59.86 ± 9.93*	55.61 ±10.00**	34.98 ± 9.24**	54.72 ± 6.82

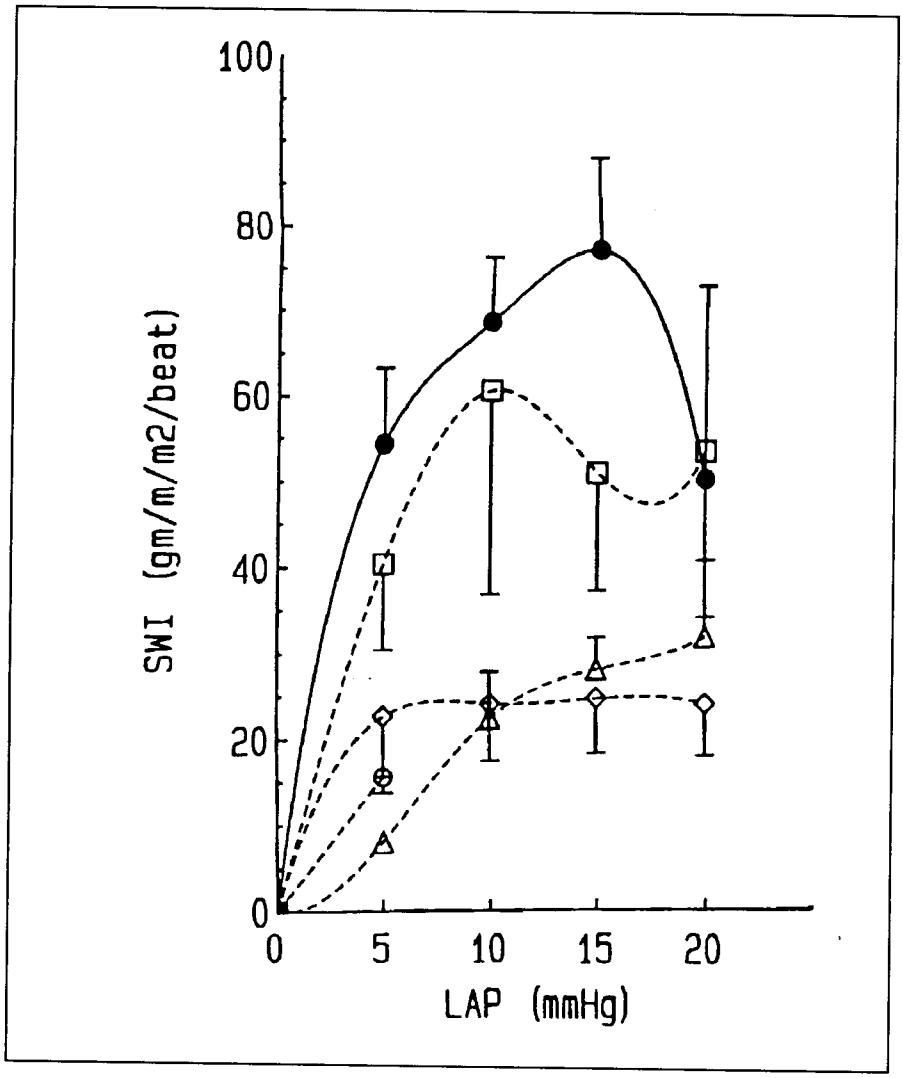
Legend: ST2 - St. Thomas' Hospital cardioplegic solution No. 2
SBX - Cardioplegic solution obtainable from SABAX Limited (Aeroton, Johannesburg)
GSH - Cardioplegic solution previously used at Groote Schuur Hospital, Cape Town
KHB - Krebs-Henseleit non-cardioplegic physiological solution

The percentage recovery of Cardiac Index (CI), Stroke Volume Index (SVI), first derivative of left ventricular developed pressure (LV dp/dt) and Stroke Work Index (SWI) compared to the pre-ischemic control, were obtained at 30 minutes after termination of cardiopulmonary bypass at a left atrial pressure of 5mmHg, following a 3-hour ischemic period. The hearts were protected with the different solutions delivered every 30 minutes at 4°C during this period in addition to topical cold saline.

* $p \leq 0.05$ vs ST2
** $p \leq 0.01$ vs ST2

Left ventricular (LV) function curves were determined at the end of the experimental protocol in some animals. The SWI was measured at different preloads by varying the left atrial pressure between 5 mmHg and 20 mmHg (Figure 5.1.). The post-ischemic LV function curve of hearts perfused with ST2 cardioplegia approximated to the basal pre-ischemic LV function curve measured in some animals prior to the 3-hour ischemic period. This finding was in contrast to the depressed post-ischemic curve for hearts protected with GSH cardioplegia or KHB buffer. This technique was not available for the animals treated with SABAX-solution due to technical reasons.

FIGURE 5.1.: POST-ISCHEMIC LEFT VENTRICULAR FUNCTION CURVES



Legend: Post-ischemic LV function of hearts protected during a 3-hour ischemic period with St. Thomas Hospital cardioplegic solution No. 2 (-□-), Groote Schuur Hospital cardioplegia (-△-), or Krebs-Henseleit non-cardioplegic physiological buffer (-❖-) compared with a pre-ischemic control LV function curve (-● -). LV function assessed as stroke work index (SWI) was measured at 30 minutes after termination of cardiopulmonary bypass at a varying left atrial pressure (LAP). For hearts treated with SABAX solution (-○-) SWI was only measured at LAP of 5 mmHg. Values are expressed as means (g/min/m^2/beat) and standard error of the means.

Recovery of high energy phosphates

The ATP and CP content of all hearts decreased during the 3-hour ischemic period despite maintenance doses of cardioplegic solution (Table 5.3). This loss of ATP was not different between the various groups. However, after releasing the aortic cross clamp there was a marked post-ischemic recovery of high energy phosphate levels. The ATP content 5 minutes post-cardiopulmonary bypass for hearts treated with ST2 was slightly better at 3.09 $\pm$ 0.45 (µmol/g wet weight) in comparison to those perfused with SABAX (2.58 $\pm$ 0.5), GSH (2.77 $\pm$ 0.5) and KHB (2.23 $\pm$ 0.34), but not statistically different . Also at 30 minutes post-bypass there was no difference in the ATP content between any of the solutions (Table 5.3a.).

The CP levels at 5 minutes and 30 minutes post-bypass were similar in all groups. Levels in the ST2 treated hearts (6.8 $\pm$ 0.8 (5min) / 6.l $\pm$ 4.0 (30 min) (µmol/g)) showed no statistical difference compared to hearts perfused with SABAX (4.9 $\pm$ 0.7 / 3.9 $\pm$ 0.7) and GSH solution (4.6 $\pm$ 1.0 / 5.2 $\pm$ 1.0). In addition, at 5 and 30 minutes, the CP content of the non-cardioplegic KHB-treated hearts was not lower than with any of the cardioplegic solutions (Table 5.3b.).

TABLE 5.3. HIGH ENERGY PHOSPHATE PRESERVATION

TABLE 5.3a.: ATP (μMOL/G WET WEIGHT)

	Control	ACC	5'	30'
ST2	4.08 ± 0.49 (5)	2.68 ± 0.23 (5)	3.09 ± 0.45 (5)	2.57 ± 0.33 (5)
SBX	3.78 ± 0.50 (5)	2.98 ± 0.45 (5)	2.58 ± 0.51 (5)	2.40 ± 0.20 (5)
GSH	3.75 ± 0.35 (7)	3.24 ± 0.45 (7)	2.77 ± 0.49 (7)	2.86 ± 0.53 (4)
KHB	3.89 ± 0.28 (7)	1.92 ± 0.21 (5)	2.23 ± 0.34 (7)	2.11 ± 0.54 (7)

TABLE 5.3b.: CP (μMOL/G WET WEIGHT)

	Control	ACC	5'	30'
ST2	6.59 ± 0.97 (5)	1.98 ± 0.53 (5)	6.78 ± 0.80 (5)	6.10 ± 0.36 (3)
SBX	7.32 ± 0.84 (5)	2.50 ± 0.74 (5)	4.88 ± 0.67 (5)	3.90 ± 0.74 (5)
GSH	7.18 ± 0.65 (7)	1.43 ± 0.44 (7)	4.61 ± 0.96 (7)	5.21 ± 0.96 (4)
KHB	6.81 ± 0.46 (7)	0.49 ± 0.27 (5)	5.69 ± 0.88 (7)	5.76 ± 0.52 (7)

Legend: Adenosine Triphosphate (ATP)(Table-5.3a.) and Creatine Phosphate (CP) (Table-5.3b.) content expressed as a mean μmoles/g wet weight of myocardial tissue and standard error of the mean. Measurements were taken prior to commencement of cardiopulmonary bypass (control), prior to the release of the aortic cross clamp (ACC) after a 3-hour ischemic period, at 5 minutes and at 30 minutes after termination of cardiopulmonary bypass. The number of hearts (n) for each mean is given in parentheses.

Ultrastructure

Only a limited number of samples have been assessed so far. All groups showed mild to moderate changes in the subendocardial region after 3 hours of ischemia (Figure 5.2a.); these were partially reversible after reperfusion (Table 5.4.). An example of the changes at the end of ischemia is shown in Figure 5.2a. and the ultrastructural appearance after one hour of reperfusion in Figure 5.2b..

TABLE 5.4.: ULTRASTRUCTURAL PRESERVATION OF MYOCARDIUM BEFORE AND AFTER PROTECTION WITH CARDIOPLEGIC SOLUTIONS USED IN SOUTH AFRICA

Time	Group	Cristae	Dense granules	Glycogen Loss
PRI	ST2 (n=2)	0 0	0 0	0 0
PRI	GSH (n=2)	0 0	1 0	0 1
PRI	SBX (n=2)	0 0	1 0	0 0
EOI	ST2 (n=2)	2 1	1 1	3 0
EOI	GSH (n=2)	2 1	2 1	1 0
EOI	SBX (n=2)	3 1	1 1	0 0
RPS	ST2 (n=2)	1 1	1 1	0 0
RPS	GSH (n=2)	0 1	1 0	0 0
RPS	SBX (n=2)	0 0	2 1	0 0

Legend: The myocardial biopsy samples from the left ventricular apical area were scored according to a semiquantitative scoring system: 0 = normal, 1 = mild abnormality, 2 = moderate abnormality, 3 = severe abnormality. PRI: Preischemia, EOI: End of ischemia (180 min), RPS: After 1hour of reperfusion.

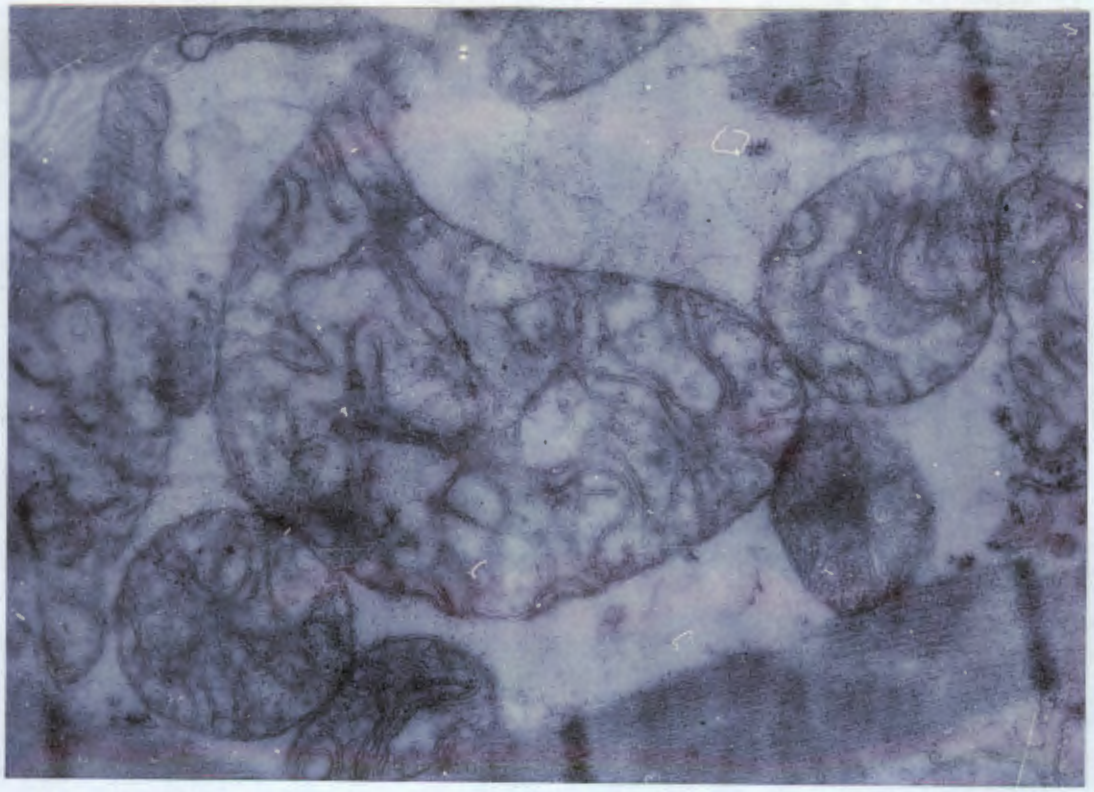


Figure 5.2.a Ultramicroscopical appearance of portion of a myocyte from a baboon heart after perfusion with Sabax-CP solution following an ischemic crossclamp period of 180 minutes. There is evidence of swelling of the mitochondria with an abnormal crystal arrangement and intracytoplasmic edema.
(Electron photomicrograph x 51000).

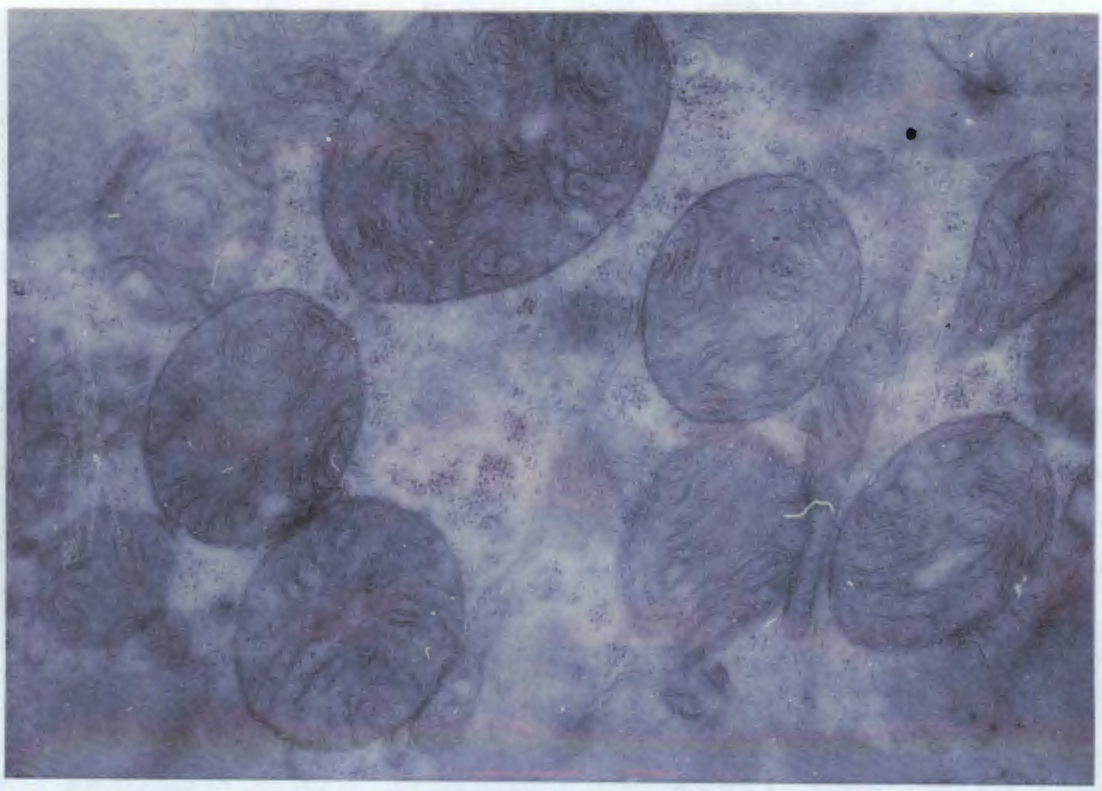


Figure 5.2.b Relatively normal appearance of mitochondria is evident in this myocyte from a baboon heart which has undergone 3 hours cardiac arrest and perfusion with Sabax-CP solution followed by 1 hour reperfusion with blood.
(Electron photomicrograph x 51000).

