Warm (32 degrees celcius) polarised arrest: towards a new cardioprotective strategy using normokalaemic adenosine-lignocaine cardioplegia

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The research methods used in this thesis received clearance from the James Cook University Experimentation Ethics Review Committee (Animal Ethics approval numbers A759, A1107, A1747).

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ABSTRACT

Background and Aims

Cardiac surgeons and anaesthetists are facing unprecedented challenges from increasing numbers of higher-risk adult and paediatric patients. Currently, cardiac surgeons rely on depolarising potassium cardioplegia and hypothermia to protect the heart during surgical cardiac arrest. A range of adverse outcomes may result from prolonged hyperkalaemia and cold temperatures, however no alternative to high potassium cardioplegia has successfully translated into clinical use. There is a compelling need for new evidence-based myocardial protection strategies. In 2004 our laboratory developed a novel polarising cardioplegia using adenosine and lignocaine (AL) as the arresting agents in a normokalaemic solution.

The main aim of this thesis was to develop normokalaemic AL cardioplegia for arrest at warm (32^{o}C) or cold (8-12^{o}C) temperatures using intermittent delivery over two hours or 'one-shot' delivery to extend the single-dose arrest interval to 50 minutes in the isolated working rat heart. The efficacy of this AL cardioplegia protocol was then compared with del Nido hyperkalaemic cardioplegia solution at warm and cold temperatures.

Methods

Experiments were conducted on male adult Sprague Dawley rats using isolated heart perfused models (Langendorff model and working heart model modified by Neely). Arrest was induced and maintained with intermittent (20 minute intervals) or continuous cardioplegia delivery for up to two hours at 32°C, or one-shot delivery for 40 and 50 minutes at 32^{o}C and 8-12^{o}C. Functional recovery was assessed during reanimation in Langendorff mode and 60 minutes of working mode reperfusion with primary endpoints of heart rate, aortic flow, coronary flow, cardiac output, systolic pressure and diastolic pressure. The alpha level of significance for all experiments was set at $p<0.05$.

Experimental design

The thesis was divided into five study arms:

Study 1: The first study compared recovery of function following 40 or 60 minutes of warm arrest with intermittent or continuous delivery of AL cardioplegia.

Study 2: The second study evaluated the effect of varying the potassium concentration in AL or potassium alone cardioplegia solution on arresting membrane potential and functional recovery following two hours of warm intermittent arrest.
Study 3: In the third study a warm AL 40-minute one-shot arrest protocol was developed, and the AL cardioplegia modified with an antioxidant adjunct to improve recovery of post-arrest function.
Study 4: In the fourth study the AL solution and arrest protocols were modified to optimise 50-minute warm and cold one-shot arrest.
Study 5: The fifth study compared recovery of function following warm and cold one-shot arrest with AL cardioplegia or hyperkalaemic del Nido solution.

Results

The first major result from this thesis was that AL cardioplegia delivered continuously or intermittently at 32°C resulted in no significant difference in coronary vascular resistance during cardioplegia delivery, or recovery of heart rate, aortic flow, coronary flow or rate pressure product following arrest.
The second study showed that increasing or decreasing the potassium concentration in AL cardioplegia above or below normokalaemia (5.9mM K^+) at 32°C led to depolarised and hyperpolarised states respectively, which increased coronary vascular resistance and decreased functional recovery. High potassium alone solutions (16 and 25mM K^+) also resulted in reduced recovery of function. After 1- or 2-hour arrest, optimal coronary vascular resistance and functional recovery (cardiac output and stroke volume) occurred when the heart was arrested at polarised membrane potentials. Notably, AL with low potassium (AL 3mM K^+) produced significantly worse outcomes than all other groups after 2-hour arrest.
In the third study, increasing the cardioplegia delivery interval from 20 to 40 minutes with warm one-shot AL arrest significantly decreased function (increased the time to reanimation at reperfusion and decreased functional recovery early in the reperfusion period) compared with continuous or intermittent AL delivery. AL one-shot hearts recovered 37 ± 13% of pre-arrest cardiac output at 10 minutes reperfusion and 76 ± 2% of cardiac output at 60 minutes reperfusion, compared with 89 ± 4% (p<0.05) and 90 ± 2% (p<0.05) recovery of cardiac output respectively for hearts arrested with intermittent AL cardioplegia. The addition of N-(2-mercaptopropionyl)-glycine (MPG), a hydroxyl radical scavenger, to the terminal flush and reperfusion solutions of the 40-minute one-shot protocol significantly improved recovery of cardiac output to 83 ± 3%

(p<0.05) at 10 minutes and 87 ± 3% (p<0.05) at 60 minutes reperfusion, which was not significantly different from recovery following intermittent warm AL arrest.
Increasing the one-shot arrest interval to 50 minutes also decreased recovery which was improved by doubling the cardioplegia adenosine and lignocaine concentrations to AL(400:1000). Functional recovery was not further improved with addition of MPG during warm arrest with higher AL concentrations. However, at cold arresting temperatures increased magnesium (2.5mM) was required in the higher AL concentrations (ALM) for comparable recovery of function to warm AL (400:1000) arrest.
Study 5 compared AL cardioplegia with hyperkalaemic del Nido solution (24mM K^+) for 50-minute one-shot arrest at warm (32^{o}C) and cold (8-12^{o}C) temperatures. Warm arrest with del Nido solution significantly reduced functional recovery with 27 ± 2% return of aortic flow and 68 ± 3% coronary flow at 60 minutes reperfusion compared with recovery of 69 ± 2% aortic flow (p<0.05) and 105 ± 4% coronary flow (p<0.05) following del Nido arrest at cold temperatures. In contrast, hearts arrested with AL cardioplegia at warm or cold temperatures recovered 76 ± 2% (p<0.05) and 73 ± 2% (p<0.05) of pre-arrest aortic flow respectively, and 92 ± 5% (p<0.05) and 121 ± 5% (p<0.05) of pre-arrest coronary flow respectively.

Conclusions

These results demonstrated that AL cardioplegia is a versatile cardioplegia when delivered continuously or intermittently at 32°C. When delivered intermittently for one or two hours, optimal coronary vascular resistance and post-cardioplegia recovery was recorded when the heart's membrane potential was close to resting value (-75mV). Higher or lower membrane potentials resulted in significantly lower functional recovery indicating that cell voltage was a key feature of AL cardioprotection in the isolated working rat heart at 32°C. When the adenosine and lignocaine concentrations were doubled (AL 400:1000) AL cardioplegia was versatile at warm (32^{o}C) and cold temperatures for 50-minute one-shot delivery. AL cardioplegia was superior to del Nido solution for one-shot delivery at warm temperatures, and at cold temperatures ALM resulted in significantly higher coronary flows and equivalent recovery of other parameters. The warm AL intermittent and one-shot arrest protocols developed in this thesis may have clinical potential as alternative cardioplegia strategies to hypothermic, hyperkalaemic cardioplegia. Further translational studies are required.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

Units

cm	centimetres
g	gram
kg	kilogram
mg	milligram
ml	millilitres
mM	millimolar
mmHg	millimetres of mercury
mV	millivolts
µM	micromolar

Abbreviations/Acronyms

A_1-receptor	adenosine$_1$-receptor
AF	aortic flow
AL	adenosine-lignocaine
ALM	adenosine-lignocaine-magnesium
ALMI	adenosine-lignocaine-magnesium-insulin
ANOVA	analysis of variance
ATP	adenosine 5'-triphosphate
CABG	coronary artery bypass grafting
Ca^{2+}	calcium ion
$CaCl_2$	calcium chloride
CF	coronary flow
CO	cardiac output
CO_2	carbon dioxide
Cont	continuous
CPB	cardiopulmonary bypass
CVR	cardiovascular resistance
DP	diastolic pressure
E_m	resting cell membrane potential
ETC	electron transport chain
GPD	glutathione peroxidase
H^+	hydrogen ion
H_2O	water
H_2O_2	hydrogen peroxide
HR	heart rate
Int	intermittent
K^+	potassium ion
K_{ATP} channels	adenosine triphosphate sensitive potassium channels
KCl	potassium chloride
KH	Krebs-Henseleit solution
LV	left ventricular
Mg^{2+}	magnesium ion
$MgCl_2$	magnesium chloride
$MgSO_4$	magnesium sulphate
MPG	N-(2-mercaptopropionyl)-glycine
Na^+	sodium ion
NaCl	sodium chloride

NAD(P)H	reduced nicotinamide adenine dinucleotide phosphate
$NaHCO_3$	sodium bicarbonate
NaH_2PO_4	sodium dihydrogen phosphate
^{31}P NMR	phosphorus-31 nuclear magnetic resonance
NO	nitric oxide
$\bullet O_2^-$	superoxide ion
•OH	hydroxyl radical
OS	one-shot
PCO	potassium channel opener
PCr	phosphocreatine
pO_2	partial pressure of oxygen
ROS	reactive oxygen species
RPP	rate pressure product
SEM	standard error of mean
SOD	superoxide dismutase
SP	systolic pressure
SV	stroke volume
TAF	time to aortic flow
TFB	time to first beat
%TTW	percent total tissue water
WM	working mode
XO	xanthine oxidase

Chapter 1

Introduction and Literature Review

1.1 Introduction

Statement of the Problem

Currently there are over 800,000 coronary artery bypass graft (CABG) surgery or cardiac valvular operations performed each year around the world, with 350,000 performed in the USA alone and more than 20,000 in Australia [1]. While mortality rates remain low at around 1-2%, the incidence of post-operative complications appears to be increasing and presents an ongoing challenge [2-4]. Some of these complications may be due to older, sicker patients presenting with multiple comorbidities, failed angioplasties and redo surgeries [5-7]. In Australia it has been estimated that about 5% (1,000 patients) each year have serious complications following surgery. There is an urgent need for improved strategies of myocardial protection during cardiac surgery and the reperfusion period.

Potential contributors linked to post-operative complications include hypothermia, high potassium cardioplegia, and the methods of rewarming the heart following surgery [8-11]. Most cardioplegia solutions contain high levels of potassium (>15mM) which arrests the heart at unnatural cell membrane voltages, and this may lead to intracellular calcium loading, mitochondrial dysfunction, generation of reactive oxygen species, post-operative arrhythmias and left ventricular dysfunction [12-14].

The following literature review will trace the development of modern cardioplegia techniques, from the earliest cardiac surgery via the discovery of arresting agents and the invention of cardiopulmonary bypass to the practice of hypothermic hyperkalaemic arrest. Next the potential problems with high potassium arrest will be discussed and the concerns regarding the level of cardioprotection afforded by high potassium cardioplegia, methods currently employed to reduce the deleterious effects of surgical cardiac arrest, and research into a novel normokalaemic cardioplegia solution using adenosine and lignocaine as the arresting agents.

1.2 Cardiac surgery – early beginnings

Prior to the 17th century, most surgeons considered that the heart was "surgically untouchable" [15-17]. Recognition for the earliest cardiac surgery is often attributed to Baron Dominique Jean Larrey, surgeon to Napoleon's Imperial Guard, and Francisco Romero, a Barcelona surgeon. In the early 19th century they each performed pericardiotomy to relieve pericardial compression due to traumatic cardiac wounds and non-traumatic effusions. These procedures were performed on beating hearts without the aid of general anaesthesia or aseptic techniques. Towards the end of the 19th century the general opinion persisted that heart surgery was next to impossible because of the time it takes to access the heart and the crucial function of the heart as a pump [16].

> *"Surgery of the heart has probably reached the limits set by Nature to all Surgery; no new method, no new discovery, can overcome the natural difficulties that attend a wound of the heart."*
>
> *Stephen Paget, 1896 (From Shumacker, HB, The Evolution of Cardiac Surgery" 1992 p3)*

Then in 1896 Ludwig Rehn successfully sutured a stab wound in the heart of a young German soldier [16,18-21], achieving a milestone in the history of cardiac surgery. By the 1940s surgery was being performed on beating hearts to repair simple congenital heart defects [22 23], and in the 1950s surgeons performed more complicated surgery on atrial septal defects and pulmonary stenosis using Bigelow's innovation of heart and whole body combined hypothermia and total body circulatory arrest [24 25]. The next goal was to find a way to stop the heart beating and provide extra protection as hypothermia alone was not sufficiently protective for longer cardiac procedures.

1.3 A short history of the development of potassium-based cardioplegia solutions

The ability to arrest the heart had been discovered around 70 years earlier in the laboratory of British physiologist Sidney Ringer. Ringer recognised the 'paralysing' properties of high potassium on heart muscle. He recorded both the incremental effect of potassium on the force of contraction until higher concentrations arrested the heart in diastole, and the opposing action of calcium which was vital for maintaining systole

[26,27]. Subsequently, studies conducted on dogs in 1929 by Hooker also demonstrated the defibrillating potential of potassium. When potassium was used to arrest a fibrillating heart normal sinus rhythm was regained on washout [28].

As mentioned above, in the 1950s, despite Bigelow's innovation of surgical hypothermia to slow whole body metabolic processes [29,30], there remained a major unmet need to safely and reversibly stop the heart for cardiac surgery. In 1950 Wiggers [31] had extended the work of Hooker and showed that potassium cardioversion in conjunction with intravenous administration of calcium led to a more stable recovery. It was not until the early-to-mid 1950s that a number of high potassium agents were being tested to induce chemical 'arrest' [32-34]. For example, Lam and colleagues used an intraventricular injection of potassium chloride (667 mEq/L) to induce cardiac arrest in hypothermic dogs [35]. However this technique was abandoned due to refractory ventricular fibrillation and heart damage during reperfusion [33,36].

In 1955 Melrose performed the first reversible cardioplegic arrest in a canine model of cardiopulmonary bypass using tri-potassium citrate which appeared safer than potassium chloride alone [34]. Unfortunately, the 'Melrose technique' resulted in high mortality and many adverse outcomes were reported such as lethal dysrhythmias [37], and lack of cardiac protection demonstrated by widespread tissue necrosis at autopsy [38-40]. Around the same time physiologists, using newly developed electrophysiological instruments to study isolated cells, reported that potassium induced membrane depolarisation in cardiac tissues [41,42] and showed the link between high potassium and contracture of the heart [43].

Due to poor clinical outcomes and concerns about the safety of cardioplegic arrest [38,44], potassium-based cardioplegia was abandoned in the 1960s, and alternative strategies sought. Aortic cross-clamping combined with topical hypothermia [45] was shown to provide superior protection compared with cross-clamping arrest at normothermic temperatures which caused ischaemic contracture and could lead to the extreme result of 'stone heart' [46]. Studies by German cardiac teams to modify cardioplegic solutions culminated in the successful development of multicomponent Bretschneider's solution [47] which induced arrest because it contained low sodium levels and was calcium free.

Interest in potassium as an arresting agent was rekindled with research commenced in the late 1960s. By the 1970s cold cardioplegia solutions containing potassium had been refined and improved by teams in America, the United Kingdom and Europe [48-51].

The original St. Thomas Hospital Solution No.1, developed by Hearse and colleagues [48] using the isolated perfused rat heart model, was then modified by Gerald Buckberg and associates [52] and used in a blood-cardioplegia mixture, with improved outcomes. Subsequent modifications to the components and administration techniques of blood–based cardioplegia solutions [53-56], and arresting temperatures [53,57-59], provided some additional protection for the heart and body, however reports of adverse effects induced by hyperkalaemia [60,61] continued.

Hyperkalaemic cardioplegia has been used at hypothermic temperatures to provide a high standard of myocardial protection for over forty years. Most surgeons, however, understand the limitations of high potassium and hypothermia, and many groups have sought alternative normokalaemic strategies of reversible myocardial arrest [13,62]. Despite their efforts very few alternatives have translated into clinical use. Given the ageing population with patients presenting with extensive degenerative heart disease complicated by comorbidities there is an urgent need for a fresh approach to cardioprotection during cardiac surgical procedures.

1.4 Surgical cardiac arrest and ischaemia-reperfusion injury

Any successful alternative to high potassium cardioplegia must address the problem of ischemia and reperfusion injury. During an ischaemic episode blood flow and oxygen delivery are inadequate to meet the energy requirements of the tissues and washout or completely oxidise metabolic products [63], resulting in a supply-demand imbalance.

1.4.1 Reversible ischaemia-reperfusion Injury

Periods of myocardial ischaemia, with no perfusion of the cardiac muscle, coronary arteries or micro-vascular networks, occur during cardioplegic arrest and surgical procedures such as coronary artery bypass grafting and angioplasty [64]. Ischaemic episodes of less than 15 minutes duration cause reversible cell changes including membrane depolarisation, potassium release, tissue hypoxia, lactate accumulation, acidosis, minor oedema and decreased levels of high-energy phosphates, adenine nucleotides and glycogen [65]. During a short no-flow, or low-flow, period there is little or no cell death or impairment of microvascular function.

At reperfusion, re-supply of oxygen and metabolic substrates leads to restoration of the myocardial cell membrane potential, ionic homeostasis, and mitochondrial oxidative phosphorylation [66]. Interstitial pH and osmolality are also rapidly normalised, however intracellular pH initially remains acidic, creating a pH gradient across the membrane. This gradient stimulates the sodium-hydrogen exchanger to extrude protons from the cell in exchange for sodium ions (Na^+). The excess cytosolic sodium is in turn extruded by the sodium/potassium ATPase (Na^+ /K^+-ATPase) if adenosine triphosphate (ATP) reserves are adequate, or Na^+ is exchanged for calcium (Ca^{2+}) by the sodium/calcium exchanger [67]. Cytosolic calcium is sequestered by calcium ATPases (Ca^{2+}-ATPase) on the sarcoplasmic reticulum, with excess calcium oscillated between the sarcoplasmic reticulum and cytosol. Providing the Na^+/K^+ ATPase has also reactivated and restored sufficient sodium gradient across the cell membrane, the Na^+ /Ca^{2+} exchanger on the sarcolemma removes the excess calcium from the cell [68-70]. Reversible ischaemic injury has been linked to myocardial 'stunning' characterised by transient impairment of myocardial contractility, arrhythmias, and microvascular dysfunction [71-73].

1.4.2 Prolonged ischaemia and irreversible ischaemia-reperfusion Injury

During prolonged ischaemia irreversible injury and cell death can occur due to activation of the processes described above which are exacerbated by delayed reperfusion. Oxidative respiration ceases [74], and anaerobic glycogenolysis, the only major pathway for ATP replenishment, produces high levels of lactate and protons (H^+). Acidosis promotes increased Na^+/H^+ exchange that leads to Na^+/ Ca^{2+} exchange and elevated intracellular sodium and calcium concentrations [67,68,75]. As the availability of glycogen becomes limiting, intracellular calcium loading continues and membrane potential continues to depolarise, with increased potassium efflux into the extracellular fluid [76,77]. Ion pumps attempt to correct the altered cellular environment, further depleting levels of high energy phosphates [78] which leads to loss of vital cell functions including inhibition of the Na^+ /K^+ ATPase and the calcium pump of the sarcoplasmic reticulum [79] resulting in further calcium overload. Cell oedema occurs as cells become energy depleted [78]. Mitochondria swell, inner membrane potential collapses and mitochondrial lipids rearrange into amorphous densities [80]. Irreversible cellular changes [81], and increasing loss of calcium homeostasis [82] appear to be associated with prolonged ischaemia.

The irreversible changes, which are not manifested until reperfusion causes a sudden restoration of blood flow and oxygen supply [69,83,84], are collectively termed ischaemia–reperfusion (IR) injury [85-88]. Irreversible damage due to abrupt re-oxygenation of previously viable ischaemic tissue was termed the 'oxygen paradox' by Hearse [89,90] and is associated with release of inflammatory mediators [91-93] and the production of reactive oxygen species (ROS) [81,94,95].

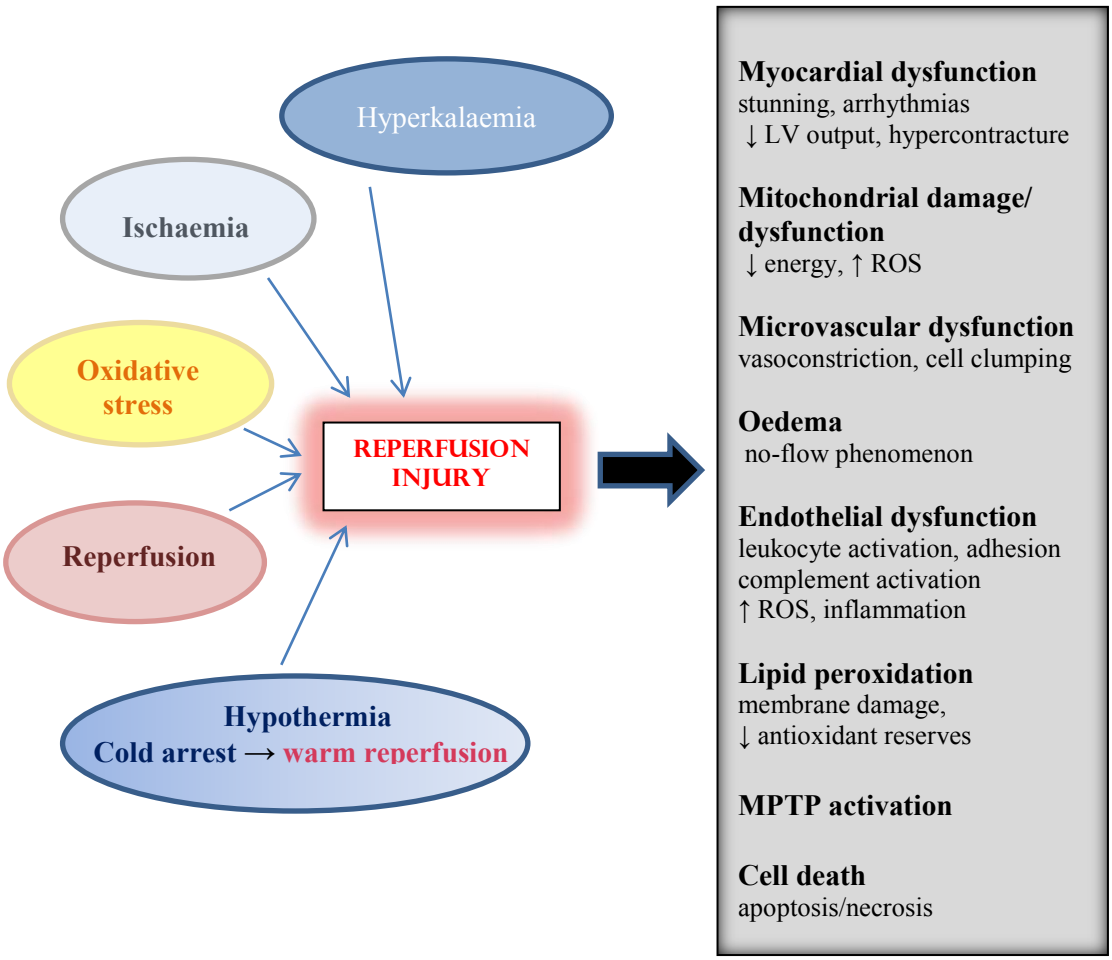


Figure 1.1: Diagram showing conditions which potentially contribute to reperfusion injury, and effects of reperfusion injury in cardiac tissues

Oxidative stress and ischaemia-reperfusion injury

Oxidative stress is recognised as a major mechanism in the process of ischaemia-reperfusion injury [96]. Oxidative stress has been demonstrated during the reperfusion period when antioxidant defences are inadequate to protect ischaemic tissue against damage from reactive oxygen species [97,98]. The increased generation of reactive oxygen species results in further depletion of antioxidant levels [99], lipid peroxidation and cell ultrastructural changes [100-102].

Reactive oxygen metabolites are partially reduced forms of molecular oxygen with an unpaired electron, making them short-lived but highly reactive. Reactive oxygen species can induce myofilament damage, lipid peroxidation of membranes, mitochondrial damage and oxidation of the sulfhydryl groups of structural proteins and enzymes [103], leading to irreversible tissue injury, calcium handling defects, reduced contractility and altered cardiac function [104-108].

During normal cellular function reactive oxygen species such as the superoxide anion ($\bullet O_2^-$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($\bullet OH$) are generated in limited amounts by mitochondrial respiration, xanthine oxidase activity, activated polymorphonuclear leukocytes, arachidonic acid metabolism and auto-oxidation of catecholamines [96]. In physiological conditions reactive oxygen species act as a cell signalling mechanism involved in homeostatic processes, and are scavenged by endogenous antioxidant enzymes such as catalase, superoxide dismutase (SOD) and glutathione peroxidase (GPD) [68,87,109] and 'organic scavengers' including ascorbate, alpha-tocopherol and glutathione [110]. Ischaemia also decreases the activities of some endogenous antioxidant enzymes (SOD, GPD) [87].

Incomplete oxidative phosphorylation during ischaemia may increase production of reactive oxygen species in the mitochondria [81,111,112], initiating damage to the electron transport chain (ETC) [68,109] which subsequently leads to a further increase in production of reactive oxygen species at reperfusion [113]. Re-oxygenation at reperfusion in an acidotic environment of increased reducing equivalents and decreased ATP levels triggers a burst of more highly reduced and reactive radical species such as the hydroxyl radical and peroxynitrite [114,115]. This rapid increase in reactive radical species peaks in the initial seconds to minutes of reperfusion [116,117], may overwhelm the cell's depleted antioxidant defences and exacerbates damage to the cell [87,118] (Figure 1.2).

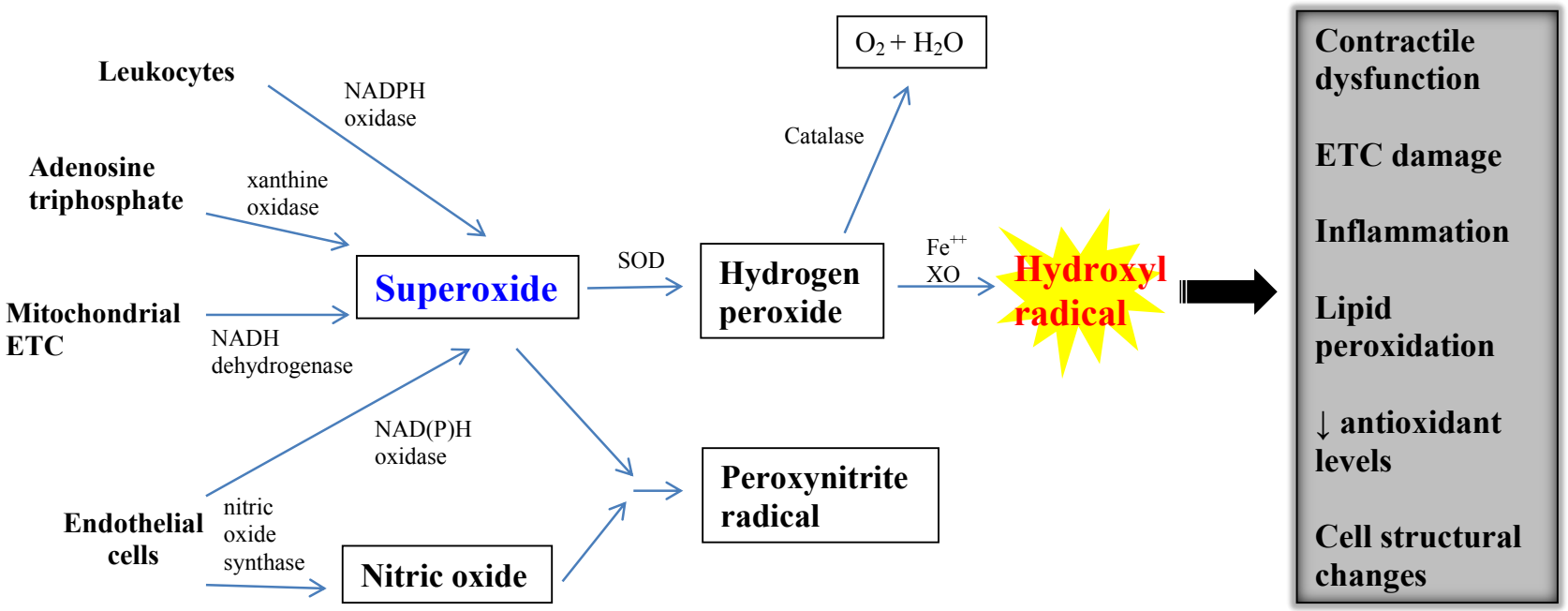


Figure 1.2 : Diagram illustrating potential pathways of superoxide, peroxynitrite and hydroxyl radical generation during ischaemia and at reperfusion with resulting reperfusion injury to cardiac tissues.
During ischaemia ATP is metabolised to hypoxanthine and xanthine which act as substrates for xanthine oxidase (XO) to generate superoxide when oxygen supply is restored at reperfusion. At resumption of mitochondrial respiration, NADH dehydrogenase generates superoxide radicals. Activated neutrophils also produce reactive oxygen species in a respiratory burst at reperfusion. Ischaemia-induced alteration of nitric oxide synthase function can result in formation of superoxide and nitric oxide which react to form the peroxynitrite radical.
Superoxide is dismuted by superoxide dismutase (SOD) to hydrogen peroxide which may be converted by catalase to oxygen and water, or superoxide may react with ferrous salts via the Fenton reaction to form hydroxyl radicals (adapted from Zweier and Talukder, 2006 [87]).

Conversely, radical species can exert a protective effect during ischaemic episodes. Increased production of nitric oxide (NO) during ischaemia [119] can inhibit other reactive oxygen species generating pathways [120,121] and cause minor depolarisation of the inner mitochondrial membrane. This limits mitochondrial calcium uptake [122] and production of superoxide [123] to preserve mitochondrial function and reduce reperfusion injury.

Hyperkalaemic conditions promote oxidative stress due to depolarisation-induced endothelial injury and inflammation [124-126], generation of reactive oxidant species such as superoxide [127-130], and exacerbation of ischaemia-reperfusion injury. These effects can be abolished by membrane hyperpolarisation [131] or the presence of a polarising adenosine triphosphate sensitive potassium (K_{ATP}) channel opener [129].

Myocardial antioxidants reduce oxidative damage to cellular molecules and structures by scavenging reactive oxygen species and their precursors, enhancing endogenous antioxidant mechanisms or inhibiting the formation of reactive oxygen species [64,132,133]. To be effective, antioxidants and ROS scavengers must be administered before, or at the onset of, reperfusion to improve recovery following cardiac arrest and minimise ischaemia-reperfusion injury due to oxidative stress [83].

A range of antioxidants has been investigated in animal studies and clinical trials for their ability to improve protection against ischaemia-reperfusion injury. In rabbit, dog and rat hearts, superoxide dismutase [99,134], allopurinol and catalase [135] effectively decreased the generation of reactive oxygen species, whereas functional and biochemical parameters were maintained by reducing hydroxyl radical formation using superoxide dismutase [136]. Antioxidant treatment with the quinone coenzyme Q10 [137,138] or supplementation with a combination of vitamin C, vitamin E and allopurinol also produced contradictory results in clinical trials, with reports varying from improved antioxidant activity and functional recovery [139,140] to no change in clinical outcome or biochemical markers of myocardial damage [141].

Treatment with thiol-containing compounds aims to maintain antioxidant reserves by preventing depletion of intracellular glutathione stores which protect against oxidative stress during myocardial ischaemia and reperfusion. N-(2-mercaptopropionyl)-glycine (MPG) is a cell-permeable synthetic thiol compound with radical species scavenging properties. MPG administered during low-flow ischaemia [142] or before and during reperfusion [83] has been reported to be effective in protecting against hydroxyl radical-induced post-ischaemic contractile dysfunction.

Extended periods of ischaemia stimulate the release of mediators of inflammation which subsequently increase the damage to cardiac tissue. Nitric oxide, reactive oxygen species, arachidonic acids, platelet-activating factor, and a range of cytokines and interleukins are released from cardiomyocytes, endothelial cells and macrophages in ischaemic myocardium and at reperfusion [143,144]. Compounding this effect is the vascular response to high potassium cardioplegia which induces vasoconstriction [126,145], endothelial cell depolarisation [129] and production of superoxide, leading to loss of nitric oxide-dependent relaxation, activation of platelets and neutrophil adhesion [124].

1.5 The contribution of hypothermia and cardio-pulmonary bypass to early advances in cardioprotection

Concurrently with the development of cardioplegia agents and solutions, clinicians were investigating methods of protecting the heart and body from ischaemic injury during elective cardiac arrest [146]. Total circulatory arrest allowed surgeons to successfully operate on small heart defects which could be rapidly repaired [22,23,147,148], but a new approach was needed for more complex surgery requiring longer procedures.

1.5.1 Cardiopulmonary bypass

After World War II, teams in several medical centres in North America were developing machines designed to take over the function of the heart and lungs during cardiac surgery to maintain oxygenated circulation and allow regulation of the temperature of the heart and body [149]. The first successful human open heart surgery using cardiopulmonary bypass (CPB) was performed by John Gibbon on May 6, 1953 to close an atrial septal defect [150]. In subsequent decades refining of cardiopulmonary bypass techniques dramatically reduced mortality rates and improved post-operative outcomes for open heart surgery [151]. Cardioplegia in conjunction with cardiopulmonary bypass is designed to produce rapid electro-mechanical arrest to reduce damage to the heart tissues, a decompressed motionless heart preventing air embolism [152] and provide operating visibility in a bloodless surgical field [60].

1.5.2 Detrimental effects of cardiopulmonary bypass

Surgical trauma and contact with the non-physiological surfaces of the bypass circuit during cardiopulmonary bypass initiates a systemic inflammatory response which potentiates ischaemia-reperfusion injury by activating leucocytes and platelets and altering normal haemostatic responses [149,153,154]. These processes may impair functional recovery [155,156] and have been implicated in graft rejection [157]. Ischaemic cardioplegic arrest with cardiopulmonary bypass has been shown to induce coronary endothelial dysfunction and production of reactive oxygen species [126,158], cerebral injury [159], and visceral complications [160,161]. Cardiopulmonary bypass has also been linked to post-operative myocardial stiffness and impaired relaxation of the left ventricle [162].

1.5.3 Cardiopulmonary bypass and hypothermic cardioplegia

At the same time as cardiopulmonary bypass techniques were evolving, researchers were investigating additional methods to improve intra-operative myocardial protection. Inspired by the survival of natural hibernators in extremely cold conditions, William Bigalow conducted animal experiments to demonstrate the use of therapeutic whole body hypothermia which reduced oxygen demand and allowed longer periods of circulatory arrest [29,30]. Initially, the use of moderate total body hypothermia (30^{o}C) as a protective strategy during transient circulatory arrest resulted in limited success [24]. However clinical expertise had improved significantly by 1955 [163,164] and favourable outcomes were reported using hypothermia in combination with extracorporeal circulation [165], or deep hypothermia (15^{o}C) combined with circulatory arrest for periods of up to 45 minutes [25]. Then in 1959, Norman Shumway's group showed that the heart's demand for oxygen was reduced, and heart muscle cells preserved, when the heart was immersed in a cold saline solution circulated through the pericardial sac during aortic cross-clamping [45]. Since the 1960s, hypothermia and total circulatory arrest have been important aspects of surgical techniques used during open heart surgery on adults [152,166] and infants [167-169]. Gradually clinical trials established guidelines for improved post-operative outcomes using hypothermia in conjunction with bypass techniques. The combination of deep hypothermia with limited bypass and a period of circulatory arrest at 22^{o}C [170] for repair of congenital defects in infants resulted in improved neurological protection [171] whereas total circulatory arrest at 18^{o}C resulted in altered cerebral metabolism, blood flow and oxygen utilisation which persisted after bypass [172].

Advantages and disadvantages of hypothermia during cardiac surgery

As a protective strategy during cardiac surgery, hypothermia may be applied as moderate systemic cooling of the blood via the cardiopulmonary bypass circuit, or as myocardial hypothermia achieved by infusing cold cardioplegic solution, applying a cooling jacket or by direct application of topical iced slush [173,174]. Hypothermia allows a longer 'safe' period of ischaemic arrest by reducing the metabolic rate and oxygen requirements of tissues [30]. Metabolic rate decreases by 8% with each degree centigrade reduction of temperature [175,176], and a reduction of 15^{o}C below normothermia lowers myocardial oxygen consumption to 0.3ml O_2/min/100g of myocardium [177]. Hypothermic temperatures also reduce cardiopulmonary bypass-induced inflammatory tissue damage [178] and increase cardioprotection when used as an adjunct to hyperkalaemic cardioplegia [179].

However the combination of cold and acidosis due to anaerobic metabolism induced by ischaemia may adversely affect enzymatic function, energy production, cellular integrity [180-182] and contractility [183], and when combined with cardiopulmonary bypass, hypothermia is also implicated in platelet dysfunction [154]**.** Deep hypothermia increases blood viscosity, reduces capillary blood flow and oxygen supply to tissues, impairs blood coagulation, and increases calcium loading of the cells and cell swelling [184]. Although increased viscosity may be counteracted by the volume of cardioplegia solution delivered, this causes haemodilution and decreased haematocrit which are associated with increased risk of perioperative stroke [185]. Systemic cooling [186] and the use of topical slush have also been linked to post-operative complications including phrenic nerve paralysis [187], pulmonary dysfunction [188,**174**] acute inflammatory response [189], and circulatory and perfusion disturbances [190].

1.6 The unsuccessful search for an alternative to high potassium cardioplegia

While potassium-based cardioplegia solutions were being developed and modified, a range of alternative pharmacological compounds were also investigated for possible use as sole or combination arresting agents, or as adjuncts to hyperkalaemic cardioplegia solutions.