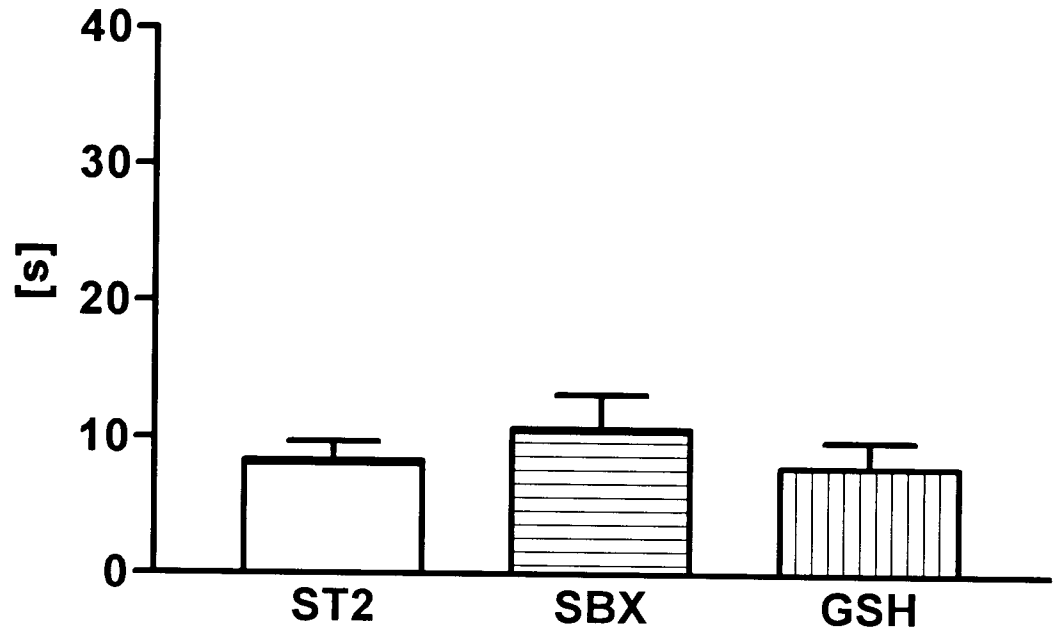
Comparison of time to induce cardiac arrest and refunction time

Comparison of the time intervals taken by each solution to induce complete cardiac arrest revealed no statistical difference, and all solutions stopped electromechanical activity within 8-11 seconds (ST2: 8.3 $\pm$ 1.4 seconds, SABAX: 10.7 $\pm$ 2.5 seconds and GSH: 7.9 $\pm$ 1.9 seconds) (N.S.) (Figure 5.3.).The average time to wean the animals off CPB, which is also referred to as "refunction time" and which is considered to be an index of myocardial protection (Sondergaard, 1967), was longest for GSH with 9.4 $\pm$ 3.9 minutes. Animals in the SABAX group had a mean refunction time of 6.9 $\pm$ 1.8 minutes compared to 4.6 $\pm$ 1.3 minutes in the ST2 group (Figure 5.4.).

Defibrillation energy

Additionally the average energy required to cardiovert the hearts back to sinus rhythm post-ischemia (defibrillation energy) was highest in the SABAX group (85.7 $\pm$ 23.8 joules) ($p \leq 0.05$ vs ST2), whereas animals in the GSH and ST2 groups required 34.3 $\pm$ 15.9 and 38.8 $\pm$ 6.7 joules respectively (Figure 5.5.).

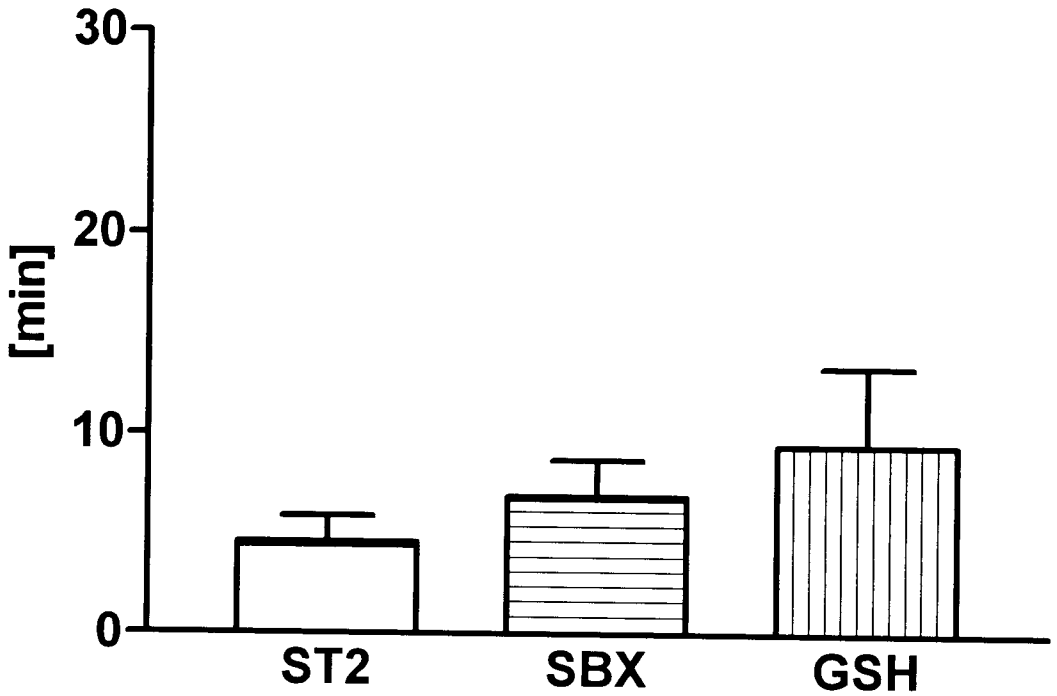
FIGURE 5.3.: COMPARRISON OF TIME TO INDUCE CARDIAC ARREST



Legend: ST2: St. Thomas' Hospital solution No. 2 (n=8)
SBX: Sabax cardioplegic solution (n=7)
GSH: Groote Schuur Hospital solution (n=7)

[s]: seconds

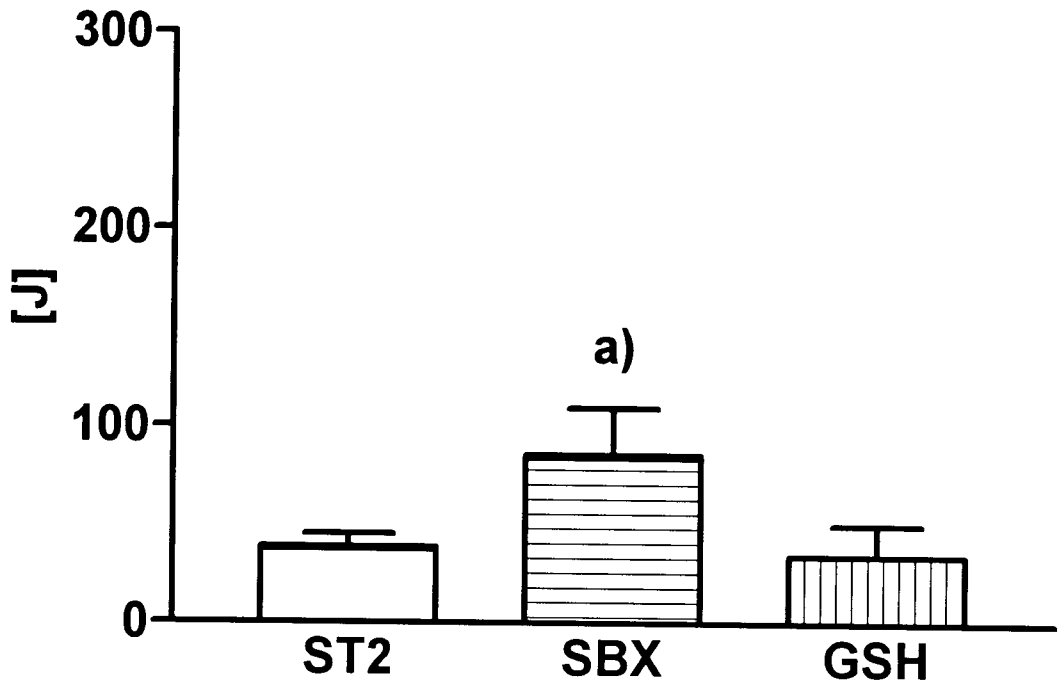
FIGURE 5.4.: COMPARISON OF REFUNCTION TIME



Legend: ST2: St. Thomas' Hospital solution No. 2 (n=8)
SBX: Sabax cardioplegic solution (n=7)
GSH: Groote Schuur Hospital solution (n=7)

[min]: minutes

FIGURE 5.5.: COMPARISON OF DEFIBRILLATION ENERGY



Legend: ST2: St. Thomas' Hospital solution No. 2 (n=8)
SBX: Sabax cardioplegic solution (n=7)
GSH: Groote Schuur Hospital solution (n=7)

a): $p \leq 0,05$ vs ST2
[J]: Joules

Electrocardiographic criteria

Transient ST-segment elevations in the post-ischemic ECG recordings were observed in all groups (4/7 cases for the GSH solution, 4/7 cases for the SBX solution and 4/8 cases for the ST2 solution). New Q waves were not discernible in any of the cardioplegic groups. All animals of the SBX treated group recovered in normal sinus rhythm after defibrillation. Of the hearts treated with GSH solution, one remained in atrial fibrillation, whereas in the group treated with ST2, one heart recovered with multiple unifocal ventricular extrasystoles for several minutes (Table 5.5.).

TABLE 5.5.: ECG CRITERIA POST ISCHEMIA

	GSH (n=7)	SBX (n=7)	ST2 (n=8)
ST-elevation	4	4	4
New Q-wave	0	0	0
NSR	4	7	6
Atrial Fibrillation	1	0	0
AV-Block	0	0	1
Ventricular ectopics	1	0	1

Legend: GSH: Groote Schuur Hospital solution; SBX: SABAX solution; ST2: St. Thomas' Hospital solution No. 2. NSR: Normal sinus rhythm. ST-segment elevation was transient in all animals and disappeared during the 30 minutes reperfusion phase. Atrial fibrillation observed in one animal in the GSH group was successfully cardioverted via direct countershock treatment. AV-block of 3rd degree recorded in one animal was gradually improved during reperfusion; at the end of 30 minutes reperfusion only 1st degree AV-block was present. Ventricular ectopics recorded in two animals were unifocal and subsided during the first ten minutes of reperfusion. Serum potassium concentrations were in the normal range.

Summary of findings

Cardioplegic solutions are routinely used to arrest and protect the myocardium during open heart surgery. Most of the hospitals performing cardiac surgery in South Africa employ various crystalloid cardioplegic solutions as opposed to blood cardioplegia. In this report we compare the locally manufactured cardioplegic solution (obtainable from Sabax Ltd.) and a modified Bretschneider's solution previously employed at Groote Schuur Hospital with the current international standard, the St. Thomas' Hospital cardioplegic solution No.2.

Chacma baboon hearts were subjected to a 3-hour in vivo ischemic period during which the myocardium was protected with each of these cardioplegic solutions. In this model, the post-ischemic functional recovery of hearts protected with the St. Thomas' Hospital cardioplegic solution No.2 was superior. There was a trend towards better maintenance of high energy phosphates in the hearts treated with St. Thomas' Hospital cardioplegic solution No. 2.

5.2. Adenosine as adjunct to a non-cardioplegic solution

Rationale

Cardioplegic solutions are routinely used to arrest and protect the myocardium during open-heart surgery. The common component of most cardioplegic solutions is a high concentration of potassium, which is intended to induce fast electromechanical arrest by depolarization of membranes (Buckberg, 1987). Membrane depolarization during ischemia has disadvantageous effects on energy metabolism and increases calcium influx. More recently, Ferguson (Ferguson, 1986) reported continuing low amplitude electrical activity during potassium cardioplegia. We therefore tested the hypothesis that adenosine, a high energy phosphate precursor which has been shown to induce fast cardiac arrest and to improve post-ischemic hemodynamic recovery in an isolated rat heart model (chapter 4) (Schubert, 1989; De Jong, 1990), might

1. act as cardioplegic agent by hyperpolarization of membranes.

2. improve post-ischemic hemodynamic recovery.

We employed an in vivo baboon model, closely resembling the clinical situation, to test this hypothesis.

Experimental procedures, materials and methods

Materials and methods are described in detail in chapter 2.2.
In order to determine the hemodynamic and high energy phosphate recovery after a prolonged period of global cardiac ischemia, Chacma baboons weighing 13-28 kg were subjected to three hours of cardiac arrest protected by three different solutions (Table 5.6.).

Group I (n=8) received St. Thomas Hospital solution No. 2 (ST2), Group II (n=7) Krebs-Henseleit Buffer (KHB) and Group III (n=8) was treated with 10 mM adenosine in KHB for induction and plain KHB for maintenance. Adenosine was dissolved in KHB for this purpose.