1.6.1 ATP-sensitive potassium channels

The discovery of adenosine triphosphate sensitive potassium channels in cardiac ventricular myocytes in 1983 [191] opened a new field of cardiac research. K_{ATP} channels have now been identified in a range of organs and tissues. Physiological concentrations of adenosine triphosphate inhibit K_{ATP} channels, but they open as ATP levels decrease during hypoxia or ischaemia, allowing potassium ions to flow out of the cell. K_{ATP} channels provide a link between metabolism and electro-mechanical coupling in muscle cells [192].

Activation of sarcolemmal [193] and mitochondrial [194] K_{ATP} channels has also been identified in the mechanism of ischaemic preconditioning, where brief periods of ischaemia enhance protection against injury during subsequent bouts of prolonged cardiac ischaemia [192,195,196].

1.6.2 Potassium channel openers as cardioplegic compounds

Potassium channel openers (PCO) provided an alternative method of achieving reversible cardiac arrest. A potassium channel opener is a compound that activates K_{ATP} channels at normal intracellular levels of ATP. This enhances the repolarising potassium current and reduces the action potential duration which decreases myocardial contractility, resulting in polarised arrest [13,62,197]. Non-depolarising potassium channel openers maintain transmembrane electrical potential at its resting level, preventing membrane ionic pump activity, which minimises sodium and calcium influx [12] and preserves cellular energy levels [198] during arrest.

The potential advantages of polarised arrest were confirmed with studies using the potassium channel openers pinacidil (50μM) and aprikalim (100μM). Alone, or in combination with hyperkalemic cardioplegia solution, these compounds improved recovery at reperfusion compared with hyperkalaemic St. Thomas' Hospital solution [199,200]. Specific demonstrated effects of PCO-induced hyperpolarisation include relaxation of vascular smooth muscle which increases coronary flow [200], improved contractile recovery [200], decreased lactate dehydrogenase release, protection of electrical coupling via gap junctions [192], reduced intracellular free calcium release [201], preservation of intracellular ATP content during ischaemia and inhibition of myocardial contracture [202].

However research into the use of potassium channel openers did not translate into a safe clinical alternative to depolarising arrest. Therapeutically, potassium channel openers exhibit a tight dose-response curve and therefore narrow therapeutic window [203] with possible toxic side-effects including reperfusion arrhythmias [204]. Potassium channel openers are pro-arrhythmic as they decrease the duration of the action potential and therefore the refractory period, which predisposes the myocardium to re-entrant ventricular arrhythmias or ventricular fibrillation [13,78] especially after warm ischaemic arrest [203]. Potassium channel openers are also associated with systemic hypotension, higher myocardial oxygen consumption during early reperfusion, and prolonged electrical and mechanical activity at induction of arrest [199,205-207].

1.6.3 Adenosine

The potential of adenosine to modify cardiac physiological responses was first recognised in 1929 when Drury and Szent-Gyorgyi **[208]** reported that adenosine had an identical physiological action to adenylic acid on heart function and vascular smooth muscle tone. Since then research has established adenosine's influence on cardiac function and regulatory role in most organ systems of the body [209].

Adenosine: actions and properties

Adenosine, an endogenous purine nucleoside, influences cellular function by binding to four receptor subtypes (A_1, A_{2a}, A_{2b}, A_3) located on the surface of cardiomyocytes, inflammatory cells and cells in the vascular and cardiac conduction systems [210,211]. Receptor activation modulates signalling pathways via a variety of cellular effectors [78,212-217] to modify cellular functions, enzyme activity and metabolic pathways.

Activation of the A_1 receptor subtype lowers heart rate (negative chronotropic effect), slows cardiac conduction (negative dromotropic effect) and reduces atrial contractility (negative ionotropic effect). Adenosine opposes the effects of catecholamines to inhibit excitability via A_1 receptors located in atria, and sinoatrial and atrioventricular nodal cells [218-220], exhibits anti-arrhythmic effects in supra-ventricular tissues [218,221], decreases metabolic demand which preserves energy reserves and delays acidosis [222,223], and reduces infarct size [224-226].

Hypoxia stimulates an increase in endogenous levels of myocardial adenosine [227,228]. This physiological protective response results in coronary vasodilation mediated by hyperpolarisation of smooth muscle [229], nitric oxide release [230], A_3 receptor stimulation [231], enhanced calcium sesquestration [232], decreased sarcolemmal permeability to calcium [233], or increases in cyclic adenosine monophosphate via stimulation of A_2 receptors [230,234] located in coronary vascular smooth muscle and endothelial cells [217].

1.6.4 Adenosine as an arresting agent

The early work of Belardinelli and colleagues in the late 1980s demonstrated that adenosine activation of A_1 receptors increases outward potassium conductance, which shortens action potential duration and hyperpolarises the membranes in the atrial nodes. The resulting inhibition of atrial action potentials leads to atrio-ventricular block and rapid arrest [218,235]. Concentrations of adenosine above 20μM caused arrest in these pacemaker cells. Indirectly adenosine also inhibits myocardial contraction by decreasing cyclic adenosine monophosphate levels and therefore calcium influx, both actions having negative inotropic effects [236].

In 1989, in the first study using adenosine instead of potassium for cardioplegic arrest, Schubert and colleagues [237] concluded that adenosine provided more rapid arrest and that polarised arrest improved protection in the isolated rat heart compared with hyperkalaemic arrest. However, they found that arrest with adenosine alone led to unpredictable recoveries, which made it problematic for translation to humans as a sole arresting agent.

Adenosine was then used to confer protection as an “adjunct” to hyperkalemic cardioplegia. Increased protection was found when adenosine was added to crystalloid cardioplegia, while results were inconclusive when added to cold or warm potassium

blood cardioplegia. Some studies did not demonstrate significant haemodynamic improvement [238,239], while others reported that functional recovery was improved [239-241]. Adenosine (400μM-2mM) added to cold hyperkalemic blood cardioplegia was reported to be well tolerated, associated with a lower incidence of myocardial infarction [242,243], and more effective in protecting the myocardium from stunning [244] by preserving myofibrillar cooperative action and increasing anaerobic gylcolysis [240]. In a clinical trial Cohen and associates [238] concluded that there was no improvement in post-operative condition when CABG patients were treated with adenosine during warm antegrade blood cardioplegia. However, these patients received high potassium (40-200mM K^+) cardioplegia containing low doses of adenosine (15, 50, or 100μM) with cardioplegia delivery interrupted for up to 40% of bypass time. In 1998 Jayawant and Damiano reported that adenosine 200μM augmentation of hyperkalemic cardioplegia was the optimal concentration for myocardial recovery from ischaemia [78].

The combination of adenosine with magnesium and procaine has also been shown to improve cardioprotection compared with hyperkalaemic cardioplegia [245] and preserve endothelium-mediated vasodilation [246]. In a clinical trial this cardioplegic formulation also improved arrest times and decreased the incidence of post-operative atrial fibrillation [247]. Further research is required to investigate this cardioplegia combination.

1.6.5 Sodium channel blockers as cardioplegic agents

Sodium channel blockers such as tetrodotoxin and the local anaesthetics procaine and lignocaine block sodium entry to the cell via voltage gated sodium channels [248,249]. These compounds inhibit the influx of sodium required to initiate the depolarisation phase of the cardiac action potential, arresting the heart by preventing conduction of the action potential through muscle and nerve cells [198,250]. Sodium channel blockers cause rapid electrical arrest and therefore can eliminate the persistent electrical activity which may be an unwanted effect of cardioplegia using potassium channel openers such as pinacidil [203].

In studies in the early 1970s, procaine was added to cardioplegia formulations in an effort to arrest the heart more safely in a non-depolarised state [50,251,252], however procaine prolongs repolarisation time which predisposes the heart to arrhythmias [253]. Bixler and colleagues showed that at normothermic temperatures procaine cardioplegia did not provide protection during ischaemic arrest [254], whereas with the addition of

hypothermia (27°C) procaine cardioplegia was more protective than hypothermia alone and equally as efficient as potassium cardioplegia [254-256]. Procaine was reported to significantly improve myocardial protection when added to crystalloid potassium cardioplegia but not blood-based potassium cardioplegia [257].

Studies using tetrodotoxin also demonstrated the protective effects of non-depolarising arrest, including decreased myocardial oxygen consumption, reduced myocardial wall tension and decreased intracellular calcium concentration [258-260]. These results suggest that the protection may involve reduced metabolic demand linked to reduced calcium influx [261]. However toxic effects due to tetrodotoxin have also been reported [262], precluding its use as an arresting agent.

1.6.6 Lignocaine

Lignocaine, a commonly used local anaesthetic and class 1b antiarrhythmic agent, has been used prophylactically and in the treatment of ventricular fibrillation since the early 1960s [263]. Lignocaine exerts anti-arrhythmic effects following myocardial ischaemia by improving regional homogeneity of action potential duration [250], reducing the magnitude of the inward sodium current, depressing automaticity and slowing conduction of the cardiac impulse [198,264-266].

Lignocaine as an arresting agent

Lignocaine also acts as a cardioplegic agent by inhibiting initiation of action potentials. By blocking inward current through voltage dependent sodium fast channels in atria, ventricles and gap junctions, lignocaine reduces the phase 0 upstroke of the cardiac action potential, preventing depolarisation [250].

As an adjunct to hyperkalaemic cardioplegia, lignocaine has been shown to improve cardioplegia delivery to cardiomyocytes by reducing vasoconstriction compared with potassium only cardioplegia [267]. Addition of lignocaine also reduced the incidence of post-operative ventricular or atrial fibrillation [268,269] and the need for defibrillation [270] with no adverse effects on post-operative clinical outcome. An optimal concentration of 0.05mM lignocaine adjunct in hyperkalaemic St. Thomas' Hospital solution was determined by Hearse and colleagues [265] to enhance cardioprotection.

Although lignocaine added to hyperkalaemic cardioplegia enhanced cardioprotection during ischaemia and reperfusion [268], combined lignocaine and magnesium cardioplegia produced only equivalent return of left ventricular systolic function compared to potassium-based cardioplegia [198]. As a sole arresting agent lignocaine has not translated into clinical use due to its pro-arrhythmic potential [271].

1.7 Hyperkalaemic cardioplegia remains the 'gold standard' despite ongoing concerns

Hyperkalaemic depolarising cardioplegia has remained the cornerstone of cardiac surgery [12,60], despite concerns that it may provide less than optimal cardioprotection [272]. A range of post-operative cardiac complications have been linked to the effects of raised cardioplegia potassium levels on cardiac cellular structure and function.

1.7.1 High potassium depolarises the heart cell membrane potential

Most cardioplegia solutions in current use contain 15-20mM potassium which depolarises the resting cell membrane potential (E_m) of myocytes and endothelial cells from -84mV to approximately -50mV [273]. At this membrane potential the majority of the fast voltage activated sodium channels, voltage dependent L-type calcium channels and the sodium/calcium (Na^+/Ca^{2+}) exchanger are inactivated [274]. However transmembrane fluxes continue through a small fraction of the voltage gated sodium channels, allowing sodium entry into the cell via the active “window” current [275] which potentially operates between -20 and -60mV [262]. Increasing sodium levels trigger reversal of the voltage dependent Na^+/Ca^{2+} exchanger, which leads to a rise in intracellular calcium [267,276,277]. Calcium leak from the sarcoplasmic reticulum also contributes to an increase in intracellular calcium [106] leading to contracture and calcium overload [62], which can be exacerbated by hypothermia [184]. Further intracellular changes include a decrease in pH, sodium accumulation via the sodium/hydrogen (Na^+/H^+) exchanger [278] and increased high energy phosphate utilization [279]. High energy phosphates are further depleted when ion pumps are activated to correct the altered cellular environment, and cells swell as water is drawn in by increased ion concentrations [78]. The metabolic imbalances induced may lead to mitochondrial damage, oedema, myocardial dysfunction and cell death [78,203,280].

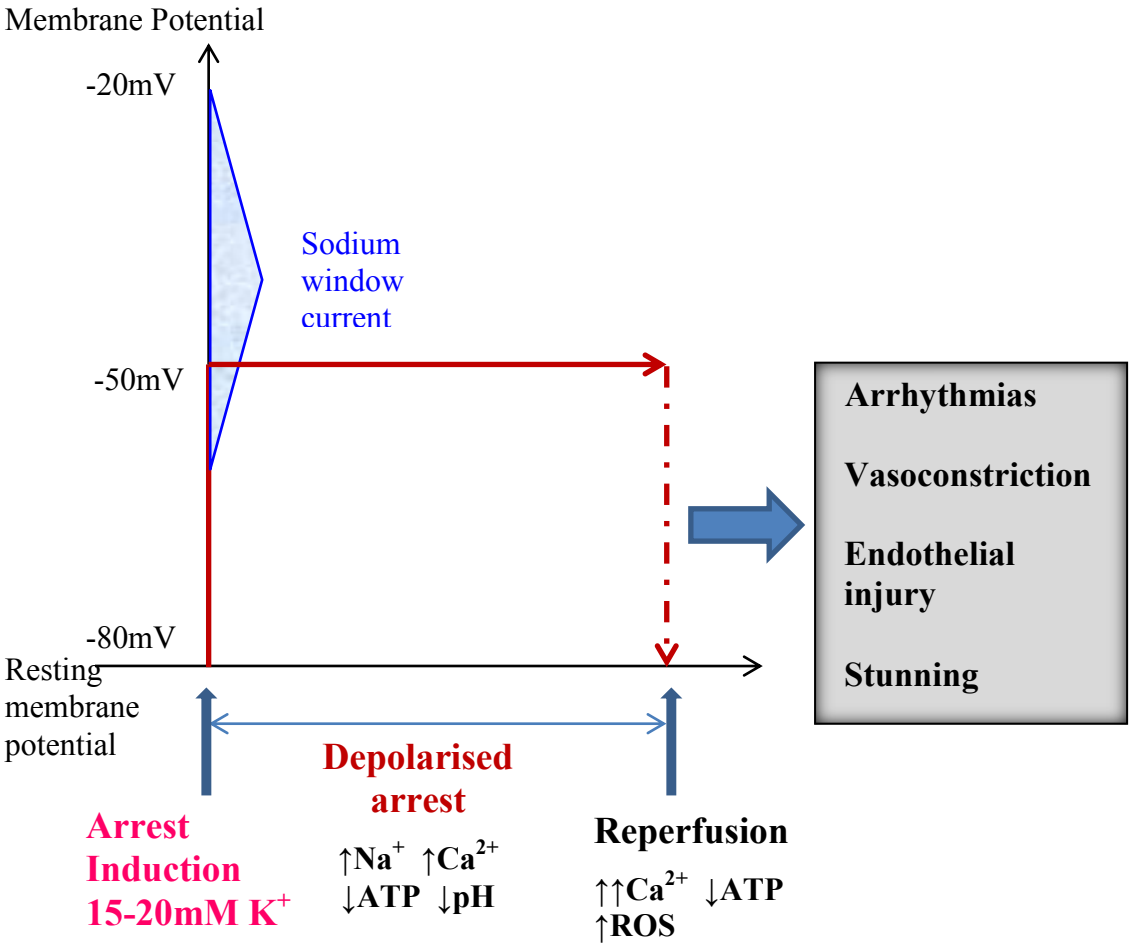


Figure 1.3 : Depolarisation of membrane potential and reperfusion injury associated with hyperkalaemic cardioplegia

1.7.2 High potassium is vasoconstrictive

The depolarising effect of potassium concentrations above 9.0mM [281] on vascular smooth muscle and endothelium has been linked to vasoconstriction [282-284], and coronary spasm [10,285] `which reduces cardioplegia flow [267,286], and may compromise perfusion of subendocardial regions [177,182,287]. Depolarising hyperkalaemia also impairs release of endothelium-derived vasodilators including nitric oxide [288], prostacyclin and endothelium-derived hyperpolarisation factor [289,290], contributing to vascular dysfunction including loss of smooth muscle relaxation and reduced vasodilation [182,291].

1.7.3 Endothelial injury linked to high potassium

Direct contact between hyperkalaemic cardioplegic solutions and the coronary vascular endothelium impairs endothelial function, promotes platelet adhesion, neutrophil activation and inflammation [124,286,292], and induces oxidative stress and generation of reactive oxygen species [129,293]. The degree of endothelial damage resulting from hyperkalaemic solutions has been shown to directly relate to the potassium concentration in the cardioplegia [294]. Endothelial injury has been linked to reduced cell viability [295], loss of endothelium-dependent vasodilation [294] and graft failure [1,296].

1.7.4 High potassium is proarrhythmic and can cause conduction disturbances

Due to potassium's effect on the electrophysiology of the heart [297], high potassium cardioplegia and the resulting decrease in membrane voltage predisposes the post-operative heart to atrial and ventricular arrhythmias and conduction disturbances at reperfusion which are exacerbated by cold arresting temperatures [9,298].

Sensitivity to potassium differs between heart regions and structures, from the atria which are the most sensitive, then the ventricles, to the conduction cells and tracts and sino-atrial node which are least sensitive. These differences in response to potassium contribute to conduction disturbances [299]. Nodal activity is more likely to persist, resulting in lack of protection for the conduction system [9] during hyperkalaemic arrest. Additional differences in potassium sensitivity between the cell layers of the ventricle wall and the regions of the ventricle [300] result in slowing of local conduction and non-uniform ventricular repolarisation [300,301] further contributing to arrhythmias during reperfusion.

While post-operative atrial fibrillation can be linked to high potassium cardioplegia, atrial fibrillation following 'off-pump' surgery has been attributed to regional ischaemia during occlusion of vessels causing local rises in extracellular potassium and intracellular calcium [302], acidosis and pro-inflammatory activation of endothelial cells [127,303].

1.7.5 High potassium can lead to left ventricular dysfunction

Transient post-operative myocardial dysfunction involving the left ventricle, or stunning, manifests as reduced contractility [304-306] with decreased cardiac output but normal electrocardiogram waveform [307]. Stunning has been linked to ischemia-reperfusion injury following exposure to high potassium concentrations [308], and temperature changes from hypothermia to normothermia at reperfusion or cross clamp removal [8,309,310].

1.8 The transition from cold to warm arrest temperatures

The majority of cardiac surgeons world-wide continue to use hyperkalemic cardioplegic solutions administered at cold temperatures to assist rapid arrest, maximise myocardial cooling and reduce metabolic demand with the aim of conserving energy reserves [273,311] and protecting the heart from ischaemic injury. However hypothermia may provide limited additional benefit as surgical cardiac arrest has been reported to decrease myocardial oxygen consumption as much as 80%, with as little as 10% extra reduction from hypothermia [177,184].

Although intermittent delivery of cardioplegia improves visibility of the surgical field, for many years investigators have reported adverse effects from hypothermic intermittent cardioplegia including reduced oxygen availability due to the rightward shift of the oxyhaemoglobin saturation curve at cold temperatures [312,313] and reduced energy production and availability of high energy phosphates [314-316]. Additional deleterious changes include loss of calcium homeostasis [317,318] and membrane stability [319,320], reduced enzyme function [321] and delayed ATP recovery [322], increased membrane peroxidation [323], longer rewarming intervals [317] and reduced contractile function at reperfusion [11,324]. In response to concerns regarding the use of hypothermia, Buckberg [325] suggested improving protection of ischaemic damaged hearts during surgery by using warm induction as a means of initiating reperfusion of the ischaemic tissue before arresting the heart at hypothermic temperatures. Subsequently a warm terminal flush or 'hot shot' was introduced to reduce the adverse metabolic changes which occurred at reperfusion following arrest with cold intermittent blood cardioplegia [326,327].

As early as 1957 continuous delivery of normothermic oxygenated blood cardioplegia had been proposed to avoid the detrimental effects of hypothermia during arrest and provide cardiac arrest with optimal myocardial protection [328], however the technique was not widely accepted at that time. Then in 1991, Lichtenstein and colleagues [181] showed that retrograde continuous blood cardioplegia at 37°C resulted in lower rates of perioperative myocardial infarction and low output syndrome, higher incidence of spontaneous return to normal sinus rhythm, shorter reperfusion time and improved cardiac output when compared with the outcome of patients treated with hypothermic cardioplegia. Similarly, the Warm Heart Investigators reported that continuous delivery of normothermic hyperkalaemic cardioplegia with brief interruptions for visualisation during construction of anastomoses was a safe and effective cardioplegia technique, providing a slightly enhanced level of intraoperative myocardial protection compared with cold continuous cardioplegia delivery [329]. Further studies in the 1990s by Philip Menasche [330] and Yau [331] and colleagues demonstrated that warm blood cardioplegia maintained aerobic myocardial metabolism compared with cold blood cardioplegia. However despite continuous delivery being most protective for high potassium cardioplegia at warm temperatures [331,332], the continuous cardioplegia technique did have several drawbacks. The constant flow impaired the surgeon's visualisation of the surgical field, especially for distal anastomoses, and increased exposure of the heart to depolarising cardioplegia solution. Difficulty in placing the coronary sinus cannula and possible inadequate distribution of cardioplegia to the right ventricle further increased concerns regarding ischaemia-reperfusion injury [333-336]. In another trial [337], normothermic cardiopulmonary bypass with continuous warm depolarising cardioplegia was compared to cardiopulmonary bypass with traditional intermittent cold blood cardioplegia. Warm cardioplegia was complicated by a higher incidence of cardiac electrical activity during aortic occlusion, lower systemic vascular resistance with higher total cardioplegia and crystalloid solution volumes and potential fluid overload, and higher serum potassium levels.

The safety of interrupting warm cardioplegia delivery to facilitate construction of distal anastomoses was confirmed in 1995 by Lichtenstein and colleagues [338]. They concluded that interruptions to cardioplegia flow of less than 13 minutes were not detrimental to myocardial protection when using the alternative technique of antegrade intermittent warm blood cardioplegia. A further study by Christakis and colleagues [8] showed that intermittent infusion of warm blood cardioplegia with interruption of flow for intervals of 11-14 minutes resulted in patients recovering a similar level of ventricular function as those treated with cold intermittent cardioplegia. In 1995 Calafiore and

colleagues reported that warm intermittent blood cardioplegia, administered every 15 minutes during coronary bypass procedures, reduced the incidence of post-surgery circulatory assistance, inotropic support and treatment for arrhythmias, and reduced mortality and length of hospital stay compared with cold intermittent blood cardioplegia [339]. Studies by the Calafiore group and Torracca and colleagues also demonstrated increased cardioprotection with improved metabolic and functional recovery and reduced oxidative stress [340,341], and decreased morbidity and mortality following warm intermittent cardioplegia [339,342].

However some surgeons expressed concerns regarding interrupting warm blood cardioplegia. Tonz and colleagues [343] advised a maximum ischaemic interval of 10 minutes to preserve myocardial function, while de Oliveira and colleagues [344] reported that interruptions to cardioplegia flow exceeding 20 minutes adversely affected metabolic and functional recovery. Matsurra and colleagues conducted a study on adult pigs comparing warm and cold intermittent cardioplegia with continuous warm cardioplegia. They reported that short interruptions (7 minutes) during delivery of warm cardioplegia resulted in increased tissue acidosis and necrosis and reduced ventricular function indicative of ischaemic injury [345], demonstrating the potential for inadequate cardioprotection with intermittent delivery of warm hyperkalaemic blood cardioplegia.

While some studies suggest that hypothermia improves cerebral protection and optimises heart recovery [184,346,347], other studies on coronary artery bypass patients treated with hypothermia or normothermia have reported no significant differences in the incidence of brain injury and stroke [329] or neurological function [348] following surgery.

More recently, normothermic cardiopulmonary bypass with intermittent hyperkalaemic cardioplegic arrest has been linked to post-operative atrial fibrillation [349]. Normothemic temperatures during cardiopulmonary bypass have also been implicated in an increased post-operative release of cytokines [350] and low systemic vascular resistance [337] requiring vasopressor intervention [350] or steroid pre-treatment to inhibit the release of cytokines [351,352]. However surgery at tepid temperatures (32^{o}C to 34^{o}C) was reported to reduce both the production of cytokines and the vasodilatory response to cardiopulmonary bypass that occur at normothermic temperatures [350], and the 5 year outcome for survival and incidence of myocardial infarction in adults was improved with warm or tepid blood cardioplegia [353].

In 2002 Ascione and associates [354] advised that cardiopulmonary bypass with cold blood cardioplegia provided better protection than warm cardioplegia for hypertrophied hearts undergoing aortic valve replacement, due to the pre-existing elevated myocardial metabolic and oxygen demands in these hearts [355]. However these researchers considered that when using hyperkalaemic cardioplegia neither the warm nor the cold technique provided sufficient protection for hypertrophied hearts [354], highlighting the inadequacy of existing hyperkalaemic arresting solutions to provide cardioprotection in the current increasingly challenging surgical situation.

Despite many animal studies and human trials, few surgeons use warm temperatures although many believe that warmer temperatures may lead to improved protection. The problem appears multifactorial: a lack of randomised trials showing superiority of warm temperatures, and no alternative to high potassium cardioplegia at warmer temperatures. The ideal temperature for cardiac surgery remains a controversial point, and opinion varies according to the surgical procedure involved, the age of the patient, the type of cardioplegia and the chosen method of administration of the arresting solution.

1.9 Alternative cardioplegia administration protocols

Surgeons and researchers have continued to investigate various cardioplegia delivery protocols with the aim of reducing both the volume of solution administered and the length of exposure to hyperkalaemic solutions, while maintaining an acceptable level of cardioprotection.

1.9.1 Mini-cardioplegia and microplegia

Small volume continuous cardioplegia, or mini-cardioplegia, was proposed by Menasche **[356]** in 1996 to maintain electromechanical arrest and oxygenation of the heart while minimising anaerobic metabolism, fluid overload and haemodilution. However, potassium all-blood miniplegia has also been reported to impair endothelial function [357].

The microplegia technique also minimises the volume of cardioplegia solution delivered during arrest, and therefore reduces haemodilution. In paediatric cardiac surgery warm microplegia with re-dosing intervals of 10-15 minutes has been reported to improve

functional recovery and reduce the length of intensive care unit stay compared with hypothermic cardioplegia [358,359].

1.9.2 Intermittent or single dose cardioplegia?

As early as the 1980s, when interest in intermittent cardioplegia delivery was increasing, animal studies were used to test the safety of increasing the duration of the ischaemic arrest interval. Isolated working heart studies using rabbits showed that the protective effects of hyperkalaemic St Thomas' Hospital solution were influenced by the administration method (single or multiple-dose) and the temperature of arrest, with recovery of function more often dependent on the temperature rather than the cardioplegia delivery method. At 10^{o}C cellular energy levels were similarly preserved, with minimal difference between the topical cooling-only group and the single-dose or multi-dose cardioplegia groups [360,361]. Kempsford and Hearse also reported that although a single-dose 4-hour arrest was equally protective at 10^{o}C or 20^{o}C (96% recovery), above 25^{o}C recovery of cardiac output following single-dose arrest decreased significantly, and at these warmer temperatures multi-dose hearts recovered significantly better cardiac output (98%) than ischaemic (21%) or single-dose (76%) hearts [360]. In another study using isolated feline hearts, Lucas and colleagues demonstrated enhanced protection at 27^{o}C. Multiple-dose administration improved preservation of ventricular function and structure compared with single-dose potassium cardioplegia or ischaemia-only arrest [362]. These studies suggested that multiple-dose cardioplegia was more protective for recovery of cardiac function than single-dose at tepid to warm temperatures. Similarly, in isolated perfused rabbit hearts, Flaherty and associates used phosphorus-31 nuclear magnetic resonance (P^{31} NMR) to demonstrate that multi-dose hyperkalaemic cardioplegia at 24^{o}C was more protective against acidosis, reduced ATP content and damage to cellular morphology and resulted in better recovery of contractile function than 24^{o}C hypothermia alone or a single-dose of cardioplegia at 10^{o}C [363].

Clinical and animal studies reported that multi-dose cold blood hyperkalaemic cardioplegia reduced the incidence of post-operative rhythm disturbances, better preserved myocardial ultrastructure and high energy phosphates and resulted in superior functional recovery compared with single dose Bretschneider's crystalloid cardioplegia [364,365] or hypothermic ventricular fibrillation alone [364]. However other studies at colder temperatures using rabbit hearts showed a progressive decrease in

myocardial protection afforded by multi-dose delivery, with reduced functional recovery and preservation of myocardial structure, compared with single-dose hyperkalaemic cardioplegia [366] or a 40-minute infusion interval regime [367]. Subsequent clinical studies verified equivalent cardioprotection with single-dose crystalloid [368] or blood [369] cardioplegia compared with multiple dose administration at cold temperatures.

Recently there has been increasing interest in single dose administration of cold cardioplegia solutions. When single-dose delivery of cold Bretschneider crystalloid solution was compared with warm (34^{o}C) intermittent blood-based potassium and lidocaine cardioplegia, Viana and colleagues reported an equivalent level of myocardial protection, and the one-shot regime was considered more convenient technically than multi-dose administration [370].

1.9.3 Development and clinical use of del Nido depolarising solution

In the early 1990s researchers at the University of Pittsburgh, led by Pedro del Nido, developed an arrest solution specifically for paediatric patients whose hearts are reported to be more vulnerable to calcium influx mediated injury and more susceptible to reactive oxygen species induced reperfusion injury [371,372] than adult hearts. The solution contained a very low calcium concentration, magnesium as a calcium competitor, and lidocaine to stabilise the myocyte membrane, with the aim of reducing calcium accumulation during arrest, and exacerbation of reperfusion injury linked to physiological levels of calcium [373] or calcium-free solutions [374-376].

Approximately 38% of paediatric cardiac surgeons in North America use blood based del Nido cardioplegia [377]. In clinical practice del Nido cardioplegia is delivered cold (8-12^{o} C), antegrade, in a single 20ml/kg dose [371], with an additional dose (10ml/kg) administered after 90 minutes if surgery is expected to exceed two hours. Demonstrated problems following del Nido arrest include reduced contraction amplitude in early reperfusion [378], slow re-establishment of rhythm and force of contraction after short cross clamp times, and an increased incidence of supraventricular tachycardia with a cardioplegia dose interval of 60 minutes. Despite the addition of the colloid Mannitol to the solution, the crystalloid component of del Nido cardioplegia leads to haemodilution and oedema, necessitating haemoconcentration via ultrafiltration [379].

Clinical studies have investigated hyperkalaemic del Nido solution delivered as cold single-dose cardioplegia for paediatric patients [371,380]. Charette and colleagues [379] reported the advantage of single-dose administration which allowed an uninterrupted surgical procedure while maintaining a similar level of cardiac protection as their adult cardioplegia regime using higher potassium concentration, multiple-dose cardioplegia. Modified del Nido cardioplegia with an increased blood component has recently been trialled for use in adult surgery [381], providing comparable cardioprotection to standard whole blood cardioplegia for surgical procedures on aged, diseased hearts [382]. However the efficacy and safety of this technique in adults requiring complex surgery with longer cross-clamp times has not been verified by randomised controlled trials [383].

1.10 Cardioprotection with adenosine or lignocaine alone

1.10.1 Adenosine cardioprotection during ischaemia

Multiple actions of adenosine contribute to its cardioprotective properties. The A_1 sub-type receptor has a high affinity for adenosine and plays a major role in the protective effect of adenosine in the heart during ischaemic episodes [384,385]. In addition to reducing calcium overload and ischaemic contracture [386], adenosine protects the ischaemic heart from catecholamine-induced increased contractility [387-390]. Adenosine also protects against endothelial damage during ischaemia by inhibiting inflammatory responses [211], and decreasing production of reactive oxygen species [391]. Activation of adenosine A_2 receptors, located in coronary vascular smooth muscle and endothelial cells [217], increases coronary blood flow and inhibits platelet and leukocyte activity in ischaemic myocardium [230]. Adenosine's ability to stimulate glycolysis and facilitate glucose uptake [392] may further contribute to preservation of myocardial ATP levels [393] and protection from irreversible myocardial cell injury [393-395].

Studies have examined the protective effect of adenosine treatment before, during and after an ischaemic event. Infusion of adenosine for ten minutes before an ischaemic insult reduced contractile dysfunction [396,397], while an adenosine bolus administered immediately preceding hyperkalaemic arrest reduced peri-operative injury and improved post-operative cardiac function [398]. In dog hearts, adenosine administered at reperfusion was reported to significantly reduce infarct size, improve blood flow, reduce neutrophil infiltration and preserve endothelial structure in the ischaemic zone [399].

Vinten-Johansen and fellow researchers [400] concluded that adenosine added at the time of reperfusion was more beneficial than as a cardioplegia supplement.

1.10.2 Lignocaine cardioprotection during ischaemia

Cardiac protection afforded by lignocaine has been attributed to conservation of high energy phosphates and mitochondrial ATP, and attenuation of acidosis [223,401,402], modulation of inflammatory responses involved in ischaemia reperfusion injury [403,404], and reduction of intracellular sodium and calcium accumulation during ischaemia [402]. These effects decrease infarct size [405] and delay ischaemic contracture [223]. The activity of lignocaine is enhanced in ischaemic cells as binding to the inactivated sodium channel is increased at depolarised membrane potentials [406]. Lignocaine also scavenges reactive oxygen species, including hydroxyl radicals associated with contractile dysfunction and endothelial injury at reperfusion [407].

1.11 Adenosine and lignocaine combined: AL cardioplegia

A novel cardioplegia concept which combined adenosine and lignocaine as the arresting agents was developed by G.P. Dobson and his laboratory at James Cook University in 1998. Adenosine was chosen for its ability to open K_{ATP} channels via A_1 receptor activation to reduce the action potential duration [218,235] and lidocaine to block sodium entry and therefore the upstroke of the cardiac action potential [250], with the aim of arresting the heart in diastole at or near the cell resting membrane potential [273]. In a study by Dobson and Jones in 2004 using isolated rat hearts [408] adenosine and lignocaine (AL) cardioplegia solution rapidly produced effective arrest, and resulted in spontaneous reanimation at reperfusion and superior functional recovery compared with hearts arrested with hyperkalaemic St. Thomas' Hospital Solution No.2 (Plegisol).

In 2005 Corvera and colleagues used a canine model of cardiopulmonary bypass to compare intermittent delivery of cold hyperkalaemic blood cardioplegia with cold or warm adenosine 400μM and lignocaine 750μM mini-cardioplegia (22 parts blood to 1 part AL) for one hour of arrest [409]. The study demonstrated a similar level of protection and recovery of post-arrest cardiac function between the groups. However arrest times in both of the AL-blood cardioplegia groups were increased compared with the hyperkalaemic blood cardioplegia group, and also with arrest times for crystalloid AL cardioplegia in an isolated heart model [408]. Corvera hypothesised that delayed arrest

and failure to achieve complete quiescence during arrest in the AL groups may have been related to rapid breakdown of adenosine by adenosine deaminase at warm temperatures [238] and inactivation of lignocaine at the cooler temperatures [410,411]. However this study, using a large animal model, provided support for the AL concept and the potential use of AL at cold or warm temperatures.

Positive results have been reported from clinical trials using the AL concept of cardioplegia for both paediatric and adult cardiac surgery. Jin and colleagues reported that cold cardioplegia containing adenosine and lignocaine with 10mM potassium improved protection of infant hearts compared with hyperkalaemic cardioplegia or adenosine-lignocaine cardioplegia with 20mM potassium [412]. However this group did not investigate potassium concentrations below 10mM. In 2013, Onorati and fellow researchers compared a 4:1 ratio of blood: Buckberg intermittent hyperkalaemic cold cardioplegia with all-blood adenosine/lignocaine/magnesium/insulin (ALMI) enriched intermittent cold potassium microplegia for coronary artery bypass grafting on high risk adults with unstable angina. Insulin was added for cardioprotection based on previous studies at their hospital [413], and increased magnesium levels are widely used in cardioplegia for reducing intracellular calcium levels during ischemia and the incidence of post-operative arrhythmias [414]. The ALMI group recovered better haemodynamic status with less myocardial damage, reduced transfusions and shorter intensive care unit and hospital stay [413]. More recently the Italian group have completed a second randomised prospective trial using normokalaemic ALM microplegia which showed superior myocardial protection compared with hyperkalaemic arrest in low risk patients undergoing coronary artery bypass grafting or aortic valve replacement [415].

1.12 Conclusion

Heart surgery is one of the great medical success stories of the 20th century. However, today's older higher risk patients and complex paediatric corrective cases present ongoing challenges. Cardiac surgeons continue to rely on hyperkalaemic cardioplegia and hypothermia [416], which may offer less than optimal cardioprotection with negative post-operative outcomes. There is an urgent need for an alternative to high potassium cardiopledia that is safe and versatile in cold and warm temperatures, with the overall goal of offering improved protection for the patient and greater predictability for the surgeon.

1.13 Thesis Aims

The overall aim of this thesis was to develop an alternative normokalaemic AL cardioplegia protocol for delivery at cold and warmer temperatures (32^{o}C) using intermittent or single-dose delivery to provide the surgeon with longer intervals of a bloodless, motionless surgical field.

The studies were designed to

1) Evaluate AL as a cardioplegia solution for warm intermittent delivery compared with warm continuous delivery
2) Investigate the importance of polarised versus depolarised arrest on functional recovery at warm temperatures, using AL cardioplegia compared with cardioplegia solutions containing varying potassium concentrations
3) Optimise AL cardioplegia solution and the arrest protocol to extend the cardioplegia delivery interval from intermittent to one-shot during warm arrest
4) Compare functional recovery following one-shot arrest with AL cardioplegia or hyperkalaemic del Nido cardioplegia at warm and cold arrest temperatures.

1.14 Addendum

Due to protracted treatment required for an ongoing medical condition there was a delay between the research presented in Chapters 1 and 2, which was published in 2007 and 2010, and the research conducted for Chapters 3, 4 and 5 of this thesis.

Chapter 2

Materials and Methods

2.1 Introduction

This chapter presents details of the experimental protocol used in this thesis, including animal care and anaesthesia, perfusion models, laboratory equipment, surgical techniques and study methods, along with measurements of study endpoints, calculations, and statistical methodology.

The experimental model used for all studies was the isolated perfused rat heart utilising both the Langendorff (non-working) perfusion method and the working heart perfusion method. The general experimental design will be described here. The specific design of each study will be presented in the corresponding chapter.

2.2 Animals

2.2.1 Care and housing

Male Sprague-Dawley rats weighing between 340 and 450g were obtained from the James Cook University breeding colony. They were housed in standard approved cages in a 14 hour light-10 hour dark cycle, with pellet food and water available *ad libitum*. Rats were transferred to the laboratory on the day of experimentation in covered cages to minimise stress.

2.2.2 Ethical Conduct

This research was conducted in compliance with the NHMRC Guidelines to promote the wellbeing of animals used for scientific purposes (2008), the Queensland Animal Care and Protection Act, 2001 (Act No.64 of 2001) and the James Cook University ethics policies and guidelines (Policy on Experimentation Ethics Standard Practices and Guidelines (2001), and Statement and Guidelines on Research Practice (2001)). Ethics approval of research methodology and the care and handling of the animals was obtained from the James Cook University Experimentation Ethics Review Committee (Animal Ethics approval numbers A759, A1107, A1747).

2.3 Materials

2.3.1 Anaesthetic agents

Rats were anaesthetised with sodium pentobarbital (Nembutal) or thiopentone sodium (Thiobarb). When supply of sodium pentobarbital became restricted in Australia, thiopentone sodium (Thiobarb, Lyppards, Queensland) was substituted as its suitability as an anaesthetic for cardiovascular research involving rodents had previously been established [417-419]. Negative ionotropic effects which may have been induced by thiopentone sodium [420] were accounted for through the exclusion criteria for stabilised hearts which required a minimum heart rate of 250 beats/min and minimum coronary flow of 10ml/min.

2.3.2 Perfusion buffers, cardioplegic solutions and reperfusion solutions

Solutions were made fresh each day as required. Buffers and solutions were not recirculated through the perfusion apparatus. The delivery temperature of solutions and buffers varied in each study and details are given in the methods section of each chapter. The composition of the Krebs Henseleit (KH) buffer, cardioplegia and reperfusion solutions and components used to modify these solutions are presented in Appendix A. All compounds used for preparation of solutions were of analytical grade purity. Adenosine (A9251), magnesium sulphate, and other chemicals listed in Appendix A were obtained from Sigma Chemical Company (Castle Hill, NSW). Lidocaine hydrochloride was purchased as a 2% solution (ilium) from Lyppards, Queensland.

The Krebs Henseleit perfusion buffer used during the Langendorff surgical set-up of isolated hearts was filtered using a one micron (1μM) filter and bubbled vigorously with 95%O_2/5%CO_2 to achieve a pO_2 greater than 600mmHg. Cardioplegic solutions were filtered with 0.2μM filters (Pall, Australia) and were not actively bubbled with 95%O_2/5%CO_2. Reperfusion solutions used during Langendorff reanimation following arrest were filtered with 0.2μM filters and bubbled vigorously with 95%O_2/5%CO_2 to achieve a pO_2 greater than 600mmHg, and buffer used for working mode perfusion was filtered with an in-line 1μM filter and actively bubbled with 95%O_2/5%CO_2 to achieve a pO_2 greater than 600mmHg.

2.4 Laboratory Equipment

2.4.1 The isolated perfused heart apparatus

The isolated heart perfusion method allows cardiovascular function to be assessed using either a non-working model or working heart model. The non-working model for mammalian hearts was designed by Oscar Langendorff in 1895 initially for studying contractile function, but was later adapted to examine the coronary circulation [421] and the heart's response to physical and pharmacological interventions [422]. Using his model Langendorff made important contributions to the understanding of cardiac physiology, including the role of the coronary circulation and the effects of vagal nerve stimulation and potassium chloride on heart rate [423].

The Langendorff model uses retrograde perfusion via the cannulated aorta. The reversed aortic flow closes the leaflets of the aortic valve, and perfusion of cardiac tissues is maintained through the ostia at the aortic root. Perfusion fluid from the coronary arteries drains into the right atrium via the coronary sinus and perfusate samples can be collected from a cannula inserted into the trunk of the pulmonary artery. As the retrograde aortic flow prevents fluid from entering the left sided heart chambers, the Langendorff perfused heart is considered a non-working model.
In the studies presented in this thesis Langendorff perfusion is used during the surgical set-up of hearts, for administration of cardioplegic solutions and in some groups during reanimation and reperfusion following arrest. Details are given in the relevant chapters.

In 1967 Neely and colleagues [424] modified the Langendorff model to an isolated working rat heart model which allowed perfusion with a physiological flow pattern through the chambers on the left side of the heart. In the working heart model oxygenated perfusion fluid enters the heart via a cannula in the left atrium, and flows into the left ventricle to be ejected via the aorta against a hydrostatic pressure head which mimics ejection into a functioning aorta. The aortic flow also maintains coronary perfusion via the coronary ostia. This model allows assessment of heart pump function via measurement of aortic and coronary flow rates and systolic and diastolic pressures. Heart function can be rapidly switched from retrograde Langendorff mode to working mode and back again by reversing the direction of perfusate flow through the aorta by means of clamps on the perfusate delivery tubing from the Langendorff and pre-

load/oxygenator columns. The working heart method is used in these studies during the stabilisation period between surgical set-up and arrest, and during working mode reperfusion following arrest (Figure 2.1).

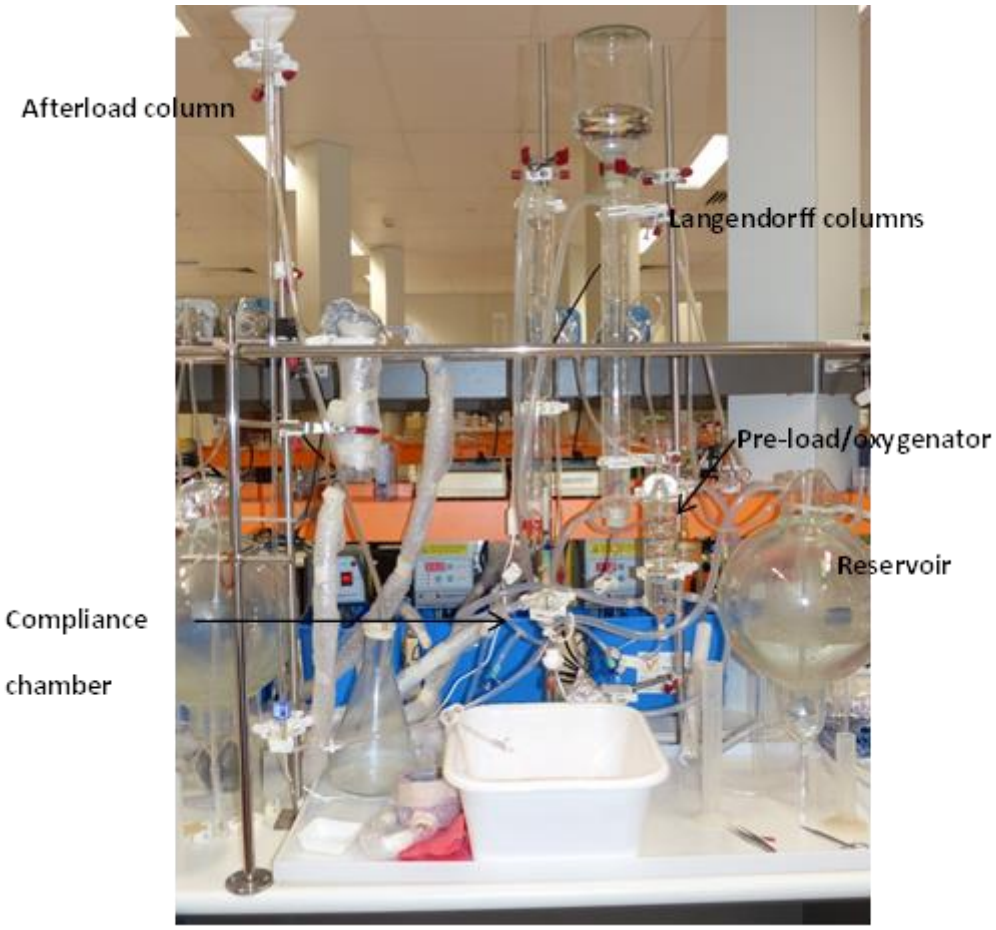


Figure 2.1: Isolated working heart apparatus

The isolated perfused heart model was chosen for these studies to assess the response of the heart to various cardioplegic solutions, arrest protocols and reperfusion methods. Functional parameters during arrest and recovery at reperfusion were monitored by recording heart rate, aortic and coronary flows and systolic and diastolic pressures for comparison with pre-arrest values.

2.4.2 Advantages and disadvantages of the isolated perfused rat heart model

The isolated perfused rat heart model is a convenient, relatively economical and reproducible preparation which is widely used for evaluating cardiac response to treatment interventions and for assessing ischaemia-reperfusion injury [425,426]. In addition to assessment of functional parameters, this model allows measurements of biochemical markers, and morphological and histological studies. Evaluation of function in working mode mimics normal cardiac physiology more closely than the Langendorff non-working model. To avoid the possibility of inadvertently causing tissue damage or preconditioning of the heart during surgical preparation rigorous exclusion criteria must be applied to identify hearts which fail to function within accepted values or maintain stable function [427].

Data generated using the isolated heart technique also allows assessment of the direct effect of chemical compounds and physiological interventions on parameters of heart function without the confounding effects present in an *in vivo* model. Conversely, the isolated heart model may also be a limitation in some research as it does not evaluate the intact body response to treatment interventions, which may involve autonomic nervous, hormonal, inflammatory and antioxidant interactions.

The use of crystalloid solutions may also be a limitation in the isolated heart model. Oedema due to lack of colloidal osmotic pressure from cell-free perfusate [418] may impact on fluid distribution to tissues and coronary flow. Raised coronary flow rates due to the low oxygen carrying capacity of crystalloid solutions can be minimised by frequent monitoring to maintain a pO_2 greater than 600mmHg in actively oxygenated perfusion solutions. Additionally, isolated hearts perfused with crystalloid buffers remain viable for a limited time. Therefore future studies to extend this research could use intact animals for a more clinically relevant model that extended the study time points for up to 24 hours of post-arrest recovery.

Cellular function, physiology and susceptibility to ischaemia-reperfusion injury vary between species due to differences in metabolic rate [428], collateral circulation [429], action potential duration [427] and expression of antioxidant enzymes, ion channels and receptors [84,430,431]. Therefore results obtained in rat studies cannot be directly extrapolated to the human clinical scenario.

Likewise, direct comparison of recovery of function at reperfusion cannot be made between the isolated heart model and the clinical situation. The isolated heart model involves reperfusion in working mode with pre-set preload and afterload pressures, whereas a dynamic transition occurs from cardiopulmonary bypass with infusion from the CPB circuit until the required filling pressure is obtained for reperfusion. In a clinical or *in vivo* situation the use of blood-based cardioplegia would also improve oxygen availability, provide buffering capacity to delay lactic acidosis, supply metabolic substrates and insulin to maintain cellular high energy phosphate levels, and oncotic components to limit cell swelling and interstitial oedema [432].

Despite these limitations, the isolated heart model is recognised as a useful technique for cardiac research which combines a variety of perfusion modes and cardioplegia protocols with quality and quantity of data output. Hearts used in these studies were from healthy adult animals without accompanying pathological conditions to allow assessment of the effect of various temperatures and cardioplegic solutions on heart function. Further studies would be required to compare the effects in older or diseased hearts.

2.4.3 Temperature regulation and monitoring

The required heart temperatures were maintained during surgical set-up, the stabilisation period, induction of arrest, the arrest period and the reperfusion period with buffers and solutions delivered at the appropriate temperature from water-jacketed columns and reservoirs connected to thermostatically–regulated circulating water baths.

During arrest the heart was placed in a water-jacketed warming or cooling chamber covered with alfoil or insulating wrap to maintain the desired temperature. The heart surface temperature was measured regularly during arrest and the Langendorff reperfusion period using a Cole-Palmer thermistor-thermometer (8402-20) as shown in Figure 2.2. Sub-auricular monitoring has previously been shown to compare closely with placement in the left heart chamber [408].

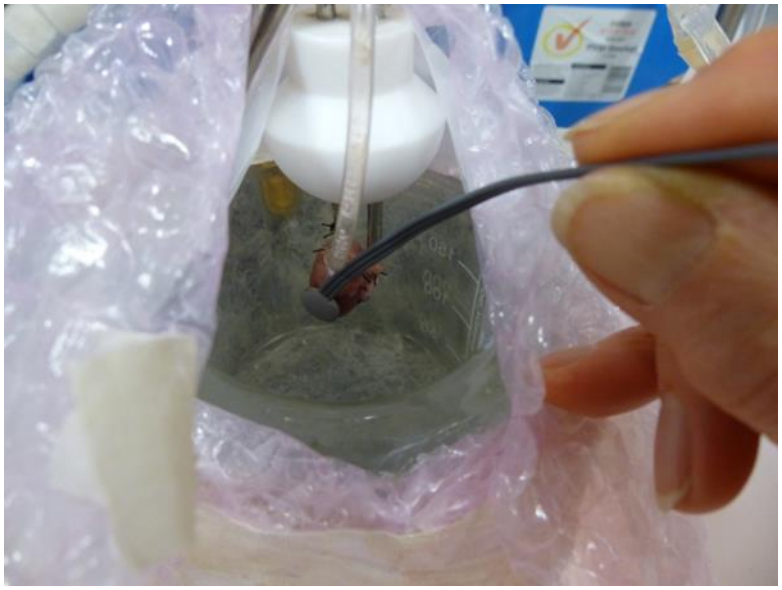


Figure 2.2: Monitoring heart temperature in the warming chamber

2.4.4 Monitoring of composition of buffers and solutions

Buffers and cardioplegic solutions were made on the day of experiment, and the pH and ion concentrations (Ca^{2+}, Cl^-, K^+ and Na^+) were checked before use with a Ciba-Corning 865 blood gas analyser (Siemens Diagnostics, Australia).

2.4.5 Functional monitoring

Aortic pressure was measured continuously using a pressure transducer (UFI Instruments, Morro Bay, CA) coupled to a MacLab 2e computer (AD Instruments, Australia). Systolic and diastolic pressures and heart rate were recorded using MacLab software. The pO_2 and pCO_2, pH and ion concentrations (Ca^{2+}, Cl^-, K^+ and Na^+) of buffers, cardioplegic solutions and coronary venous perfusate samples were measured during the stabilisation period, induction of arrest, cardioplegia flushes during the arrest period and during the reperfusion period using a Ciba-Corning 865 blood gas analyser. Coronary venous flow and aortic flow were measured in volumetric cylinders.

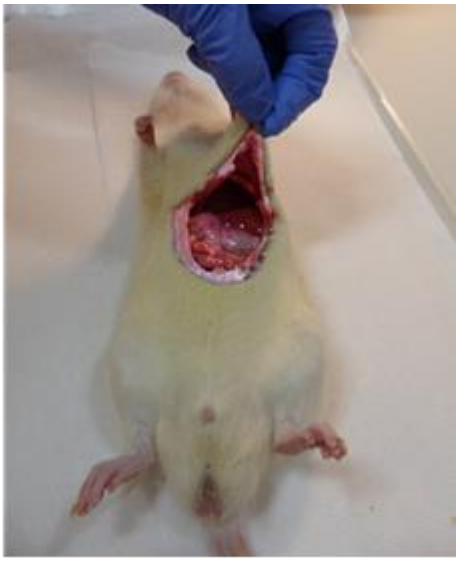
The perfusion apparatus was flushed after each use with 10 litres of 18 megaohm water and allowed to air-dry, and regularly flushed with 5 litres of 1% glacial acetic acid solution followed by 10 litres of 18 megaohm rinsing water.

2.5 Preparation of the isolated perfused rat heart model

2.5.1 Anaesthesia

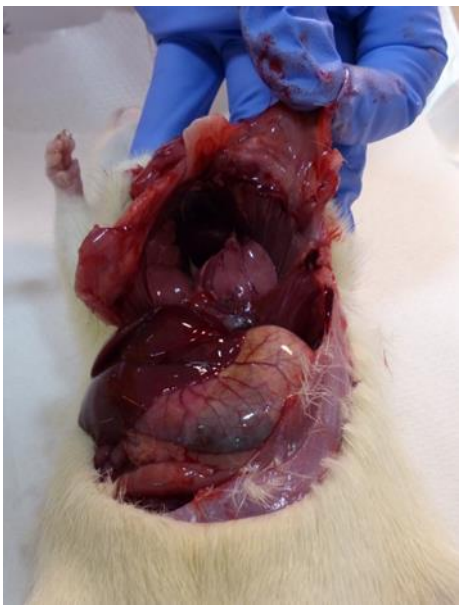
Animals were kept in a covered cage and handling minimised to reduce stress before general anaesthesia was induced with an intraperitoneal injection of thiopentone sodium (80mg/kg) [417]. Before surgery commenced animals were checked for deep anaesthesia by complete lack of response to painful stimuli (pedal withdrawal reflex). A further incremental dose of 0.2ml was given if required to achieve satisfactory anaesthesia.

2.5.2 Surgical preparation of hearts for the isolated heart technique



Rats were positioned in a dorsal supine position and the abdominal cavity opened with a transverse incision immediately below the diaphragm (Figure 2.3).

Figure 2.3: Transverse abdominal incision



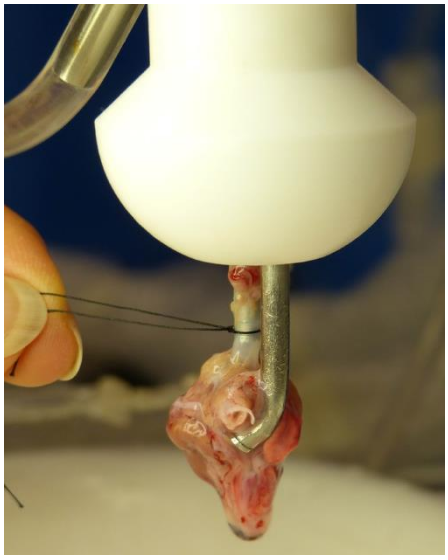
The chest cavity was opened by transecting the diaphragm and cutting through each side of the rib cage to allow the sternum and ribs to be lifted and folded back exposing the organs in the chest cavity (Figure 2.4).

Figure 2.4: Chest cavity opened and anterior wall folded back

The aorta and trachea, vena cava and pulmonary arteries were identified and incised and the heart and lung block was rapidly removed and immediately placed into 40ml of cold Krebs-Henseleit buffer in a plastic dish resting in ice slush. Contractions ceased within 5 seconds (Figure 2.5).

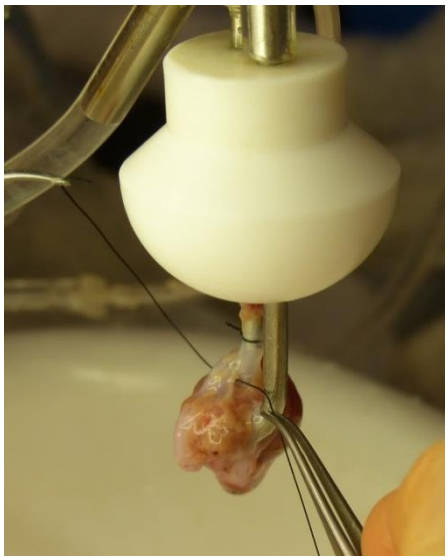
Figure 2.5: Heart arrested in cold KH buffer

Excess connective and fatty tissue, and the thymus gland, oesophagus and larger lung lobes were rapidly removed from the heart. The aortic root was slipped on to the aortic cannula of the Langendorff apparatus and held with a non-traumatic artery clip to allow immediate retrograde perfusion with oxygenated KH buffer at 37^{o}C and a constant hydrostatic perfusion pressure of 80cm water (60mmHg) [427]. The flow of buffer washed blood out of the capillaries and warmed the heart which resumed beating within a few seconds.



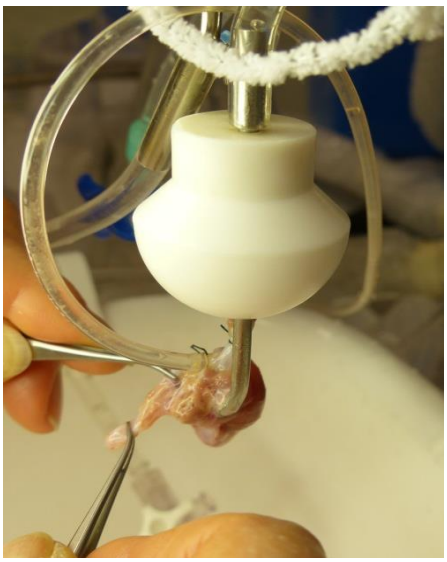
The position of the heart was then adjusted if necessary ready for placement of atrial and pulmonary cannulas, and a silk suture used to secure the aorta onto the cannula (Figure 2.6).

Figure 2.6: Heart positioned on aortic cannula



The pulmonary trunk was freed from underlying heart structures and a silk suture positioned under the vessel (Figure 2.7).

Figure 2.7: Suture placed under pulmonary trunk



The pulmonary trunk was cannulated at the bifurcation of the pulmonary arteries and the cannula secured with the silk suture to enable monitoring of coronary flow and collection of perfusate samples for analysis (Figure 2.8).

Figure 2.8: Pulmonary trunk cannulated; removal of fatty tissue

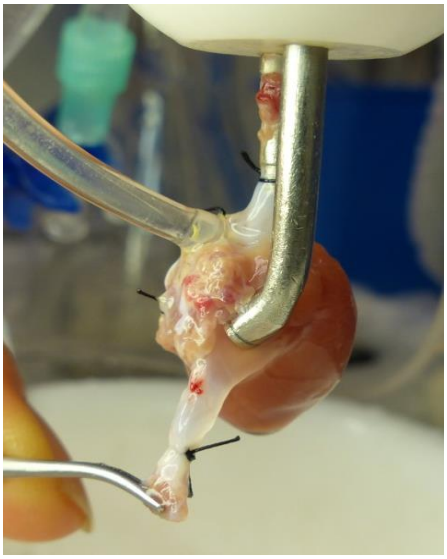


Figure 2.9: Inferior vena cava tied off

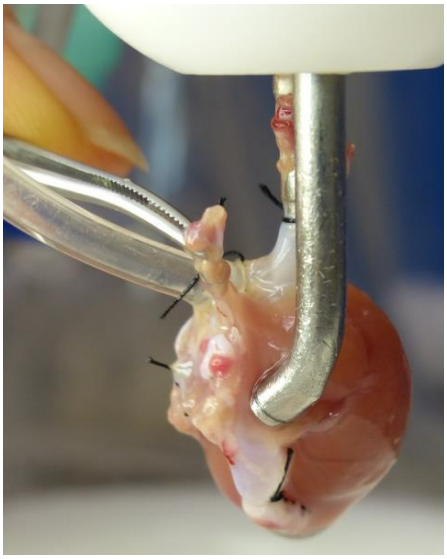
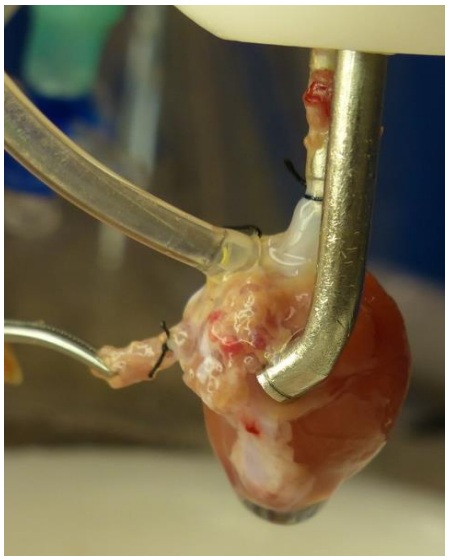


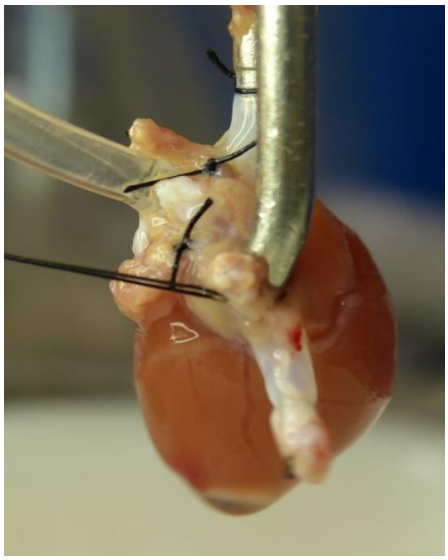
Figure 2.10: Superior vena cava tied off

Any remaining fatty or connective tissue was removed from external heart structures. The superior vena cava and inferior vena cava were identified and tied off with silk suture approximately 5mm from their junction with the right atrium (Figures 2.9 and 2.10).



The pulmonary veins were identified and the lower veins tied off with silk suture close to their junction with the left atrium (Figure 2.11).

Figure 2.11: Lower pulmonary veins tied off



The remaining pulmonary vein was excised close to the surface of the atrium, creating an opening into the atrium. A cannula was introduced through the opening into the atrium and secured in place with a silk suture (Figure 2.12).

Figure 2.12: Placement and suturing of atrial cannula

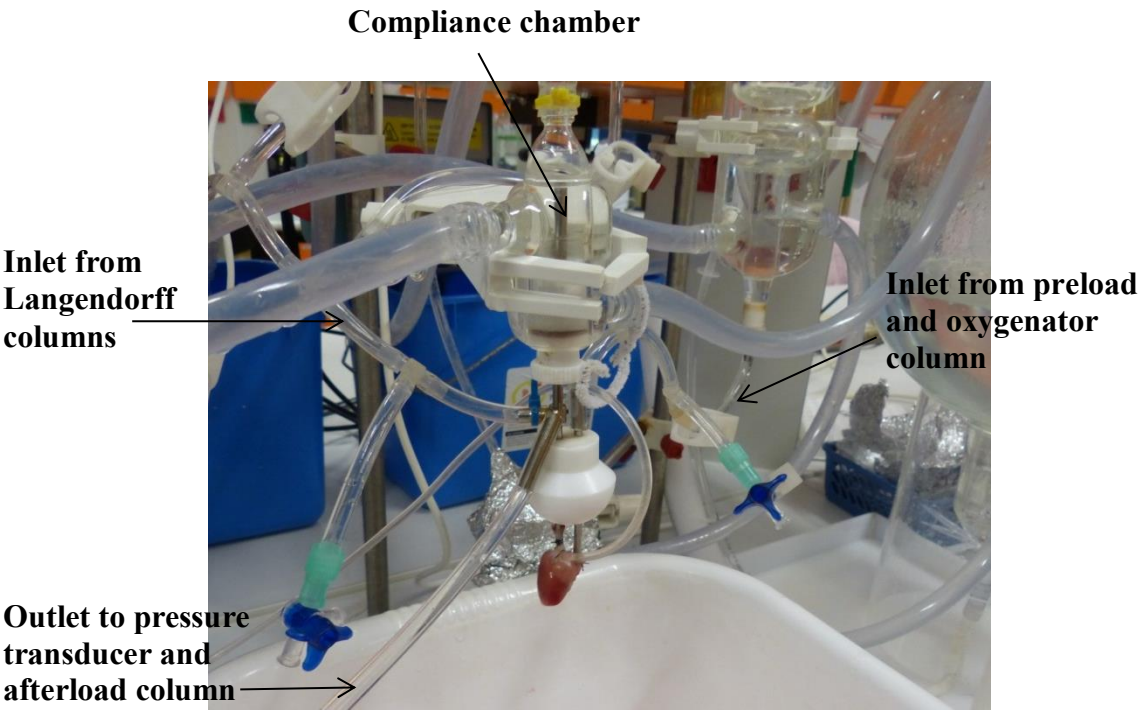


Figure 2.13: Completed set-up for Langendorff or working mode perfusion of the isolated rat heart

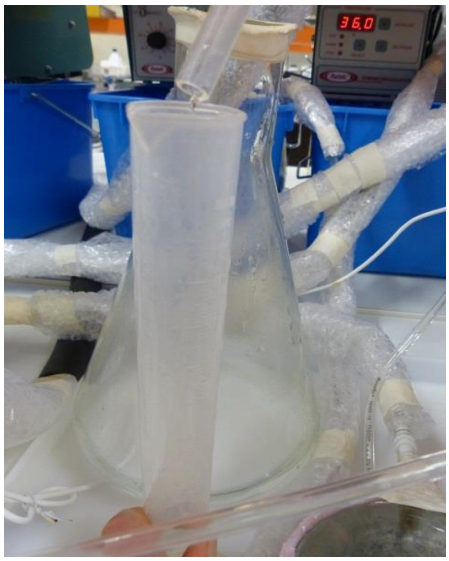
Cannulas and suturing sites were checked for leaks and re-sutured if necessary to minimise leakage of buffer from the heart to less than 1ml per minute. In preparation for perfusion in working mode, 2ml of air was introduced into the compliance chamber. This air bubble compensates for elasticity usually provided by blood vessel walls when intra-aortic pressure increases during contraction of the left ventricle (Figure 2.13).

2.5.3 Perfusion of hearts in working mode

After surgical set-up hearts were switched from Langendorff mode to working mode perfusion with KH buffer at 37^{o}C by clamping the tube from the Langendorff column and unclamping the tube from the preload column supplying oxygenated perfusate from the reservoir to the left atrium. Preload (left atrial filling pressure) was set at 10cm H_2O (7.6 mmHg) and afterload (aortic resistance) at 100cm H_2O (76 mmHg).

2.5.4 Stabilisation period

Hearts were perfused in working mode for a 10 minute stabilisation period during which function was monitored and any heart rhythm irregularities noted.



Baseline measurements of heart rate, systolic and diastolic pressures, aortic and coronary flows were recorded (Figure 2.14), and levels of gases, ions and pH in the perfusion buffer and venous effluent were assessed in the final minute of the stabilisation period before induction of arrest.

Figure 2.14: Measurement of aortic flow

2.5.5 Exclusion criteria

The criteria for exclusion of working hearts during the 10 minute equilibration period were: a heart rate less than 250 beats/minute, a systolic pressure less than 110mmHg and coronary flow less than 10ml/minute.

2.6 Treatment protocols

2.6.1 Induction of arrest

For studies involving arrest at warm temperatures the cardioplegic solution was administered at 32°C. This temperature has been reported to provide optimal heart recovery and brain protection during cardiac surgery [346,347]. Hearts arrested at cold temperatures received cardioplegic solution at 8-12°C as in previously published studies [371].

Following the stabilisation period in working mode, hearts were converted back to Langendorff mode to administer the arrest solution at the appropriate temperature for the study, and the coronary venous flow rate was recorded. The time to complete

cessation of ventricular beating and occurrence of escape beats were noted. Heart rhythm was not monitored with an electrocardiogram.

2.6.2 Cardioplegia delivery during arrest period

Cardioplegic solution was administered via the aorta by continuous, intermittent or single dose (one-shot) delivery. Solution was delivered from the temperature-controlled Langendorff column at a constant 80cm H_2O pressure.

During continuous cardioplegia delivery the solution was allowed to flow for the duration of the arrest period. The coronary flow rate was measured for 2 minutes of every 20 minutes and effluent samples collected for analysis.

For intermittent delivery the flow of cardioplegic solution was stopped after the initial 50ml induction dose and the aorta was clamped with a non-traumatic artery clip. After 18 minutes of arrest the clip was released and cardioplegic solution was administered for 2 minutes (18 minutes no flow: 2 minutes cardioplegia flush) and effluent samples collected for analysis. These 2-minute flushes were repeated every 20 minutes until the terminal flush that was administered immediately before reperfusion was commenced.

Hearts arrested with the one-shot method received an initial cardioplegia induction dose, the flow of cardioplegic solution was stopped and a non-traumatic artery clip was applied to the aorta. The volume of cardioplegic solution administered at induction and the use of a 2-minute terminal flush varied in some groups in the one-shot studies. Details of the variation in methods used for these groups are given in the relevant chapters.

2.6.3 Reanimation in Langendorff mode

Some groups of hearts arrested for 50 minutes using one-shot cardioplegia delivery were initially reperfused in Langendorff mode to allow gradual rewarming and reanimation after the extended arrest period. Oxygenated reperfusion solution was delivered at 80cm H_2O pressure for 10 minutes and the time to first ventricular beat noted. Details of the reperfusion solutions administered in Langendorff mode are given in the relevant chapters. Functional measurements of heart rate and coronary flow, the

temperature of the heart, and the pH, ion and gas levels of buffers and venous perfusate samples were recorded at 2, 5 and 10 minutes of Langendorff reperfusion.

2.6.4 Reperfusion in working mode

Hearts were reperfused in working mode at 37^{o}C, either after the terminal cardioplegia flush of the arrest period, or following Langendorff reperfusion for those groups arrested for 50 minutes. Functional measurements (heart rate, systolic and diastolic pressures, aortic and coronary flows) and analysis of buffer and venous perfusate samples (pH, gases and ion levels) were recorded at 5, 10, 15, 30, 45 and 60 minutes of working mode reperfusion. Recorded functional values were also expressed as a percentage recovery of the pre-arrest values, and used to calculate cardiac output (CO), stroke volume (SV) and rate pressure product (RPP). No pacing or cardiac massage was employed during the working mode reperfusion phase as recovery of heart rate was assessed for comparison between studies.

2.7 Calculations

2.7.1 Rate-pressure product

Rate- pressure product, an indication of myocardial oxygen consumption [433], is calculated during working mode perfusion as per equation 1.

Rate-pressure product (RPP) (mmHg/min) = heart rate x systolic pressure (1)

2.7.2 Coronary vascular resistance during cardioplegia delivery and Langendorff reperfusion

Coronary vascular resistance (CVR) in megadyne.sec.cm^{-5} during Langendorff perfusion was calculated by dividing delivery pressure (mmHg) by flow (ml/sec) as per equation 2.

$$CVR = \frac{1333 \text{ x mm Hg}}{(ml/\text{sec})} \text{ x } 10^{-6} \quad (2)$$

where 1 mmHg = 1333 dynes cm^{-2} and 10^{-6} is a conversion factor from dynes to megadynes [408].

2.7.3 Calculation of percent total tissue water

Hearts were freeze clamped at liquid nitrogen temperatures and stored at -80^{o}C until analysed. Heart tissue was ground to a powder, weighed and then dried to a constant weight at 85^{o}C for up to 48 hours. The percent total tissue water (%TTW) was calculated from the difference between wet and dry weight, divided by the wet weight, and multiplied by 100 [434,435].

2.7.4 Estimation of myocardial membrane potential

Hearts were freeze clamped at liquid nitrogen temperatures pre-arrest and immediately following induction of arrest. Left ventricular tissue was ground under liquid nitrogen in a mortar and stored at -80^{o}C. Samples of tissue (50-100mg) were digested with nitric acid and analysed for total tissue potassium (mg/kg) with a Varian Liberty Series II Inductively Coupled Plasma Atomic Emission Spectrometer (Melbourne, Australia).

Membrane potential (V_M or ψ in millivolts) was calculated from the Nernstian distribution of K^+ ion between the extracellular and intracellular phases (equation 3).

$$V_M = E_{K^+} = \frac{RT}{z_{K^+} F} \times \ln \frac{[K^+]_{OUT}}{[K^-]_{IN}} \qquad (3)$$

where R is the universal gas constant (8.31 J mol^{-1} K^{-1}), F is Faraday's constant (96.49 KJ mol^{-1} V^{-1}), T is absolute temperature (305.15 K), *z* is the valence of potassium ion (+1), and $[K^+]_{IN}$ and $[K^+]_{OUT}$ are the intracellular and extracellular concentrations of potassium ion in mol/L, respectively [408]. The $[K^+]_{IN}$ was calculated from the equation: $[K^+]_{TOTAL} = x\,[K^+]_{IN} + y\,[K^+]_{OUT}$ where x is the intracellular space and y is the extracellular space, respectively. In the perfused working rat heart, the distribution of total tissue water is 41% intracellular and 59% extracellular [435]. It was assumed that $[K^+]_{OUT}$ was equal to the potassium concentrations in the various cardioplegic solutions (0.1, 3, 5.9, 10, 16 or 25mM K^+).

2.7.5 Calculation of percentage recovery of functional parameters

Percentage recovery of functional parameters (% recovery) was calculated by dividing the recovered value by the pre-arrest value of the parameter and multiplying the result by 100.

2.8 Statistical analysis

Results are expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed with SPSS software (versions 16.0, 17.0, 22.0). Data was checked for normality using Levene's and Shapiro-Wilks tests. Discrete variables measured pre-arrest and at specific time points (e.g. heart rate, aortic flow, systolic pressure) were compared for significant difference between groups using one-way analysis of variance (ANOVA). Data from the 40-minute and 60-minute protocols in Chapter 3 were analysed separately. Post-arrest non-parametric functional data was analysed using Mann-Whitney U or t-tests. Parametric functional data over multiple time points during the arrest period and 60 minutes recovery for the different treatment groups was analysed using two-way ANOVA with repeated measures. If significant differences were found, post hoc analysis was performed using Tukey and Dunnett (2 sided) post hoc tests. Statistical significance was defined as $p<0.05$.

Chapter 3

Developing warm adenosine-lignocaine cardioplegia: continuous versus intermittent delivery

This chapter is based on the publication:

Sloots, K. L., Vinten-Johansen, J., & Dobson, G. P. (2007). Warm nondepolarizing adenosine and lidocaine cardioplegia: continuous versus intermittent delivery. *The Journal of thoracic and cardiovascular surgery*, *133*(5), 1171-1178.