TABLE 5.6.: COMPOSITION OF SOLUTIONS TESTED.

mMl/L	ST2	ADO	KHB
Glucose		11.1	11.1
Adenosine		10	-
Na^+	120	133	133
K^+	16	6	6
Ca^{2+}	1.2	1.25	1.25
Mg^{2+}	16	1.2	1.2
HCO_3^-	10	25	25

Legend: ST2: St. Thomas' Hospital Solution No. 2
ADO: Adenosine (10mM) in Krebs-Henseleit Buffer
KHB: Krebs-Henseleit Buffer

Results

Comparison of time to induce cardiac arrest, refunction time and total defibrillation energy:

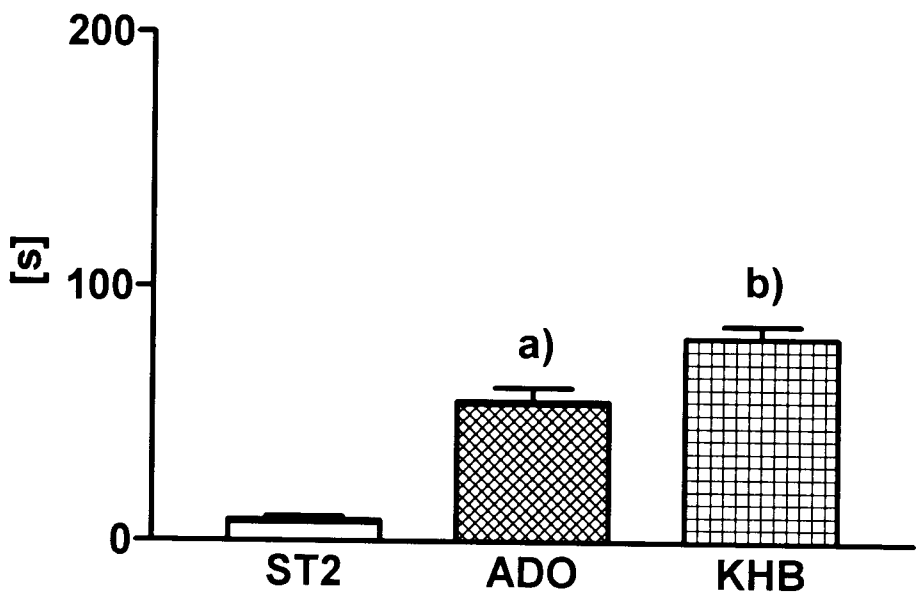
Comparison of the time intervals taken by each solution to induce complete arrest showed a shorter period for ST2 (8.3 ± 1.4 sec) when compared to ADO (55.0 ± 5.6 sec) ($p \leq 0.001$) and KHB (80.0 ± 5.4 sec) ($p \leq 0.001$) (Figure 5.7.).

The average time to wean the animals off CPB, which is also referred to as refunction time and which is considered to be an index of myocardial protection, was

longest for KHB treated animals (13.2 ± 3.7 min) in comparison to ST2 (4.6 ± 1.3 min) ($p < 0.05$) and ADO (5.1 ± 1.7 min) (N.S.) (Figure 5.8.).

Defibrillation energy required to reverse the hearts back to sinus rhythm postischemia was lowest for the ST2 treated groups (50,0 ± 15,0 joules) compared to KHB (250,0 ± 45,0 joules) ($p \leq 0.001$). ADO treated hearts required 140,07 ± 67,3 joules (Figure 5.9.).

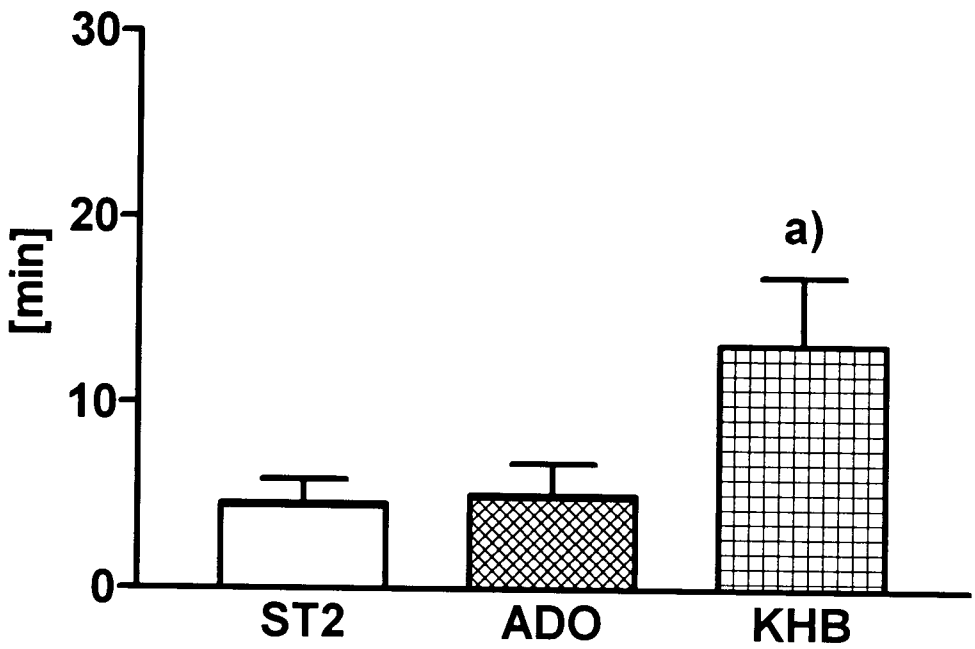
FIGURE 5.7.: COMPARISON OF TIME TO INDUCE CARDIAC ARREST



Legend: ST2: St. Thomas' Hospital solution No 2 (n=8)
ADO: Adenosine 10 mM in ST2 (n=8)
KHB: Krebs Henseleit Buffer (n=7)

a) $p \leq 0,05$ vs ST2
b) $p \leq 0,001$ vs ST2
[s]: seconds

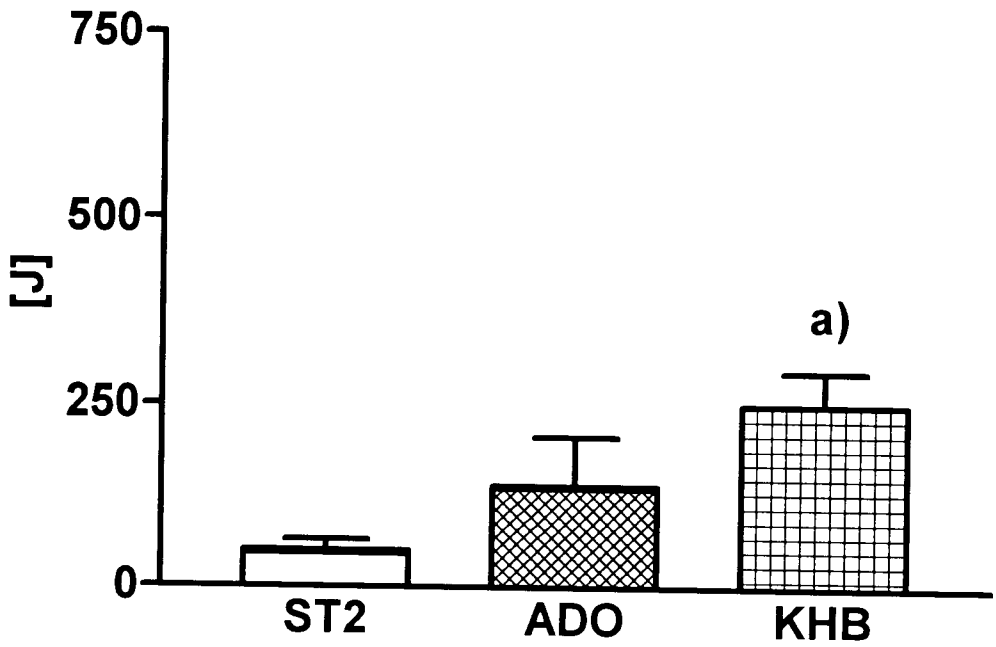
FIGURE 5.8.: COMPARISON OF REFUNCTION TIME



Legend: ST2: St. Thomas' Hospital solution No 2 (n=8)
ADO: Adenosine 10 mM in ST2 (n=8)
KHB: Krebs Henseleit Buffer (n=7)

a) $p \leq 0{,}001$ vs ST2
[min]: minutes

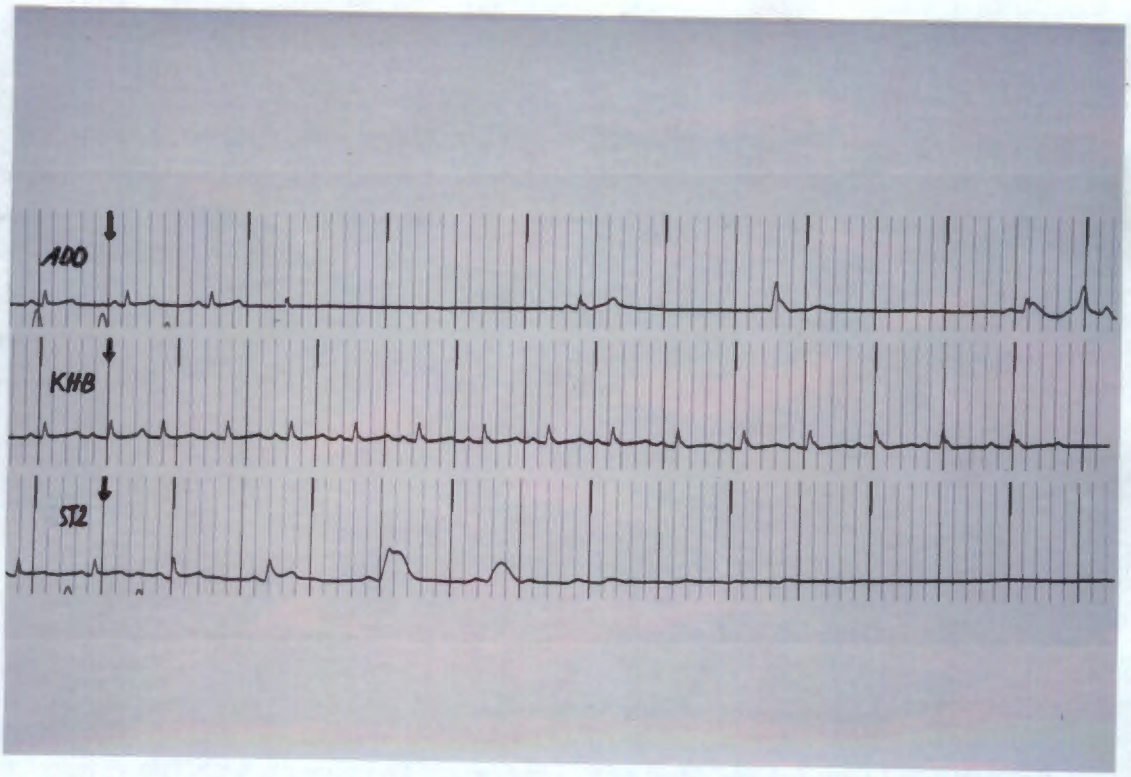
FIGURE 5.9.: COMPARISON OF DEFIBRILLATION ENERGY



Legend: ST2: St. Thomas' Hospital solution No 2 (n=8)
ADO: Adenosine 10 mM in ST2 (n=8)
KHB: Krebs Henseleit Buffer (n=7)

a) $p \leq 0{,}001$ vs ST2
[J]: Joules

FIGURE 5.10.: ECG RECORDING OF INDUCTION OF CARDIAC ARREST FOR THE THREE DIFFERENT TREATMENT GROUPS



Legend: ADO: adenosine 10 mM in KHB; KHB: Krebs Henseleit buffer ; ST2: St. Thomas' Hospital cardioplegia No. 2. Vertical arrows indicate the start of cardioplegic infusion; bold vertical lines indicate 1 second intervals. Fast electrical arrest is recorded with adenosine cardioplegia within 1.5 seconds with only two arrest beats. However, there are several escape beats in this recording and soon after termination of the registration the hearts regularly started with ventricular fibrillation. In the recording of an KHB treated animal unmodified electrical activity is seen until hypothermia causes arrest. High potassium solutions as ST2 cause fast arrest, within 6 seconds in this recording. After two normal beats, two deformed complexes are recorded and for about two seconds ongoing atrial electrical activity is present until complete arrest is achieved. No ventricular escape beats and no ventricular fibrillation are seen in hearts treated with ST2.

Hemodynamics

No differences between hemodynamic recovery at 5 and at 30 minutes within each group after termination of cardiopulmonary bypass were discernible by two way analysis of variance. Therefore, only the functional recovery at 30 minutes is presented (Table 5.6.).

Functional recovery 30 minutes after termination of CPB as assessed by CI and SVI was significantly better for the ST2 and ADO treated hearts than in the KHB control (Table 5.6). Left ventricular dP/dt was best for ST2 (87.0 ± 12.4 %) and ADO (73.1 ± 9.9 %) in comparison to KHB (54.7 ± 6.8 %) although this did not reach statistical significance (Table 5.6.).

High energy phosphate recovery

The ATP and CP content of all hearts decreased during the three hour ischemic period despite maintenance doses of cardioplegic solution. ATP was well preserved in the ADO group (103.5 % ± 21.1) in comparison to KHB (48.5 % ± 8.7) and ST2 (67.9 % ± 9.3), but not statistically different 30 minutes after termination of CPB (Table 5.6.). CP recovery was also not different between the three treatment groups at 30 minutes (Table 5.6.).

TABLE 5.6.: HEMODYNAMIC AND HIGH ENERGY PHOSPHATE RECOVERY 30 MINUTES AFTER WEANING OFF CPB (% RECOVERY OF PRE-ISCHEMIC CONTROL ± SEM).

CPS	n	CI	SVI	dP/dt	ATP	CP
ST2	8	94.8 (± 5.8) •	114.0 (± 8.3)°	87.0 (± 12.4)	67.9 (± 9.3)	103.0 (± 22.2)
KHB	7	59.9 (± 9.9)	55.6 (± 10.0)	54.7 (± 6.8)	48.5 (± 8.7)	88.7 (± 12.6)
ADO	8	91.6 (± 7.2)■	101.6 (± 8.9)°	73.1 (± 9.9)	103.5 (± 21.1)	86.1 (± 5.6)

Legend: ■: $p \leq 0.05$ vs KHB, •: $p \leq 0.025$ vs KHB, °: $p \leq 0.01$ vs KHB
CI = Cardiac Index, SVI = Stroke Volume Index, dP/dt = Left Ventricular pressure derivative, ATP = Adenosine triphosphate, CP = Creatine phosphate.

Ultrastructure

Initial analysis of cardiac ultrastructure showed mild to moderate changes in mitochondrial structure in all treatment groups at the end of ischemia. After reperfusion, only adenosine-treated hearts showed a normal ultrastructural appearance, whereas residual changes remained in the other groups (Table 5.7.).

TABLE 5.7.: ULTRASTRUCTURAL PRESERVATION OF MYOCARDIUM BEFORE AND AFTER PROTECTION WITH THREE DIFFERENT CARDIOPLEGIC SOLUTIONS

Time	Group	Cristae	Dense granules	Glycogen Loss
PRI	ST2 (n=2)	0 0	0 0	0 0
PRI	ADO (n=2)	0 0	1 0	0 0
PRI	KHB (n=2)	0 0	1 0	1 0
EOI	ST2 (n=2)	2 1	1 1	3 0
EOI	ADO (n=2)	0 0	0 1	0 0
EOI	KHB (n=2)	2 3	0 0	0 0
RPS	ST2 (n=2)	1 1	1 1	0 0
RPS	ADO (n=2)	0 0	0 1	0 0
RPS	KHB (n=2)	0 3	0 2	0 0

Legend: The myocardial biopsy samples from the left ventricular apical area were scored according to a semiquantitative scoring system: 0 = normal, 1 = mild abnormality, 2 = moderate abnormality, 3 = severe abnormality. PRI: Preischemia, EOI: End of ischemia (180 min), RPS: After 1 hour of reperfusion.