3.1 Introduction

Despite an increasing number of investigators advocating warm surgery [330,337,359,436,437], the majority of surgeons worldwide continue to use hypothermic arrest [377,438]. However, there are a number of legitimate concerns with arrest at hypothermic temperatures including its association with myocardial calcium loading and ischaemia-reperfusion injury [184,439,440], microvascular injury [177,184] and myocardial stunning [314,340,441,442]. A fundamental problem that confronts surgeons is the fine balance between the need to interrupt cardioplegia delivery to perform surgery, and the need to provide continuous perfusion of the heart for adequate protection at higher temperatures. However continuous delivery of cardioplegia obscures the operating field. Improved visibility is one reason for the majority of surgeons preferring cold arresting temperatures.

3.1.1 Aims

The aims of this study were to:

1) examine the efficacy of normokalaemic, non-depolarising adenosine-lignocaine (AL) cardioplegia delivered at 32°C using both continuous and intermittent delivery protocols in the isolated working rat heart
2) compare functional recovery of hearts arrested with AL with functional recovery of hearts arrested with lignocaine only cardioplegia which has also shown protection at normothermic temperatures in animal models [266,443,444]. Adenosine alone cardioplegia was not included because the high doses required to rapidly arrest the heart and maintain quiescence impair post-ischaemic recovery [239].

3.1.2 Hypothesis

It was hypothesised that continuous and intermittent delivery of AL cardioplegia at 32°C would result in comparable post-arrest functional recovery which was significantly better than recovery following arrest with warm intermittent lignocaine solution.

3.2 Methods

3.2.1 Buffers and arrest solutions

1) Adenosine 200μm + Lignocaine 500μm (AL) cardioplegia
2) Krebs Henseleit buffer (KH)
3) Krebs Henseleit buffer + lignocaine 500μm (lignocaine cardioplegia)

3.2.2 Experimental groups

Rats were randomly assigned to 5 groups (Figure 3.1, n = 6 each group):

1) 40-minute arrest with AL cardioplegia continuous delivery (AL 40 Cont)
2) 40-minute arrest with AL cardioplegia intermittent delivery (AL 40 Int)
3) 60-minute arrest with AL cardioplegia continuous delivery (AL 60 Cont)
4) 60-minute arrest with AL cardioplegia intermittent delivery (AL 60 Int)
5) 60-minute arrest with Lignocaine cardioplegia intermittent delivery (Lig 60 Int)

3.2.3 Arrest and reperfusion protocols

As detailed in Chapter 2: Materials and Methods Section 2.5, hearts were rapidly removed from anaesthetised rats and immediately placed in ice-cold Krebs-Henseleit buffer. Hearts were connected via the aorta to a standard Langendorff apparatus at a perfusion pressure of 80cm H_2O (60mmHg). The pulmonary artery was cannulated for collection of coronary venous effluent. After the left atrium was cannulated the preparation was switched to working mode. Hearts were stabilised for 10 minutes before converting back to Langendorff (non-working) mode to administer the arrest solution at 32^{o}C. Heart rate, aortic pressures, coronary flow, aortic flow, inflow and coronary venous oxygen content were measured pre-arrest and during reperfusion (Figure 3.1).

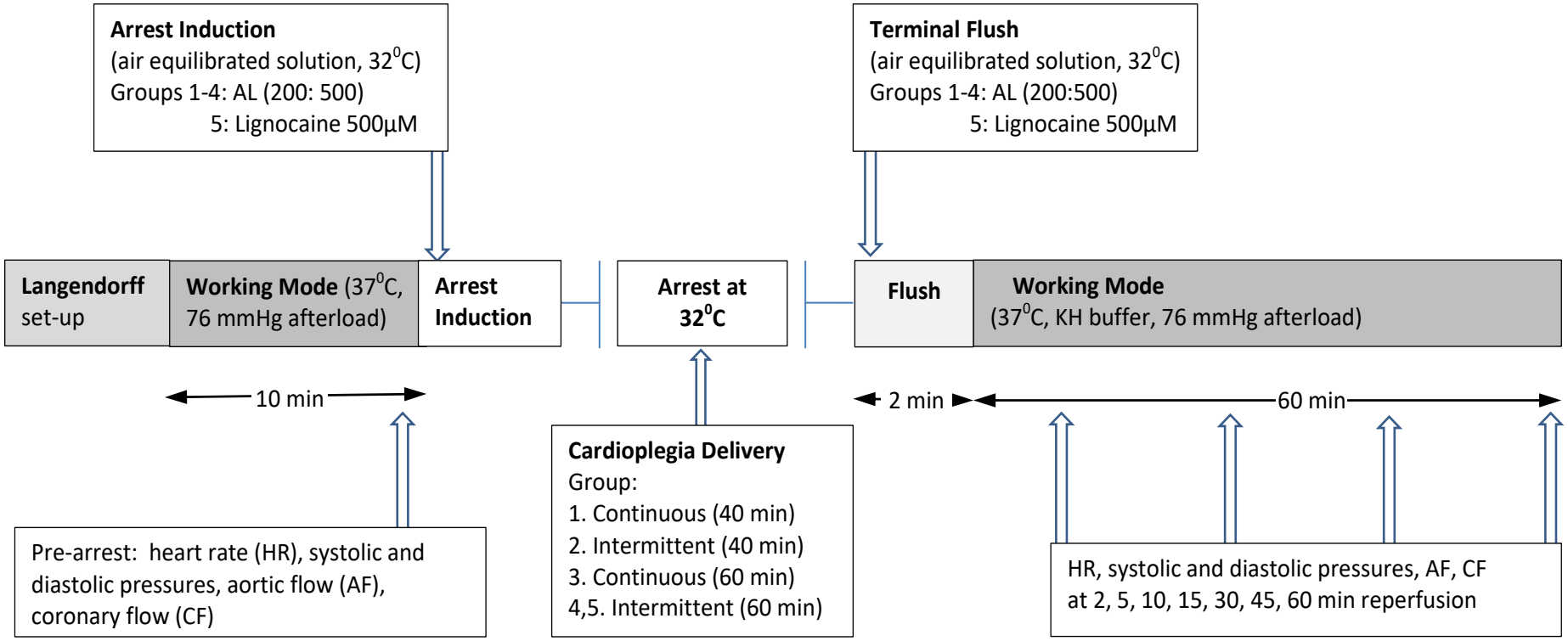


Figure 3.1: Timeline for arrest and reperfusion protocol using continuous or intermittent delivery of adenosine and lignocaine (AL) or lignocaine cardioplegia for 40-minute or 60-minute arrest

Continuous cardioplegia (Groups 1 and 3)

Cardioplegia solution was delivered continuously for 40 or 60 minutes of arrest. The perfusate volume was measured after 18 minutes and 38 minutes, and also after 58 minutes in the 60 minute arrest groups, for calculating coronary vascular resistance. Hearts were switched to working mode and reperfused with KH buffer at 37^{o}C for 60 minutes and functional measurements taken at 15, 30, 45 and 60 minutes of reperfusion (Figure 3.1).

Intermittent cardioplegia (Groups 2, 4 and 5)

A 50ml cardioplegia induction dose was administered and the aorta was cross-clamped. The cross-clamp was removed and a 2-minute cardioplegia infusion was given after 18 minutes and 38 minutes in the 40-minute arrest groups, and also after 58 minutes in the 60-minute arrest groups. The perfusate volume was measured for calculating coronary vascular resistance. After the terminal cardioplegia infusion hearts were switched to working mode and reperfused for 60 minutes as detailed in the continuous cardioplegia protocol for groups 1 and 3 above and Figure 3.1.

3.3 Results

Functional measurements prior to and during arrest and reperfusion are shown in Tables 3.1 to 3.3 and Figures 3.2 and 3.3. There were no significant differences between the 5 groups in stabilised functional parameters (heart rate, systolic and diastolic pressures, aortic and coronary flows, cardiac output, rate pressure product) measured pre-arrest.

3.3.1 Effect of cardioplegia protocol on arrest times, cardioplegia solution volumes and coronary vascular resistance

Arrest times are shown in Table 3.1. No significant differences were found between the different AL arrest protocols, with arrest times ranging from 7.2 ± 0.8 to 10.0 ± 1.8 seconds (n = 24). Hearts receiving lignocaine cardioplegia took 102 ± 27 seconds to arrest (p<0.05) with values ranging from 25 to 200 seconds. In addition, ventricular arrest occurred before atrial arrest in two hearts receiving lignocaine cardioplegia, and escape beats occurred for 2 minutes before full electrochemical arrest was achieved in one heart receiving lignocaine cardioplegia.

The total volume of cardioplegia solution delivered to hearts during the 40-minute arrest period was 699.4 ± 0.5ml during continuous delivery and 121.5 ± 0.6ml during intermittent delivery (Table 3.1). For the 60-minute arrest protocol, the total cardioplegia volume was 922.1 ± 0.3ml for continuous flow and 159.3 ± 0.8ml for intermittent delivery. There was a slight (<5%) decrease in AL cardioplegia volume per minute delivered during the 40-minute and 60-minute arrest periods, but this was not significant. The volume of lidocaine cardioplegia delivered per minute during the 60-minute arrest period decreased significantly from 31.7 ± 2.1ml/min to 25.5 ± 1.4ml/min (p<0.05).

Coronary vascular resistances are shown in Figure 3.2. At 18 minutes arrest there was no significant difference in coronary vascular resistance between the continuous and intermittent AL arrest groups (data not shown), or at 38 minutes (0.28 ± 0.01 megadyne.sec.cm^{-5} for both 40-minute AL arrest groups (Figure 3.2a) or at 38 minutes for the two 60-minute AL arrest groups (0.32 ± 0.01 vs. 0.27 ± 0.02 megadyne.sec.cm^{-5} (Figure 3.2a).

Table 3.1 Arrest times and delivered cardioplegia volumes during 40-minute or 60-minute arrest using continuous or intermittent delivery of adenosine and lignocaine (AL) or lignocaine only cardioplegia at 32°C, (n=6).

Cardioplegia delivery regimen	Arrest Time (sec)	Induction Volume (ml)	Cardioplegia Volumes (2 min @18 min) (ml)	Cardioplegia Volumes (2 min @ 38 min) (ml)	Cardioplegia Volumes (2 min @ 58 min) (ml)	Total Cardioplegia Volume (ml)	Time to first beat (min)	Time to aortic flow (min)
AL 40 min Continuous	10.0 ± 1.8	n/a	35.6 ± 1.6	34.3 ± 1.2	n/a	699.4 ± 0.5	1.7 ± 0.6	4.0 ± 0.8
AL 40 min Intermittent	8.2 ± 0.8	50	37.0 ± 1.9	34.5 ± 1.7	n/a	121.5 ± 0.6	1.8 ± 0.5	7.5 ± 1.3
AL 60 min Continuous	8.0 ± 0.4	n/a	31.7 ± 1.4	30.7 ± 1.1	30.0 ± 1.0	922.1 ± 0.3	2.5 ± 0.2	6.1 ± 0.7
AL 60 min Intermittent	7.2 ± 0.8	50	37.3 ± 2.2	37.0 ± 2.1	35.3 ± 2.4	159.3 ± 0.8	3.5 ± 0.6	10.0 ± 2.0
Lignocaine 60 min Intermittent	102 ± 27 §	50	31.7 ± 2.1	29.0 ± 2.1	25.5 ± 1.4 †	136.1 ± 0.7	3.3 ± 1.3	11.7 ± 2.7

§ $p<0.05$ lignocaine group arrest time with all other groups
† $p<0.05$ lignocaine group cardioplegia volume at 58 minutes compared with 18 minutes (paired-samples t test), and lignocaine group cardioplegia volume at 58 minutes compared with AL intermittent group cardioplegia volume at 58 minutes

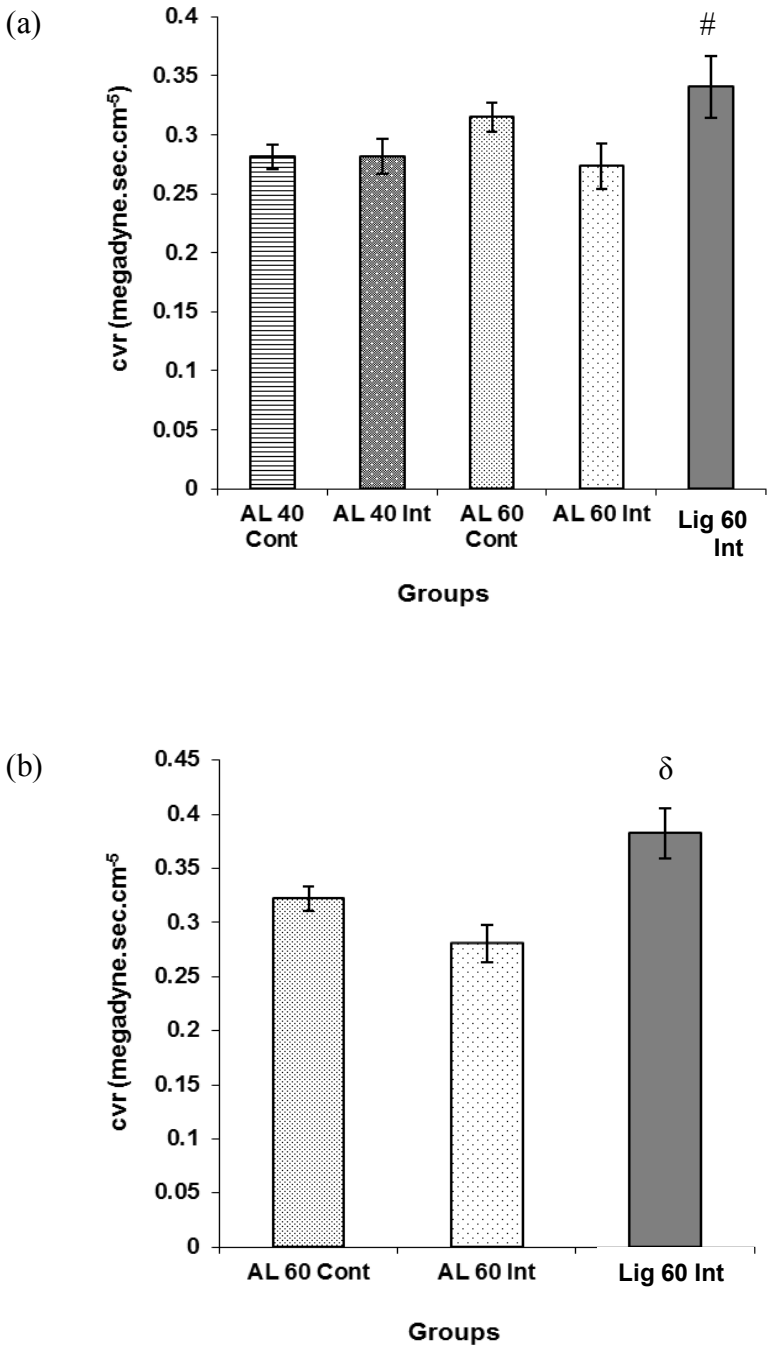


Figure 3.2: Coronary Vascular Resistance measured after (a) 38 minutes arrest and (b) 58 minutes arrest in hearts arrested for 40 or 60 minutes with continuous (Cont) or intermittent (Int) delivery of AL or lignocaine cardioplegia.

# $p<0.05$ lignocaine group compared with AL intermittent group after 38 min arrest,
δ $p<0.05$ lignocaine group compared with AL intermittent group after 58 min arrest

Similarly, there was no significant difference at 58 minutes for the continuous and intermittent AL groups (0.32 ± 0.02 vs. 0.27 ± 0.02 megadyne.sec.cm^{-5} respectively) (Figure 3.2b). In contrast, coronary vascular resistance in the 60-minute intermittent lignocaine group (0.34 ± 0.03 megadyne.sec.cm^{-5}) was significantly different from the 60-minute intermittent AL group after 38 minutes of arrest (p<0.05) (Figure 3.2a), and during the terminal delivery of cardioplegia after 58 minutes of arrest (0.38 ± 0.02 megadyne.sec.cm^{-5}) (p<0.05) (Figure 3.2b).

The time taken for the hearts to spontaneously resume beating following arrest is shown in Table 3.1. There were no significant differences between the AL groups or the lignocaine group. Although the time to achieve aortic flow varied from 4.0 ± 0.8 min for the AL 40-minute continuous group to 11.7 ± 2.7 minutes for the lignocaine 60-minute intermittent group, this difference was not significant due to the range of data within both the 60-minute intermittent AL and lignocaine groups (Table 3.1).

3.3.2 Effect of cardioplegia protocol on functional profiles during reperfusion

40-minute arrest groups: Heart rate, developed pressures, aortic flow, coronary flow and rate-pressure product for the 40-minute arrest groups during 60 minutes working mode reperfusion are shown in Table 3.2. Percentage recovery of aortic flow and coronary flow during the reperfusion period are shown in Figure 3.3a and b. Hearts arrested with continuous AL for 40 minutes recovered 89 ± 6% of heart rate, 85 ± 5% of pre-arrest aortic flow and 100 ± 7% of pre-arrest coronary flow after 15 minutes of reperfusion. Hearts arrested with intermittent AL for 40 minutes recovered 91 ± 4% of heart rate, 82 ± 3% of pre-arrest aortic flow and 106 ± 10% of pre-arrest coronary flow after 15 minutes of reperfusion. After 30 minutes of reperfusion the flows had increased to a maximum of 105 ± 7% of pre-arrest aortic flow and 108 ± 4% of pre-arrest coronary flow in the continuous group, and 94 ± 3% of pre-arrest aortic flow and 99 ± 3% of pre-arrest coronary flow in the intermittent group. There was a subsequent slight decrease in aortic flows by 60 minutes of reperfusion, but there was no significant difference in heart rate, aortic flow, coronary flow or rate-pressure product between groups during the recovery period.

Table 3.2: Functional parameters of isolated rat hearts during pre-arrest and reperfusion (working mode) using continuous or intermittent delivery of adenosine-lidocaine (AL) cardioplegia for 40-minute arrest at 32°C, (n=6).

Arrest Protocol	40 min cardioplegia delivery regimen	Heart Rate (beats/min)	Systolic Pressure (mmHg)	Diastolic Pressure (mmHg)	Aortic Flow (ml/min)	% of pre-arrest AF	Coronary Flow (ml/min)	% of pre-arrest CF	Rate Pressure Product (mmHg/min)
Pre-Arrest	Continuous	297 ± 18	140.8 ± 0.8	65.0 ± 3.4	60.3 ± 0.5	n/a	20.8 ± 1.2	n/a	41733 ± 2339
	Intermittent	313 ± 16	136.7 ± 3.1	63.3 ± 2.1	57.7 ± 2.9	n/a	19.2 ± 1.0	n/a	42700 ± 1835
Reperfusion 15 min	Continuous	263 ± 20	140.0 ± 2.2	65.0 ± 2.6	51.3 ± 2.9	85 ± 5	20.8 ± 1.9	100 ± 7	36600 ± 2623
	Intermittent	282 ± 11	133.3 ± 6.3	61.7 ± 4.0	47.8 ± 3.9	82 ± 3	20.0 ± 1.5	106 ± 9	37425 ± 1799
Reperfusion 30 min	Continuous	281 ± 15	137.5 ± 2.1	65.8 ± 3.3	53.8 ± 1.1	105 ± 7	22.3 ± 1.2	108 ± 4	38513 ± 1713
	Intermittent	321 ± 14	127.5 ± 5.3	63.3 ± 4.0	54.0 ± 1.9	94 ± 3	19.0 ± 1.3	99 ± 3	40783 ± 2013
Reperfusion 45 min	Continuous	300 ± 17	133.3 ± 2.5	65.0 ± 3.4	54.2 ± 0.4	90 ± 1	23.2 ± 1.2	112 ± 4	39913 ± 1940
	Intermittent	325 ± 12	126.7 ± 5.1	65.0 ± 5.0	52.8 ± 1.8	92 ± 3	21.0 ± 1.7	109 ± 4	41021 ± 1658
Reperfusion 60 min	Continuous	306 ± 13	133.3 ± 2.5	65.0 ± 3.4	53.3 ± 0.7	88 ± 1	22.7 ± 0.9	109 ± 3	40729 ± 1594
	Intermittent	322 ± 8	127.5 ± 4.6	66.0 ± 4.9	51.0 ± 1.7	89 ± 2	20.7 ± 0.9	109 ± 4	40958 ± 1607

Table 3.3a: Functional parameters of isolated rat hearts during pre-arrest and working mode reperfusion (15,30 minutes) using continuous or intermittent delivery of adenosine-lidocaine (AL) or lignocaine cardioplegia for 60-minute arrest at 32°C, (n=6).

Arrest Protocol	60 min cardioplegia delivery regimen	Heart Rate (beats/min)	Systolic Pressure (mmHg)	Diastolic Pressure (mmHg)	Aortic Flow (ml/min)	Coronary Flow (ml/min)	Rate Pressure Product (mmHg/min)
Pre-Arrest	AL Continuous	309 ± 10	126.7 ± 3.6	71.7 ± 4.0	56.7 ±1.6	20.7 ± 0.8	39025 ± 892
	AL Intermittent	317 ± 14	140.0 ± 3.9	75.0 ± 3.2	61.0 ± 2.4	21.3 ± 1.7	44275 ± 2155
	Lignocaine Intermittent	339 ± 16	134.2 ± 2.7	70.0 ± 2.2	51.7 ± 0.8	19.8 ± 1.4	45413 ± 1949
Reperfusion 15 min	AL Continuous	254 ± 17	126.7 ± 4.2	72.5 ± 4.4	44.0 ± 3.1	20.3 ± 1.7	31875 ± 1295
	AL Intermittent	276 ± 12	135.0 ± 3.4	75.8 ± 2.7	46.8 ± 4.3	22.2 ± 2.3	37292 ± 2124
	Lignocaine Intermittent	226 ± 34	124.3 ± 14.0	70.0 ± 5.0	27.5 ± 6.2	14.2 ± 2.8	30327 ± 5526
Reperfusion 30 min	AL Continuous	288 ± 14	122.5 ± 4.6	74.2 ± 4.0	47.8 ± 2.5	20.7 ± 1.1	34938 ± 966
	AL Intermittent	304 ± 6	132.5 ± 3.6	76.7 ± 2.5	54.3 ± 2.1	20.7 ± 1.1	40271 ± 1179
	Lignocaine Intermittent	293 ± 17	127.5 ± 1.7	72.5 ± 3.1	37.2 ± 2.5	16.2 ± 0.9	37329 ± 2242

Table 3.3b: Functional parameters of isolated rat hearts during working mode reperfusion (45, 60 minutes) using continuous or intermittent delivery of adenosine-lidocaine (AL) or lignocaine cardioplegia for 60-minute arrest at 32°C, (n=6).

Arrest Protocol	60 min cardioplegia delivery regimen	Heart Rate (beats/min)	Systolic Pressure (mmHg)	Diastolic Pressure (mmHg)	Aortic Flow (ml/min)	Coronary Flow (ml/min)	Rate Pressure Product (mmHg/min)
Reperfusion 45 min	AL Continuous	297 ± 18	120.8 ± 4.5	73.3 ± 4.4	46.3 ± 2.7	21.0 ± 1.8	35496 ± 1331
	AL Intermittent	310 ± 10	132.5 ± 4.2	75.8 ± 3.3	53.5 ± 2.0	22.8 ± 1.2	40929 ± 1041
	Lignocaine Intermittent	290 ± 13	126.7 ± 1.7	71.7 ± 2.8	37.5 ± 1.9	16.3 ± 0.9	36817 ± 2042
Reperfusion 60 min	AL Continuous	297 ± 16	119.2 ± 4.4	73.3 ± 4.4	43.5 ± 3.6	20.5 ± 1.9	35008 ± 826
	AL Intermittent	317 ± 12	132.5 ± 4.2	76.7 ± 2.5	52.6 ± 1.9 #	23.0 ± 1.1 δ	41854 ± 1538
	Lignocaine Intermittent	302 ± 13	124.2 ± 1.5	70.8 ± 2.7	36.3 ± 2.2	16.8 ± 0.7	37513 ± 1946

# $p<0.05$ aortic flow for lignocaine intermittent group compared with AL intermittent group

δ $p<0.05$ coronary flow for lignocaine intermittent group compared with AL intermittent group

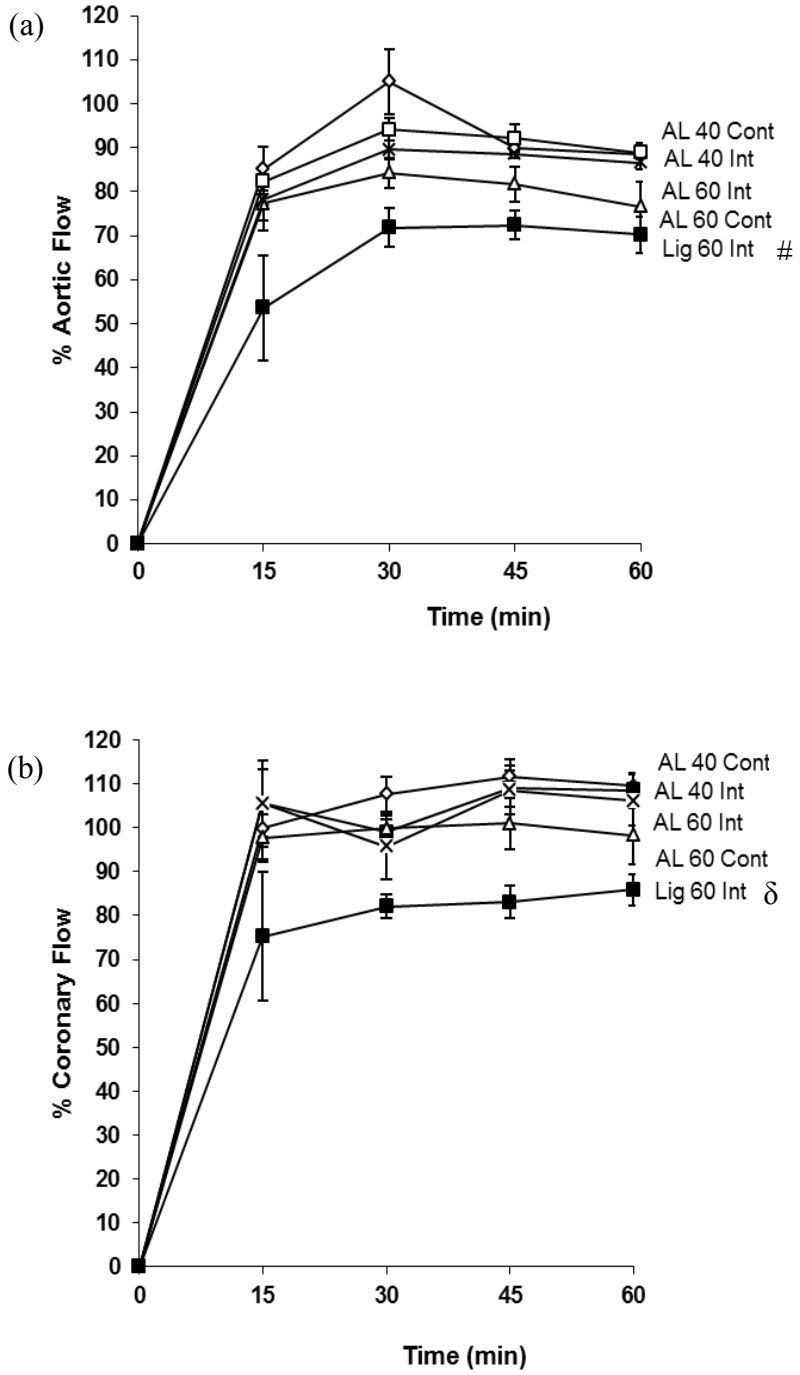


Figure 3.3: Percentage return of (a) aortic flow and (b) coronary flow during working mode reperfusion following arrest for 40 or 60 minutes with intermittent or continuous delivery of AL or lignocaine cardioplegia

(a) aortic flow
# $p<0.05$ 60-minute lignocaine intermittent group compared with 60-minute AL intermittent group and 60-minute AL continuous group

(b) coronary flow
δ $p<0.05$ 60-minute lignocaine intermittent group compared with 60-minute AL intermittent group

60-minute arrest groups: Functional parameters during recovery in hearts arrested for 60 minutes are shown in Table 3.3a and b and Figure 3.3a and b. By 15 minutes of reperfusion, heart rate recovered to 82 ± 4% and 88 ± 5% of pre-arrest values in hearts arrested with AL continuous and AL intermittent methods respectively, and 68 ± 11% of pre-arrest value in hearts arrested with lignocaine. There were no significant differences between these groups in heart rate or rate-pressure product during the recovery period. After 15 minutes of reperfusion hearts arrested with AL solution had recovered 77 ± 4% of pre-arrest aortic flow and 98 ± 5% of pre-arrest coronary flow in the continuous group and 77 ± 7% of pre-arrest aortic flow and 104 ± 8% of pre-arrest coronary flow in the intermittent group, compared with 54 ± 12% recovery of aortic flow and 75 ± 15% recovery of coronary flow in the lignocaine group. There was a significant difference in the percentage recovery of aortic flow between the lignocaine group and the AL intermittent and AL continuous groups ($p<0.05$). Recovery of coronary flow was also significantly different between the lignocaine group and the AL intermittent group ($p<0.05$).

When data from AL groups were compared, there was a trend in the intermittent group towards increased coronary flow and lower coronary vascular resistance during arrest, and improved recovery of aortic flow and coronary flow during the recovery period compared with the continuous group, but these differences did not reach statistical significance.

3.4 Discussion

Although hypothermic cardioplegia remains a popular method of myocardial protection [68,272,377,438,445,446], the use of tepid and warm cardioplegia is increasingly being investigated to establish the safe temperature and optimal procedure for protecting the heart and the brain during cardiac surgery [332,409,447,448].

The main findings of this chapter were:

1) Intermittent (re-dosing for 2 minutes every 20 minutes) and continuous delivery of AL cardioplegia produced equivalent functional recovery profiles after 60-minute arrest at 32°C.
2) Intermittent AL delivery reduced cardioplegia volume administered during arrest by more than 80% compared with continuous delivery.
3) Intermittent AL cardioplegia led to significantly shorter time to arrest, lower cardiovascular resistance and higher left ventricular recovery profiles compared to intermittent lignocaine cardioplegia.

These findings will now be discussed.

3.4.1 Intermittent and continuous delivery of warm AL cardioplegia resulted in equivalent functional recovery

This study showed that intermittent AL cardioplegia was equivalent to continuous AL cardioplegia despite the total cardioplegia volume for the intermittent group being less than 20% of the continuous infusion group (Table 3.1). These results demonstrated that greater cardioplegia volume does not improve functional outcome in this model.

Coronary vascular resistance

There was no significant difference in coronary vascular resistance between the intermittent and continuous AL groups with values ranging from 0.27 ± 0.02 to 0.32 ± 0.02 megadyne.sec.cm^{-5} (Figure 3.2a and b). A relatively constant coronary vascular resistance suggests that there were no differences in smooth muscle reactivity, vascular endothelial function or extravascular compression from oedema between the AL groups during 40 or 60 minutes of global ischemic arrest.

Reduced coronary vascular resistance in AL arrested hearts [408] may improve distribution of cardioplegia solution, delivery of oxygen and metabolites, and removal of wastes at reperfusion. In contrast, Ericsson and colleagues [449] showed in pigs undergoing cardiopulmonary bypass that coronary vascular resistance increased 1.7 times from approximately 0.33mmHg.min.ml^{-1} at 5 minutes to 0.55mmHg.min.ml^{-1} at 45 minutes arrest, and proposed that this increase may have been linked to increased endothelial dysfunction or perivascular oedema associated with warm continuous hyperkalemic cardioplegia. Similarly, Torchiana et al. [450] reported that in their canine model coronary vascular resistance increased slowly to a maximum of 2.5 fold in the last minute of receiving antegrade warm hyperkalemic blood cardioplegia. They postulated that the increased coronary vascular resistance was likely due to the depolarising potassium in the cardioplegia, an observation previously reported by Kucich in 1987 [451], and this effect of potassium may be more pronounced at higher temperatures. Furthermore, in 1991 Mankad and colleagues reported that high potassium levels in St. Thomas' hospital solution or Bretschneider solution resulted in endothelial damage, and that this deleterious effect of potassium was concentration dependent [294]. Increased coronary vascular resistance during hyperkalaemic arrest may also compromise the distribution of the cardioplegic solution and increase the risk of ischemic injury. For many decades, high potassium concentrations have been used in aortic ring studies and artery conduits to induce maximum vasoconstriction response when studying the effect of new drugs on coronary vasoreactivity [452]. AL cardioplegia with physiological plasma potassium levels may offer an alternative to the possible detrimental effects of depolarising high potassium concentrations on the microvasculature [408].

Functional recovery during working mode reperfusion

There were also no significant differences in functional recovery during reperfusion following 40- and 60-minute global ischaemic arrest with AL (Tables 3.2 and 3.3). For example, hearts arrested for 60 minutes with intermittent or continuous AL recovered similar percentages of pre-arrest values in developed pressures, heart rate, aortic flow, coronary flow and rate-pressure product after 60 minutes of reperfusion (Table 3.3). These data suggest that the myocardium and coronary microvasculature were protected during the 40- or 60-minute arrest period using AL cardioplegia at 32°C. While there was a trend for intermittent delivery of AL cardioplegia to improve functional recovery after 60 minutes arrest, these differences were not significant.

Additional cardioprotective properties of adenosine and lignocaine may have contributed to the comparable functional recovery following continuous or intermittent warm (32°C) AL cardioplegia delivery. Apart from promoting vasodilation, adenosine and lignocaine protect against ischaemia-induced ventricular arrhythmias and exhibit anti-inflammatory properties [211,224,250,265,453,454] and reduce free radical damage and ischaemia by attenuating calcium loading of myocardial cells [455-457]. The combination of adenosine and lidocaine also down-regulates myocardial metabolism and preserves high energy phosphates which delays intracellular acidosis during ischaemia [222,223,458]. By opposing the stimulatory effect of catecholamines and inhibiting norepinephrine release from cardiac sympathetic nerves [211], adenosine further assists in reducing myocardial metabolism and oxygen needs [459,460] during ischaemic arrest intervals, promoting improved functional recovery.

Although not investigated in the present study, similar levels of cardioprotection during intermittent or continuous AL delivery may be due to the ability of adenosine and lignocaine to improve homeostatic ionic balance, reduce ischemia during arrest and reperfusion, and decrease production of radical species during reperfusion. Further studies are required to investigate these mechanisms.

3.4.2 Warm intermittent AL cardioplegia improved functional recovery compared to lignocaine cardioplegia

Intermittent AL cardioplegia was also compared with intermittent lignocaine cardioplegia for 60-minute arrest at 32°C. Lignocaine acts on voltage–dependent sodium fast channels in atrial and ventricular cells and gap junctions to prolong the inactivation state of the channels [248-250]. Lignocaine exerts a negative inotropic effect by shortening the action potential duration [461], and at high concentrations blocks the flow of sodium into the cell, preventing conduction of the action potential which results in arrest of the heart.

In this study intermittent lignocaine cardioplegia was not as effective as intermittent AL cardioplegia, with variable and significantly longer arrest times (102 ± 27 sec vs 7.2 ± 0.8 to 10.0 ± 1.8 sec, respectively) (Table 3.1), significantly higher coronary vascular resistance during arrest (~20% higher at 38 and 58 minutes arrest) (Figure 3.2) and significantly lower returns in aortic and coronary flows (p<0.05) (Table 3.3 and Figure 3.3).

Lignocaine cardioplegia has been advocated as an alternative to depolarising potassium cardioplegia by a number of groups using Langendorff perfused rat and rabbit heart models [443,444,462]. However, a study by Yamaguchi and colleagues [198] using a canine model of cardiopulmonary bypass showed that lignocaine was not satisfactory as a sole arresting agent, and in combination with magnesium was only equivalent to potassium-based cardioplegia.

While lignocaine at 500μM arrests the rat heart faster than clamping a heart receiving Krebs-Henseleit alone (1.7 ± 0.5 vs 10.7 ± 2.2 minutes (n=4, unpublished data), the addition of 200μM adenosine resulted in more rapid arrest and improved protection of the myocardium and coronary vasculature compared to lignocaine only cardioplegia. Therefore the combination of adenosine and lignocaine appears to provide cardioprotection during polarised arrest at 32^{o}C.

3.5 Conclusion

This study has shown that AL cardioplegia can be delivered continuously or intermittently for 40-or 60-minute arrest at 32°C with no significant differences in post-arrest functional recovery. Lignocaine only cardioplegia was not as effective as AL cardioplegia in the time to arrest, maintenance of coronary vascular resistance or functional recovery during reperfusion. The combination of adenosine and lidocaine led to faster arrest times and conferred greater protection during and following cardioplegic arrest.

Chapter 4

Effect of varying potassium levels in AL cardioplegia: the search for optimal potassium concentration

This chapter is based on the publication:

Sloots, K. L., & Dobson, G. P. (2010). Normokalemic adenosine–lidocaine cardioplegia: importance of maintaining a polarized myocardium for optimal arrest and reanimation. *The Journal of thoracic and cardiovascular surgery*, *139*(6), 1576-1586.