Summary of findings

Hyperkalemia-induced hypopolarization of the sarcolemmal membrane during standard crystalloid cardioplegic arrest potentiates calcium influx during ischemia and reperfusion and is associated with depletion of high energy phosphate reserves. Adenosine has been shown to induce rapid cardiac arrest whilst preserving membrane hyperpolarization in an isolated rat heart model (Schubert, 1989). In this study we compared the efficacy of adenosine, both as an arresting agent and as an ultrastructural, hemodynamic and high energy phosphate preserving agent, in an in situ global ischemia model in the baboon with St. Thomas' Hospital No. 2 solution (ST2)(n=8) and with Krebs-Henseleit buffer (KHB)(n=7). The addition of 10 mM/L adenosine to the non-cardioplegic KHB (ADO)(n=8) improved hemodynamic recovery significantly in terms of cardiac index (91.6% ± 7.2 vs 59.9% ± 9.9) and stroke volume index (101.6% ± 8.9 vs 55.6% ± 10.0) and was not statistically distinguishable from the ST2 with regard to cardiac index (91.6% ± 7.2 vs 94.8% ± 5.8), stroke volume index (101.6% ± 8.9 vs 114.0% ± 8.3) or left ventricular dP/dt (73.1% ± 9.9 vs 87.0% ± 12.4). However adenosine did not induce rapid complete electromechanical arrest in this model. Comparison of the time intervals taken by each solution to induce complete arrest showed a shorter period for ST2 (8.3 ± 1.4 sec) when compared to ADO (55.0 ± 5.6 sec) ($p \leq 0.001$) and KHB (80.0 ± 5.4 sec) ($p \leq 0.001$). ATP was best preserved with ADO (103.5% ± 21.1 vs 67.9% ± 9.3 and 48.5% ± 8.7) although this was not statistically significant. This suggests therefore that the mechanism of cardioprotection by adenosine depends on factors other than its role as high energy phosphate precursor or fast arresting agent.

5.3. Adenosine as adjunct to St. Thomas Hospital solution

Rationale

There are several studies in experimental models of myocardial ischemia showing the effect of exogenous adenosine on prevention of endogenous breakdown and on the preservation of ATP, but reports on functional recovery are few (Bolling, 1989; Bolling, 1990). This is especially important since recent evidence suggests no direct correlation between high energy phosphate content at the end of ischemia and functional recovery during reperfusion (Hohlfeld, 1989). Furthermore there has been only one report in an isolated rabbit heart model concerning the optimal concentration of adenosine (Bolling, 1990). Obviously the optimal dose will be important because in suboptimal concentration adenosine will be unable to exert its beneficial properties, whereas in an excessive concentration unwanted side effects, such as negative chronotropy or renal vasoconstriction will be more predominant (Belhassen, 1984; Spielman,1987).

All the above mentioned properties might render adenosine an ideal cardioplegic substance, but as we have previously shown (see chapter: Adenosine as adjunct to a non-cardioplegic solution), it does not act as a cardioplegic agent in an in-vivo baboon model via its inhibitory action on SA and AV conduction. This finding is at variance with an experimental study in the isolated rat heart (Schubert, 1989). But even while it failed to inhibit ventricular fibrillation, with adenosine as adjunct to a non-cardioplegic solution such as Krebs-Henseleit-Buffer (KHB) myocardial protection was significantly improved and was as effective as the protection achieved with St. Thomas' Hospital Cardioplegia No 2, whereas recovery with KHB alone was poor. We have previously shown (see chapter: Comparison of cardioplegic solutions used in South Africa), that compared with various crystalloid cardioplegic solutions St. Thomas' Hospital No.2 cardioplegia resulted in the best recovery in the same model.

The objective of the present study was to assess the potential benefits of adenosine on functional and metabolic recovery as an adjunct to a high potassium cardioplegic formulation such as the St. Thomas' solution No 2 and to optimize the dose. We employed an in-vivo baboon model closely resembling the clinical setting in hearts undergoing an extended period of hypothermic global ischemia.

Experimental procedures, materials and methods

Materials and methods are described in detail in chapter 2.2.

Treatment Groups

Group I (n=7) received St. Thomas' solution No. 2 (ST2), Group II (n=6) ST2 with adenosine 0.1 mM/L, Group III (n=9) ST2 with adenosine 1.0 mM/L and Group IV (n=4) ST2 with adenosine 10 mM/L.
Adenosine was added for induction cardioplegia as well as for maintenance cardioplegia, and the solution was infused every 30 minutes throughout the ischemic period.

Results

Hemodynamic recovery

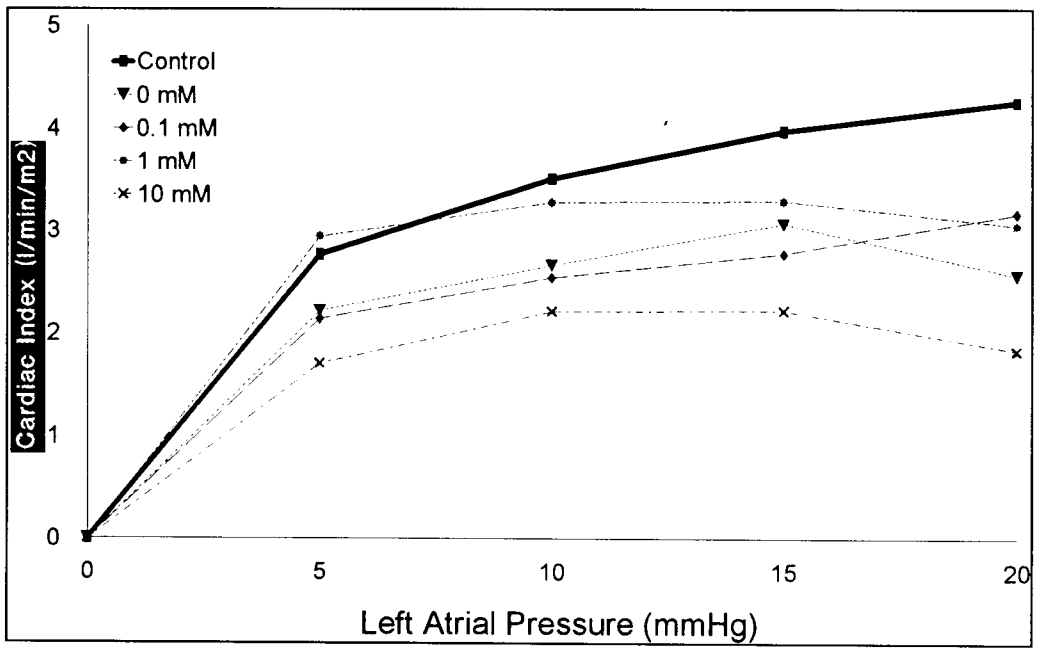
Mean hemodynamic recovery, as expressed by cardiac index, stroke volume index, stroke work index and minute work index, was best in hearts perfused with St. Thomas' Hospital cardioplegic solution No.2 modified with 1mM adenosine (Group III) (Table 5.8.). Recovery of these parameters was 100% or more in this group, whereas recovery was not so good in the remaining groups: Recovery of cardiac index was 115.6% ± 8.6% in Group III vs 87.5% ± 8.2% in Group I, 89.9% ±

8.75% in Group II and 62.9% ± 7.25% in Group IV. There was a similar distribution of recovery for the derived parameters stroke volume index (SVI) and stroke work index (SWI) (Table 5.8.). There were no changes in the above parameters when measured at 30 minutes compared to the 5 minute measurement (CI: 80.3% ± 5.7% (Group I), 77.4% ± 7.6% (Group II), 106.5% ± 10.5% (Group III) and 61.7% ± 9.3% (Group IV) (Table 5.8.).

Left ventricular dP/dt/DP recovery at 5 minutes was 111.4% ± 4.6% for group I, 101.0% ± 4.7% for group II, 100.4% ± 3.9% for group III and 95.8% ± 6.5% for group IV ($p < 0.05$ vs group I) (Table 5.8.).

Left ventricular function assessed by volume loading at 30 minutes revealed adequate recoveries for 1mM Ado and ST2 compared to depressed function obtained for 10mM/L Ado, whereas the hearts treated with ST2 and ST2 + 0.1mM/L Ado showed intermediate recovery (Figure 5.11).

FIGURE 5.11.: LEFT VENTRICULAR FUNCTION CURVES FOR THE DIFFERENT TREATMENT GROUPS



Legend: Normalized percentage recovery to an integrated preischemic control of the four different treatment groups (as described in chapter 2.2.). The group treated with 1mM adenosine approximates the preischemic control best. Inferior recovery is calculated for the group treated with 10mM adenosine, whereas the groups with 0mM and 0,1mM adenosine show intermediate recovery of ventricular function.

Calculated systemic vascular resistance indices (SVRI) showed statistical differences between group I and III at 5 minutes reperfusion : 112.0% ± 15% for group I compared to 73.0% ± 6.2% for group III ($p < 0.05$) (Table 5.8.).

TABLE 5.8.: HEMODYNAMIC RECOVERY

Parameter	Time	0 mM	0.1 mM	1 mM	10 mM	
Cardiac index ($l.min^{-1}.m^{-2}$)	**Control**	**3.15** ±0.50 n=7	**3.08** ±0.29 n=6	**2.17** ±0.17 n=9	**2.96** ±0.30 n=4	**N.S.**
% Recovery	**5 min**	87.50 ±8.18 n=6	89.92 ±8.75 n=6	115.63 ±8.57 n=9	62.90 ±7.25 n=4	a,b,c
% Recovery	**30 min**	80.34 ±5.68 n=7	77.42 ±7.58 n=6	106.49 ±10.52 n=9	61.69 ±9.34 n=4	d,e
Stroke Volume index ($ml.beat^{-1}.m^{-2}$)	**Control**	**27.19** ±4.38 n=7	**29.14** ±3.42 n=6	**20.10** ±1.93 n=9	**28.22** ±3.38 n=4	**N.S.**
% Recovery	**5 min**	86.65 ±9.00 n=6	79.89 ±4.70 n=6	102.23 ±6.72 n=9	68.65 ±7.78 n=4	c
% Recovery	**30 min**	86.96 ±9.76 n=7	77.58 ±5.18 n=6	107.12 ±11.38 n=9	69.16 ±16.50 n=4	d,e
Stroke Work index ($g.m.beat^{-1}.m^{-2}$)	**Control**	**40.07** ±7.42 n=7	**49.29** ±7.14 n=6	**29.51** ±4.29 n=9	**39.99** ±10.99 n=4	**N.S.**
% Recovery	**5 min**	80.16 ±7.95 n=6	67.16 ±6.21 n=6	99.87 ±15.05 n=9	50.89 ±10.64 n=4	c
% Recovery	**30 min**	83.05 ±6.81 n=7	62.08 ±4.60 n=6	105.40 ±17.09 n=9	54.45 ±17.04 n=4	c,d
Left Vent. dP/dt/DP (sec^{-1})	**Control**	**9.42** ±0.32 n=7	**9.97** ±0.58 n=6	**9.96** ±0.43 n=9	**8.98** ±0.48 n=4	**N.S.**
% Recovery	**5 min**	111.37 ±4.58 n=6	100.97 ±4.65 n=6	100.39 ±3.88 n=9	95.76 ±6.45 n=4	f
% Recovery	**30 min**	104.35 ±4.18 n=7	94.09 ±4.11 n=6	98.85 ±3.80 n=9	96.29 ±11.95 n=4	N.S.
Heart Rate ($beat.min^{-1}$)	**Control**	**117.36** ±5.19 n=7	**108.62** ±8.63 n=6	**110.67** ±6.14 n=9	**105.75** ±3.25 n=4	**N.S.**
% Recovery	**5 min**	102.42 ±5.99 n=6	112.46 ±7.79 n=6	113.76 ±5.26 n=9	92.35 ±6.48 n=4	c
% Recovery	**30 min**	96.01 ±6.27	100.00 ±7.05	101.03 ±4.55	96.24 ±16.46	N.S.
Systemic Vascular Resistance index ($Dynes.sec^{-1}$)	**Control**	**2514.31** ±534.45 n=7	**2650.86** ±303.35 n=6	**3142.61** ±125.96 n=8	**2740.42** ±138.03 n=4	N.S.
% Recovery	**5 min**	112.01 ±15.13 n=6	89.70 ±7.40 n=6	72.98 ±6.23 n=8	80.22 ±10.06 n=4	c
% Recovery	**30 min**	119.35 ±9.48 n=7	107.82 ±10.30 n=5	89.35 ±7.62 n=8	99.92 ±25.21 n=4	N.S.

Legend: a: $p<0.05$; 0mM vs 1mM c: $p<0.05$; 1mM vs 10mM e: $p<0.01$; 1mM vs 10mM
b: $p<0.05$; 0.1mM vs 10mM d: $p<0.05$; 0,1mM vs 1mM f: $p<0.05$; 0mM vs 10mM

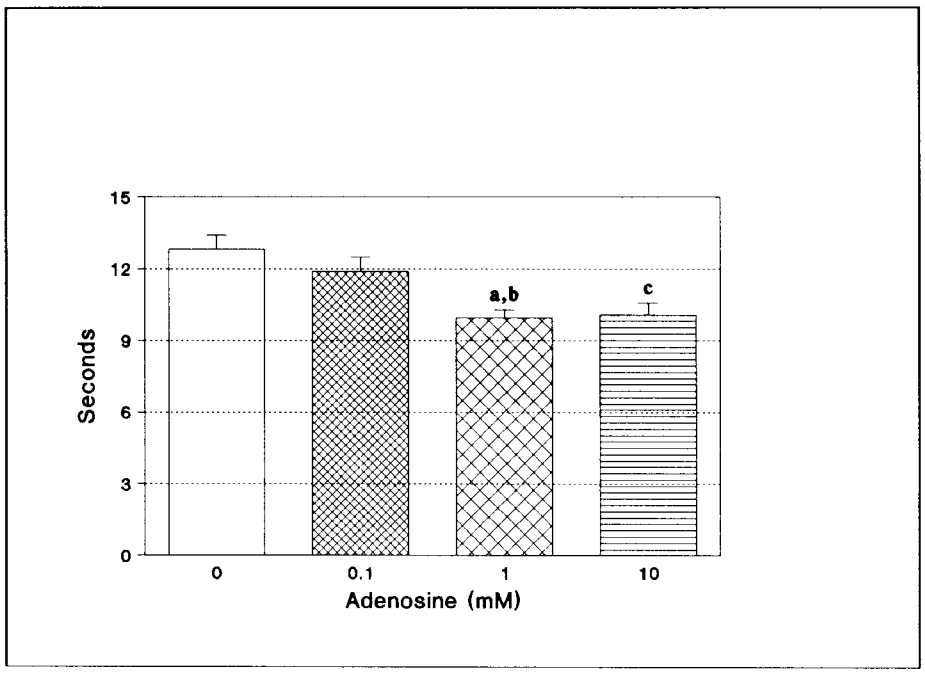
Time to induce cardiac arrest:

Comparison of the time intervals taken by each solution to induce complete electromechanical arrest showed no differences between the various groups: 10.2 sec ± 2.6 sec in group I, 9.7sec ± 1.3 sec in group II, 7.1 sec ± 1.3 sec in group III and 10.0 sec ± 1.9 sec in group IV (N.S.).

Cardioplegic infusion times:

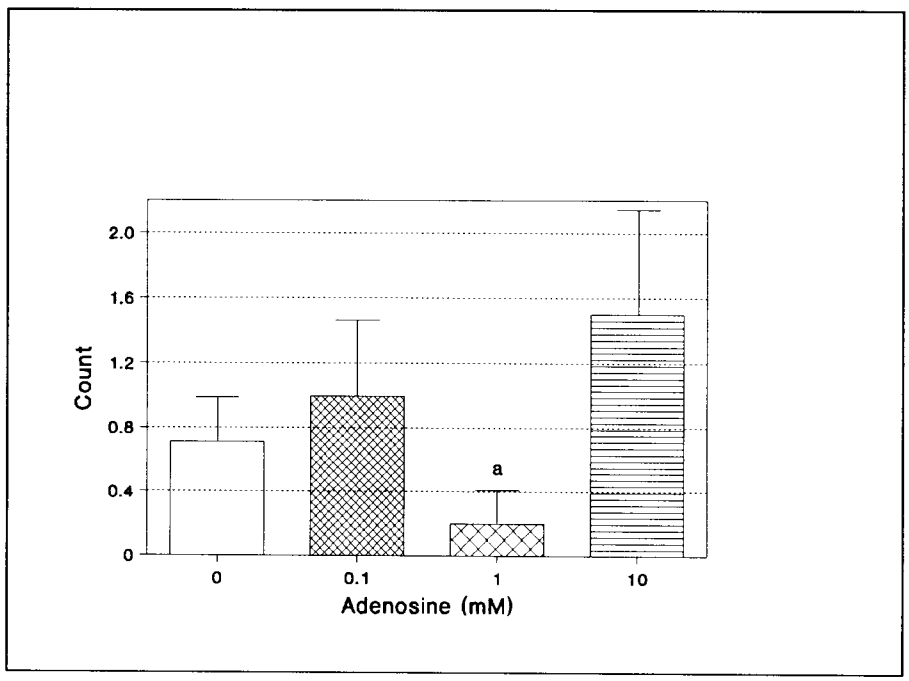
The measurement of time intervals required for infusion of maintenance cardioplegia represent an indirect measurement of coronary vasodilator tone as the delivery pressure was maintained at 80 mmHg. All measurements per group were pooled and averaged. The infusion times for each application of maintenance cardioplegia of 100ml on average were significantly shorter for the groups treated with 1mM and 10mM adenosine concentration (9.95 $\pm$ 0.32 sec and 10.07 $\pm$ 0.50 sec respectively) compared to plain ST2 and ST2 plus 0.1mM adenosine (12.8 $\pm$ 0.52 sec and 11.87 $\pm$ 0.62 sec respectively) (Figure 5.12.).

FIGURE 5.12.: MEAN INFUSION TIMES OF MAINTENANCE CARDIOPLEGIA IN THE DIFFERENT TREATMENT GROUPS



Legend: a: $p \leq 0.05$ vs 0 mM b: $p \leq 0.01$ vs 0.1 mM c: $p \leq 0.05$ vs 0 mM

FIGURE 5.13.: MEAN POST-ISCHEMIC DEFIBRILLATION COUNT PER ANIMAL IN THE DIFFERENT TREATMENT GROUPS TO REVERT THE HEARTS BACK TO SINUS RYTHM



Legend: a: $p \leq 0.05$ vs 0, 0.1, 10 mM

Defibrillation requirement:

Defibrillation requirement as expressed by the defibrillation count per animal was high for the hearts treated with St. Thomas' Hospital solution modified with 10 mM adenosine (Group IV, 1.5 ± 0.7) and low in hearts treated with St. Thomas' Hospital solution modified with 1mM adenosine (Group III, 0.2 ± 0.2, $p < 0.05$ vs group I: 0.71 ± 0,3) (Figure 5.13).

Metabolic recovery:

Tissue adenosine triphosphate (ATP) levels were lowered in all groups at the end of the ischemic period. (Table 5.9.). However, there was a trend towards better maintenance of ATP-levels in all hearts treated with adenosine. Five minutes after termination of cardiopulmonary bypass ATP-levels were 3.54 ± 0.34 µmol/g for group III compared to 2.53 ± 0.42 µmol/g for group I, 2.12 ± 0.27 µmol/g for group II ($p \leq 0.05$ vs group III) and 2.78 ± 0.25 µmol/g for group IV. (Table 5.9.). At 30 minutes reperfusion there were no statistical differences.

Tissue creatine phosphate (CP) levels were reduced in all groups at the end of ischemia with no statistical differences between the different groups. Five minutes after discontinuation of cardiopulmonary bypass, CP levels were better preserved in group III (7.78 ± 1.23 µmol/g) compared to group II (3.42 ± 0.88 µmol/g, $p \leq 0.05$ vs group III). At thirty minutes of reperfusion there were no differences in CP-content between the groups (Table 5.9.).

TABLE 5.9.: HIGH ENERGY PHOSPHATES

Parameter	Time	0 mM	0.1 mM	1 mM	10 mM	
Adenosine triphosphate ($\mu mol.g^{-1}$ wet weight)	**Control**	3.20 ±0.52 n=6	3.31 ±0.42 n=6	4.00 ±0.20 n=4	3.24 ±0.65 n=3	N.S.
	End ischemia	1.93 ±0.35 n=6	2.39 ±0.26 n=6	2.98 ±0.21 n=4	2.37 ±0.57 n=4	N.S.
	5 min	2.53 ±0.42 n=5	2.12 ±0.27 n=6	3.54 ±0.34 n=5	2.78 ±0.25 n=4	a
	30 min	2.23 ±0.30 n=5	3.13 ±0.40 n=6	2.92 ±0.36 n=5	2.52 ±0.76 n=3	N.S.
Creatine phosphate ($\mu mol.g^{-1}$ wet weight)	**Control**	4.19 ±0.77 n=6	5.28 ±0.84 n=5	7.17 ±1.10 n=3	5.04 ±0.90 n=3	N.S.
	End ischemia	2.33 ±1.30 n=6	1.32 ±0.32 n=6	1.74 ±0.56 n=4	3.61 ±1.73 n=4	N.S.
	5 min	5.51 ±1.05 n=5	3.42 ±0.88 n=6	7.78 ±1.23 n=5	5.15 ±0.77 n=4	b
	30 min	5.43 ±0.98 n=5	5.78 ±0.85 n=6	5.85 ±1.44 n=5	4.16 ±1.02 n=3	N.S.

Legend: a: $p \leq 0.05$; 0.1mM vs 1mM
b: $p \leq 0.01$; 0.1mM vs 1mM

Ultrastructure:

Ultrastructural studies have so far been performed on only two animals per group. The limited number of results show no differences in mitochondrial preservation or glycogen loss between the groups (Table 5.10.).

TABLE 5.10.: ULTRASTRUCTURAL PRESERVATION OF MYOCARDIUM BEFORE AND AFTER PROTECTION WITH ADENOSINE SUPPLEMENTED ST. THOMAS' HOSPITAL CARDIOPLEGIA NO. 2

Time	Group	Cristae	Dense granules	Glycogen Loss
PRI	ADO 0.1mM (n=2)	1.5 0	0 0	0.5 0
PRI	ADO 1mM (n=2)	0 0	1 0	1.5 0
PRI	ADO 10mM (n=2)	0 0.5	0 0	1 0
EOI	ADO 0.1mM (n=2)	0.5 0.5	0 0	0.5 0
EOI	ADO 1mM (n=2)	1.5 0	0 0	1.5 0
EOI	ADO 10mM (n=2)	1.5 0.5	0 0	1 0
RPS	ADO 0.1mM (n=2)	0 0	0 0	1.5 0
RPS	ADO 1mM (n=2)	0 1	1 0	1.5 0
RPS	ADO 10mM (n=2)	1 0.5	0 0	1 0

Legend: The myocardial biopsy samples from the left ventricular apical area were scored according to a semiquantitative scoring system: 0 = normal, 1 = mild abnormality, 2 = moderate abnormality, 3 = severe abnormality. PRI: Preischemia, EOI: End of ischemia (180 min), RPS: After 1 hour of reperfusion.

Summary of findings

Adenosine has numerous protective properties in the ischemic myocardium. We have previously shown that adenosine added to Krebs-Henseleit buffer for induction cardioplegia improved hemodynamic recovery, as measured by cardiac output and dP/dt in an in-vivo baboon model. The aim of this study was to test the hypothesis that adenosine added to St. Thomas' Hospital solution No.2 (ST2) for induction and maintenance cardioplegia would improve hemodynamic recovery in a dose-dependent manner. Four different treatment groups were studied in an in-vivo baboon model closely resembling the clinical setting: Group I: ST2 (control), Group II: ST2 + 0.1mM/L adenosine, Group III: ST2 + 1mM/L adenosine and Group IV: ST2 + 10mM/L. After three hours of cardiac arrest hemodynamic recovery as measured by cardiac index (CI) was 115.6 ± 8.6% for group III ($p \leq 0.05$ vs group I), 87.5 ± 8.2% for group I, 89.9 ± 8.8% for group II and 62.9 ± 7.3% for group IV ($p \leq 0.05$ vs group III). Reperfusion left ventricular function curves for group III best approximated to the preischemic control curves, while all other groups showed depressed postischemic function, which was most pronounced in group IV. However, systemic vascular resistance index (SVRI) at 20 minutes reperfusion was significantly lower in group III compared to group I, indicating systemic vasodilatation induced by higher concentrations of adenosine, resulting in improved cardiac output. Postischemic defibrillation requirements to revert the hearts back to sinus rhythm were reduced in group III compared to group I (0.2 ± 0.2 counts/animal vs 0.7 ± 0.3 counts/animal respectively). In groups III and IV coronary vasodilatation was induced as indirectly measured with the time intervals required for infusion of maintenance cardioplegia at a defined perfusion pressure of 80 mmHg. ATP-levels were best preserved in group III at early reperfusion. In summary, adenosine 1 mM/L added to ST2 improved hemodynamic recovery , which was mediated by a drop in SVRI, as dP/dt values were not different between the various groups. However, further benefits of adenosine included improved rhythm stability during the early reperfusion period and coronary vasodilatation.

CHAPTER 6

IN-VITRO STUDIES

6.1. Adenosine deaminase (ADA) concentration in various species

Rationale

Like inosine, adenosine is a catabolite of adenine nucleotide metabolism. In contrast to inosine, which has a relatively long half-life in blood, adenosine exists in blood plasma for less than one second (Moeser, 1989). In the circulation ist life may be even shorter because of the high activity of adenosine deaminase in endothelial cells (de Jong, 1990). Consequently the adenosine concentration in blood is very low (ca. 0.01µM) (Dawicki, 1988) and difficult to quantitate. In coronary effluent from the normoxic isolated rat heart, the adenosine concentration is about 0.1µM (Huizer, 1987). Adenosine is rapidly broken down by adenosine deaminase and after cell entry mainly phosphorylated to AMP (Doloretta, 1988) in the erythrocytes and in the capillary endothelium (Gerlach, 1985). It is doubtful whether its incorporation into myocytes is also rapid, since the adenosine transport across the membrane, which is a carrier-mediated process, is the rate-limiting step (Bodwitch, 1985; Dow, 1987).
As we found differences in the rat heart model and in the baboon model with regard to induction of cardiac arrest, we tried to determine whether there are species differences in blood adenosine deaminase. At the time of the study there were no comparative data on blood purine catabolism in various species.

Experimental procedures, materials and methods

We compared adenosine breakdown in whole blood (diluted 10x with water: hemolysate) of baboons, pigs and rats with that in man. Fresh blood from these species and from healthy volunteers was taken and immediately processed. A volume of 0.1 ml of the sample was mixed with 50mM Na-phosphate buffer (pH 7.4) and 45 µM adenosine, adding up to a final volume of 3.0 ml. The Δ absorption at 265 nm was measured for 15-30 seconds at 20°C.

Results

TABLE 6.1.: ADENOSINE DEAMINASE ACTIVITY (units/l)

SPECIES	n	PLASMA	HEMOLYSATE
MAN	**4**	**7.3 ± 2.0**	**74 ± 7**
BABOON	**4**	**6.7 ± 0.7**	**231 ± 16***
PIG	**4**	**7.5 ± 1.8**	**158 ± 17***
RAT	**7**	**11.3 ± 2.8**	**172 ± 43**

Legend: mean + SE, * $p<0.005$ vs man (modified t-test);

Summary of findings

Adenosine deaminase found in erythrocytes is relatively inactive in human blood. To avoid overdosing, care must be exercised if clinical trials are planned, using animal data on cardioprotection by adenosine.

CHAPTER 7

DISCUSSION, SUMMARY AND CONCLUSIONS

7.1. Limitations of the animal models used

The isolated rat heart model several limitations. The most important are that a non-blood perfusate and small mammalian "normal" hearts are used.

Species

Small mammalian hearts, especially the rat's, guinea pig's and rabbit's have been widely used to study the effect of cardioplegic solutions, because of low cost, ease of handling, and the possibility of studying large numbers (Hearse, 1981). However, physiological differences clearly exist between species (Blank, 1989; Galinanes, 1990; Hearse, 1976) and tolerance to normothermic ischemia varies between species (Galinanes, 1990). Furthermore, cardioplegic protection may be beneficial in one species (rat), but provide no protection in others (ferret and guinea pig) (Galinanes, 1990), and susceptibility to reperfusion damage also differs (Hearse, 1976).

Major physiological differences also exist between these smaller mammals and larger animals (pig, dog, primate); e.g. smaller animals have high resting heart rates (rat - 355 beats/min, guinea pig - 273 beats/min), and the oxidative capacity of the myocardium of smaller animals is significantly higher, although the glycolytic potential of mammalian myocardium seems to be fairly uniform (Blank, 1989). Smaller animals also have higher levels of basal metabolism and oxygen consumption (Hudson, 1971), and may have considerably different electrophysiological properties (Bretschneider, 1980). This might be phylogenetically related to the much higher heart rate and includes a considerably shorter plateau of the action potential, a different relationship of the fast sodium to the slow calcium and sodium channels of the external membrane, and probably a quantitatively different source of calcium- producing electromechanical coupling (Bretschneider,

1980). Therefore, before extrapolating from the results found in small animals, these results need to be confirmed in large animals or preferably in species more closely related to man.

Amongst larger animals there are also differences; the pig has extremely limited collateral flow between different regions of the myocardium and develops cardiac edema rapidly. The calf heart exhibits considerably higher resistance to ischemia than the dog heart; in canine hearts resistance is somewhat less than in a healthy human heart (Bretschneider, 1980). The canine heart is less tolerant to cardiopulmonary bypass and develops myocardial edema more predictably and frequently than the human heart (Rovetto, 1973).