4.1 Introduction

Chapter 3 results showed that normokalaemic AL cardioplegia delivered intermittently (re-dosing 2 minutes every 20 minutes) provided equivalent protection to continuous delivery for 60-minute arrest at 32^{o}C, despite an 80% reduction in infused volume. Although, as previously mentioned, an increasing number of investigators advocate warm surgery [330,337,359,436,437], hypothermic arrest remains the most popular choice of surgeons worldwide partly due to concerns with potassium-linked damage at higher temperatures [284,294,308,463-466]. This chapter examines the question of the optimal potassium level for cardioprotection using AL cardioplegia containing potassium levels ranging from 0.1mM to 16mM, or 16mM and 25mM potassium-alone cardioplegia.

4.1.1 Aims

This study was designed to assess the effect of varying the concentrations of extracellular potassium in AL cardioplegia and hyperkalaemic solutions for arrest for 1 and 2 hours at 32^{o}C on

1) estimated cellular membrane potential during arrest
2) time to arrest and cardiovascular resistance during intermittent cardioplegia delivery
3) time to reanimate, aortic and coronary flows, cardiac output, and stroke volume during 60 minutes of working mode reperfusion

4.1.2 Hypothesis

The hypothesis for this chapter was that normokalaemic AL cardioplegia would provide significantly improved cardioprotection during warm (32^{o}C) intermittent arrest compared with hypokalaemic or hyperkalaemic arresting solutions.

4.2 Methods

4.2.1 Buffers and arrest solutions

1) Krebs Henseleit buffer (KH)
2) AL arrest solutions: Adenosine 200μm and lignocaine 500μm added to Krebs Henseleit buffer containing 0.1, 3, 5.9, 10 or 16mM potassium with 10mM glucose at pH 7.7
3) Hyperkalaemic arrest solutions: Krebs Henseleit buffer containing 16mM or 25mM potassium and 10mM glucose at pH 7.7

4.2.2 Experimental groups

Rats were randomly assigned to one of three experiments and 20 groups (Figure 4.1):

Cardiac Membrane potential: 8 groups (n = 6 each group):
Control (non-arrest); and immediately after arrest induction: AL (0.1, 3, 5.9, 10 or 16mM K^+), and 16 and 25mM K^+ alone.

One-hour arrest protocol: 7 groups (n = 8 each group):
AL 0.1, 3, 5.9, 10 and 16mM K^+, and 16 and 25mM K^+ alone.

Two-hour arrest protocol: 5 groups (n = 8 each group):
AL 3, 5.9, 10, and 16mM K^+, and 16mM K^+ alone.
The AL 0.1mM K^+ and 25mM K^+ groups were not included in the two-hour arrest experiment because 40% of these hearts failed to recover after 1-hour arrest.

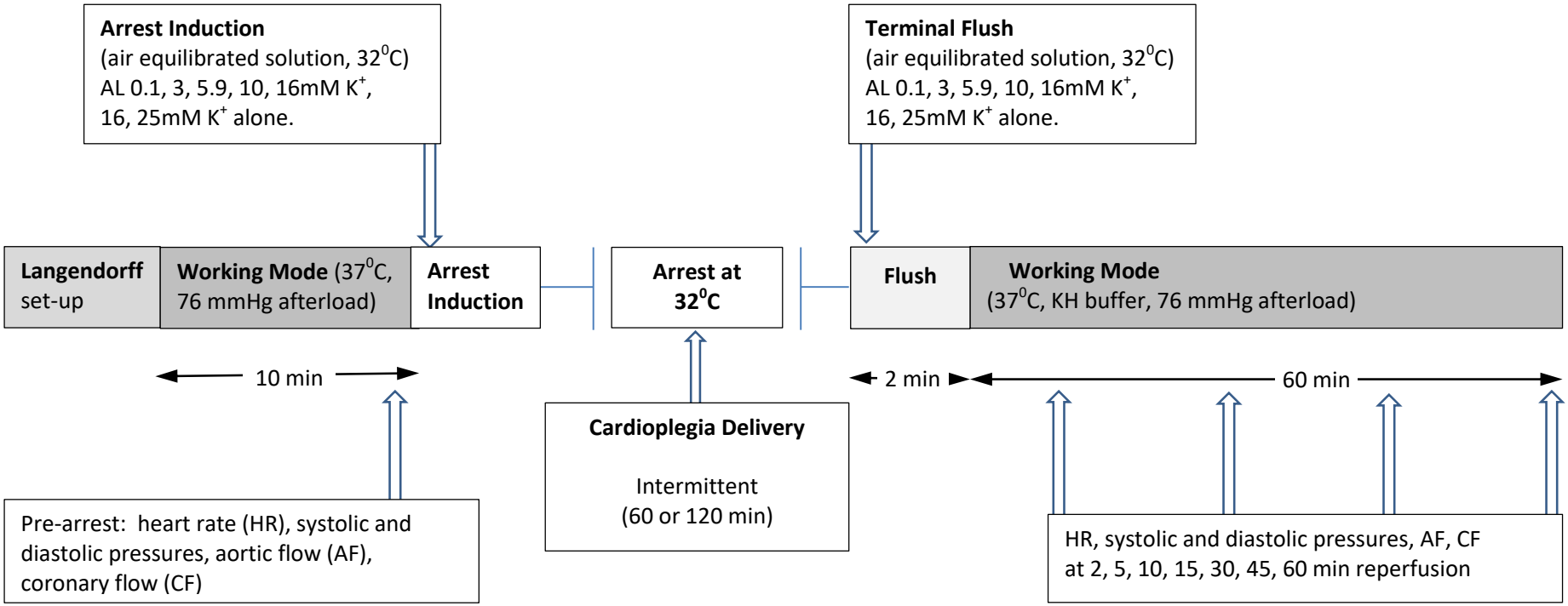


Figure 4.1: Timeline for arrest and reperfusion protocol using warm intermittent delivery of AL cardioplegia with varying concentrations of potassium, or potassium-based cardioplegia, for 1- or 2-hour arrest

4.2.3 Arrest and reperfusion protocols

Hearts were prepared for working mode perfusion as detailed in Chapter 2: Materials and Methods Section 2.5.

A 50ml cardioplegia induction dose was administered at 32^{o}C in Langendorff mode and the aorta was cross-clamped. The cross-clamp was removed and a 2-minute cardioplegia infusion was given after 18, 38 and 58 minutes in the 1-hour arrest groups, and also after 78, 98 and 118 minutes in the 2-hour arrest groups. The perfusate volume was measured for calculating coronary vascular resistance. Hearts in all groups were maintained at 32^{o}C during arrest. After the terminal cardioplegia infusion hearts were switched to working mode and reperfused with KH buffer at 37^{o}C for 60 minutes and functional measurements taken at 15, 30, 45 and 60 minutes of reperfusion (Figure 4.1).

Table 4.1: Arrest parameters during 1-hour or 2-hour warm intermittent arrest.

Arrest Solution	Diastolic Membrane Potential	1-hour arrest			2-hour arrest		
		Total Cardioplegia Flush Volume (ml) #	Coronary Vascular Resistance (megadyne.sec.cm^{-5})		Total Cardioplegia Flush Volume (ml) #	Coronary Vascular Resistance (megadyne.sec.cm^{-5})	
			End of Induction	At 58 min Arrest		End of Induction	At 118 min Arrest
AL (0.1mM K^+)	-183 ± 1 mV	74 ± 2	0.25 ± 0	0.53 ± .02 †	ND	ND	ND
AL (3.0mM K^+)	-94 ± 1 mV	87 ± 3	0.26 ± .01	0.40 ± .03	138 ± 5	0.25 ± .01	0.65 ± .06
AL (5.9mM K^+)	-75 ± 2 mV	104 ± 1 *	0.24 ± .01	0.29 ± .01	203 ± 3 §	0.24 ± .01	0.31 ± .01 §§§
AL (10mM K^+)	-65 ± 1 mV	109 ± 3 **	0.24 ± .01	0.28 ± .02	155 ± 4	0.27 ± .01	0.47 ± .03
AL (16mM K^+)	-49 ± 1 mV	89 ± 3	0.26 ± .01	0.40 ± .06	157 ± 6	0.28 ± .01	0.57 ± .07
16mM K^+	-51 ± 1 mV	71 ± 2	0.35 ± .02 ¤	0.46 ± .03 ††	139 ± 5	0.40 ± .03 §§	0.57 ± .05
25mM K^+	-39 ± 1 mV	56 ± 2 ***	0.38 ± .02 ¤¤	0.61 ± .05 †††	ND	ND	ND

The diastolic membrane potential of the left ventricle was calculated from the Nernstian distribution of potassium ions (see Materials and Methods).
Volume of cardioplegia solution administered during 2-minute flushes during arrest period
ND: not determined as approximately 40% of AL (0.1mM K^+) or 25mM K^+ hearts failed to recover function after 1-hour arrest.

* $p<0.01$ compared with AL (0.1mM K^+) and 16mM K^+ groups
** $p<0.01$ compared with AL (0.1mM K^+) and 16mM K^+ groups, and $p<0.05$ compared with AL (3, 16mM K^+) groups
*** $p<0.01$ compared with AL (3, 5.9, 10, 16mM K^+) and 16mM K^+ groups, and $p<0.05$ compared with AL (0.1mM K^+) group
¤ $p<0.01$ compared with all groups except 25mM K^+ groups
¤¤ $p<0.01$ compared with all groups except 16mM K^+ groups
† $p<0.05$ compared with AL (5.9, 10mM K^+) and 25mM K^+ groups
†† $p<0.01$ compared with AL (5.9, 10mM K^+) groups
††† $p<0.05$ compared with all other groups
§ $p<0.05$ compared with AL (3mM K^+) and 16mM K^+ groups
§§ $p<0.01$ compared with all other groups
§§§ $p<0.01$ compared with AL (3, 16mM K^+) and 16mM K^+ groups

4.3 Results

Functional measurements prior to and during arrest and reperfusion are shown in Tables 4.1 and 4.2, and Figures 4.2 to 4.4. There were no significant differences between groups in stabilised functional parameters (heart rate, systolic and diastolic pressures, aortic and coronary flows, cardiac output, rate pressure product) measured pre-arrest.

4.3.1 Effect of cardioplegia potassium concentration on arrest times and membrane potentials

Times to arrest for the AL cardioplegia groups were not significantly different and ranged from 7.4 ± 0.6 to 11 ± 1.3 seconds. Arrest times increased significantly in hearts perfused with 16mM K^+ alone (22 ± 2 to 28 ± 8 seconds, $p < 0.05$ with all other groups) but then decreased in the 25mM K^+ group to 13.4 ± 1.1 seconds. Nearly all hearts perfused with hypokalemic AL solutions (0.1 and 3mM K^+) failed to maintain arrest; escape beats occurred for 9.8 ± 1.3 minutes and 7.1 ± 0.6 minutes, respectively. Similarly, hearts perfused with 16mM K^+ alone and 25mM K^+ alone had escape beats lasting for 2.5 to 25 minutes, and for 8 seconds to 11 minutes, respectively, after which arrest occurred.

The membrane potential for each group is shown in Figure 4.2a and Table 4.1. The pre-arrest value was -78 ± 1mV at 37°C. Total tissue water content was not significantly different among the groups (86.0 ± 0.4% to 87.1 ± 0.5%).

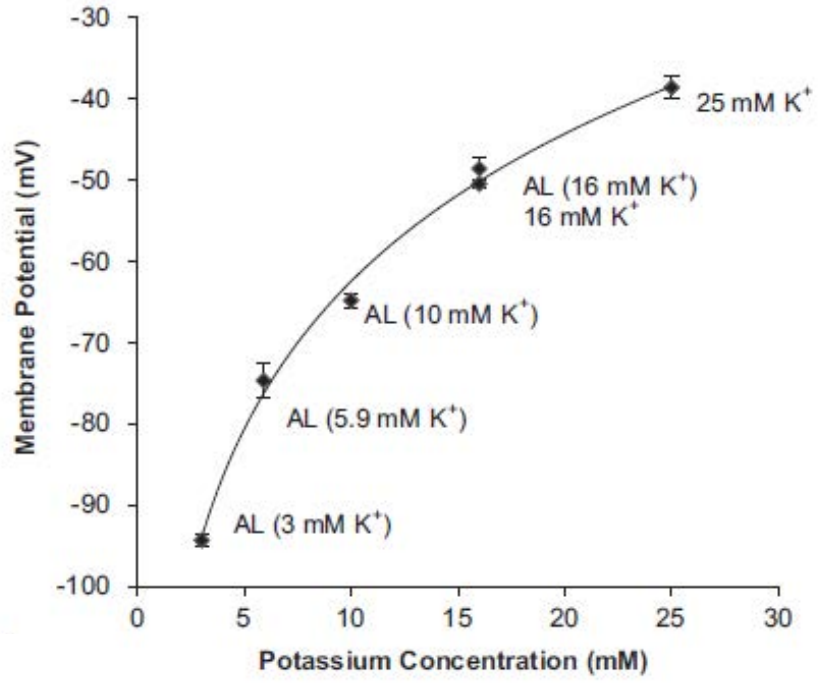


Figure 4.2a: The effect of increasing potassium (K+) concentrations on the left ventricular diastolic membrane potential in the isolated rat heart arrested with AL cardioplegia 3, 5.9, 10, and 16mM K+ or 16mM and 25mM K+ alone in Krebs–Henseleit buffer.

Membrane potentials (φ) were calculated from the Nerstian distributions of K^+ (as detailed in Chapter 2 Section 2.7.4). The relationship between diastolic membrane potential and extracellular K^+ is: φ (mV) = 26.23 ln $[K^+]$ − 123.44 (R^2 = 0.99), where $[K^+]$ is the extracellular potassium concentration in millimoles per liter.

4.3.2 Relationship between potassium concentration and coronary vascular resistance during induction and 2-minute cardioplegia flushes

During induction, there were no significant differences in rate of coronary outflow in the AL groups, although a 5% increase occurred in the AL 5.9mM K^+ group. In contrast, the rates in the 16mM and 25 K^+ alone groups were significantly decreased (24% and 35% decrease, respectively, $p<0.05$) (Table 4.1). The significant increase in coronary vascular resistance at the "End of Induction" for the 16mM K^+ group ($p < 0.05$) is shown on the Y-axis of Figure 4.2b.

Maintenance cardioplegia volumes and coronary vascular resistance measured during 2-minute cardioplegia flushes over the 1-hour and 2-hour arrest periods are shown in

Table 4.1 and Figure 4.2b. Significantly greater volumes were delivered to the AL 5.9mM K^+ and AL 10mM K^+ groups. After 1-hour arrest the AL 0.1mM K^+ and 25mM K^+ alone groups had the highest coronary vascular resistance. Nearly 40% of hearts (3/8) in each of these two groups subsequently failed to recover aortic/coronary flows after 1-hour arrest, therefore these groups were not included in the longer arrest study. After 2-hour arrest the AL 5.9mM K^+ group had the lowest coronary vascular resistance (Figure 4.2b). As the membrane potential deviated from near resting voltage (ie from AL 5.9mM K^+) the coronary vascular resistance increased, and this effect was magnified as arrest time increased (Figure 4.2c).

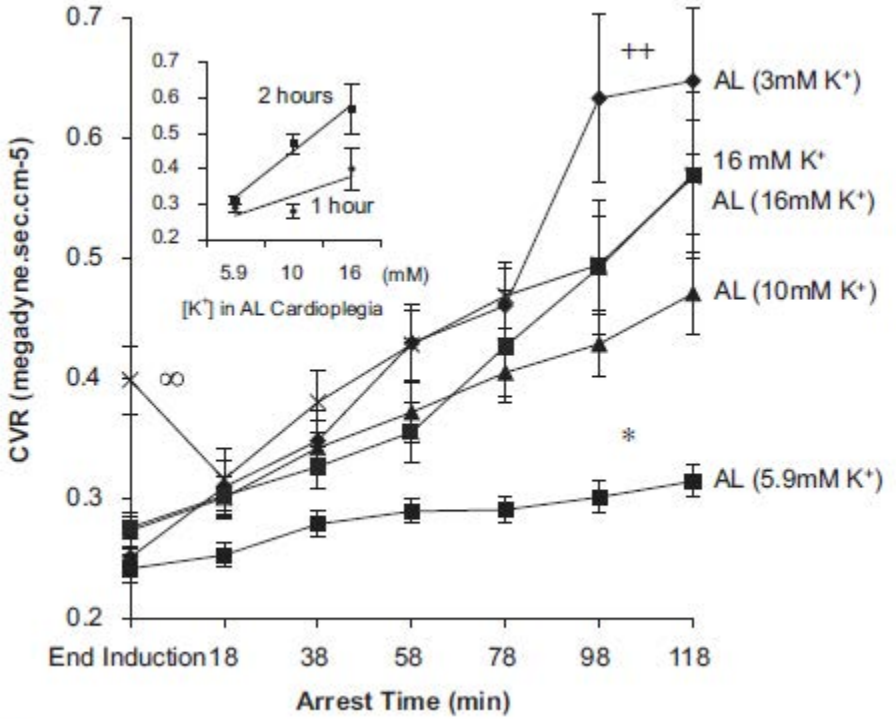


Figure 4.2b: The effect of arrest time on coronary vascular resistance (CVR) during intermittent cardioplegic flushes over a 2-hour arrest period (1-hour arrest groups not shown)

∞ $p<0.01$ 16mM K^+ alone compared with AL 3, 5.9, 10, and 16mM K^+

++ $p<0.01$ AL 3mM K^+ compared with AL 10mM K^+

* $p<0.05$ AL 5.9mM K^+ compared with AL 3 and 16mM K^+ and 16mM K^+ alone

<u>Insert</u>: Comparison of CVR after 1-hour or 2-hour arrest with AL 5.9, 10 or 16mM K^+. After 1-hour arrest: CVR = 0.0116 [K^+, mM] + 0.2004 (R^2 = 0.78), and after 2-hour arrest: CVR = 0.0251 [K^+] + 0.1834 (R^2 = 0.94)

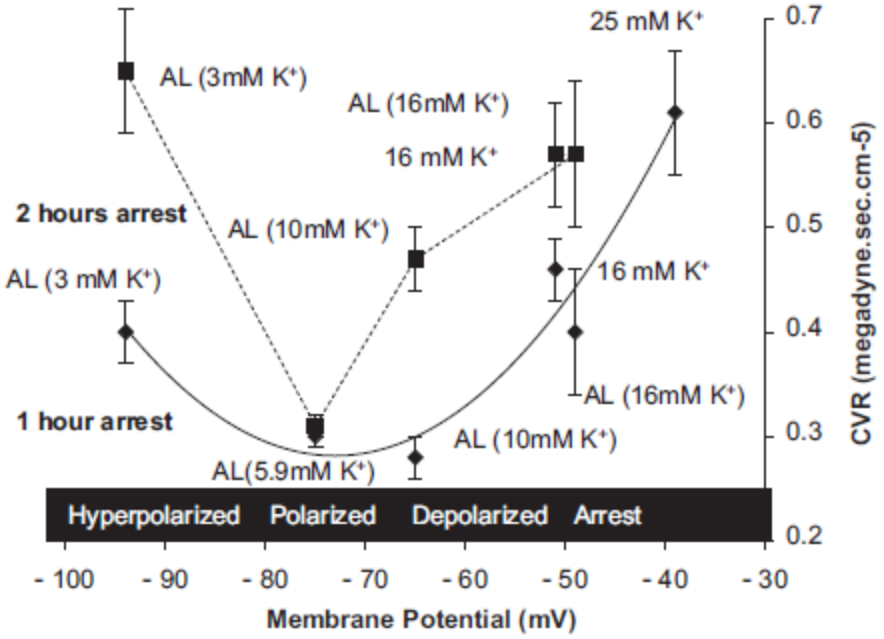


Figure 4.2c: The effect of variation in the membrane potential on coronary vascular resistance for groups arrested for 1 and 2 hours at 32°C.

The AL 0.1mM K^+ group (φ = −183 mV) and the 25mM K^+ alone group (φ = −39 mV) were not included in the 2-hour arrest groups because nearly 40% failed to return aortic flow after 1-hour arrest.

4.3.3 Effect of cardioplegia potassium concentration on time to first spontaneous beat and time to aortic flow

After 1-hour arrest, times to first beat increased from 0.9 minutes (AL 0.1mM K^+) to 5.2 minutes (25mM K^+ alone) (Fig 4.3a). Doubling of the cross-clamp time led to longer times to first beat, with the exception of AL 5.9mM K^+ and AL 3.0mM K^+ groups (Figure 4.3a). Time to aortic flow increased from 3.1 minutes (16mM K^+ alone) to 13.9 minutes (AL 16mM K^+) (Figure 4.3c). Time to generate aortic flow increased up to 2.4 fold with the increase in duration of arrest from 1 to 2 hours (Figure 4.3c). Nineteen hearts failed to achieve aortic flow at reperfusion: AL 0.1mM K^+ (3 hearts after 1-hour arrest), AL 3mM K^+ (6 hearts after 2-hour arrest), AL 16mM K^+ (4 hearts after 2-hour arrest), 16mM K^+ alone (3 hearts after 2-hour arrest) and 25mM K^+ alone (3 hearts after 1-hour arrest). AL 5.9mM K^+ and AL 10mM K^+ were the only two groups in which all hearts recovered aortic flow after 1- and 2-hour arrest at 32°C. The effect of membrane potential and increasing K^+ in AL cardioplegia on times to first beat and aortic flow are shown in Figures 4.3b and 4.3d.

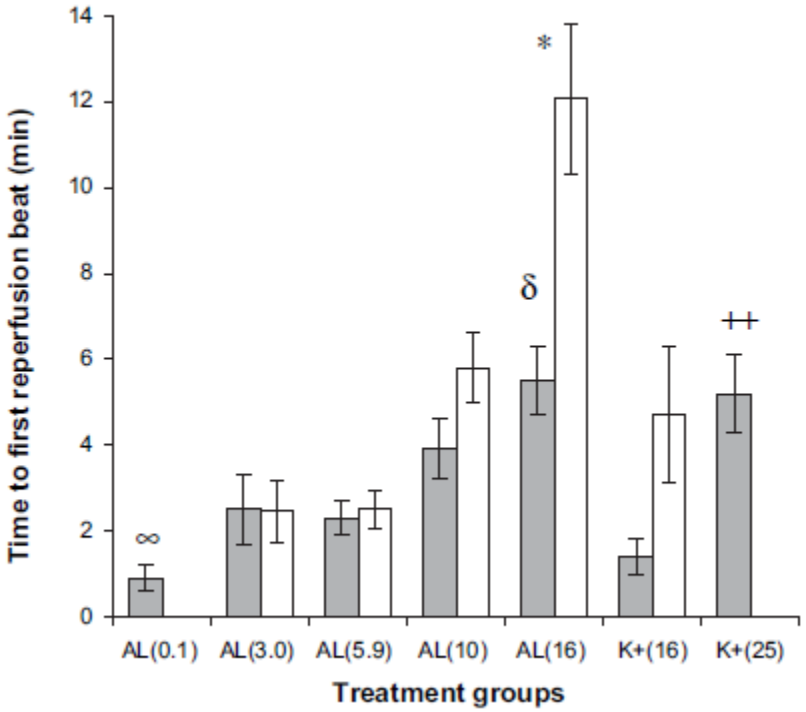


Figure 4.3a: Time to first ventricular beat at reanimation in working mode after 1-hour (shaded) or 2-hour (not shaded) arrest.

AL 0.1mM K^+ and 25mM K^+ alone were not included in the 2-hour arrest study because approximately 40% failed to recover function after 1-hour arrest.

∞ $p<0.05$ AL 0.1mM K^+ compared with AL 10, 16mM K^+

δ $p<0.05$ AL 16mM K^+ compared with AL 3, 5.9mM K^+ and 16mM K^+ alone

* $p<0.01$ AL 16mM K^+ compared with all other groups

++ $p<0.05$ 25mM K^+ alone compared with AL 0.1, 5.9mM K^+ and 16mM K^+ alone

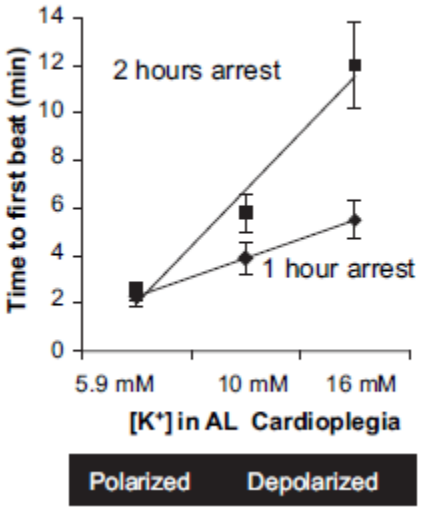


Figure 4.3 b: Relationship between the time to first beat at reanimation after 1-hour and 2-hour arrest and the concentration of K+ in AL cardioplegia solution (5.9, 10, 16mM K+).

Time to first beat after 1 hour was:

TFB (min) = 0.3131 $[K^+]$ + 0.5703 (R^2 = 0.99),

and after 2 hours was:

TFB (min) = 0.9474 $[K^+]$ − 3.3076 (R^2 = 0.99).

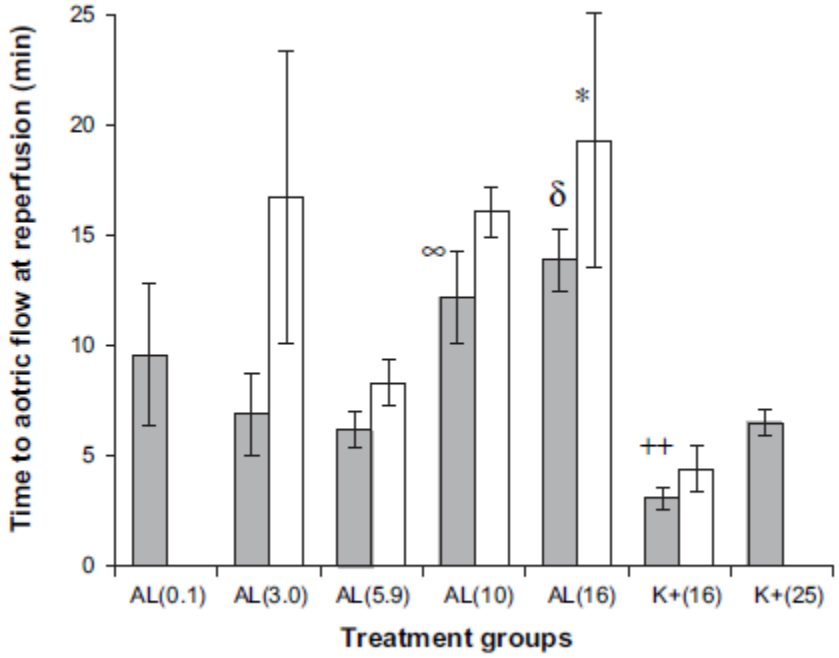


Figure 4.3c: Time to aortic flow at reperfusion after 1-hour (shaded) or 2-hour (not shaded) arrest.

∞ $p<0.01$ AL 10mM K^+ compared with 16mM K^+ alone

δ $p<0.05$ AL 16mM K^+ compared with AL 3, 5.9mM K^+

* $p<0.05$ AL 16mM K^+ compared with AL 5.9mM K^+ and 16mM K^+ alone

++ $p<0.01$ 16mM K^+ alone compared with AL 10, 16mM K^+

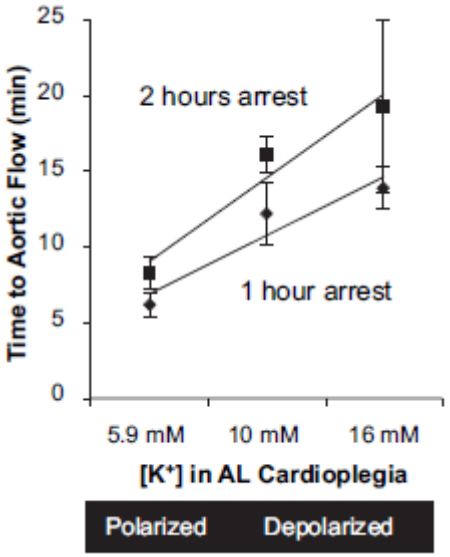


Figure 4.3d: Relationship between the time to aortic flow after 1-hour or 2-hour arrest and the concentration of K+ in AL cardioplegia solution (5.9, 10, 16mM K+).

Time to aortic flow after 1 hour was:

TAF (min) = 0.727 $[K^+]$ + 3.3035 (R^2 = 0.84),

and after 2 hours was:

TAF (min) = 1.057 $[K^+]$ + 3.290 (R^2 = 0.88).

4.3.4 Early reperfusion and ventricular arrhythmias

During reanimation and before aortic flow was achieved, abnormal rhythms were observed in hearts from the AL 0.1mM K^+ (2 hearts), 16mM K^+ alone (1 heart) and 25mM K^+ alone (2 hearts) groups arrested for 1 hour, and after 2-hour arrest in the AL 10mM K^+ (3 hearts), AL 16mM K^+ (4 hearts) and 16mM K^+ alone (2 hearts) groups.

4.3.5 Effect of cardioplegia potassium concentration on functional recovery during 60 minutes of working mode reperfusion

Recovery of heart rate, systolic and diastolic pressures, aortic and coronary flows, cardiac output and stroke volume for the 1- and 2-hour arrest groups are shown in Table 4.2a and b and Fig 4.4a-c.

Recovery of heart rate

After 1- and 2-hour arrest, the AL 5.9mM K^+ group recovered 107 to 110% of pre-arrest heart rate at 60 minutes reperfusion (94% to 98% of recovery occurred at 15 minutes), which was significantly higher than recovery in the AL 3, 10 and 16mM K^+ groups after 2-hour arrest. After 1-hour arrest, the AL 3mM,10mM, 16mM, K^+ and 16mM K^+ alone groups recovered 79%, 84%, 70% and 99% respectively of pre-arrest heart rate at 15 minutes reperfusion, and 99%, 102%, 103% and 94% of heart rate respectively at 60 minutes reperfusion. After 2-hour arrest, the AL 10mM K^+ group recovered 95% at 60 minutes, however at 15 minutes only 36% recovery occurred. The 16mM K^+ alone, AL 16mM K^+ and AL 3mM K^+ groups recovered 79%, 28% and 39% heart rate at 15 minutes reperfusion, and 82%, 67% and 45% of their respective pre-arrest heart rate by 60 minutes reperfusion (Table 4.2a and b). The relationship between heart rate and increasing extracellular K^+ in AL cardioplegia at 15 minutes reperfusion after 1-hour arrest was:

HR (percentage recovery) = -3.8406 [K^+ mM] + 128.84 (R^2 = 0.92);

and after 2-hour arrest was

HR (percentage recovery) = -6.1517 [K^+ mM] + 118.08 (R^2 = 0.75).

Table 4.2a: Functional parameters of hearts pre-arrest and at 60 minutes working mode reperfusion after 1-hour or 2-hour arrest at 32°C.

Treatment Groups (n = 8)	Heart Rate		Systolic Pressure		Diastolic Pressure		Aortic Flow		Coronary Flow		Cardiac Output		Stroke Volume	
	beats.min^{-1}	% PA	mmHg	% PA	mmHg	% PA	ml.min^{-1}	% PA	ml.min^{-1}	% PA	ml.min^{-1}	% PA	ml.beat^{-1}	% PA
AL (0.1mM K^+)														
Pre-arrest	338 ± 6		133 ± 2		75 ± 3		56 ± 2		22 ± 1		78 ± 2		0.23 ± 0.1	
1 hour arrest	206 ± 61	61	71 ± 21	54	51 ± 15	66	16 ± 6 §§	28	9 ± 3 **	41	25 ± 9 §§	32	0.07 ± .02 §§	32
AL (3mM K^+)														
Pre-arrest	336 ± 15		129 ± 4		71 ± 3		57 ± 2		20 ± 1		77 ± 2		0.23 ± 0.1	
1 hour arrest	329 ± 7	99	118 ± 3	92	74 ± 2	105	46 ± 2	81	18 ± 1	90	64 ± 3	83	0.19 ± .01	84
Pre-arrest	304 ± 10		131 ± 3		61 ± 1		60 ± 2		20 ± 1		79 ± 3		0.26 ± 0.1	
2 hour arrest	133 ± 43 *	45	32 ± 18 *	23	20 ± 10 *	32	6 ± 5 §	11	4 ± 2 §	17	10 ± 7 §	13	0.04 ± .02 §	13
AL (5.9mM K^+)														
Pre-arrest	301 ± 19		131 ± 4		73 ± 2		56 ± 2		19 ± 1		75 ± 3		0.25 ± 0.1	
1 hour arrest	327 ± 16	110	124 ± 4	94	73 ± 2	100	51 ± 3	91	19 ± 1	101	70 ± 3	93	0.22 ± .01	86
Pre-arrest	291 ± 11		130 ± 1		61 ± 1		59 ± 2		18 ± 1		77 ± 2		0.26 ± 0.1	
2 hour arrest	309 ± 9	107	120 ± 2	92	61 ± 1	100	48 ± 2 þ	81	21 ± 1 þ	113	69 ± 2 þ	89	0.22 ± .01 þ	84
AL (10mM K^+)														
Pre-arrest	320 ± 10		132 ± 2		71 ± 2		60 ± 1		20 ± 1		79 ± 1		0.25 ± 0.1	
1 hour arrest	325 ± 9	102	124 ± 2	94	78 ± 3	110	47 ± 1	79	19 ± 1	97	66 ± 2	84	0.21 ± .01	83
Pre-arrest	338 ± 15		124 ± 1		60 ± 1		56 ± 1		19 ± 1		75 ± 2		0.23 ± 0.1	
2 hour arrest	317 ± 16	95	114 ± 2	92	64 ± 1	107	40 ± 2	71	16 ± 1	84	56 ± 1	74	0.18 ± .01	80

AL: adenosine and lidocaine. % PA: percentage of pre-arrest value.

* $p<0.01$ compared with AL 5.9, 10mM K^+ groups
** $p<0.05$ compared with AL 3, 5.9, 10, 16mM K^+ groups
þ $p<0.01$ compared with AL 3, 16mM K^+ and 16mM K^+ alone groups
§ $p<0.01$ compared with AL 10mM K^+ group
§§ $p<0.01$ compared with all groups except 25mM K^+ alone group

Table 4.2b: Functional parameters of hearts pre-arrest and at 60 minutes working mode reperfusion after 1-hour or 2-hour arrest at 32°C.

Treatment Groups (n = 8)	Heart Rate		Systolic Pressure		Diastolic Pressure		Aortic Flow		Coronary Flow		Cardiac Output		Stroke Volume	
	beats.min^{-1}	% PA	mmHg	% PA	mmHg	% PA	ml.min^{-1}	% PA	ml.min^{-1}	% PA	ml.min^{-1}	% PA	ml.beat^{-1}	% PA
AL (16mM K^+)														
Pre-arrest	309 ± 15		131 ± 2		71 ± 2		56 ± 1		19 ± 1		75 ± 1		0.25 ± 0.1	
1 hour arrest	318 ± 15	103	122 ± 3	93	72 ± 3	101	45 ± 2	81	18 ± 2	94	63 ± 2	84	0.20 ± .01	82
Pre-arrest	309 ± 14		139 ± 2		61± 1		57 ± 2		19 ± 1		76 ± 2		0.25 ± 0.1	
2 hour arrest	208 ± 33	67	74 ± 23	53	39 ± 11	65	20 ± 8	34	8 ± 3	42	28 ± 11	36	0.10 ± .04	39
16mM K^+														
Pre-arrest	329 ± 6		133 ± 2		73 ± 3		59 ± 1		19 ± 1		78 ± 2		0.24 ± 0.1	
1 hour arrest	306 ± 25	94	117 ± 3	88	78 ± 3	107	40 ± 5	69	15 ± 1	81	55 ± 5	72	0.18 ± .01	76
Pre-arrest	304 ± 9		134 ± 3		61 ± 1		56 ± 1		18 ± 1		75 ± 2		0.25 ± 0.1	
2 hour arrest	248 ± 47	82	74 ± 21	56	41 ± 12	65	25 ± 8	44	10 ± 3	56	35 ± 10	46	0.11 ± .03	43
25mM K^+														
Pre-arrest	306 ± 12		136 ± 3		66 ± 3		59 ± 1		20 ± 1		79 ± 1		0.26 ± 0.1	
1 hour arrest	189 ± 56 **	63	78 ± 23 †	57	44 ± 13 ††	66	26 ± 8 **	43	11 ± 3 **	54	37 ± 11 þþ	46	0.12 ± .04 **	46

AL: adenosine and lidocaine. % PA: percentage of pre-arrest value.

** $p<0.05$ compared with AL 3, 5.9, 10, 16mM K^+ groups
† $p<0.05$ compared with AL 5.9, 10mM K^+ groups
†† $p<0.05$ compared with AL 3, 10mM K^+ and 16mM K^+ alone groups
þþ $p<0.01$ compared with AL 3, 5.9, 10, 16mM K^+ groups

Recovery of systolic and diastolic pressures

After 1- and 2-hour arrest, hearts in the AL 5.9mM K^+ group demonstrated a rapid return of systolic and diastolic pressures with nearly 100% return at 15 minutes reperfusion, and 92% to 100% recovery at 60 minutes (Table 4.2a). After 1-hour arrest, the AL 10mM K^+ and 16mM K^+ alone groups recovered 90% to 100% developed pressures at 15 minutes; however, in contrast to the AL 5.9mM K^+ group, the AL 10mM K^+ was slow to recover after 2-hour arrest, with only 50% developed pressures at 15 minutes reperfusion. After 2-hour arrest, the AL 16mM K^+ and 16mM K^+ alone groups recovered 43% to 66% developed pressures at 15 minutes, and the AL 3mM K^+ group recovered only 17% to 26% at 15 minutes; developed pressures for these groups changed little over the remaining 45 minutes of reperfusion (Table 4.2a and b).