In addition, differences in susceptibility to ischemic injury also depend upon the maturity of the animal (Pridjian, 1987), and cardioplegic solutions used in adult hearts may not have equivalent efficacy in neonatal hearts (Baker, 1988; Baker, 1990) .

The baboon has a close evolutionary relationship to man, and is therefore probably the closest available experimental model to man for evaluating the efficacy of cardioplegic solutions. Nevertheless, direct extrapolation of animal experiments to the human clinical situation requires caution, especially as we have shown that adenosine deaminase activity is five times higher in baboon erythrocytes than in human samples.

Ex vivo models

Experimental conditions can be rigidly controlled in ex vivo models (Hearse, 1981). However, the "unphysiological" crystalloid perfusates used in the majority of these models allow only limited recovery after ischemic contracture, and also increase compliance and cause slow deterioration of the heart with time (Galinanes, 1990; Neely, 1967). Furthermore, bubble oxygenation of the crystalloid perfusate denatures added proteins and may cause microemboli. Trace amounts of heavy

metals in the reagents of the perfusate can also affect the stability of the model (Neely, 1967), and Neely et al added Ca-EDTA 0.5 mM/L to the Krebs-Henseleit perfusate to improve the stability of their model. Thus, alternative paracorporeal and in vivo models using blood perfusates are more physiological, although more complex and expensive (Warnecke, 1980).

In vivo models, in contrast to ex vivo models, maintain the involvement of metabolic, nervous, hormonal and other factors such as noncoronary collateral flow. However, metabolic, hormonal, biochemical factors such as ionized calcium concentration (Drop, 1980) and hematological parameters (hematocrit) in these paracorporeal models can also effect myocardial function and therefore outcome. Cardiac output can also be altered by heart rate, preload and afterload in the in vivo model. Although the former two variables can be kept constant, afterload can be difficult to keep stable and can thus affect the results. Therefore, isovolumic measurements of contractility may be more relevant in this model, and a more sensitive indicator of ischemic damage may be postischemic oxygen utilization (Krukenkamp, 1986).

Nevertheless, the clinical situation of cardiopulmonary bypass, with its associated damaging effects, can be closely simulated with in vivo models.

Experimental protocol

The experimental protocol used to evaluate the efficacy of cardioplegic solutions is extremely important. Cardioplegic solutions should be evaluated in models that simulate the clinical situation, and two fundamental aspects are the temperature during the ischemic period and the energy state of the experimental heart.

Hypothermia alters the physiological environment; „fluidity" of proteins and lipids, activity of enzymes (Pullmann, 1960), calcium homeostasis (Keon, 1988; Shattock, 1983), coronary autoregulation (Chitwood, 1979; Mendler, 1984), and the neutral pH of water (White, 1981). Therefore, one should critically question the validity of normothermic evaluations of cardioplegic solutions, destined to be used in

hypothermic conditions (Jynge, 1978; Kempsford, 1988). Nevertheless, it is important to also be aware of the efficacy of cardioplegic solutions at different temperatures (Jynge, 1978), as temperatures may vary during open-heart procedures. In all studies in this thesis, the cardioplegic solutions were evaluated at hypothermia (10 °C).

The majority of experimental animals have "normal hearts", whereas in the clinical situation operations are performed on diseased hearts. Thus models simulating coronary stenoses to study cardioplegic distribution (Gundry, 1984; Lazar, 1988), or models using energy depleted hearts (by adding an initial ischemic insult) (Rosenkranz, 1982), for instance via preischemic ventricular fibrillation (Boehm,1993) or using hypertrophied hearts (Blandergroen, 1990), and cyanotic hearts (Fujiwara, 1988) are extremely relevant and important (Buckberg, 1990). However, when studying the effects of cardioplegic solution composition on functional recovery, an initial ischemic insult may impose an additional reperfusion injury which itself induces additional physiological changes. Nevertheless, because of the excellent performance of better formulated cardioplegic solutions, an initial normothermic ischemic insult may be necessary.

Other experimental variables

The preparation of the cardioplegic solution itself can introduce experimental variables. For example, particle-induced coronary vasoconstriction must be prevented (Hearse, 1985; Robinson, 1984), and therefore all experimental cardioplegic solutions should be filtered through an 0.8 μm sized cellulose filter (Millipore corporation; Bedford, Massachusetts, USA).

Oxygen content of cardioplegic solutions

Most of the solutions prepared in the laboratory are routinely gassed with 95 % O_2 5 % CO_2 during their preparation, in order to prevent precipitation of calcium salts.

Thereafter, depending upon how these solutions are stored and the length of elapsed time prior to use, the oxygen content can vary significantly. Thus "non-oxygenated" cardioplegic solutions may contain significant quantities of dissolved oxygen if equilibration to atmospheric pressure has not yet occurred. Furthermore, some commercial cardioplegic solutions (Bretschneider's HTK 4) are stored in vacuum in glass bottles, and are therefore anoxic if infused immediately upon opening the container. Hence, the amount of oxygen dissolved in „non-oxygenated" solutions can result in experimental variations. Oxygenation of crystalloid cardioplegic solutions has been shown to improve myocardial protection via the amount of physically dissolved oxygen in several models (de Wit, 1988; Ledingham, 1988; von Oppell, 1991).

Processing of samples for electron microscopy

Morphological studies have consistently been in broad agreement with both enzymatic and functional assessments (Hearse, 1981) . Characteristic mitochondrial changes correlate well with functional recovery and adequacy of myocardial preservation (Sjostrand, 1986) . However, processing of tissue for transmission electron microscopy is critical, as fixation techniques may alter structural findings (Sjostrand, 1986; Van Winkle, 1989). Fixation of tissue samples in these studies was with 5 % buffered glutaraldehyde and processing was by routine techniques employed in our laboratory (osmic acid and dehydration in graded alcohols and embedding in Araldite) (Rose, 1972).

Extrapolation of results to the clinical situation

Extrapolation of experimental results obtained in animal models should be done with care for the reasons mentioned. This is underlined by the fact that we could not confirm the induction of cardiac arrest with a high dose of adenosine in the baboon compared to the isolated rat heart. This finding stresses the fact, that before extrapolating from animal models distant from man, results must be confirmed in

animal species closer to man. Nevertheless, while adenosine failed to induce electromechanical arrest in the in vivo study it did improve hemodynamic recovery and electropysiologic and ultrastructural preservation significantly when added to a normokalemic solution (KHB). However, improvement of hemodynamic parameters is limited in the isolated rat heart model to adenosine as adjunct to a non-cardioplegic (normokalemic) solution.

Likewise, in the baboon model hemodynamic recovery, as measured and calculated with ejection phase parameters, is only evident when adenosine is added to a non-cardioplegic (normokalemic) solution. The improvement in postischemic hemodynamic recovery found in the dose response study, where adenosine was added to St. Thomas Hospital solution No. 2, was mainly mediated via a reduction of systemic vascular resistance. Improvement of postischemic electrophysiological stability, however, could still be demonstrated in this study.

Therefore, if extrapolating from the experimental to the clinical setting, the full potential benefit of adenosine in the context of cardioplegia is only to be anticipated if adenosine is used as adjunct to normokalemic solutions. This would, however, require additional measures to achieve electromechanical arrest such as high magnesium, low sodium or calcium depletion.

Nevertheless, supplementation of high potassium cardioplegic solutions with adenosine is beneficial in the baboon. In a recent study performed in a canine model adenosine-supplemented blood cardioplegia was compared to standard cardioplegia (Hudspeth, 1994). The authors found no change in dP/dt recorded before and after ischemia and between the two different groups. However, enddiastolic pressure volume relations as measured via impedance catheter technique were improved in the adenosine-supplemented group, indicating that more sensitive parameters of cardiac performance, especially of diastolic function, might be necessary to detect improvements when adenosine is used as adjunct to high potassium solutions. A study published in 1996 (Alekseev, 1996) employing the perforated patch clamp method applied to single guinea-pig ventricular myocytes revealed that adenosine 1mM did not significantly reduce the magnitude of K^+ induced membrane depolarization. Yet adenosine significantly slowed the rate of membrane depolarization with a subsequent reduction of net inward Ca^{2+} current. This finding supports the view that adenosine may play a role in hyperkalemic cardioplegia via

reduction of K^+ - induced intracellular Ca^{2+} loading. This is an important finding in view of the idea that intracellular Ca^{2+} loading in cardiomyocytes has been considered to represent a precipitating factor that could lead to diastolic dysfunction and cellular impairment (Harman,1995; Tani, 1990). This finding in an isolated myocardial cell preparation supports the observed improvements found especially in diastolic myocardial function in the study of Hudspeth. Our dose response study did not, however, employ sensitive parameters for diastolic cardiac performance.

Initial clinical trials have shown, that adenosine can safely be added to existing crystalloid cardioplegic solutions (Mentzer, 1996; Fremes, 1996). The clinically added concentrations of 200 µM of adenosine to blood cardioplegia (Hudspeth, 1994) are, however, five times lower than the optimal concentration found at 1 mM in this study. Considering the fact that we also found a five times lower activity of adenosine deaminase in human, compared to baboon erythrocytes, this concentration might well be optimal for the human heart.

7.2. Adenosine vs high potassium in the isolated rat heart

Adenosine has been reported to depress impulse formation and conduction in the sinus and AV node respectively (Szentmiklosi,1980; Urthaler,1972) via hyperpolarization of the sarcommal membrane of these pacemaker cells. In combination with an adenosine desaminase inhibitor, adenosine was shown to enhance myocardial recovery after ischemia (Humphrey,1982), when used as pretreatment. While the investigators emphasized slowed ATP degradation and faster ATP and phosphocreatine resynthesis, our experiments were additionally based on the idea of energy preservation by a rapid cardiac arrest.

Effect on cardiac arrest

Comparing the features of the induction of cardiac arrest with adenosine cardioplegia with commonly used arresting agents like high potassium, one outstanding difference is obvious: there is nearly immediate heart arrest with adenosine cardioplegia. The hearts stop beating about 20 times faster in the Langendorff and about 10 times faster in the working mode. The number of arrest beats differed accordingly. The addition of adenosine to potassium cardioplegia enhanced cardiac arrest. Increasing the adenosine adjunct enhanced cardiac arrest even more. Potassium plus 10 mM of adenosine was nearly as effective as adenosine in causing cardiac arrest.

Effect on hemodynamic recovery

In the experiments with 30 minutes of hypothermic ischemia, adenosine cardioplegia was superior to the control and there was a trend for better recovery compared to the other treatment groups. As the ischemic time was relatively short, more than 80 % of the control group hearts recovered, with no statistical significance between the groups. Furthermore it was impossible to judge the effectiveness of the combination of adenosine and potassium. Therefore, the experiments were repeated with an extended ischemic period of 90 minutes. Again adenosine cardioplegia showed superior recovery. In these experiments a significant difference between the control group and the potassium group was demonstrated. Interestingly, the combination of adenosine and potassium showed depressed recovery for aortic output and for heart rate-mean pressure product compared with potassium cardioplegia.

It is important to point out that all of our adenosine cardioplegic solutions had to be washed out immediately after infusion. If this was omitted, all hearts began beating with a low heart rate and a low cardiac output immediately after reperfusion, indicating a persistent action of adenosine on the conduction system. Accordingly, the ATP and phosphocreatine stores were depressed and recovery parameters diminished.

Effect on energy status

The energy status after infusion of the various cardioplegic solutions prior to the onset of ischemia showed no significant differences for ATP, but there were differences in phosphocreatine levels. The phosphocreatine levels were superior for adenosine in both concentrations as well as with the adenosine supplement compared with potassium cardioplegia. After 30 minutes of ischemia phosphocreatine was again superior to all other cardioplegic groups. Now even ATP stores were significantly increased. The lowest values were reached for the combination of adenosine and potassium for either group.

Possible mechanisms

The mechanism by which adenosine improves myocardial preservation after global ischemic heart arrest is not clear. Adenosine hastens cardiac arrest and thus shortens the period of ischemic myocardial contractions after aortic cross-clamping. Regarding the energy status prior to ischemia, ATP stores were not significantly different, whereas phosphocreatine seems to be a better parameter for preservation, as suggested in previous studies (Rosenkranz,1986). Along with an increase of adenosine and with an increasing concentration of adenosine supplemented to potassium cardioplegia, phosphocreatine levels prior to ischemia rose. In trials with pure potassium cardioplegia phosphocreatine levels even dropped below the pre-cardioplegia control values.

While these experiments emphasize the effectiveness of adenosine and adenosine admixture respectively, our hemodynamic data confirmed superior recovery only for pure adenosine cardioplegia. Potassium cardioplegic solutions supplemented with various amounts of adenosine showed worse recovery than adenosine alone and were not different from high potassium.

The fact that the combination of adenosine and high potassium accelerated cardiac arrest in a similar way as pure adenosine excludes this effect as an explanation for the improved preservation. It seems more likely that high potassium limits the protective effects of adenosine. Adenosine has been linked to preconditioning via

stimulation of A_1 receptor-mediated G_i protein induction and opening of K_{ATP} channels. Opening of ATP-sensitive potassium channels with selective channel openers is, however, only cardioprotective in the absence of high potassium cardioplegia (Galinanes,1992). This indicates that adenosine A_1-mediated cardioprotection via activation of ATP or ADO-sensitive potassium channels might be equally limited in the presence of high potassium. This suggests that part of the cardioprotective qualities could be lost when adenosine is used in conjunction with high potassium cardioplegia. This would depolarize sarcolemmal membranes and antagonize the hyperpolarizing forces of adenosine, as shown by de Jong in studies of isolated SA nodes (de Jong,1990). Several authors have stated in the past that cardiac membrane polarization during cardiac ischemia is energetically advantageous, as it represents the normal resting state of the myocardium and decreases energy consuming transmembrane ionic exchange system activity (Sternbergh,1989). Similarly, a study by Ely (Ely,1990), using adenosine as pretreatment in an isolated rat heart model, demonstrated superior functional and metabolic recovery compared to non-treated normothermic ischemia.

From these experiments we conclude that high adenosine cardioplegia, i.e. 10 mM of adenosine, is an efficient cardioplegic solution in this model. It induces cardiac arrest more rapidly than cardioplegic solutions based on high potassium and preserves myocardial function safely. The combination of potassium and adenosine cardioplegia is less effective than expected.

7.3. Comparison of cardioplegic solutions used in South Africa

Comparison of hemodynamic recovery

This study, carried out under conditions which closely resemble the clinical situation, shows that the indices of hemodynamic recovery (cardiac index, stroke volume index and stroke work index) in hearts protected with St. Thomas' Hospital solution No.2 was above 95%, whilst in hearts protected with SABAX solution, recovery was between 70-85%, with the GSH solution between 51-80% and with Krebs-Henseleit

buffer between 34-60%. The St. Thomas' Hospital solution No.2 cardioplegia was superior to the SABAX solution in stroke volume index and to the GSH solution in the majority of measured hemodynamic variables. Left ventricular curves of stroke work index calculated at left atrial pressures of 5, 10, 15 and 20 mmHg showed that hearts protected with St. Thomas' Hospital solution No. 2 had good recovery in contrast to the hearts perfused with Groote Schuur Hospital solution or Krebs Henseleit buffer. These findings confirm the importance of thorough laboratory testing of any cardioplegic solution. The St. Thomas' Hospital solution No.2 was extensively investigated in two different animal species and the electrolyte content was formulated according to dose-response curves for each ion (Jynge, 1981; Jynge, 1980; Jynge, 1978; Hearse, 1978).

Comparison of metabolic recovery

Although there was no significant difference in the various series in the post-ischemic recovery of ATP at 5 and 30 minutes, previous studies have shown that ATP levels are not the only factor controlling viability (Rosenkranz, 1986), and that the rate of ATP synthesis may be more important.

Although ATP and CP levels decreased during the ischemic period, they were quickly restored to acceptable levels during reperfusion in hearts protected with ST2 cardioplegia. However, post-ischemic ATP and CP levels observed in the other groups in this study do not correlate with the functional recoveries.

Comparison of composition

The Plasmalyte B with added potassium used in some centers in South Africa has a similar electrolyte content to Ringer's lactate except that Plasmalyte B is calcium-free. Coronary perfusion with a calcium-free solution may lead to increased sarcolemmal permeability to calcium, and when the myocardium is reperfused with a calcium-containing solution a massive influx of calcium can occur causing severe cellular damage (Jynge, 1977; Ruigrok, 1983; Torchiana, 1987, Yamamoto, 1984).

The addition of 200 ml pump blood to the maintenance Plasmalyte B cardioplegic solution (thus introducing a minimal concentration of calcium), as practised by one unit, could prevent this calcium paradox and consequently be beneficial. Furthermore, part of the beneficial actions of calcium-free Plasmalyte B may be attributed to the fact that hypothermia protects against the calcium paradox. In addition, the Plasmalyte B cardioplegia contains a high potassium concentration. It has been shown that the optimal potassium concentration for a crystalloid cardioplegic solution is approximately 15 mM/L and that higher potassium concentrations may be damaging (Rousou, 1981; Dewar, 1987; Chiavarelli, 1982). For these reasons this solution was not included in this study.

Clinical situation

In the clinical situation, because of the large number of variables such as coronary artery anatomy, LV dysfunction, cross clamp time, non-collateral flow and the extent of the procedure, it is difficult to objectively evaluate the performance of any one myocardial protection regimen. Variability of non-collateral coronary flow in the diseased heart may influence the rate of "wash-out" of the cardioplegic solution so diminishing its advantages; likewise the "wash-in" of substances (e.g. calcium) may be beneficial when using a calcium-free cardioplegic solution. It is not yet known whether undetected myocardial damage, resulting from poor myocardial protection, will result in late myocardial fibrosis and decreased left ventricular function. Therefore, we should continue to seek improvements in our techniques of myocardial preservation.

The result of this comparative control study, taken in conjunction with other reports in the literature, lead us to propose that the optimum crystalloid cardioplegic solution for use in South Africa today should be based on the St. Thomas' Hospital cardioplegic solution No. 2. Even minor alterations to this solution should be fully evaluated experimentally before clinical use. The Cardio-Thoracic Department at Groote Schuur Hospital now uses St. Thomas' solution No. 2 cardioplegic solution.

All further studies in the primate model were conducted with St. Thomas' Hospital solution No. 2 and modifications thereof.

7.4. Adenosine as cardioplegic agent in the in-vivo baboon model

Effect on hemodynamic recovery

These results obtained in an in-vivo baboon model confirm findings in the isolated rat heart (De Jong, 1990; Schubert, 1989), that adenosine improved post-ischemic hemodynamic recovery when added to a non-cardioplegic solution such as Krebs-Henseleit buffer for induction. The improvement was sufficient to make the ADO solution comparable to the internationally used St. Thomas' Hospital solution No 2 in the hemodynamic recovery of CI and SVI. ADO and ST2 were significantly better in all measured parameters than KHB alone.

Effect on cardiac arrest

However, in contradistinction to the isolated rat heart model (De Jong, 1990; Schubert, 1989), adenosine did not act by inducing fast cardiac arrest since it was ineffective in preventing ventricular fibrillation or arresting the fibrillating heart. Complete electromechanical arrest was delayed by 47 seconds with adenosine when compared with potassium arrested hearts ($p < 0.001$), but 25 seconds faster than with the low potassium KHB alone (55.0 ± 5.6 sec vs 80.0 ± 6.4 sec) ($p < 0.01$) respectively. This finding, which is at variance with results obtained in the isolated rat heart (Schubert, 1989), could be due to the fact that the effects of adenosine on heart rate and AV-nodal conduction are species-dependent and there might be a species-specific sensitivity (Fabiato, 1978). The possibility of a higher fibrillation threshold in rat hearts might account for this difference.

Effect on energy status

Recovery of high energy phosphates showed no significant advantage from adding adenosine although ATP and CP levels were well maintained in the adenosine group. This is in contradistinction to Bolling (1989) who attributes the beneficial effect of adding adenosine to cardioplegic solutions to an increase in myocardial ATP. Significant differences between the various groups may, however, have been hindered by the energy-wasting fibrillation seen in the KHB and adenosine groups.

Effect on ultrastructure

Interpretation of the preliminary ultrastructural studies showing normal cardiac ultrastructure in the adenosine-treated hearts at reperfusion suggests that adenosine might be beneficial by other mechanisms than restoring high energy phosphates (Petsikas, 1990).
It has been shown by Cronstein (1986) to inhibit neutrophil-mediated injury to endothelial cells and to modulate superoxide anion generation by human neutrophils (Cronstein, 1983).
Olafsson confirmed these in vitro findings by showing a reduction of reperfusion injury in a canine preparation by intracoronary adenosine (Olafsson, 1987).

Interpretation of the preliminary ultrastructural studies showing normal cardiac ultrastructure in the adenosine treated hearts at the end of ischemia and after reperfusion suggests that adenosine might be beneficial by other mechanisms than restoring high energy phosphates (Ledingham, 1990; Olafsson, 1987; Petsikas, 1990; Babbitt, 1989). Further beneficial effects of adenosine could be the reduction of platelet aggregation and prevention of the "no-reflow" phenomenon through prevention of plugging of the microvascular bed (Pitarys, 1991). Additionally the calcium antagonistic properties (Kato, 1990) of adenosine might be beneficial in preventing intracellular calcium overload, which is the one of the contributing factors to reperfusion injury apart from oxygen free radicals (Opie, 1989).

A further possible mechanism of adenosine is its action as a potent coronary vasodilator (Ledingham, 1990; Wilson, 1990). This could have improved, on the one hand, cardioplegia delivery with more rapid and uniform cooling as evidenced by the shorter infusion times for cardioplegia in the treatment groups with 1 and 10 mM of adenosine added to St. Thomas' Hospital cardioplegia No. 2. On the other hand, it could maximize coronary flow in the reperfusion period, which was not measured in this study. This effect, in addition to the prevention of microvascular plugging, might improve post-ischemic flow and delivery of oxygen and substrates substantially. Ledingham concluded from his study, in which he used adenosine during reperfusion in an isolated rat heart model, that the beneficial effect is likely to be due to an increase in coronary flow rather than to an increase in myocardial ATP.

Of further importance is that no adverse effects from adenosine at the concentration used were observed in the treated animals. However, systemic hypotension, renal vasoconstriction and AV-block have been reported when using high concentrations (Owall,1988; Osswald, 1978; Richardt, 1987).

In conclusion, adenosine was effective in improving post-ischemic hemodynamic recovery when added to a non-cardioplegic solution. However, it did not prove to be a cardioplegic agent in this model due to its failure to prevent ventricular fibrillation. Preliminary ultrastructural findings together with reports in the literature suggest that adenosine might modulate reperfusion and endothelial cell injury (Babitt, 1989; Crostein, 1983; Cronstein, 1986; Ledingham, 1990; Olafsson, 1987).
Further studies are, however, required to test the beneficial effects of adenosine in the presence of high potassium concentrations and to elucidate the mechanism or mechanisms by which adenosine exerts its beneficial effects. Once these studies are complete, adenosine, in an appropriate concentration, may well prove to be a beneficial additive to cardioplegic or reperfusion solutions.

7.5. Adenosine as cardioplegic adjunct in the in-vivo baboon model

Effect on hemodynamic recovery

These results were obtained in an *in vivo* baboon model closely simulating the clinical procedure. They are similar to findings in the isolated rat heart (De Jong, 1990) and isolated rabbit heart (Bolling, 1989) confirming that adenosine improves post-ischemic hemodynamic recovery when used as adjunct to a hyperkalemic cardioplegic formulation such as the St. Thomas' Hospital Solution. Addition of adenosine at a moderate concentration of 1 mM/L resulted in marked improvement of cardiac output and derived ejection phase indices when compared to St. Thomas' Hospital cardioplegia No. 2 alone: the measured and derived parameters were close to 100% or above. Addition of adenosine at the lower concentration of 0.1 mM/L, however, did not improve recovery compared to St. Thomas' Hospital solution No. 2 alone and adenosine supplementation at a concentration of 10 mM/L even resulted in inferior recovery.

However, the improvement in postischemic ejection phase indices was not related to a shortened time interval for induction of electromechanical cardiac arrest as demonstrated in the isolated rat heart model (Schubert, 1989; De Jong, 1990). It seemed to be mediated by a dose-dependent reduction in afterload as measured by systemic vascular resistance (SVR) and mean arterial blood pressure (MAP), especially since contractile function measured with dP/dt/DP was not different between the treatment groups. We realize that dP/dt is pre- and afterload dependent, but since we kept the preload constant and corrected for changes in afterload by calculating dP/dt over the developed pressure DP, the index should give a fairly reliable indication of contractile function (Mason, 1971).

Adenosine, apart from being a coronary vasodilator (Berne, 1980), also causes systemic vasodilatation (Oewall, 1988). The pronounced effect on systemic resistance in our model might have been aggravated by two important factors:

1. The adenosine -containing cardioplegic solution was not released from the right atrium, but was drained into the cardiopulmonary bypass circuit and subsequently into the systemic circulation. Although adenosine was not detected in arterial blood samples during reperfusion using a spectrophotometric assay (detection threshold 0.01mM) (Boehm, unpublished data) trace concentrations of adenosine may have been present. Normal arterial and venous plasma levels of adenosine in humans are in the range of 0.1- 0.3 μM/L (Sollevi, 1984). Sollevi reported that intravenous adenosine infusion of 150-350 μg/kg/min induced a marked reduction of the mean arterial blood pressure of 40-50%, mediated by a 55-65% reduction in systemic vascular resistance (Sollevi, 1984). This effect was used therapeutically to induce hypotension during anesthesia in patients with peripheral vascular disease and during neurosurgical procedures(Oewall, 1988; Sollevi, 1984). However, rapid breakdown would be expected via adenosine deaminase, an enzyme which was found to have higher activity in baboon blood than in human or rat blood (chapter 6) (De Jong, 1992). Metabolism could, however, have been delayed by the hypothermic temperatures. Another option is the possible binding of adenosine to vascular receptors or to fat tissue explaining its long-lasting effect which was still present 50 minutes after infusion of adenosine containing-cardioplegic solution.

2. Systemic ionized calcium concentrations during cardiopulmonary bypass and reperfusion varied between 0.3 and 0.5 mM/L, which is one half to one third of the normal range measured in the baboon. This was due to large priming volumes in relation to body size and to additional volume loading for determination of Starling function curves with a calcium-free crystalloid solution (Plasmalyte B). A similar observation has been noted by clinicians in pediatric cardiac surgery (Mayer, 1990). However, any attempt at correction was avoided due to a potential beneficial effect on preventing calcium overload during ischemia and reperfusion. Adenosine in conjunction with low calcium levels has an even stronger vasodilatory effect on the coronary system (Humphrey, 1982). The same effect could be expected for the systemic circulation.

Nevertheless, adenosine proved to be beneficial in several isolated heart studies (Bolling, 1989; Bolling, 1991) when given during the ischemic period, an effect which was not related to coronary or systemic vasodilatation. To separate the influence of systemic vasodilatation from other potentially beneficial properties in our model it might be necessary to drain the adenosine-containing cardioplegic solution or to correct the calcium concentration of the systemic perfusate. Recent evidence from our laboratory indicates that adenosine 1mM/L as adjunct to St. Thomas' Hospital cardioplegia No. 2 in the same model does not reduce systemic vascular resistance if the systemic calcium concentration is normalized (Boehm, unpublished data)

However, in the previous study we were able to show an improvement in hemodynamic recovery when adenosine was added to Krebs-Henseleit-Buffer as an induction cardioplegia. This effect was demonstrated in terms of ejection phase parameters such as cardiac output as well as in terms of contractile function measured with dP/dt. The results of the above mentioned study revealed no change in systemic vascular resistance, probably because adenosine was given once only for induction. On the other hand, there were no differences in postischemic high-energy phosphate content, possibly due to the long period of ventricular fibrillation prior to electromechanical arrest and/or because of the single application of adenosine.

Possible mechanisms

Several facts may explain why adenosine failed to improve cardiac contractile function in this study:

1. As evidenced when using the control solution (ST2) recovery of contractile function (dP/dt) was 100% or above. This demonstrates adequate protection of these baboon hearts during three hours of global hypothermic ischemia. Takahasi and Hearse (1990) emphasize that the control group should ideally recover to only approximately 50% of preischemic control values in order to reveal any beneficial or deleterious effects of any given intervention. Therefore, to lower the

recovery of control hearts in this study, a more severe ischemic insult would have been required. In our previous study recovery with Krebs Henseleit buffer was poor (55%) when compared to the treatment group with adenosine-containing Krebs Henseleit buffer, indicating that more ischemic/reperfusion damage occurred than in this dose-response study. Reviewing the literature, beneficial effects of adenosine are reported when the recovery with the control solution was poor, as for instance 40% in the study of Bolling (Bolling, 1989). Therefore a contractile recovery of more than 100% as in this study can hardly be expected after a prolonged period of global cardiac ischemia.

2. Our measurements of cardiac contractile function might have been not sensitive enough to detect any differences between the various treatment groups. Most investigators make use of pressure volume relationships for determination of systolic and diastolic cardiac function. A recent study performed at the University of North Carolina using adenosine-supplemented blood cardioplegia in an in vivo dog model showed decreased left ventricular systolic pressure at reperfusion compared to standard blood cardioplegia (Hudspeth, 1994). There was no change in dP/dt recorded before and after ischemia or between the different groups. However, end-diastolic pressure volume relations as measured via impedance catheter technique were improved in the adenosine-supplemented group, indicating that more sensitive parameters of cardiac contractile function might be necessary to detect differences in myocardial performance.