Recovery of aortic flow

After 1- and 2-hour arrest, the AL 5.9mM K^+ group recovered 81% to 91% of its pre-arrest aortic flow at 60 minutes (Table 4.2a), and nearly 100% return occurred at 15 minutes. After 2-hour arrest, this recovery for AL 5.9mM K^+ group was significantly higher than for all other groups (Table 4.2a). After 1-hour arrest, the AL 3mM, 10mM and 16mM K^+ and 16mM K^+ alone groups recovered 71%, 57%, 44% and 75% respectively of pre-arrest aortic flow at 15 minutes, and 81% 79%, 81% and 69% at 60 minutes (Table 4.2a and b). Hearts in the 25mM K^+ alone group recovered significantly less, with 43% aortic flow at 60 minutes (49% return at 15 minutes). Hearts in the AL 0.1mM K^+ group recovered 28% (15% at 15 minutes). After 2-hour arrest, the AL 10mM K^+ group recovered 71% at 60 minutes but recorded only 26% return at 15 minutes. The 16mM K^+ alone, AL 16mM K^+ and AL 3mM K^+ hearts recovered 33%, 15% and 4% respectively, of their pre-arrest aortic flow at 15 minutes reperfusion (Table 4.2a and b). The relationship between aortic flow and increasing extracellular K^+ concentrations in AL cardioplegia at 15 minutes reperfusion after 1-hour arrest was:

AF (percentage recovery) = -4.4704 [K^+ mM] + 111.53 (R^2 = 0.88);

and after 2-hour arrest was:

AF (percentage recovery) = -6.1885 [K^+ mM] + 106.47 (R^2 = 0.79).

Recovery of coronary flow

Recovery of rate and percentage of coronary flow showed profiles similar to those of aortic flows (Table 4.2a and b). After 1- and 2-hour arrest, the AL 5.9mM K^+ group recovered 101% to 113% of its pre-arrest coronary flow at 60 minutes, and 95% of this recovery was achieved at 15 minutes reperfusion. After 1-hour arrest, the AL 3mM, 10mM and 16mM K^+ and 16mM K^+ alone groups recovered 72%, 72%, 65% and 78% respectively of pre-arrest coronary flow at 15 minutes reperfusion, and 90%, 97%, 94% and 81% at 60 minutes reperfusion. Hearts arrested with 25mM K^+ alone recovered only 54% (49% at 15 minutes), and those arrested with AL 0.1mM K^+ recovered 41% (45% at 15 minutes) of their pre-arrest coronary flow. After 2-hour arrest, the AL 10mM K^+ group recovered 84% at 60 minutes, and only 40% of recovery occurred at 15 minutes. The 16mM K^+ alone, AL 16mM K^+, and AL 3.0mM K^+ groups recovered 44%, 29% and 15% respectively of their pre-arrest coronary flow at 15 minutes, and 56%, 42% and 17% respectively at 60 minutes (Table 4.2a and b). The relationship between coronary flow and increasing extracellular K^+ in AL cardioplegia at 15 minutes reperfusion after 1-hour arrest was:

CF (percentage recovery) = -2.8375 [K^+ mM] + 107.51 (R^2 = 0.84);

and after 2-hour arrest was:

CF (percentage recovery) = -6.1885 [K^+ mM] + 120.47 (R^2 = 0.79).

Recovery of cardiac output and stroke volume

The recovery of cardiac output and stroke volume are shown in Table 4.2a and b and Figure 4.4a, b and c. After 1- and 2-hour arrest, the AL 5.9mM K^+ group recovered 89% to 93% of its pre-arrest cardiac output at 60 minutes (85% to 93% of this recovery at 15 minutes) (Figure 4.4a). After 1-hour arrest, the AL 3mM,10mM and 16mM K^+ and 16mM K^+ alone groups recovered 71%, 61%, 49% and 76% of pre-arrest cardiac output at 15 minutes, and 83%, 84%, 84% and 72% at 60 minutes reperfusion, respectively. Hearts arrested with 25mM K^+ alone recovered only 46% (49% at 15 minutes), and those arrested with AL 0.1mM K^+ recovered 32% pre-arrest cardiac output. The relationship between cardiac output and increasing extracellular K^+ in AL cardioplegia at 15 minutes reperfusion after 1-hour arrest was:

CO (percentage recovery) = -4.1829 [K^+ mM] + 112.15 (R^2 = 0.87).

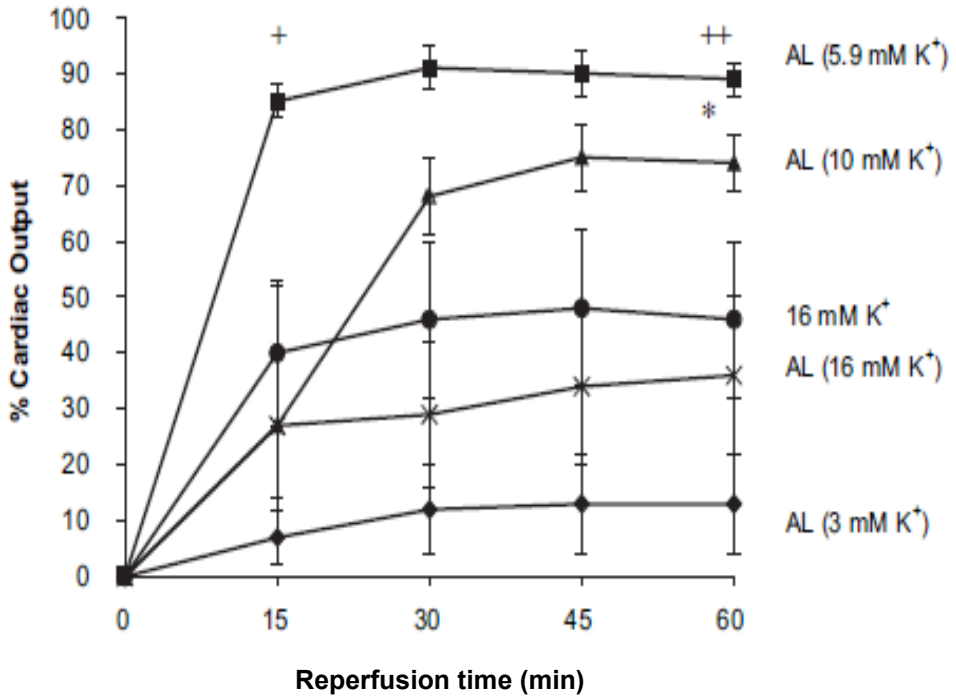


Figure 4.4a: Recovery of cardiac output (percentage of pre-arrest values) during 60 minutes reperfusion after 2-hour arrest at 32°C.

\+ $p<0.01$ AL 5.9mM K^+ compared with AL 3, 10, 16mM K^+ and 16mM K^+ alone

++ $p<0.01$ AL 5.9mM K^+ compared with AL 3mM K^+, $p<0.05$ compared with AL 3, 16mM K^+ and 16mM K^+ alone

* $p<0.01$ AL 10mM K^+ compared with AL 3mM K^+

After 2-hour arrest, the AL 10mM K^+ group recovered 74% of pre-arrest cardiac output, and 27% at 15 minutes reperfusion, which was significantly less than in the AL 5.9mM K^+ group ($p < 0.05$). The 16mM K^+ alone, AL 16mM K^+, and AL 3.0mM K^+ groups recovered 40%, 27% and 7% of pre-arrest cardiac output after 15 minutes, and 43%, 39%, and 13% at 60 minutes reperfusion, respectively (Figure 4.4a, Table 4.2a and b).

Similar recovery profiles were recorded with stroke volume (Figure 4.4b and c). After 2-hour arrest the AL 5.9mM K^+ group recovered 0.22 ± 0.1 ml/beat (84% of pre-arrest stroke volume), and 0.24 ± 0.1 ml/beat (90% recovery) was achieved at 15 minutes. This recovery was significantly higher than the AL 3mM K^+, AL 16mM K^+ and 16mM K^+ alone group

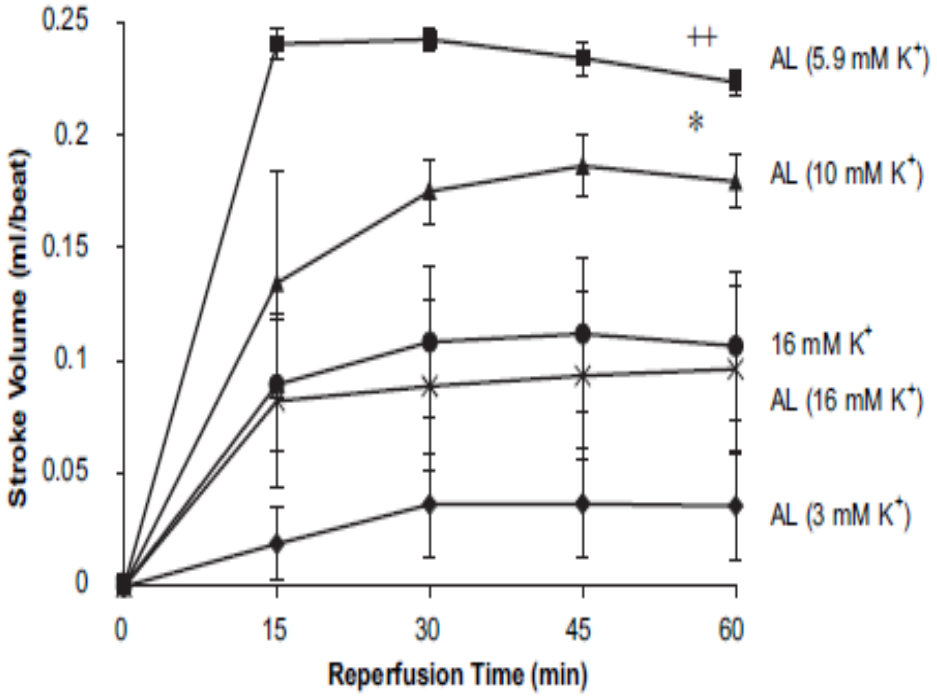


Figure 4.4b: Recovery of stroke volume (in milliliters per beat) during 60 minutes reperfusion after 2-hour arrest at 32°C.

++ $p<0.01$ AL 5.9mM K^+ compared with AL 3, 16mM K^+ and 16mM K^+ alone

* $p<0.01$ AL 10mM K^+ compared with AL 3mM K^+

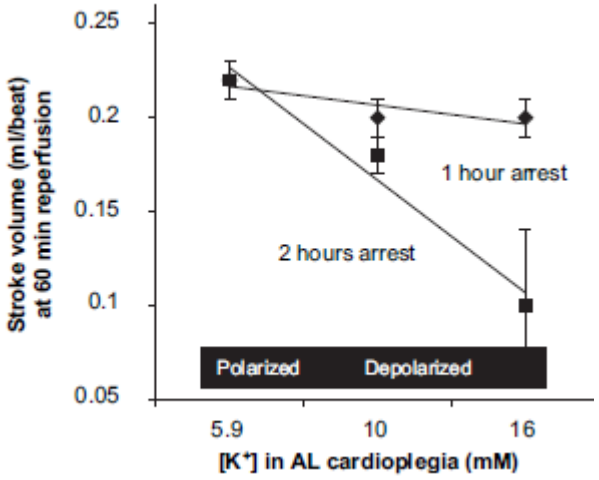


Figure 4.4c: Relationship between stroke volume and increasing concentrations of K+ in AL cardioplegia solution (5.9, 10, 16 mM K+) at 60 minutes reperfusion for 1-hour and 2-hour arrest groups.

Stroke volume after 1 hour was:

SV = -0.0018 [K^+, mM] + 0.2262

(R^2 = 0.65), and after 2 hours was:

SV = -0.012 [K^+] + 0.294 (R^2 = 0.99).

4.4 Discussion

This chapter in the development of warm AL cardioplegia addresses the question of the importance of polarised arrest, the effect of variations in potassium concentration on post- arrest recovery, and the ideal potassium level in AL cardioplegia to confer optimal protection during arrest at 32°C. The major finding of this chapter was that polarised membrane potentials close to resting voltage provided the greatest cardioprotection. Arrest with low potassium levels (3mM K^+) or high potassium levels (16, 25mM K^+) adversely affected performance with significantly higher coronary vascular resistance during arrest, slower times to reanimate and achieve aortic flow, severe arrhythmias and functional losses during 60 minutes reperfusion. These major findings will now be discussed.

4.4.1 Normokalaemic AL cardioplegia (5.9mM K+) maintains membrane potentials close to natural resting voltage at warm arresting temperatures

The results of this study showed that raising or lowering the potassium concentration of the cardioplegia solution above or below physiological level resulted in depolarised or hyperpolarised membrane potentials, respectively, compared with normokalaemic AL cardioplegia which maintained cell membrane potential close to natural resting voltage.

The theoretical relationship between resting membrane potential and transmembrane potassium concentration gradient was first proposed in 1902 by German physiologist Julius Bernstein [467], from ideas and equations originally developed by Ostwald and Nernst. A Nernstian relationship was demonstrated between changing potassium concentrations and membrane potential (φ) in the arrested rat heart:

φ (mV) = 26.23 ln [K^+, mM] - 123.44 (R^2 = 0.99) (Figure 4.2a).

This finding is in good agreement with previous work on the rat heart [408,435] and with independent microelectrode measurements made on *in vivo*, isolated heart and myocyte preparations [274,468,469]. For example, Snabaitis and colleagues measured -50mV in the isolated rat heart perfused with 16mM K^+ alone in K-H buffer [468] and Kleber measured -82 ± 2mV in the isolated guinea-pig heart perfused with 4.5mM potassium [274]. The values from this study, at these identical potassium concentrations, are -50.7mV and -84mV respectively (Figure 4.2a).

Similarly, at lower potassium concentrations (3.0mM), Wan and colleagues using microelectrodes measured –93mV in ventricular myocytes of guinea-pig [469], which compares closely with the value of –94mV shown in Figure 4.2a, given the limitations of both methods. Although membrane potential was estimated only at induction in this study, Snaibaitis and colleagues [468] showed that membrane potential remained relatively constant during polarised and nonpolarised arrest in the rat heart receiving tetrodotoxin or 16mM K^+ solution, respectively. Future studies could compare microelectrode measurements and Nernstian potassium distributions during warm arrest induction and maintenance, and recovery at reperfusion.

Since the Nernst equation strictly describes an equilibrium state, this data lends support to the concept that the resting membrane potential in heart is not a diffusion potential, but arises from a measure of electrical work [470,471]. The data indicates that the net myocardial transmembrane flux of potassium over the range of 0.1 to 25mM K^+ is zero, which means that during diastolic arrest the net inward currents (e.g. sum of rectifier I_{K1}) [472] is equal to the net reversal potential for K^+ ions where $\Delta G' [K^+]_{o/i} = 0$ [435,471]. Therefore, despite lowering the transmembrane gradient by increasing extracellular potassium from 5.9 to 25mM (increasing depolarisation), these results show that the membrane potential follows a Nernstian relationship.

This study has also shown that polarised membrane potentials are not maintained by adenosine and lignocaine concentrations of 200μM and 500μM, respectively, when administered in conjunction with raised potassium concentrations. This effect has implications in the clinical situation where adenosine and/or lignocaine have been used as adjuncts in an attempt to reduce the adverse sequelae of hyperkalaemia. Addition of adenosine or lignocaine may improve cardioprotection during hyperkalaemic arrest, for example by providing more stable arrest [239,250]. However if the raised potassium levels trigger membrane depolarisation, loss of ionic homeostasis during arrest may lead to intracellular calcium loading [276,277], and post-arrest oxidative stress [129,293], myocyte [301,308] and vascular [286,288] dysfunction, and inflammatory reactions [124,292] as described previously for potassium alone arrest..

4.4.2 Variations in potassium concentration and membrane potential influence coronary vascular resistance during warm arrest

The coronary vascular resistance for normokalaemic AL cardioplegia (5.9mM K^+) increased by only 30% during 2-hour warm (32^oC) arrest (0.24 to 0.31 ± 0.01 megadyne.sec.cm^{-5}). Brace and colleagues reported that a small window exists between ~ 5mM to 10mM K^+ where reduced coronary vascular resistance and dilation occurs [473], however as most hyperkalaemic cardioplegia solutions contain potassium levels higher than 10mM, the effect of potassium concentrations between 5mM and 10mM were not assessed here. In this study 10mM K^+ added to AL cardioplegia resulted in doubling of the coronary vascular resistance after 2-hour arrest (Table 4.1, Figure 4.2b).

In contrast, hypokalaemic AL arrest (0.1 and 3mM K^+) led to significantly higher coronary vascular resistance values (up to 2.6 fold after 2-hour arrest) compared with AL normokalaemia (Table 4.1, Fig 4.2b). In 1974 Brace and associates [473] reported that an intracoronary hypokalaemic (2.1mM K^+) infusion into the canine heart *in vivo* under constant pressure led to reduced blood flow with a concomitant decrease in coronary sinus oxygen tension and increased oxygen consumption. They concluded that the increase in coronary vascular resistance was either the result of active vasoconstriction (direct vascular smooth muscle contraction), passive vasoconstriction (extravascular compression), or both [473].

For many decades, hyperkalaemia has been linked to increased coronary vascular resistance, endothelial dysfunction and vasospasm [10,294,449]. The results in this chapter confirm earlier studies in the isolated rat heart which showed that hyperkalaemia increases coronary vascular resistance during warm arrest [408,474]. This study demonstrated that coronary vascular resistance was linearly related to the extracellular potassium level in AL cardioplegia (from 5.9 to 16mM) (Fig 4.2b, insert), and more specifically, that the rate of change in coronary vascular resistance with increasing potassium (slope) was more than 2-fold with doubling of arrest duration (Table 4.1, Figure 4.2b). In terms of membrane potential, hyperpolarisation (-183 and -94mV) or depolarisation (-65, -49, -39mV) increased coronary vascular resistance to a significantly greater extent than normokalaemic AL membrane polarisation (Fig 4.2c), demonstrating that diastolic arrest voltages outside the normal physiological range have an untoward effect on coronary vascular resistance. Vasoconstriction may reduce the homogeneity of cardioplegia distribution and compromise myocardial

protection during surgical arrest and reperfusion, especially in aged hearts affected by occluded or atherosclerotic coronary arteries [416].

4.4.3 Effect of arresting voltage on early reperfusion parameters: time to first spontaneous beat, time to aortic flow and arrhythmias

Time to first spontaneous ventricular beat also increased linearly with the extracellular potassium concentration in AL cardioplegia (Figure 4.3b). After 1-hour arrest, time to first beat increased from 2.3 to 5.5 minutes, and after 2-hour arrest time to first beat increased from 2.5 to 12 minutes (Figure 4.3a). After 1-hour arrest:
TFB (min) = 0.3131 [K^+] + 0.5703, (R^2 = 0.99); and after 2-hour arrest:
TFB (min) = 0.9474 [K^+] - 3.3076 (R^2 = 0.99).
The difference in slopes in Figure 4.3b shows that time to first beat (in minutes) per millimole per litre of potassium is three times longer after 2-hour arrest, and importantly that time to first beat for AL 5.9mM K^+ hearts was unchanged after the extended duration of arrest (Figure 4.3a and b). Similar relations were found for time to first aortic flow (Figure 4.3c and d). Increasing potassium also increased the incidence of abnormal heart rhythms during reanimation. Hearts arrested with AL (5.9mM K^+) cardioplegia for 1-hour and 2-hour arrest were the only groups in which all hearts showed continuous and consistent spontaneous beating with no arrhythmias in early reperfusion (data not shown).

4.4.4 Functional recovery is optimal after arresting with AL containing physiological potassium levels

Hearts arrested with normokalaemic AL 5.9mM K^+ cardioplegia were also superior in functional recovery of heart rate, developed pressures, aortic flow, coronary flow, cardiac output and stroke volume compared with those hearts receiving hypokalaemic or hyperkalaemic AL cardioplegia, or potassium alone (16mM or 25mM K^+) cardioplegia (Table 4.2a and b, Figure 4.4a, b and c).

After 1- and 2-hour warm arrest, the AL 5.9mM K^+ cardioplegia groups recovered rapidly and predictably with 90% to 110% of pre-arrest heart rate, developed pressures, aortic and coronary flows and cardiac output (Table 4.2a, Figure 4.4a). After 2-hour arrest, hearts receiving depolarising AL 10mM K^+ cardioplegia recovered function more slowly, with significantly lower cardiac output at 15 minutes reperfusion (Table 4.2, Figure 4.3a). Increasing the K^+ concentration to 16mM in AL cardioplegia

led to further functional loss in recovery of heart rate, developed pressures, aortic and coronary flows and cardiac output (Table 4.2b, Figure 4.4a). Nearly all metrics changed in a linear fashion with increasing potassium concentrations (from 5.9 to 16mM) at 15 and 60 minutes of reperfusion (Table 4.2a and b, Fig 4.4a, b and c), and doubling the arrest time further exacerbated the damaging effects of potassium. The lower functional recoveries for the AL 10mM and 16mM K^+ or 16mM and 25mM K^+ alone groups in early reperfusion suggests potassium-induced myocardial (and vascular) stunning possibly involving H^+ ion imbalances, oxidative stress, calcium overload and mitochondrial oxidative dysfunction [71,84,306,308,309]; and irreversible ischaemia-reperfusion injury may have occurred in those hearts which either failed to reanimate or did not improve in functional recovery later in the reperfusion period.

The effect of increasing potassium concentrations in AL cardioplegia on loss of functional recovery was clearly illustrated by a significant reduction in stroke volume (Fig 4.4b). Stroke volume (in millilitres per beat) was calculated by dividing cardiac output by heart rate, and because the preload (10cm H_2O) and afterload (100cm H_2O) are pre-set in the working heart preparation, a decrease in stroke volume may indicate a decrease in left ventricular contractility. The relationship between stroke volume and increasing potassium concentrations in AL cardioplegia (Figure 4.4c) after 1-hour arrest and 15 minutes reperfusion was:
SV (ml/beat) = -0.0085 [K^+ mM] + 0.2771 (R^2 = 0.84); and after 2-hour arrest was:
SV (ml/beat) = -0.0152 [K^+ mM] + 0.3103 (R^2 = 0.88).
After 2-hour arrest stroke volume had decreased by up to 67% at 15 minutes and 60% at 60 minutes reperfusion with increasing potassium, and this rate of decrease was more than 10-fold compared with the decrease recorded in the 1-hour arrest group at 60 minutes reperfusion (Figure 4.4c). Increasing the potassium concentration from 5.9 to 10 and 16mM in AL cardioplegia resulted in a significant loss of cardiac output, stroke volume and left ventricular contractility, and the decrease was more pronounced the longer the arrest time. Pressure-volume loops or an intra-ventricular balloon would be required to quantify the effects of varying potassium concentrations in AL on changes in contractility (e.g. degree of sarcomere stretch) during recovery. In cell voltage terms, the more the cardiomyocyte, heart conduction cells and cells of the coronary vasculature appear to deviate from their natural resting membrane potentials during cardioplegic arrest, the greater the loss of cardiac function during arrest, reanimation and reperfusion.

Combined adenosine and lignocaine blood cardioplegia has also previously been reported to be protective, in a canine *in vivo* cardiopulmonary-bypass model, when delivered intermittently at warm or cold temperatures [409]. However, in comparison to the present study, hyperkalaemic cardioplegia (20mM) was reported to be equally protective of left ventricular systolic function and endothelial function, and the hyperkalaemic solution induced more rapid arrest than the AL (400:750) cardioplegia. The discrepancy in results between the two studies may be due to use of a different research animal, an *in vivo* versus isolated heart model, use of cardiopulmonary bypass and blood-based cardioplegia compared with crystalloid cardioplegia, and the different concentrations and ratio of adenosine and lignocaine in the cardioplegia solutions.

In agreement with the results of this study, AL (10mM K^+) crystalloid cardioplegia was shown to be more protective than AL (20mM K^+) or non-AL 20mM K^+ cardioplegia in 134 paediatric patients undergoing correction of ventricular septal defect [412]. With decreasing potassium concentrations and correspondingly more polarised membrane potentials (i.e. from -46 to -67 mV), there was reduced use of inotropes, significantly lower release of troponin I following surgery, and one day less of hospitalisation. The data from the present study helps to explain some of the advantages of maintaining physiological potassium levels (above 3mM K^+) in AL cardioplegia.

4.5 Conclusion

Warm (32^{o}C) normokalaemic AL cardioplegia provided optimal arrest conditions, with the myocardial cell membrane potential close to its resting state. Hearts arrested with lower potassium levels (hyperpolarising AL 3mM K^+ cardioplegia) or higher potassium levels (depolarising AL 16mM K^+ cardioplegia) recorded significantly higher coronary vascular resistance during arrest, experienced reanimation arrhythmias, and were 'slow-to-recover' with significant losses in cardiac output, stroke volume and contractility during reperfusion. Nearly 40% of hearts arrested with AL 0.1mM K^+ or 25mM K^+ alone failed to recover heart rate, developed pressures or cardiac output after 1-hour arrest.

Chapter 5

Developing a warm AL ‘One-Shot’ Arrest Protocol

5.1 Introduction

The previous chapters have shown that AL cardioplegia can be delivered continuously or intermittently (re-dosing every 20 min) for 40- or 60-minute arrest at 32°C with no significant differences in post-arrest functional recovery (Chapter 3); and that physiological potassium levels (5.9mM K^+) in AL cardioplegia provided optimal "polarised" protection during 1- or 2-hour arrest compared to solutions containing hyperpolarising or depolarising potassium concentrations (Chapter 4). To further develop the warm AL cardioplegia concept, single-dose delivery of AL was compared to the intermittent delivery protocol used in previous chapters.

In this chapter the efficacy of normokalaemic AL cardioplegia administered as a single dose (one-shot) at 32^{o}C for induction of 40-minute warm arrest was tested. Recovery of function was compared with hearts arrested with continuous, intermittent or hypothermic (20^{o}C) one-shot AL arrest protocols. Magnesium or N-(2-mercaptopropionyl)-glycine (MPG) was added to examine the effect on functional recovery following the extended periods of ischaemic arrest. Magnesium was added because it is a naturally occurring calcium antagonist. Magnesium limits calcium entry into the cell via sarcolemmal calcium channels [475-477], inhibits efflux of calcium from the mitochondria [478] and sarcoplasmic reticulum [479], and alters sodium and potassium fluxes across membranes [478,480-482] helping to maintain intracellular calcium concentrations [477,483,484]. MPG was selected because it is a potent cell-permeable antioxidant which scavenges hydroxyl and peroxynitrite radicals [83,142,485,486], and can reduce ischaemia-reperfusion injury due to reactive oxygen and nitrogen species [487,488]. As mentioned in the main introduction, calcium loading and free radical production are two key processes leading to myocardial and microvascular dysfunction as a result of prolonged ischaemic periods.

5.1.1 Aims

The aims of this chapter were

1) to develop a 40-minute one-shot arrest protocol using a single dose of warm (32^{o}C) AL (200:500) cardioplegia for induction and arrest
2) to compare recovery at reperfusion following 40-minute warm one-shot AL arrest with recovery after warm continuous or intermittent AL arrest, or one-shot AL arrest at 20^{o}C
3) to assess whether addition of MPG or magnesium to the cardioplegia and/or reperfusion buffers improved functional recovery.

5.2 Methods

5.2.1 Buffers and arrest solutions

4) Adenosine 200μm + Lignocaine 500μm (AL) cardioplegia
5) Adenosine 200μm + Lignocaine 500μm + N-(2-mercaptopropionyl)-glycine 1mM (AL+MPG) cardioplegia or terminal flush
6) Krebs Henseleit buffer (KH)
7) Krebs Henseleit buffer + N-(2-mercaptopropionyl)-glycine 1mM (KH+MPG)
8) Adenosine 10μm + Lignocaine 25μm + magnesium 16mM in KH (AL + Mg^{2+}) terminal flush

5.2.2 Experimental groups

A total of 9 experimental groups were used (n = 8 each group). The 40-minute warm AL one-shot arrest protocol (Group 3) was compared with the continuous and intermittent arrest protocols (Groups 1 and 2) previously used in the studies in Chapters 3 and 4, and with 40-minute one-shot hypothermic arrest (Group 4) to examine the effect of the one-shot arrest protocol on recovery of function at reperfusion. Groups 5-9 were then included to assess the effect of the adjuncts magnesium or MPG on recovery of function following warm AL one-shot arrest. Details of these treatment groups and experimental methods are shown in Table 5.1 and Section 5.2.3.

Table 5.1: Experimental groups and treatment aims

Group	Arrest protocol	Abbreviation	Treatment / Aim
1	AL continuous delivery	Cont	Control group
2	AL intermittent delivery	Int	Control group
3	AL one-shot arrest, AL terminal flush	OS	Single dose AL induction of arrest
4	AL one-shot induction,20^{o}C arrest, AL terminal flush	20^{o}C	Single dose AL induction, hypothermic arrest
5	AL one-shot arrest, AL + Mg^{2+} terminal flush	Mg^{2+}Fl	Magnesium administered before reperfusion
6	AL + MPG one-shot arrest, AL + MPG terminal flush	MPG OS + Fl	Antioxidant treatment during ischaemic arrest and before reperfusion
7	AL one-shot arrest, AL + MPG terminal flush	MPG Fl	Antioxidant treatment before reperfusion only
8	AL one-shot arrest, AL + MPG terminal flush, KH + MPG working mode reperfusion	MPG Fl + WM	Antioxidant treatment commenced before reperfusion and continued in early working mode reperfusion
9	AL one-shot arrest, KH + MPG working mode reperfusion	MPG WM	Antioxidant treatment during early working mode reperfusion only

5.2.3 Arrest and reperfusion protocols for the nine treatment groups

Hearts were prepared for Langendorff and working mode perfusion, stabilised, and pre-arrest functional parameters assessed as detailed in Chapter 2: Materials and Methods Section 2.5.
Hearts in all groups were arrested with cardioplegia solution at 32°C. During arrest hearts were maintained at 32°C in all groups except Group 4 which was maintained at 20°C during arrest. The following protocols were used (Figures 5.1a and b):

Continuous cardioplegia (Cont, Group 1)

AL cardioplegia was delivered continuously at 32°C for 40 minutes of arrest, and the perfusate volume measured after 18 minutes and 38 minutes of arrest for calculating coronary vascular resistance. Hearts were switched to working mode and perfused with KH buffer at 37°C for 60 minutes and functional measurements taken at 2, 5, 10, 15, 30, 45 and 60 minutes of reperfusion.

Intermittent cardioplegia (Int, Group 2)

A 50ml AL cardioplegia induction dose was administered at 32°C and the aorta was cross-clamped. After 18 minutes and 38 minutes of arrest the cross-clamp was removed, a 2-minute AL cardioplegia infusion was given, and the perfusate volume measured for calculating coronary vascular resistance. The heart was reperfused in working mode for 60 minutes as detailed for Group 1.

One-shot arrest + Terminal Flush (OS, Group 3)

A 50ml AL cardioplegia induction dose was administered at 32°C and the aorta was cross-clamped. After 38 minutes arrest a 2-minute AL cardioplegia infusion was given (terminal flush) at 32°C, and the perfusate volume measured for calculating coronary vascular resistance. The terminal flush was included in the one-shot method to allow assessment of coronary vascular resistance, and to administer AL at the end of the arrest period. Adenosine adjunct, administered when the aortic cross-clamp is released, has been shown to reduce ischaemia-reperfusion injury and improve immediate post-operative cardiac function [489]. The heart was reperfused for 60 minutes as for Group 1.

One-shot induction, 20°C arrest (20°C, Group 4)

Following 50ml AL one-shot induction of arrest at 32°C, heart temperature decreased to room temperature (20 ± 0.3°C*)* for the remainder of the arrest period. The terminal flush and reperfusion protocol as for Group 3 one-shot arrest was then followed.

One-shot arrest, AL + Mg^{2+} terminal flush (Mg Fl, Group 5)

After AL one-shot induction, and arrest at 32°C for 38 minutes, adenosine 10µm + Lignocaine 25µm + magnesium 16mM was added to KH for the terminal 2-minute flush at 32°C prior to working mode reperfusion with KH as for Group 3.

AL + MPG one-shot arrest and AL + MPG terminal flush (MPG OS+Fl, Group 6)

N-(2-mercaptopropionyl)-glycine **(**MPG) 1mM was added to the AL arrest solution for 38 minutes one-shot arrest at 32°C, and to the 2-minute AL terminal flush at 32°C, followed by working mode reperfusion with KH buffer as for Group 3.

AL one-shot arrest, AL + MPG terminal flush (MPG Fl, Group 7)

Following AL one-shot induction and 38 minutes arrest at 32°C, MPG 1mM was added to the 2-minute AL terminal flush at 32°C prior to working mode reperfusion with KH as for Group 3.

AL one-shot arrest, AL + MPG terminal flush, KH + MPG working mode reperfusion (MPG Fl + WM, Group 8)

Following AL one-shot induction and 38 minutes arrest at 32°C, MPG 1mM was added to the 2-minute AL terminal flush at 32°C. MPG 1mM was also added to the KH reperfusion solution for the first 20 minutes of working mode reperfusion, followed by a further 40 minutes reperfusion with KH buffer.

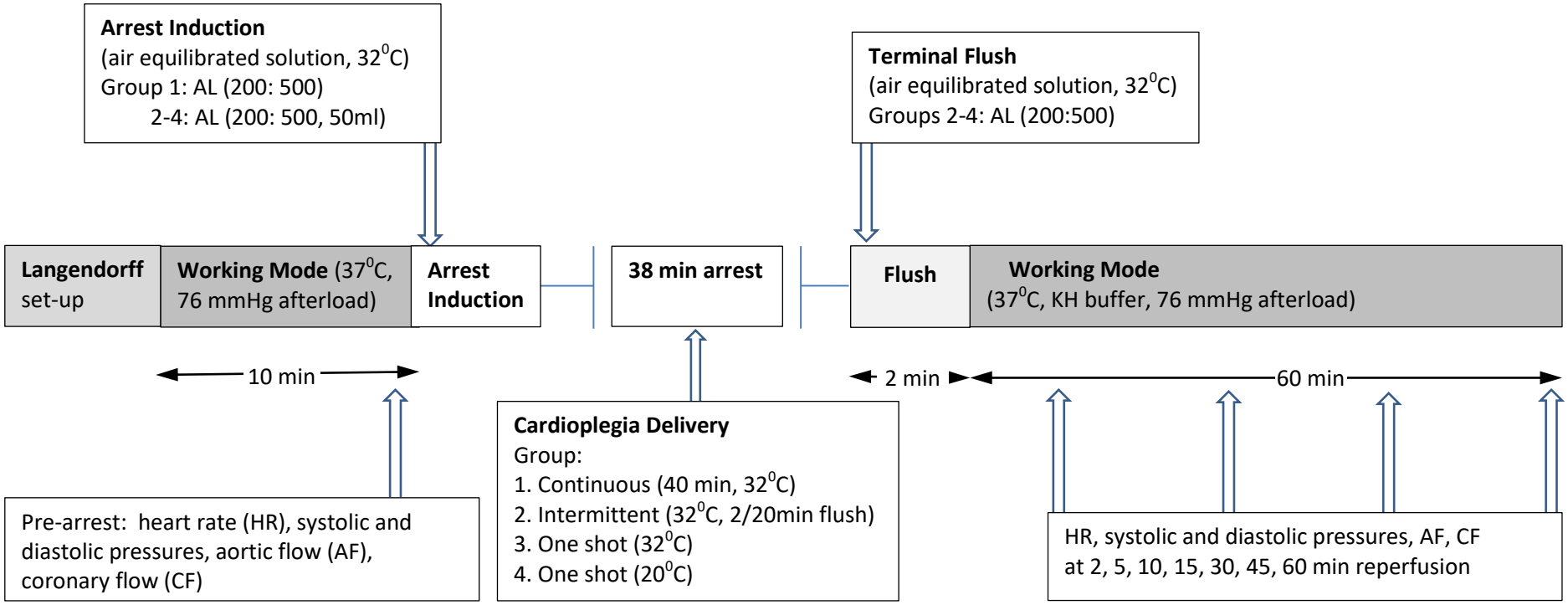


Figure 5.1a: Timeline for 40-minute arrest and reperfusion protocol using continuous, intermittent or one-shot delivery of AL cardioplegia solution

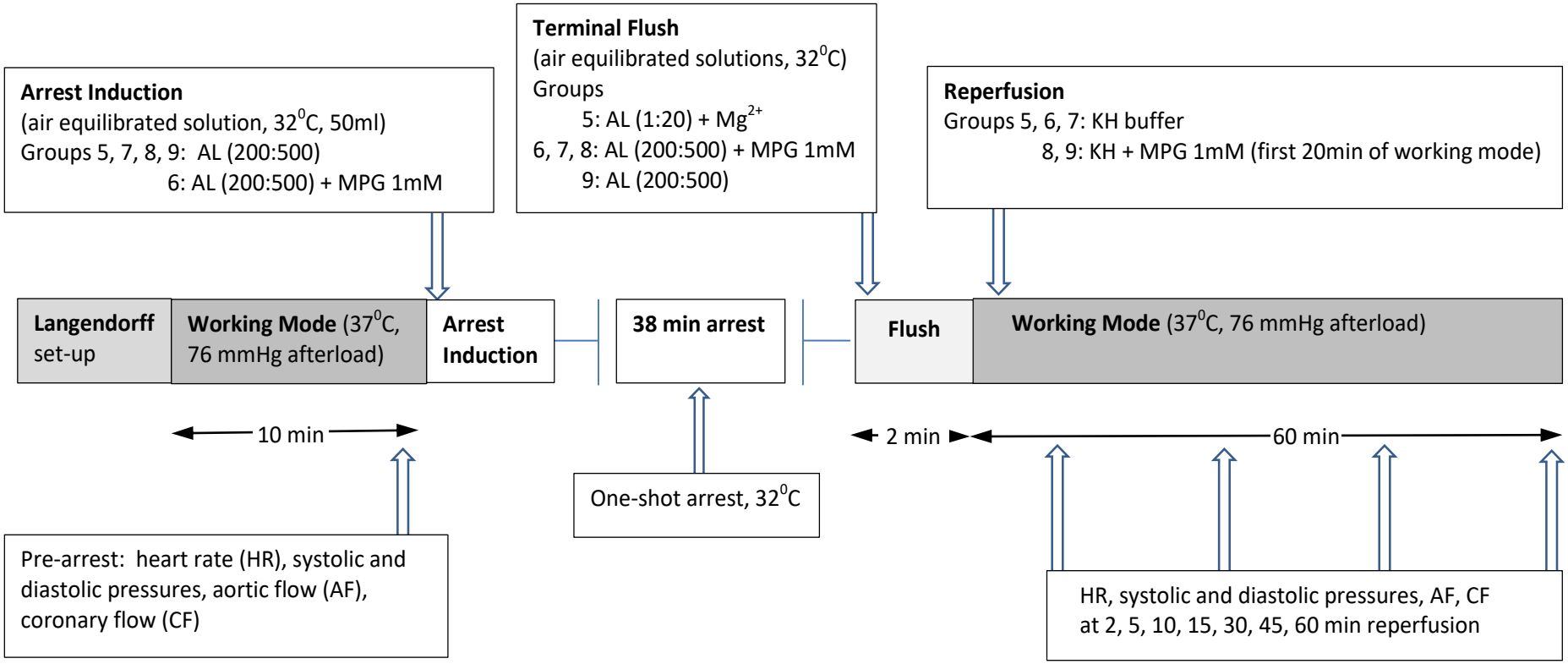


Figure 5.1b: Timeline for 40-minute one-shot AL arrest and reperfusion protocol with MPG or magnesium adjunct

AL one-shot arrest, KH + MPG working mode reperfusion (MPG WM, Group 9)

After AL one-shot induction and arrest for 38 minutes at 32^{o}C, a 2-minute AL terminal flush was administered. MPG 1mM was added to the KH reperfusion solution for the first 20 minutes of working mode reperfusion, followed by a further 40 minutes reperfusion with KH buffer.