3. Adenosine has been linked to preconditioning via stimulation of A1 receptor-mediated Gi protein induction and opening of KATP channels. However, opening of ATP-sensitive potassium channels with selective channel openers is only cardioprotective in the absence of high potassium cardioplegia (Galinanes, 1992). This indicates that adenosine A1 mediated cardioprotection via activation of ATP or ADO sensitive potassium channels might be equally limited in the presence of high potassium. In Schubert's study using the isolated rat heart, the combination of adenosine and high potassium was not superior to potassium arrest alone in contrast to low potassium adenosine cardioplegia which showed superior hemodynamic and biochemical refunction (Schubert, 1989). This finding suggests

that part of the cardioprotective qualities could be lost in conjunction with high potassium cardioplegia, leading to depolarization of sarcolemmal membranes and antagonizing the hyperpolarizing forces of adenosine, as shown by de Jong in studies of isolated SA nodes (de Jong, 1990). Several authors have stated in the past that cardiac membrane polarization during cardiac ischemia is energetically advantageous, as it represents the normal resting state of the myocardium and decreases energy consuming transmembrane ionic exchange mechanisms (Sternbergh, 1989; Cohen, 1993,1994). However, a very recent study by Alekseev (1996) demonstrated that adenosine slowed the rate of K+-induced membrane depolarization and reduced K+-induced intracellular Ca2+ loading in ventricular myocytes during hyperkalemic cardioplegia. Blocking of the ATP-sensitive K+ channels with glyburide did not modify this effect, suggesting no involvement of opening of ATP-sensitive K+ channels in this action of adenosine. The authors concluded that at least in part a decrease in the net Ca2+ influx during K+ mediated depolarization might be the mechanism involved, although the phenomenon needs further investigation. This report supports our findings of an improved recovery with adenosine as adjunct to St. Thomas' Hospital cardioplegia, but it was less pronounced than in the studies with normokalemic solutions(Krebs Henseleit buffer) and those of other investigators showing superior recovery with adenosine and high K+ (Hudspeth, 1994; Mentzer, 1995; Fremes, 1996).

Other mechanisms

However, we cannot exclude the possibility that adenosine may offer additional benefits as suggested in this study by the reduced postischemic requirement for defibrillation in the group treated with 1mM/L adenosine. This improvement cannot be attributed to reduced afterload, but rather indicates amelioration of myocardial stunning, which Forman (1991) suggested as the likely role of adenosine. The previously cited study of Bolling found a 100% spontaneous defibrillation rate in adenosine treated rabbit hearts compared to a rate of only 87% in the control group.

However, only the intermediate adenosine concentration of 1mM/L gave superior results, whereas the lower (0.1mM/L) concentration gave no improvement and the higher (10mM/L) concentration resulted in increased requirements for postischemic defibrillation, when compared to St. Thomas' solution alone. This indicates a dose-dependent mechanism and stresses the importance of optimizing the concentration. The optimal dose found in our experiments seems to differ from the 200µM/L established in an isolated rabbit heart preparation (Bolling, 1989).

Considering that isolated heart models employ a blood-free perfusate , our five times higher concentration for optimal effect might reflect the necessity for administering higher doses in in-vivo models in order to anticipate the adenosine breakdown via adenosine deaminase. Lasley and Mentzer also stress the importance of a high dose in in vivo models in a similar way (Mentzer, 1993).
In group IV (10 mM/L adenosine) mean arterial blood pressure five minutes after termination of cardiopulmonary bypass was 54 ± 8,4 mmHg. This might have reduced coronary perfusion pressure below the critical level necessary to maintain adequate cardiac perfusion and function. Therefore reduced dP/dt/DP in this group can be either a result of the decreased perfusion or the result of a direct negative inotropic effect. Adenosine exerts this indirect negative inotropic effect on ventricular myocardium only when simultaneous ß-adrenergic stimulation is present (Schrader, 1977), which is the case in our experiments during reperfusion, rewarming and weaning from cardiopulmonary bypass. Under baseline conditions, adenosine is reported to have a mild positive or no inotropic effect on ventricular myocardium (Belardinelli, 1988). An additional negative chronotropic effect is evident with the 10mM/L concentration as reflected in the heart rate having a recovery of 92% ($p < 0.05$ vs control) of preischemic control. Two animals out of four (50%) had transient AV-conduction delay upon reperfusion as diagnosed on limb lead ECG.

Effect on energy status

High energy phosphate content as measured by tissue ATP and CP content failed to show a direct statistical correlation to functional recovery; although ATP preservation was best in the group treated with 1mM/L at early reperfusion, the group which also had the best hemodynamic recovery. Influence of the reduced afterload, resulting in reduced myocardial work, in this group on high-energy-phosphate preservation is possible, but seems unlikely as preservation tended to be better already at the end of the ischemic period. However, Mentzer suggested that higher ATP levels during reperfusion associated with adenosine pretreatment are at least in part the result of improved ischemic protection (Mentzer, 1993). It now seems established that total tissue ATP content per se is of limited value in predicting postischemic recovery of function (Mallet, 1990; Neely, 1984; Hohlfeld, 1989)

There have been conflicting reports about the ability of intracoronary administered adenosine to enhance intra-myocardial high energy phosphates depending on the model and preservation temperature used. Bolling (Bolling 1989,1990,1991) attributed the improved functional recovery found in an isolated rabbit heart model to the role of adenosine as a substrate for nucleotide resynthesis. Foker (1980) found improved high energy phosphate levels in a canine model of global normothermic ischemia only when adenosine supplementation was combined with an adenosine deaminase inhibitor (EHNA). Ledingham (1990) found no improvement on high energy phosphate content when adenosine was added during reperfusion, whereas Silverman (1983) detected improved high energy phosphates and increased coronary blood flow during reperfusion even without inhibition of adenosine catabolism in a dog model of global hypothermic ischemia. Ely (1985), in an isolated rat heart model of global normothermic ischemia, reported a reduction in net degradation of ATP during ischemia and found that adenosine facilitated repletion during reperfusion when it was infused throughout the experiment. Mentzer concluded in 1993 (Mentzer, 1993) that adenosine does not improve postischemic ventricular function via enhanced ATP content but rather via improvement of the creatine phosphate phosphorylation potential. Hohlfeld (1989) found an increase in an isolated rat heart model, but concluded that "total tissue ATP is not necessarily a good indicator of functional capabilities under conditions of normothermic ischemia

and reperfusion in the isolated rat heart". The author claims, however, the uncontradicted value of ATP as a parameter of ischemia-related tissue damage.
Other studies found that increased interstitial adenosine levels by intracoronary application of adenosine facilitated ATP recovery only late in reperfusion (Mentzer, 1993). However, our experiment was not designed to evaluate cardiac biochemical recovery in the late reperfusion phase.
If the main action of adenosine in protecting the ischemic heart is not the maintenance and restoration of an adequate intramyocardial ATP level above a postulated critical level, there might be other mechanisms involved:

Adenosine is a physiological coronary vasodilator (Berne, 1963). The small arteries are most sensitive, and especially when they have been preconstricted with potassium adenosine still mediates this vasodilating effect (Sabouni, 1990). This might enhance cardioplegic delivery and the velocity and uniform distribution of hypothermia. During the reperfusion period this effect might be essential in maintaining an improved oxygen supply - demand ratio. Forman (1991) and Ledingham (1990) confirmed this theory in isolated rat hearts where improvement of post-ischemic functional recovery was mainly attributed to a substantial increase in flow in the coronary micro-vasculature rather than to an increase in intramyocardial ATP when adenosine was used in the reperfusion period.
Our results indicate an increase in coronary vasodilatation during ischemia as indirectly measured by the cardioplegic infusion times for adenosine concentrations of 1 and 10 mM/L, whereas the 0.1 mM/L concentration had no additional effect compared to ST2. We had, however, no facilities to investigate whether the coronary vasodilatation was maintained in the reperfusion period. There is experimental evidence that adenosine leads to maximal coronary vasodilatation in the reperfusion period (Ledingham, 1990), which might be substantially more than the reactive hyperemia expected. This is supported by other studies, which have shown (Saldanha, 1989) that regulation of vasomotor tone is impaired postischemia especially in conjunction with high potassium cardioplegia (Lefer,1991)

Effect on ultrastructure

In addition to maximal coronary vasodilatation a further benefit of adenosine might contribute to the improved recovery: adenosine has been shown to reduce platelet aggregation and to inhibit neutrophil-mediated damage to endothelial cells via inhibition of oxygen-free radicals (Cronstein, 1983; Cronstein, 1986; Gruber, 1989). This mechanism might lead to prevention of the "no-reflow" phenomenon especially in the microvasculature (Kitikaze, 1991; Olafsson, 1987; Grisham, 1989). Furthermore, it might be responsible for the better ultrastructural preservation with regard to mitochondrial integrity in the adenosine-treated hearts as found in our previous study (Table 5.7.).

Stimulation of K-ATP-channels

A further mechanism by which adenosine might reduce myocardial stunning could be that it reduces post- and intraischemic calcium overload through stimulation of A_1 receptors, either by inhibiting potassium-dependent calcium uptake or by impeding further calcium entry through blockade of calcium-dependent channels (Kato, 1990). Additionally, adenosine can reduce myocardial oxygen consumption by protecting the myocardium from endogenous catecholamine stimulation via its action on A_1 receptors (Forman, 1991). The resulting negative chronotropic and dromotropic effect would result in a lower myocardial oxygen consumption. In our experiments, however, only the 10mM/L adenosine concentration had a significant negative chronotropic effect.

Side effects

A significant finding of this study is the sustained effect on systemic vasomotor tone as well as the importance of using the optimal adenosine concentration. Negative systemic effects such as constriction of renal arteries and decrease in glomerular filtration rate (Osswald, 1978), as measured indirectly from total urine output, were not observed. Inhibition of AV-conduction (Bellardinelli, 1990) was only detected in the 10mM/L group, where 2/4 animals had transient AV-conduction delay in the reperfusion period.

In conclusion, adenosine might be a beneficial additive to cardioplegic solutions in an appropriate concentration. However, part of its cardioprotective effect might be lost in conjunction with high potassium cardioplegia. It appears to be advisable to drain either the adenosine-containing cardioplegic solution or to normalize systemic calcium concentrations in order to avoid systemic vasodilatation in the reperfusion period. On the other hand, however, this could be a desirable effect during clinical reperfusion of the cardioplegically arrested heart and during weaning from cardiopulmonary bypass, if it improves cardiac output and myocardial energy consumption.

Findings of improved electrophysiological stability in the early reperfusion period in conjunction with reports in the literature suggest that adenosine might modulate reperfusion and endothelial cell injury. Further potential beneficial effects include coronary vasodilatation, improved O_2 supply/demand ratio and prevention of calcium overload, all of which are unlikely to be mediated via maintenance of intramyocardial high-energy phosphate content or facilitated restoration in the early reperfusion phase in this model. However, further studies are required to test and to elucidate the mechanisms by which adenosine exerts its beneficial effects.

7.6 Summary and conclusions

Intraoperative myocardial protection has evolved in parallel with the evolution of cardiac surgery from a technique to provide a still and bloodless field for the cardiac surgeon to an extensively investigated and clinically used method to additionally protect the myocardial, vascular and conductive tissues during global ischemic arrest. In view of the multitude of experimentally and clinically used cardioplegic solutions there is still an ongoing quest for further improvements in cardioplegic protection, especially as the extent of cardiac surgical repair is increasing and existing techniques still offer only limited time for cardiac repair and still lead to depressed postoperative myocardial function.

Adenosine, a physiologic purine compound, has been shown to be an endogenous cardioprotective agent via multiple mechanisms during episodes of induced or spontaneous global or regional ischemia. This study was therefore designed to investigate the role of adenosine as cardioplegic agent and as cardioplegic additive to high potassium solutions. To achieve this aim two animal models in two species, rat and baboon, were developed. The isolated rat heart was used as a rapid screening model and to test the hypothesis that adenosine would induce electromechanical arrest and improve hemodynamic, metabolic and ultrastructural recovery after a prolonged period of ischemia. The in-vivo baboon model was designed to approximate the clinical situation as close as possible and to test the interventions in a species close to man.

The study was restricted to the use of adenosine during ischemia and was not aimed at preischemic treatment with adenosine or adenosine supplementation during the reperfusion phase, representing episodes which might lead to improved preservation with adenosine supplementation.

The results obtained in an isolated rat heart model and in an in vivo primate model show that adenosine cardioplegia had a significant effect on improvement of hemodynamic function post-ischemia when used without high potassium cardioplegia. However, the combination of adenosine and high potassium cardioplegia was less effective than expected.

In the isolated rat heart adenosine in high concentrations (10 mM) is able to induce complete electromechanical cardiac arrest and appears to be a cardioplegic agent in

this model. In the in vivo baboon model, however, there was no induction of cardiac arrest because of the failure to limit ventricular fibrillation, but in both models improvements in hemodynamic recovery were demonstrated. That excludes the acceleration of cardiac arrest as the main mechanism involved. It appears possible that adenosine without high potassium acts as a preconditioning agent via activation of A_1 receptors and opening of ATP-sensitive potassium channels.

In the in vivo model with normal baboon hearts the improvement of cardiac ejection phase indices was mediated via a reduction of systemic vascular resistance with the intermediate concentration of 1mM of adenosine added to St. Thomas' Hospital solution No. 2. Additionally we also demonstrated an improvement in electrophysiologic stability and coronary vasodilatation during cardioplegic perfusion with the addition of 1mM adenosine. The addition of 0.1 mM did not improve hemodynamic recovery compared to St. Thomas' hospital solution, whereas addition of 10 mM showed depressed recovery of hemodynamic function.

The results of this study show that the concentration of adenosine is important in relation to its effect in improving and protecting myocardial function during ischemia. Other studies have shown an improvement with 200 µM of adenosine added to high potassium solutions. Our rationale for using high doses of adenosine was based on the idea of inducing cardiac arrest.

It appears, however, that in the primate and possibly also in man, cardiac arrest with adenosine can only be achieved with additional measures. That does not exclude, however, the possibility that a low dose of adenosine as mentioned in other studies with 200 µM might be beneficial as an additive to high potassium solutions. The optimal concentration is also species-dependent. Our in-vitro studies showed that baboon erythrocytes contain a five times higher rate of adenosine deaminase than human cells, which could explain the five times higher concentration found to be optimal in the baboon. On the other hand, Mentzer (1993) stresses the value of adding high doses of adenosine in cardiac preservation solutions such as the University of Wisconsin solution. However, as mentioned in previous chapters, there is a difference between storage and in vivo cardioplegic protection, and adenosine might be metabolized less at the low temperatures involved with storage of 4° C.

To conclude, adenosine alone is not effective as a cardioplegic agent in the primate, but it improves hemodynamic recovery post-ischemia. The combination of adenosine and high potassium improved hemodynamic recovery in terms of ejection phase indices mainly via reduction of systemic vascular resistance. However, the combination of high potassium and adenosine improved postischemic electrical stability, as shown by the reduced requirements for defibrillation to revert the hearts back to sinus rhythm. It appears that in the clinical setting adenosine in high concentrations can only be used as an induction agent for cardioplegia with additional measures or as a pre-treatment before ischemia. The combination of adenosine and high potassium is less beneficial in terms of hemodynamic recovery, but improved rhythm stability and coronary vasodilatation are desirable factors in clinical practice. Clinical studies should, however, employ a lower concentration of adenosine.

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Full length articles

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