5.3 Results

Functional measurements prior to and during arrest and reperfusion are shown in Tables 5.2a-g and Figures 5.2 – 5.7. There were no significant differences between groups in stabilised functional parameters (heart rate, systolic and diastolic pressures, aortic and coronary flows, cardiac output) measured pre-arrest.

5.3.1 The effect of cardioplegia protocols on arrest times and coronary vascular resistance during 2-minute terminal flush

Times to arrest for the 9 experimental groups were not significantly different, and ranged from 6.6 ± 0.4 to 10.9 ± 1.7 seconds.

There were no significant differences between the groups in coronary flow or coronary vascular resistance recorded after 38 minutes of arrest. Coronary flow measured between 15 ± 1.2 and 17 ± 0.7 ml/min, and coronary vascular resistance ranged from 0.28 ± 0.01 to 0.34 ± 0.03 megadyne.sec.cm $^{-5}$.

Table 5.2a: Functional parameters of isolated rat hearts before 40-minute arrest at 32°C or 20°C, (n=8).

Arrest Protocol	40 min cardioplegia delivery regimen	Heart Rate (beats/min)	Systolic Pressure (mmHg)	Diastolic Pressure (mmHg)	Aortic Flow (ml/min)	Coronary Flow (ml/min)	% of pre-arrest CO	Stroke volume (ml/beat)	Rate Pressure Product (mmHg/min)
Pre-Arrest	Continuous	299 ± 11	128 ± 4	70 ± 3	56 ± 2	19 ± 1	n/a	0.25 ± 0.1	38313 ± 2232
	Intermittent	326 ± 13	124 ± 3	68 ± 3	58 ± 2	20 ± 1	n/a	0.24 ± 0.1	40516 ± 2272
	One Shot	302 ± 11	133 ± 4	65 ± 2	53 ± 2	20 ± 1	n/a	0.24 ± 0.1	39903 ± 1450
	20°C	300 ± 12	127 ± 3	63 ± 1	53 ± 1	19 ± 1	n/a	0.24 ± 0.1	38128 ± 1899
	MPG Flush	319 ± 14	127 ± 3	69 ± 2	51 ± 3	18 ± 1	n/a	0.22 ± 0.01	40263 ± 1478
	MPG OS + Flush	301 ± 12	128 ± 3	65 ± 2	53 ± 3	21 ± 1	n/a	0.25 ± 0.01	38369 ± 1122
	MPG WM	323 ± 10	127 ± 3	66 ± 2	54 ± 3	20 ± 1	n/a	0.23 ± 0.01	40913 ± 1274
	MPG Flush + WM	296 ± 10	132 ± 2	64 ± 2	57 ± 3	19 ± 1	n/a	0.26 ± 0.01	39014 ± 1252
	Magnesium Flush	296 ± 12	131 ± 2	68 ± 3	53 ± 2	20 ± 1	n/a	0.25 ± 0.01	38756 ± 1414

Table 5.2b: Functional parameters of isolated rat hearts at 5 minutes working mode reperfusion following 40-minute arrest at 32°C or 20°C, (n=8).

Arrest Protocol	40 min cardioplegia delivery regimen	Heart Rate (beats/min)	Systolic Pressure (mmHg)	Diastolic Pressure (mmHg)	Aortic Flow (ml/min)	Coronary Flow (ml/min)	% of pre-arrest CO	Stroke Volume (ml/beat)	Rate Pressure Product (mmHg/min)
Reperfusion 5 min	Continuous	π 221 ± 8	ππ 145 ± 5	π 68 ± 3	πππ 47 ± 2	π 22 ± 1	πππ 93 ± 1	πππ 0.32 ± 0.02	π 31969 ± 1458
	Intermittent	† 162 ± 41	††† 79 ± 17	† 51 ± 8	ʒʒʒ 24 ± 9	† 14 ± 4	ʒʒʒ 48 ± 17	π 0.18 ± 0.05	††† 16556 ± 5630
	One Shot	* 6 ± 4	* 26 ± 6	* 25 ± 5	* 0	* 1 ± 1	* 1 ± 1	* 0.01 ± 0.01	* 303 ± 222
	20°C	*** 254 ± 12	πππ 134 ± 4	π 63 ± 2	πππ 43 ± 2	π 21 ± 1	π 88 ± 3	πππ 0.26 ± 0.01	ʃʃ 33944 ± 1771
	MPG Flush	131 ± 48	70 ± 19	45 ± 8	13 ± 6	9 ± 4	36 ± 17	0.09 ± 0.03	14287 ± 6579
	MPG OS + Flush	31 ± 21	30 ± 13	23 ± 7	4 ± 4	3 ± 3	11 ± 10	0.05 ± 0.04	2799 ± 2521
	MPG WM	13 ± 10	22 ± 4	21 ± 4	0	1 ± 1	0	0.01 ± 0.01	243 ± 165
	MPG Flush + WM	** 159 ± 32	†† 82 ± 22	45 ± 10	†† 17 ± 2	*** 12 ± 4	ʒʒ 39 ± 16	ʃ 0.13 ± 0.04	ʒ 17594 ± 5936
	Magnesium Flush	69 ± 32	ʒ 52 ± 14	ʒ 41 ± 5	4 ± 4	5 ± 3	11 ± 8	0.05 ± 0.03	6353 ± 4437

π $p<0.05$ compared with MPG OS+Fl, MPG WM, Mg^{2+} Fl

ππ $p<0.05$ compared with MPG OS+Fl, MPG WM, MPG Fl, Int, Mg^{2+} Fl

πππ $p<0.05$ compared with MPG OS+Fl, MPG WM, MPG Fl, Mg^{2+} Fl

* $p<0.05$ compared with Cont , Int , 20°C

** $p<0.05$ compared with OS, 20°C, MPG WM, MPG OS+Fl

*** $p<0.05$ compared with OS, MPG OS+Fl, MPG WM, Mg^{2+} Fl

† $p<0.05$ compared with MPG OS+Fl, MPG WM

†† $p<0.05$ compared with Cont

††† $p<0.05$ compared with MPG OS+Fl, MPG WM, 20°C

ʒ $p<0.05$ compared with MPG OS+Fl, MPG WM, OS

ʒʒ $p<0.05$ compared with OS, Cont, MPG OS+Fl, MPG WM, Mg^{2+} Fl

ʒʒʒ $p<0.05$ compared with MPG WM

ʃ $p<0.05$ compared with OS, Cont, MPG OS+Fl, MPG WM, Mg^{2+} Fl, 20°C

ʃʃ $p<0.05$ compared with MPG OS+Fl, MPG WM, Mg^{2+} Fl, Int

Table 5.2c: Functional parameters of isolated rat hearts at 10 minutes working mode reperfusion following 40-minute arrest at 32°C or 20°C, (n=8).

Arrest Protocol	40 min cardioplegia delivery regimen	Heart Rate (beats/min)	Systolic Pressure (mmHg)	Diastolic Pressure (mmHg)	Aortic Flow (ml/min)	Coronary Flow (ml/min)	% of pre-arrest CO	Stroke Volume (ml/beat)	Rate Pressure Product (mmHg/min)
		π	**π**	***	**ππ**	**π**	ψψψ	**ππ**	**π**
Reperfusion 10 min	Continuous	251 ± 12	136 ± 5	69 ± 3	49 ± 2	20 ± 1	93 ± 2	0.28 ± 0.01	34319 ± 2253
		***	***	***	**ψψ**	ψψψ	###	ψψψ	***
	Intermittent	285 ± 15	126 ± 5	70 ± 3	47 ± 1	23 ± 1	89 ± 4	0.25 ± 0.01	35694 ± 2007
		*			#	ψ	#	**δ**	
	One Shot	145 ± 41	88 ± 23	50 ± 10	16 ± 7	11 ± 3	37 ± 13	0.17 ± 0.04	18237 ± 6731
		***	***	***	###	**þþ**		**δδδ**	***
	20°C	292 ± 16	120 ± 3	64 ± 1	42 ± 3	18 ± 1	82 ± 5	0.21 ± 0.01	34963 ± 1887
	MPG Flush	176 ± 47	84 ± 16	56 ± 7	20 ± 8	9 ± 3	45 ± 17	0.11 ± 0.04	19265 ± 6425
	MPG OS + Flush	98 ± 35	49 ± 18	31 ± 7	10 ± 7	6 ± 3	22 ± 14	0.08 ± 0.04	8870 ± 5004
	MPG WM	56 ± 26	40 ± 14	32 ± 7	4 ± 4	4 ± 3	9 ± 8	0.06 ± 0.03	4537 ± 3633
		**	**þþþ**	***	##	ψψ	**πππ**	**δδ**	**π**
	MPG Flush + WM	261 ± 9	133 ± 3	68 ± 3	44 ± 2	19 ± 2	83 ± 3	0.24 ± 0.01	34625 ± 778
		***	***		**þ**	**þ**		**þþ**	
	Magnesium Flush	222 ± 37	110 ± 16	70 ± 7	26 ± 4	15 ± 3	58 ±11	0.22 ± 0.06	28099 ± 5778

π	p<0.05 compared with MPG OS+Fl, MPG WM	#	p<0.05 compared with Cont, Int, 20°C
ππ	p<0.05 compared with MPG OS+Fl, MPG WM, MPG Fl, Mg^{2+} Fl	##	p<0.05 compared with OS, MPG OS+Fl, MPG WM, MPG Fl, Mg^{2+} Fl, 20°C
πππ	p<0.05 compared with OS, Cont, MPG OS+Fl, MPG WM, Mg^{2+} Fl	###	p<0.05 compared with MPG OS+Fl, MPG WM, Mg^{2+} Fl
		ψ	p<0.05 compared with Cont, Int
*	p<0.05 compared with Int, 20°C	ψψ	p<0.05 compared with MPG OS+Fl, MPG WM, MPG Fl
**	p<0.05 compared with OS, MPG WM, MPG OS+Fl	ψψψ	p<0.05 compared with MPG OS+Fl, MPG WM, MPG Fl, Mg^{2+} Fl
***	p<0.05 compared with MPG OS+Fl, MPG WM	δ	p<0.05 compared with Cont
þ	p<0.05 compared with MPG WM	δδ	p<0.05 compared with MPG OS+Fl, MPG WM, MPG Fl, Mg^{2+} Fl, 20°C
þþ	p<0.05 compared with Int, MPG WM	δδδ	p<0.05 compared with Int, MPG OS+Fl, MPG WM, MPG Fl
þþþ	p<0.05 compared with MPG OS+Fl, MPG WM, MPG Fl, 20°C		

Table 5.2d: Functional parameters of isolated rat hearts at 15 minutes working mode reperfusion following 40-minute arrest at 32°C or 20°C, (n=8).

Arrest Protocol	40 min cardioplegia delivery regimen	Heart Rate (beats/min)	Systolic Pressure (mmHg)	Diastolic Pressure (mmHg)	Aortic Flow (ml/min)	Coronary Flow (ml/min)	% of pre-arrest CO	Stroke volume (ml/beat)	Rate Pressure Product (mmHg/min)
					π	ππ	πππ	π	
Reperfusion 15 min	Continuous	271 ± 16 **	128 ± 4 †††	72 ± 3	45 ± 2 ##	22 ± 1 ψψ	90 ± 3 πππ	0.25 ± 0.01 π	34438 ± 2223
	Intermittent	297 ± 18	121 ± 2 †	71 ± 3	50 ± 2 #	21 ± 2 ψ	90 ± 4 #	0.24 ± 0.01 ###	36006 ± 2246
	One Shot	253 ± 13 *	123 ± 13 †††	68 ± 5	30 ± 5 †††	16 ± 2 ψ	63 ± 7 †††	0.18 ± 0.02 ψ	31313 ± 3696
	20°C	296 ± 15	118 ± 3	65 ± 1	44 ± 2	18 ± 1	85 ± 3	0.21 ± 0.01	34638 ± 1671
	MPG Flush	262 ± 24	120 ± 10	73 ± 4	32 ± 6	15 ± 2	70 ± 11	0.17 ± 0.02	32130 ± 3688
	MPG OS + Flush	201 ± 34	105 ± 19	56 ± 8	29 ± 8	16 ± 4	58 ± 13	0.18 ± 0.04	25484 ± 5704
	MPG WM	250 ± 20	132 ± 5 ††	71 ± 3	40 ± 4 ##	17 ± 1 ψ	67 ± 11 ΨΨΨ	0.20 ± 0.03 π	33115 ± 3059
	MPG Flush + WM	272 ± 12	128 ± 2	68 ± 3	49 ± 2	17 ± 1	87 ± 2	0.25 ± 0.01	34613 ± 1447
	Magnesium Flush	282 ± 14	126 ± 2	71 ± 3	36 ± 2	18 ± 1	35 ± 3	0.19 ± 0.01	35491 ± 1617

π $p<0.05$ compared with MPG Fl), Mg^{2+} Fl
ππ $p<0.05$ compared with MPG Fl, MPG WM, Mg^{2+} Fl
πππ $p<0.05$ compared with MPG OS+Fl, MPG WM, Mg^{2+} Fl
* $p<0.05$ compared with MPG OS+Fl
** $p<0.05$ compared with MPG OS+Fl, MPG WM
† $p<0.05$ compared with 20°C
†† $p<0.05$ compared with Int, 20°C
††† $p<0.05$ compared with Mg^{2+} Fl
\# $p<0.05$ compared with Cont, Int, 20°C
$p<0.05$ compared with MPG OS+Fl, MPG Fl, MPG WM, Mg^{2+} Fl
$p<0.05$ compared with Cont, Int
Ψ $p<0.05$ compared with Cont
ΨΨ $p<0.05$ compared with MPG Fl
ΨΨΨ $p<0.05$ compared with MPG OS+Fl, Mg^{2+} Fl

Table 5.2e: Functional parameters of isolated rat hearts at 30 minutes working mode reperfusion following 40-minute arrest at 32°C or 20°C, (n=8).

Arrest Protocol	40 min cardioplegia delivery regimen	Heart Rate (beats/min)	Systolic Pressure (mmHg)	Diastolic Pressure (mmHg)	Aortic Flow (ml/min)	Coronary Flow (ml/min)	% of pre-arrest CO	Stroke volume (ml/beat)	Rate Pressure Product (mmHg/min)
Reperfusion 30 min	Continuous	282 ± 15	126 ± 4	73 ± 3	π 47 ± 2	ππ 23 ± 1	ππ 95 ± 2	πππ 0.25 ± 0.01	35469 ± 1906
	Intermittent	315 ± 16	118 ± 2 †	71 ± 3	πππ 52 ± 2 #	π 20 ± 1 ψ	ʒʒʒ 93 ± 2 ʒ	δδ 0.23 ± 0.01	37216 ± 2000
	One Shot	282 ± 15	128 ± 2 †††	70 ± 3 δ	41 ± 1 ###	16 ± 1 ψψψ	78 ± 5 †††	0.20 ± 0.01	35919 ± 1491
	20°C	311 ± 12	116 ± 3	64 ± 2	45 ± 2	20 ± 1	89 ± 2	0.21 ± 0.01	35822 ± 1276
	MPG Flush	295 ± 10	121 ± 2	73 ± 3	36 ± 4	15 ± 1	75 ± 7	0.17 ± 0.01	35569 ± 1393
	MPG OS + Flush	274 ± 15	119 ± 8	64 ± 4	42 ± 5	17 ± 2	79 ± 6	0.21 ± 0.02	32919 ± 2906
	MPG WM	279 ± 15	126 ± 4 ††	71 ± 3	40 ± 4 ##	16 ± 2 ψψ	76 ± 6 ʒʒ	0.20 ± 0.01 ##	35016 ± 1562
	MPG Flush + WM	291 ± 12	128 ± 2	66 ± 2	51 ± 3	19 ± 2	91 ± 1	0.24 ± 0.01	37091 ± 1501
	Magnesium Flush	295 ± 17	124 ± 2	71 ± 3	41 ± 1	17 ± 1	81 ± 2	0.20 ± 0.01	36363 ± 1840

π	$p<0.05$ compared with MPG Fl, Mg^{2+} Fl	ψ	$p<0.05$ compared with Cont, Int, 20°C
ππ	$p<0.05$ compared with MPG OS+Fl, MPG Fl, MPG WM, Mg^{2+} Fl	ψψ	$p<0.05$ compared with Cont
πππ	$p<0.05$ compared with MPG Fl, MPG WM, Mg^{2+} Fl	ψψψ	$p<0.05$ compared with Cont, MPG Fl, Mg^{2+} Fl
†	$p<0.05$ compared with Int, MPG Fl, 20°C	ʒ	$p<0.05$ compared with Cont, Int
††	$p<0.05$ compared with Int, 20°C	ʒʒ	$p<0.05$ compared with OS, MPG WM, Mg^{2+} Fl
†††	$p<0.05$ compared with MPG WM, Mg^{2+} Fl	ʒʒʒ	$p<0.05$ compared with MPG OS+Fl, MPG WM, Mg^{2+} Fl
#	$p<0.05$ compared with Int	δ	$p<0.05$ compared with Cont, MPG Fl
##	$p<0.05$ compared with OS, MPG Fl, MPG WM, Mg^{2+} Fl	δδ	$p<0.05$ compared with MPG Fl
###	$p<0.05$ compared with Int, MPG Fl		

Table 5.2 f: Functional parameters of isolated rat hearts at 45 minutes working mode reperfusion following 40-minute arrest at 32°C or 20°C, (n=8).

Arrest Protocol	40 min cardioplegia delivery regimen	Heart Rate (beats/min)	Systolic Pressure (mmHg)	Diastolic Pressure (mmHg)	Aortic Flow (ml/min)	Coronary Flow (ml/min)	% of pre-arrest CO	Stroke Volume (ml/beat)	Rate Pressure Product (mmHg/min)
					π	**ππ**		**πππ**	
Reperfusion 45 min	Continuous	291 ± 14	124 ± 4	72 ± 3	47 ± 2	23 ± 1	94 ± 2	0.24 ± 0.01	36147 ± 1876
					###	**ππ**	**ππ**	**δδ**	
	Intermittent	327 ± 16	117 ± 2	71 ± 3	51 ± 2	21 ± 1	92 ± 2	0.22 ± 0.01	38141 ± 1786
			†		**#**	**Ψ**	**ʒ**	**ΨΨ**	
	One Shot	279 ± 15	126 ± 3	69 ± 3	41 ± 1	17 ± 1	80 ± 3	0.21 ± 0.01	34894 ± 1350
				◊	**##**	**ΨΨΨ**	**ʒʒʒ**	**ΨΨΨ**	
	20°C	312 ± 12	114 ± 2	64 ± 1	43 ± 1	20 ± 1	86 ± 1	0.20 ± 0.01	35581 ± 1152
			†††	**◊◊**	**ϱ**			**δδδ**	
	MPG Flush	305 ± 13	114 ± 3	72 ± 2	34 ± 2	15 ± 1	72 ± 6	0.16 ± 0.01	34663 ± 944
	MPG OS + Flush	284 ± 16	119 ± 5	65 ± 2	42 ± 4	17 ± 1	79 ± 4	0.21 ± 0.01	33781 ± 2549
	MPG WM	284 ± 19	124 ± 5	72 ± 3	37 ± 3	16 ± 2	72 ± 5	0.19 ± 0.01	34816 ± 2126
			††		**ʒʒ**	**ΨΨ**	**ʒʒ**	**δ**	
	MPG Flush + WM	286 ± 14	124 ± 2	66 ± 2	49 ± 3	18 ± 1	86 ± 2	0.24 ± 0.01	35544 ± 1585
					δδ	**δδ**	**δδ**	**δδ**	
	Magnesium Flush	299 ± 18	122 ± 2	71 ± 3	42 ± 1	18 ± 1	83 ± 2	0.20 ± 0.01	36200 ± 1812

π	*p*<0.05 compared with MPG FI, MPG WM	###	*p*<0.05 compared with MPG FI, MPG WM, Mg^{2+} FI
ππ	*p*<0.05 compared with MPG OS+FI, MPG FI, MPG WM, Mg^{2+} FI	Ψ	*p*<0.05 compared with Cont, Int, 20°C
πππ	*p*<0.05 compared with MPG FI, MPG WM, Mg^{2+} FI	ΨΨ	*p*<0.05 compared with Cont
†	*p*<0.05 compared with Int, 20°C	ΨΨΨ	*p*<0.05 compared with Cont, MPG FI
††	*p*<0.05 compared with Int, MPG FI, 20°C	ʒ	*p*<0.05 compared with Cont, Int
†††	*p*<0.05 compared with OS	ʒʒ	*p*<0.05 compared with OS, MPG FI), MPG WM, Mg^{2+} FI
◊	*p*<0.05 compared with Cont, MPG FI, MPG WM	ʒʒʒ	*p*<0.05 compared with Cont, Int, MPG WM
◊◊	*p*<0.05 compared with MPG OS+FI	δ	*p*<0.05 compared with MPG FI, 20°C, Mg^{2+} FI
#	*p*<0.05 compared with Int	δδ	*p*<0.05 compared with MPG FI
##	*p*<0.05 compared with MPG FI, 20°C	δδδ	*p*<0.05 compared with OS, MPG OS+FI

Table 5.2g: Functional parameters of isolated rat hearts at 60 minutes working mode reperfusion following 40-minute arrest at 32°C or 20°C, (n=8).

Arrest Protocol	40 min cardioplegia delivery regimen	Heart Rate (beats/min)	Systolic Pressure (mmHg)	Diastolic Pressure (mmHg)	Aortic Flow (ml/min)	Coronary Flow (ml/min)	% of pre-arrest CO	Stroke Volume (ml/beat)	Rate Pressure Product (mmHg/min)
Reperfusion 60 minutes	Continuous	297 ± 13	124 ± 4 πππ	72 ± 3	46 ± 2 π	22 ± 1 ππ	90 ± 2 ππ	0.23 ± 0.01 ππ	36741 ± 1979
	Intermittent	325 ± 15 #	115 ± 2 †	73 ± 3	49 ± 2 ππ #	21 ± 1 π Ψ	90 ± 2 ππ ʒ	0.22 ± 0.01 π	37344 ± 1707
	One Shot	280 ± 16	126 ± 2 †††	72 ± 2	39 ± 1 ##	16 ± 1 ΨΨ	76 ± 2 ʒʒʒ	0.20 ± 0.01 δδ	34972 ± 1504
	20°C	318 ± 10	113 ± 2 ###	67 ± 2	41 ± 2 δδδ	19 ± 1 ΨΨΨ	82 ± 2	0.19 ± 0.01 ΨΨΨ	35906 ± 1295
	MPG Flush	307 ± 12	110 ± 4	69 ± 2	29 ± 2	14 ± 1	65 ± 6	0.14 ± 0.01	33519 ± 1150
	MPG OS + Flush	286 ± 14	118 ± 5	65 ± 2	42 ± 4	18 ± 1	81 ± 5 ΨΨΨ	0.21 ± 0.01	33891 ± 2381
	MPG WM	299 ± 14	119 ± 4 ††	72 ± 3	34 ± 3 ππ	16 ± 1 ΨΨ	67 ± 5 ʒʒ	0.17 ± 0.01 δ	35341 ± 1318
	MPG Flush + WM	290 ± 13	123 ± 2	68 ± 2	47 ± 3	19 ± 2	87 ± 3	0.23 ± 0.01	35706 ± 1682
	Magnesium Flush	311 ± 17	119 ± 2	71 ± 3	40 ± 1	19 ± 1	81 ± 2	0.19 ± 0.01	36956 ± 1627

π	*p*<0.05 compared with MPG Fl, MPG WM	Ψ	*p*<0.05 compared with Cont, Int, 20°C, Mg^{2+} Fl
ππ	*p*<0.05 compared with MPG Fl, MPG WM, Mg^{2+} Fl	ΨΨ	*p*<0.05 compared with MPG Fl
πππ	*p*<0.05 compared with 20°C, MPG Fl	ΨΨΨ	*p*<0.05 compared with MPG OS+Fl, Mg^{2+} Fl
†	*p*<0.05 compared with Int, 20°C	ʒ	*p*<0.05 compared with Cont, Int
††	*p*<0.05 compared with Int, 20°C, MPG Fl	ʒʒ	*p*<0.05 compared with OS, MPG WM, MPG Fl
†††	*p*<0.05 compared with Mg^{2+} Fl	ʒʒʒ	*p*<0.05 compared with Int, MPG Fl, MPG WM
#	*p*<0.05 compared with Int	δ	*p*<0.05 compared with MPG WM, MPG Fl, 20°C, Mg^{2+} Fl
##	*p*<0.05 compared with Int, MPG Fl	δδ	*p*<0.05 compared with Cont, MPG Fl
###	*p*<0.05 compared with OS	δδδ	*p*<0.05 compared with OS, MPG OS+Fl, Mg^{2+} Fl

5.3.2 The effect of cardioplegia protocols on time to first spontaneous beat and time to aortic flow at reperfusion

There was no significant difference in time to first spontaneous reperfusion beat between the continuous (Cont) (1.6 ± 0.3 min), intermittent (Int) (2.3 ± 0.3 min), MPG flush (MPG Fl) (3.1 ± 0.7 min) and MPG flush + working mode (MPG Fl+WM) (2.7 ± 0.3 min) groups, and these groups all had a significantly faster time to first beat than the one-shot (OS) group (5.2 ± 0.7 min) (p<0.05 all groups). The MPG Fl+WM group also had a significantly shorter time to first beat than the MPG working mode (MPG WM) (5.5 ± 0.5 min) and MPG one-shot + flush (MPG OS+Fl) (5.5 ± 0.8 min) group (p<0.05 both groups) (Figure 5.2). The one-shot 20^{o}C arrest (20^{o}C) group had a significantly shorter time to first beat (1.0 ± 0.3 min) than all other groups except the Cont group (p<0.05 all groups).

The time to aortic flow in working mode reperfusion for the one-shot group (12 ± 1.2 min) was significantly slower than the Cont (3.6 ± 0.5 min), Int (5.9 ± 0.9 min), 20^{o}C (2.4 ± 0.8 min) and MPG Fl+WM (6.4 ± 0.6 min) groups (p<0.05 all groups). The time to aortic flow was not significantly different between the Int group and the MPG Fl (9.9 ± 2.3 min) and MPG Fl+WM and one-shot + magnesium flush (Mg^{2+} Fl) (9.0 ± 1.1 min) groups. The MPG Fl+WM group also had a significantly shorter time to aortic flow than the MPG WM (12 ± 1.1 min) and MPG OS+Fl (13 ± 1.8 min) groups (p<0.05 all groups). The time to aortic flow for the 20^{o}C group (2.4 ± 0.8 min) was significantly less than all other groups except Cont (p<0.05 all groups) (Figure 5.3).

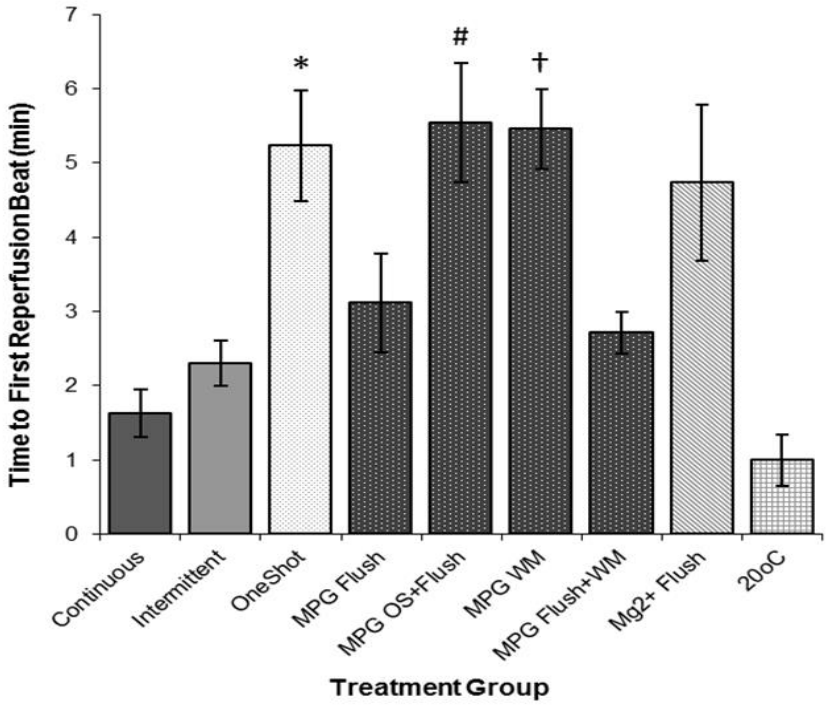


Figure 5.2: Time to first beat (minutes) at reperfusion in working mode

* $p<0.05$ One-shot compared with continuous, intermittent, MPG flush + working mode, 20°C

#, † $p<0.05$ MPG one-shot + flush and MPG working mode compared with continuous, intermittent, MPG flush + working mode, MPG flush

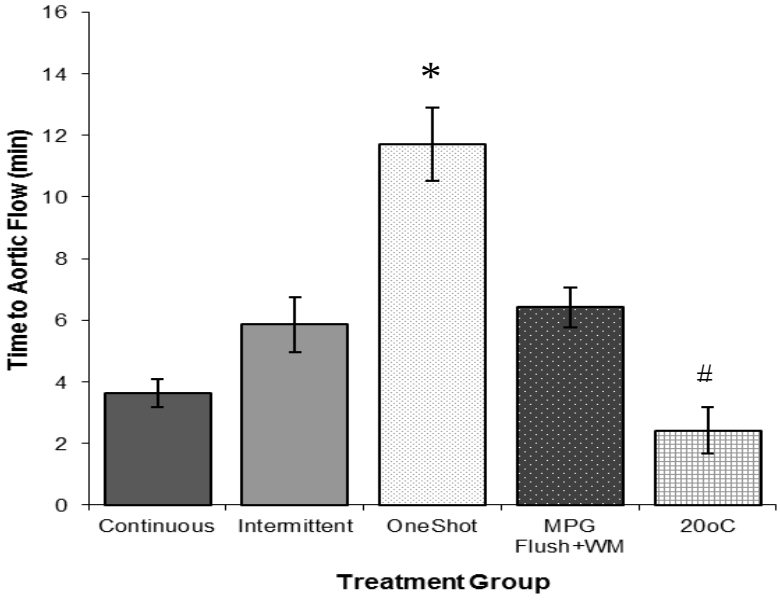


Figure 5.3: Time to aortic flow (minutes) at reperfusion in working mode

* $p<0.05$ One-shot group compared with continuous, intermittent, MPG flush + working mode, 20°C

\# $p<0.05$ 20°C compared with one-shot, intermittent, MPG flush + working mode

5.3.3 The effect of cardioplegia protocols on functional recovery during 60 minutes of working mode reperfusion

Recovery of heart rate

Heart rate recovered most rapidly in the 20°C group, while the Cont, Int and MPG Fl+WM groups also recovered rapidly with no significant difference in heart rate at 5 minutes reperfusion (159 ± 32 to 254 ± 12 bpm), and these groups were all significantly different from the one-shot group (6 ± 4 bpm) at this time (p<0.05 all groups).
At 10 minutes reperfusion the MPG WM group recorded a heart rate of 56 ± 26 bpm, which was significantly lower than all other groups except the MPG OS+Fl group (98 ± 35 bpm) (p<0.05 all groups).
No significant differences in heart rate were recorded between any of the groups after 15 minutes of reperfusion (Tables 5.2a-g).

Recovery of systolic and diastolic pressures

Hearts in the Cont and 20°C groups recovered systolic pressure rapidly and consistently in working mode reperfusion. Variability in the recovery of pressure in hearts within the other groups precluded significant differences between these groups in early reperfusion.

At 5 minutes reperfusion the Cont group had recovered 114 ± 2% of pre-arrest systolic pressure, which was significantly different from all other groups (p<0.05).
The one-shot group (19 ± 4%) was also significantly different from the Int (65 ± 14%), 20°C (105 ± 2%) and Mg^{2+}Fl (40 ± 11%) groups (p<0.05). There was no significant difference between the Int and MPG Fl+WM (63 ± 17%) groups, and although both of these groups had a higher return of systolic pressure than the one-shot group and the other three MPG groups (OS+Fl, Fl, WM), the difference was not significant due to within group variability.

By 10 minutes reperfusion hearts in the MPG Fl+WM (101 ± 2%) group had consistently recovered to pre-arrest systolic pressure, but recovery of the one-shot group (66 ± 17%) remained variable, preventing significant difference. However the MPG Fl+WM group was significantly different from the MPG WM and MPG OS+Fl groups (17 ± 3% and 24 ± 11% respectively) (p<0.05). After 15 minutes of reperfusion systolic pressure had recovered to ≥81% of the pre-arrest value in all groups, however

the systolic pressure of the MPG FI+WM group was significantly higher than the Int and 20°C groups for the remaining reperfusion time (Tables 5.2 a-g)

At 5 minutes reperfusion, recovery of diastolic pressure in the MPG FI+WM group (69 ± 13%) was significantly better than the MPG WM group (30 ± 5%), but not significantly different from any other group. The diastolic pressure of the one-shot group (39 ± 9%) was significantly different from the Cont (97 ± 3%), Int (73 ± 11%), 20°C and Mg^{2+}FI (61 ± 9%) groups (p<0.05). After 10 minutes of reperfusion, diastolic pressure in the one-shot group was not significantly different from any other group.

Recovery of rate-pressure product

At 5 minutes reperfusion the rate-pressure product of the Cont, Int, 20°C and MPG FI+WM groups was not significantly different, and these four groups had significantly higher rate-pressure product than the one-shot, MPG OS+FI and MPG WM groups (p<0.05 for all groups). After 10 minutes of reperfusion there were no significant differences in rate-pressure product between any of the groups (Tables 5.2a-g).

Recovery of aortic flow

Recovery of aortic flow in the one-shot group was significantly different from the Int group at every time point in the reperfusion period, and from the Cont, 20°C and MPG FI+WM groups for the first 15 minutes of reperfusion (p<0.05 for all groups). Aortic flow in the one-shot group at 10 and 15 min reperfusion was 31 ± 13% and 55 ± 10% of pre-arrest value respectively, compared with 77 ± 3% and 86 ± 3% respectively for the MPG FI+WM group and 81 ± 4% and 86 ± 4% respectively for the Int group at the same time points. By 60 minutes reperfusion the one-shot hearts had recovered aortic flow to comparable to the 20°C, Cont and MPG FI+WM groups (Figure 5.4).

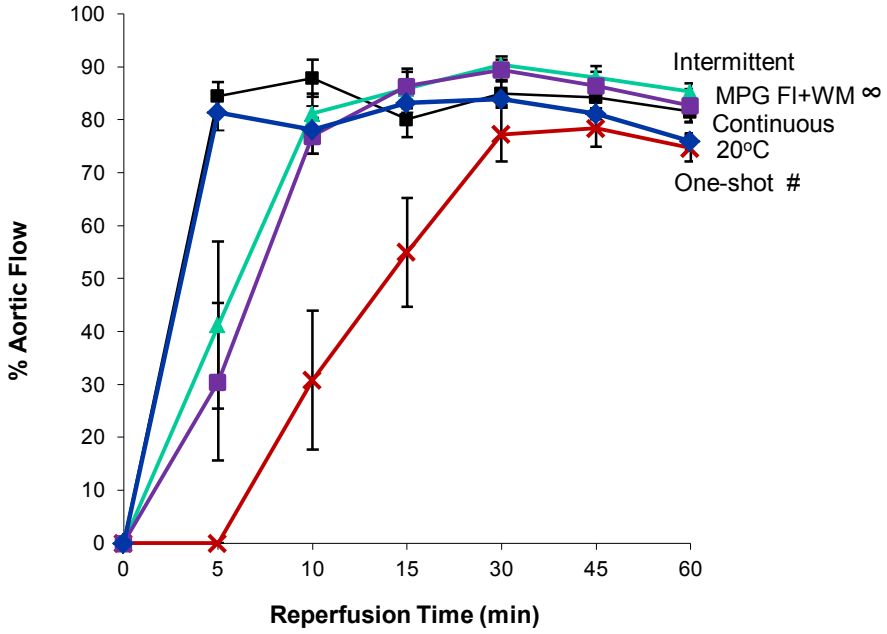


Figure 5.4 Percentage return of aortic flow (one-shot, continuous, intermittent, 20°C and MPG flush + working mode groups) during 60 minutes reperfusion

\# $p<0.05$ One-shot X compared with intermittent ▲ (5-60 min), MPG flush + working mode ■ (5-15 min), continuous ■ (5-15, 60 min)

∞ $p<0.05$ MPG flush + working mode compared with continuous (10 min)

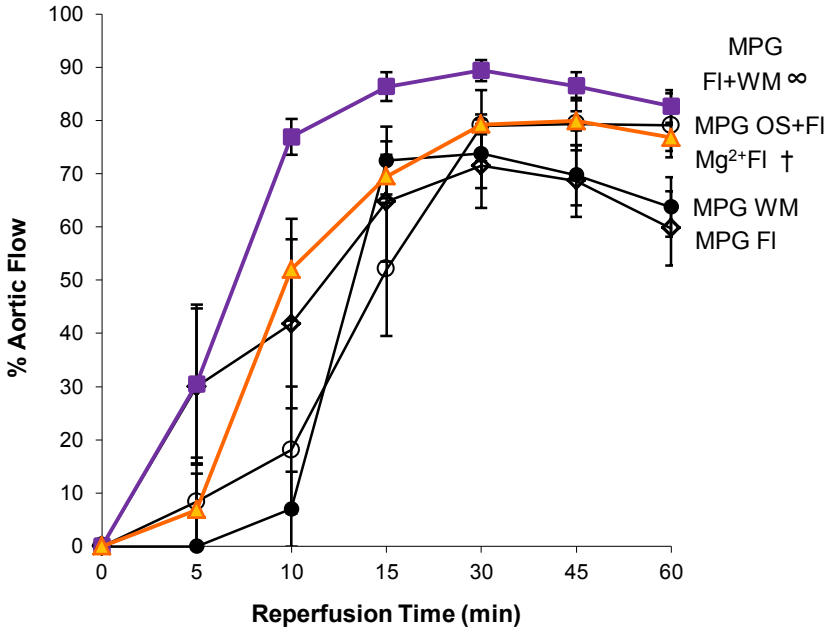


Figure 5.5: Percentage return of aortic flow (MPG and magnesium groups) during 60 minutes reperfusion

∞ $p<0.05$ MPG flush + working mode ■ compared with MPG one-shot + flush ○ (10,15 min), MPG flush ◇ (30-60 min), MPG working mode ● (10-60 min)

† $p<0.05$ Magnesium flush ▲ compared with MPG flush (60 min), MPG working mode (10 min), MPG one-shot + flush (10 min)

The addition of MPG to the terminal flush and reperfusion buffer (MPG FI+WM group) led to significantly improved recovery of aortic flow in the first 15 minutes of the reperfusion period, compared with the one-shot group. Percentage recovery of aortic flow for the MPG FI+WM group was not significantly different from the Int group at any time point, or from the 20°C group between 10 and 60 minutes reperfusion. Return of aortic flow in the MPG FI+WM group was significantly higher than the MPG FI, MPG OS+FI, MPG WM groups and the Mg^{2+} FI group (p<0.05 for all groups) (Figure 5.5).

There were no significant differences in percentage recovery of aortic flow between the MPG FI, MPG OS+FI, MPG WM groups or the Mg^{2+} FI group, or between those groups and the one-shot group at any time point (Figures 5.4 and 5.5 , and Tables 5.2a-g).

Recovery of coronary flow

Return of coronary flow at 5 minutes reperfusion was not significantly different between the Cont (118 ± 5%), Int (67 ± 21%), 20°C (108 ± 3%), and MPG FI+WM (64 ± 20%) groups, and coronary flow for these four groups was significantly higher than the one-shot (3 ± 1%) group (p<0.05 for all groups). The MPG FI+WM group also recovered significantly higher coronary flow than the MPG OS+FI (18 ± 14%) and MPG WM (2 ± 1%) groups, but not the MPG FI (50 ± 23%) group (p<0.05 for all groups) (Figures 5.6 and 5.7).

Between 10 and 30 minutes reperfusion the return of coronary flow for hearts in the one-shot group was very variable, preventing significant difference from all other groups except the Int group. There was no significant difference in coronary flow between the one-shot group and the MPG FI, MPG OS+FI, or MPG WM groups at any time point during reperfusion.

At 30 minutes and 60 minutes of reperfusion coronary flow in the MPG FI+WM group (97 ± 5%; 98 ± 5% respectively) was significantly higher than the one-shot (81 ± 4%; 80 ± 2% respectively) group, and the MPG FI, MPG OS+FI, or MPG WM groups (p<0.05 for all groups). Return of coronary flow for the MPG FI+WM group was not significantly different from the 20°C or Int groups at any time point during the 60 minutes of working mode reperfusion. (Figures 5.6 and 5.7, and Tables 5.2a-g). A significant difference in coronary flow between the one-shot and Mg^{2+}FI groups was recorded at 60 minutes reperfusion only.

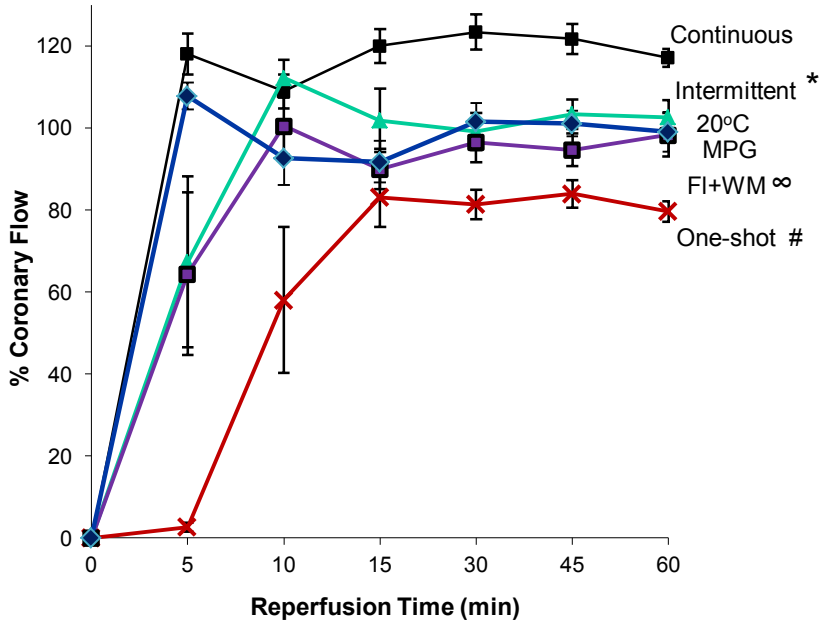


Figure 5.6: Percentage return of coronary flow (one-shot, continuous, 20°C, intermittent, and MPG flush + working mode groups) during 60 minutes reperfusion

\# $p<0.05$ One-shot X compared with intermittent▲ (5,10,30-60 min), MPG flush + working mode ■ (5,30,60 min), continuous■ (5-60 min)
∞ $p<0.05$ MPG flush + working mode compared with continuous (15-60 min)
* $p<0.05$ Intermittent compared with continuous (30-60 min)

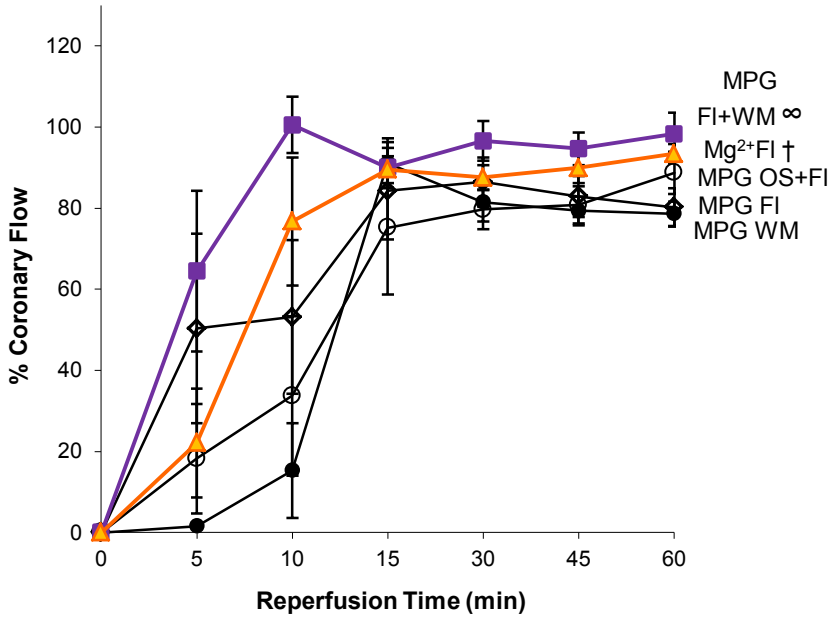


Figure 5.7: Percentage return of coronary flow (MPG and magnesium groups) during 60 minutes reperfusion

∞ $p<0.05$ MPG flush + working mode ■ compared with MPG one-shot + flush ○ (5,30,45 min), MPG flush ◇ (5,10,30-60 min), MPG working mode ● (5,10,30-60 min)
† $p<0.05$ Magnesium flush▲ compared with MPG flush (60 min), MPG working mode

Recovery of stroke volume

The Cont group had recovered a significantly higher stroke volume of 0.32 ± 0.02 ml/beat at 5 minutes reperfusion compared with the 20°C (0.26 ± 0.01 ml/beat), Int (0.18 ± 0.05 ml/beat), MPG FI+WM (0.13 ± 0.04 ml/beat), MPG FI (0.09 ± 0.03 ml/beat), Mg^{2+} FI and MPG OS+FI (0.05 ± 0.03 ml/beat each), and one-shot and MPG WM (0.01± 0.01 ml/beat each) groups (p<0.05 for all groups).

By 10 minutes reperfusion the stroke volume of the MPG FI+WM group had increased to 0.24 ± 0.01 ml/beat and was not significantly different from the Cont or Int groups for the remainder of the reperfusion time.

After 30 minutes of reperfusion the MPG FI+WM group had a significantly higher stroke volume than the one-shot, 20°C, Mg^{2+} FI, MPG FI and MPG WM groups (p<0.05 for all groups), but by 60 min the MPG FI+WM group was not significantly different from the one-shot group (Tables 5.2a-g).

Recovery of cardiac output

During the first 15 minutes of reperfusion there was no significant difference in cardiac output between the Cont, Int, 20°C and MPG FI+WM groups, and these four groups all had significantly higher cardiac output than the one-shot, MPG OS+FI , MPG WM and Mg^{2+} FI groups (p<0.05 for all groups).

Between 30 and 60 minutes reperfusion the cardiac output of the Cont, Int and MPG FI+WM groups was not significantly different, and significant difference remained between these three groups and the one-shot, MPG OS+FI and MPG WM groups during the reperfusion period (p<0.05 for all groups) (Tables 5.2a-g).

5.4 Discussion

This chapter investigated whether a one-shot AL(200:500) cardioplegia induction for 40-minute arrest at 32^{o}C offered comparable cardioprotection to the warm continuous and intermittent protocols used in the previous chapters, and the effect of hypothermia during one-shot arrest on recovery. Addition of MPG or magnesium was then assessed for effect on functional recovery following warm one-shot arrest.

The key findings of this chapter were:

1) warm 40-minute AL one-shot arrest resulted in significantly reduced functional recovery compared with warm intermittent or continuous arrest
2) lowering the arrest temperature to 20^{o}C or adding 16mM magnesium to the terminal flush following one-shot arrest improved recovery in the early reperfusion period, however the difference in recovery was not maintained
3) addition of MPG to the terminal cardioplegia flush and reperfusion buffer following warm one-shot arrest significantly improved functional recovery at reanimation and during the reperfusion period to comparable with recovery in the warm intermittent group.

5.4.1 AL cardioplegia maintained coronary flow during warm one-shot arrest

Warm one-shot arrest with AL(200:500) did not significantly alter the coronary flow and coronary vascular resistance during the terminal cardioplegia flush compared with the other 8 treatment groups. This result demonstrates AL protection of the coronary vasculature during all of the arrest protocols, with comparable coronary flow maintained following 40 minutes arrest with continuous, intermittent or one-shot cardioplegia delivery at 32^{o}C or 20^{o}C.

5.4.2 Warm AL one-shot arrest resulted in myocardial stunning at reanimation which was partly reversed by arrest at 20°C

In this study, the significantly slower time to first beat and time to aortic flow in the one-shot group compared with the intermittent and continuous groups is an indication of myocardial stunning at reperfusion [72,84]. This suggests that a single dose of AL (200:500) may not provide adequate protection of the contractile components and energy reserves of myocytes during 40-minute warm (32°C) arrest.

Lowering the arresting temperature to 20°C during AL one-shot arrest significantly improved the time to first beat and time to aortic flow compared with AL one-shot warm arrest and all other groups. This demonstrated an additional protective effect conferred by hypothermia during 40-minute one-shot AL arrest which resulted in superior recovery of contractile function early in the reperfusion period. Intermittent delivery of AL did not significantly change the time to first beat compared with continuous delivery, which supports the findings in Chapter 4.

5.4.3 Warm one-shot arrest led to functional loss during 60 minutes reperfusion which was partly reversed in 20°C group

Hearts in the warm one-shot group recorded an irregular, significantly slower heart rate, and significantly lower developed pressures, and aortic and coronary flows during early reperfusion (Figures 5.4 and 5.6, Table 5.2b) compared with the continuous, intermittent and 20°C groups, which indicates reperfusion injury [64]. This data, in conjunction with the significantly longer times to first beat and to aortic flow reported in 5.4.2 above, would be consistent with the symptoms of stunning. Further results supporting myocardial stunning in the warm one-shot group were the improved recovery of heart rate and pressures after 30 minutes working mode reperfusion. At 60 minutes reperfusion the one-shot group recorded a heart rate of 280 ± 16 beats/min, systolic pressure of 126 ± 2mmHg and diastolic pressure of 72 ± 2mmHg. After 60 min reperfusion the warm one-shot group had recovered 75 ± 3% aortic flow and 80 ± 2% coronary flow, however these flows remained significantly lower than the continuous and intermittent groups which recorded 82 ± 2% and 85 ± 2% aortic flow respectively, and 117 ± 4% and 103 ± 2% coronary flow respectively. The cardiac output of the warm one-shot group continued to improve during working mode reperfusion to a maximum of 80 ± 3% at 45 minutes (Table 5.2f). This also suggests stunning of one-shot hearts arrested with AL for 40 minutes at 32°C, rather than the irreversible

damage to mitochondria and the contractile apparatus which has been reported in rats during unprotected ischaemia [490]. Contractile function may have improved during the reperfusion period following AL arrest as adenosine activates K_{ATP} channels triggering protection against ischaemia-reperfusion injury [95,491] and stunning if administered during both ischaemia and at reperfusion, but not if given during reperfusion only [492]. Further studies are required to understand the underlying mechanisms of myocardial stunning, and possibly micro-vascular stunning, in this model.

5.4.4 MPG prevented stunning at reanimation following warm one-shot arrest

When MPG was added to the terminal flush plus working mode reperfusion buffer (MPG FI+WM group) the times to first beat and aortic flow significantly improved compared to times in the warm one-shot group without MPG, and were comparable to the continuous and intermittent groups (Figures 5.2 and 5.3). In the present study, it was concluded that the MPG FI+WM protocol was the most effective MPG treatment strategy for reversing stunning after 40-minute warm one-shot arrest.

Reasons for improvement during early reperfusion are not known, but possibly related to the ability of MPG to scavenge hydroxyl radicals. In 1989 Bolli and colleagues conducted *in vitro* studies showing that MPG was a powerful scavenger of $^{\bullet}OH$ (rate constant = 8.1×10^{9} M^{-1} sec^{-1} by pulse radiolysis) with no significant effect on $^{\bullet}O_2^-$ (rate constant $<10^3$ M^{-1} sec^{-1}), H_2O_2 (rate constant = 1.6 M^{-1} sec^{-1}), or non-$^{\bullet}OH$-initiated lipid peroxidation [83]. This data suggests that in the present study the scavenging of hydroxyl radicals was a major mechanism of reversal of stunning in hearts arrested with warm one-shot AL with MPG added to the terminal flush and working mode solutions.

Bolli and colleagues also reported that myocardial stunning after reversible ischemia in dogs was associated with the release of hydroxyl radicals which commenced within the initial seconds of reperfusion [83]. Their data showed that to suppress production of radicals and attenuate stunning and mechanical dysfunction, antioxidant therapy needed to be administered before reperfusion was commenced, which is consistent with the effectiveness of MPG administered during the 2-minute terminal flush before commencing reperfusion in the present study. Delaying MPG administration until reperfusion (MPG WM group) failed to attenuate stunning in the present study, which is also in agreement with results reported in the study by Bolli and colleagues.

5.4.5 MPG significantly improved functional recovery during working mode reperfusion

MPG treatment was continued during early working mode reperfusion to provide possible antioxidant protection for the first 20 minutes of reperfusion, based on studies by Culcasi [493] and Tosaki [494] using isolated rat hearts, and the study by Bolli [83]. These spin trapping studies confirmed the production of hydroxyl and superoxide radicals at reperfusion following global ischaemia for 30 minutes, with peak production of reactive oxygen species occurring between 30 seconds and three minutes of reperfusion [493,494], and then declining release during the first 20 minutes of reperfusion [83].

In the present study, MPG added to the terminal flush and reperfusion solutions led to significantly improved recovery of heart rate, developed pressures and aortic and coronary flows during reperfusion compared with the warm one-shot group without MPG (Table 5.2a-g, Figures 5.4 to 5.7). For example, at 5 minutes reperfusion the MPG FI+WM group had recovered 30 ± 15% aortic flow, 64 ± 20% coronary flow and 39 ± 16% cardiac output compared with 0%, 3 ± 1% and 1 ± 1% respectively for the one-shot group. At 60 minutes reperfusion the MPG FI+WM group recorded 83 ± 3% aortic flow, 98 ± 5% coronary flow and 87 ± 3% cardiac output. Recovery in the one-shot group had improved to 75 ± 3%, 80 ± 2% and 76 ± 2% respectively, with significant difference in coronary flow and cardiac output remaining between these two groups.

Tanonaka and colleagues [495] concluded that MPG pre-treatment alone was effective in reducing sodium overload and preserving mitochondrial function to improve post-ischaemic recovery of myocardial function. However in the present study, MPG administered in the cardioplegia solution at arrest and in the terminal flush (MPG OS+FI group) did not improve recovery of contractile function compared to the one-shot group without MPG. Similarly, MPG added to the terminal flush only improved functional recovery in early reperfusion, however the improvement was not maintained for the entire reperfusion period. There was also no functional improvement when MPG was added to reperfusion solution only (MPG WM group) (Table 5.2a-g, Figures 5.5 and 5.7) suggesting that MPG administered after reperfusion commenced did not protect the myocardium and coronary vasculature from hydroxyl radicals released within seconds of reperfusion. The MPG had to be administered in the 2-minute

terminal flush and reperfusion solution for the prevention of stunning and sustained improved recovery during working mode reperfusion.

In addition to the earlier research linking radicals and myocardial ischaemia and reperfusion injury, more recent studies have found that MPG treatment administered pre-ischaemia and during reperfusion attenuates ischaemia-reperfusion injury by reducing sodium [495] and calcium overload [496], protecting contractile proteins [142,497] and cytoskeletal elements [103], preserving antioxidant reserves, and reducing lipid peroxidation [495,498] and preserving mitochondrial structure and function [495,499] to improve recovery of myocardial contractile function [500].

In summary, the data showed that MPG administered before reperfusion commenced and during early working mode reperfusion provided optimal protection from myocardial stunning after 40-minute warm (32^{o}C) AL one-shot arrest. Future spin-trapping studies are required to assess the nature and timing of the radicals released after 40-minute warm one-shot AL arrest, and identify if the hydroxyl radical is a major contributor to ischemic-reperfusion injury after warm AL arrest.

5.5 Conclusion

This study demonstrated that 40-minute one-shot arrest with AL(200:500) cardioplegia at 32^{o}C resulted in stunning with reduced recovery of function at reperfusion compared with warm intermittent (20 minute re-dosing) AL arrest. The addition of MPG 1mM to the terminal flush and reperfusion buffer of the one-shot arrest protocol improved recovery of function to equivalent to the warm intermittent arrest protocol. Therefore antioxidant adjuncts may be required to maintain cardioprotection during one-shot warm delivery of AL(200:500) cardioplegia.

Chapter 6

Optimising functional recovery following warm and cold AL one-shot arrest

6.1 Introduction

The results of Chapter 5 demonstrated that addition of the antioxidant MPG reversed myocardial, and possibly microvascular, stunning following 40-minute warm (32^{o}C) AL one-shot arrest. The improvement that occurred with added MPG suggests that adenosine 200μM and lignocaine 500μM may not be the optimal concentrations at warm temperatures. The present chapter investigated the effect of increasing adenosine and lignocaine concentrations on myocardial and coronary vascular recovery, and modifications to the arrest and reperfusion protocols to optimise protection.

6.1.1 Aims

The major aims of the present chapter were to:

1) investigate the effect of increasing adenosine and lignocaine in AL cardioplegia during one-shot arrest at 32^{o}C (Study A)
2) optimise protection during warm AL one-shot arrest by modifying the arrest and reperfusion protocol (Study A)
3) compare functional recovery when magnesium or MPG were used as adjuncts during 50-minute warm one-shot arrest and reperfusion (Study B)
4) assess the effect of cold AL one-shot arrest on functional recovery (Study C).

Note on study design: Due to the large number of groups involved, some pilot studies involving 2 to 4 hearts were used to assess changes to the experimental protocol and AL cardioplegia solution in Study A. Data from larger groups treated with the modified arrest and reperfusion methods were collected in Study B of this chapter and in the next chapter (Chapter 7) for further comparison of post-arrest recovery of function using the modified methods.

6.2 Methods

6.2.1 Study A: Examining the effect of increased adenosine and lignocaine concentrations in AL cardioplegia on recovery of function following warm one-shot arrest

AL cardioplegia solutions:

1) Adenosine 200μM + Lignocaine 500μM (AL 200:500)
2) Adenosine 400μM + Lignocaine 1000μM (AL 400:1000)
3) Adenosine 600μM + Lignocaine 1500μM (AL 600:1500)

Experimental groups

Rats were randomly assigned to three experimental groups to compare functional recovery following one-shot arrest at 32^{o}C with AL cardioplegia containing varying concentrations of adenosine and lignocaine using the following protocols:

Table 6.1: Experimental groups in Study A.

Group	Arrest protocol	Reperfusion protocol
1 (n=2)	AL (400:1000) 38 min arrest AL (400:1000) 2 min terminal flush	Working mode, 60 min, KH buffer
2 (n=3)	AL (400:1000) 48 min arrest AL (200:500) 2 min terminal flush	Langendorff mode, 10 min, KH buffer Working mode, 60 min, KH buffer
3 (n=3)	AL (600:1500) 48 min arrest AL (200:500) 2 min terminal flush	Langendorff mode, 10 min,, KH buffer Working mode, 60 min, KH buffer

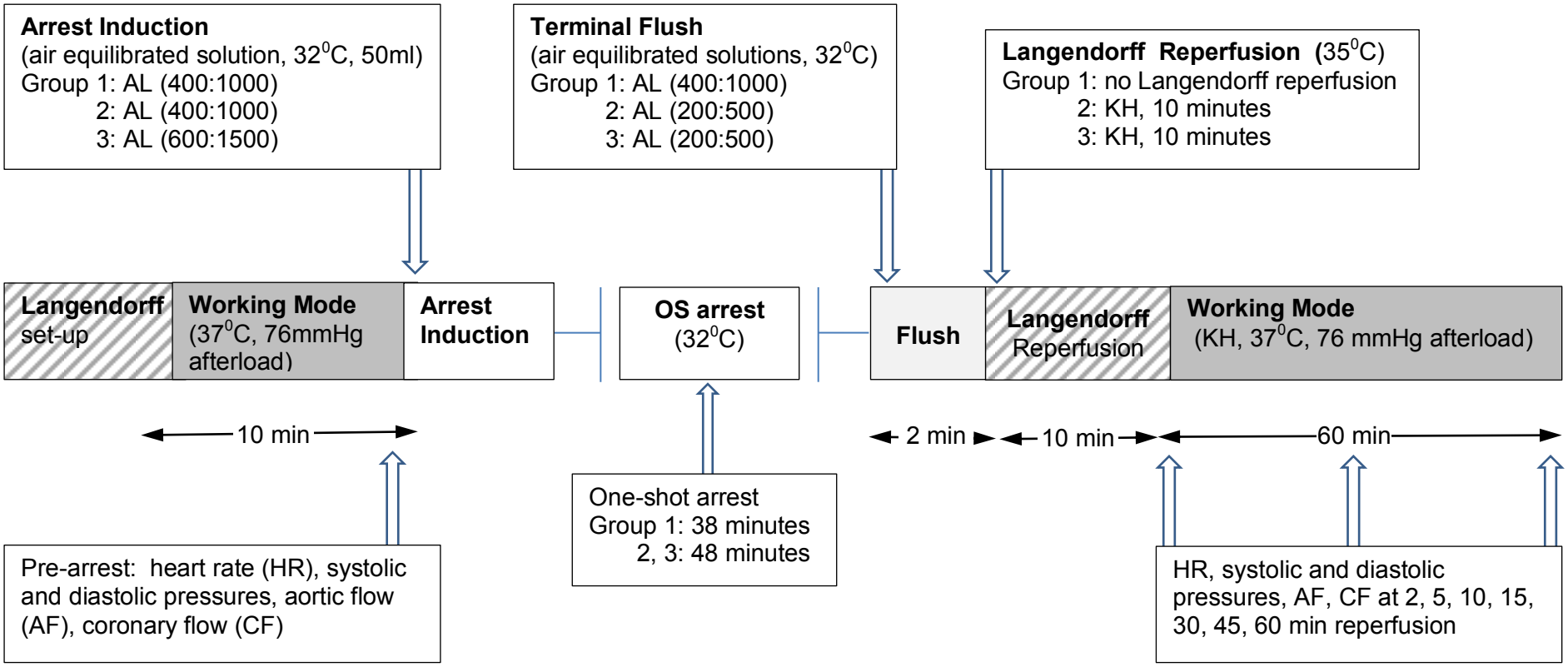


Figure 6.1: Timeline of experimental protocol for Study A: Examining the effect of different adenosine and lignocaine concentrations on recovery of function following 50-minute warm AL one-shot arrest

Arrest and reperfusion protocol for Study A

Hearts were prepared and perfused in working mode as detailed in Chapter 2: Materials and Methods Section 2.5. Arrest solutions were delivered as a single dose via Langendorff mode at 32^{o}C, and the hearts maintained at 32^{o}C during arrest using a water-perfused warming chamber. The terminal flush solution was delivered at 32^{o}C for 2 minutes and the coronary flow measured for determination of coronary vascular resistance.

Hearts in Groups 2 and 3 were initially reperfused in Langendorff (non-working) mode with KH buffer at 35^{o}C for a 10 minute period. Function (heart rate and coronary flow) was monitored at 2, 5 and 10 minutes of Langendorff reperfusion.

All hearts were reperfused in working mode with KH buffer at 37^{o}C for 60 minutes and functional measurements taken at 2, 5, 10, 15, 30, 45 and 60 minutes of reperfusion (Figure 6.1).

6.2.2 Study B: Optimising warm 50-minute AL one-shot arrest

AL cardioplegia and reperfusion solutions:

1) Adenosine 200μm + Lignocaine 500μm (AL 200:500)
2) Adenosine 400μm + Lignocaine 1000μm (AL 400:1000)
3) Adenosine 200μm + Lignocaine 500μm (AL 200:500) + magnesium (Mg^{2+}) (4mM, 2mM, 1mM or 0.075mM)
4) Adenosine 200μm + Lignocaine 500μm (AL200:500) + MPG 1mM
5) Krebs Henseleit buffer + MPG 1mM

Experimental groups

Rats were randomly assigned to six experimental groups (Table 6.2) to compare functional recovery following 50-minute one-shot arrest at 32^{o}C with AL cardioplegia or AL containing MPG or varying concentrations of magnesium using the following protocols.

Table 6.2: Experimental groups in Study B

Group	Arrest protocol	Reperfusion protocol
4 (n=2)	AL (400:1000) 48 min arrest, AL (200:500) + Mg^{2+} 4mM (total Mg^{2+} 4.5mM) terminal flush	Langendorff mode, 10 min,, KH buffer Working mode, 60 min, KH buffer
5 (n=3)	AL (400:1000) 48 min arrest, AL (200:500) + Mg^{2+} 2mM (total Mg^{2+} 2.5mM) terminal flush	Langendorff mode, 10 min, KH buffer Working mode, 60 min, KH buffer
6 (n=2)	AL (400:1000) 48 min arrest, AL (200:500) + Mg^{2+} 1mM (total Mg^{2+} 1.5mM) terminal flush	Langendorff mode, 10 min,, KH buffer Working mode, 60 min, KH buffer
7 (n=2)	AL (400:1000) 48 min arrest, AL (200:500) + Mg^{2+} 0.075mM (total Mg^{2+} 0.575mM) terminal flush	Langendorff mode, 10 min,, KH buffer Working mode, 60 min, KH buffer
8 (n=8)	AL (400:1000) 48 min arrest, AL (200:500) + MPG 1mM terminal flush	Langendorff mode, 10 min,, KH buffer + MPG 1mM Working mode, 60 min, KH buffer
9 (n=8)	AL (400:1000) 48 min arrest, AL (200:500) terminal flush	Langendorff mode, 10 min,, KH buffer Working mode, 60 min, KH buffer

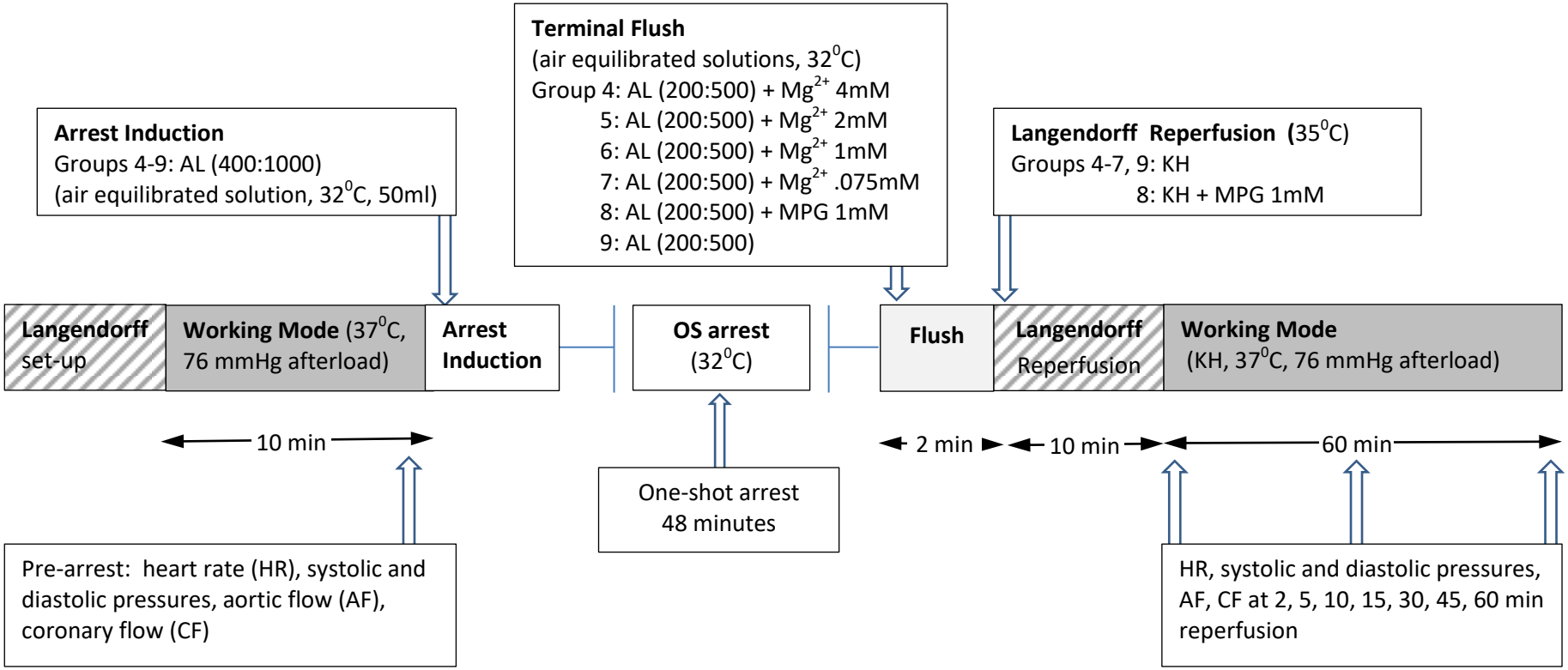


Figure 6.2: Timeline of experimental protocol for Study B: Optimising 50-minute warm AL one-shot arrest

Arrest and reperfusion protocol for Study B

Hearts were prepared and perfused in working mode as detailed in Chapter 2: Materials and Methods Section 2.5. The AL (400:1000) arrest solution was delivered as a single dose via Langendorff mode at 32^{o}C, and the hearts maintained at 32^{o}C during arrest. The terminal flush solution, (AL 200:500) or AL (200:500) with magnesium or MPG adjunct, was delivered at 32^{o}C for 2 minutes and the coronary flow measured for determination of coronary vascular resistance.

Hearts were reperfused in Langendorff mode for 10 minutes at 35^{o}C: groups 4-7 and group 9 were reperfused with KH buffer and hearts in Group 8 with KH buffer + MPG 1mM. Heart rate and coronary flow were recorded at 2, 5 and 10 minutes. All hearts were then reperfused in working mode with KH buffer at 37^{o}C for 60 minutes and functional measurements taken at 2, 5, 10, 15, 30, 45 and 60 minutes of reperfusion (Table 6.2, Figure 6.2).

6.2.3 Study C: Developing a cold 50-minute one-shot AL arrest protocol

AL cardioplegia solutions

1) Adenosine 200μm + Lignocaine 500μm (AL 200:500)
2) Adenosine 400μm + Lignocaine 1000μm (AL 400:1000)
3) Adenosine 400μm + Lignocaine 1000μm (AL 400:1000) + Mg^{2+} 2mM
4) Adenosine 20μm + Lignocaine 50μm (AL 20:50)

Experimental groups:

Rats were randomly assigned to three experimental groups to compare functional recovery following one shot arrest at 8-12^{o}C with AL (400:1000) cardioplegia or AL (400:1000) with magnesium adjunct using the following protocols:

Table 6.3: Experimental groups in Study C

Group	Arrest protocol	Reperfusion protocol
10 (n=3)	AL (400:1000) induction at 32^{o}C, 48 min arrest at 8-12^{o}C, AL (200:500) terminal flush at 26^{o}C	Langendorff mode, 10 min, KH warming from 26^{o}C to 35^{o}C, Working mode, 60 min, KH, 37^{o}C
11 (n=2)	AL (400:1000) induction at 32^{o}C, 48 min arrest at 8-12^{o}C	Langendorff mode, 10 min, AL (20:50) warming from 26^{o}C to 35^{o}C, Working mode, 60 min, KH, 37^{o}C
12 (n=4)	AL (400:1000) + Mg^{2+} 2mM (total Mg^{2+} 2.5mM) induction at 32^{o}C, 48 min arrest at 8-12^{o}C	Langendorff mode, 10 min,, AL (20:50) warming from 26^{o}C to 35^{o}C, Working mode, 60 min, KH buffer

Arrest and reperfusion protocol for Study C

Hearts were prepared and perfused in working mode as detailed in Chapter 2: Materials and Methods Section 2.5. The cardioplegia solution (AL (400:1000) or AL (400:1000) + Mg^{2+} 2mM) was delivered as a single dose via Langendorff mode at 32°C, and the hearts were maintained at 8-12°C during arrest using a cooling chamber water-perfused at 4°C. The cooling chamber was removed after 43 minutes of arrest to allow the hearts to start warming towards room temperature.

A 2-minute AL (200:500) terminal flush was delivered at 26°C to group 10 hearts and the coronary flow measured for determination of coronary vascular resistance. Group 10 was perfused in Langendorff mode with KH buffer for 10 minutes while warming from 26°C to 35°C, and heart rate and coronary flow were recorded at 2, 5 and 10 minutes reperfusion.

Groups 11 and 12 did not receive a terminal flush. Hearts in these two groups were reperfused in Langendorff mode with AL (20:50) for 10 minutes while warming from 26°C to 35°C, and heart rate and coronary flow were recorded at 2, 5 and 10 minutes reperfusion.

All hearts were then reperfused in working mode with KH buffer at 37°C for 60 minutes and functional measurements taken at 2, 5, 10, 15, 30, 45 and 60 minutes of reperfusion (Table 6.3, Figure 6.3).

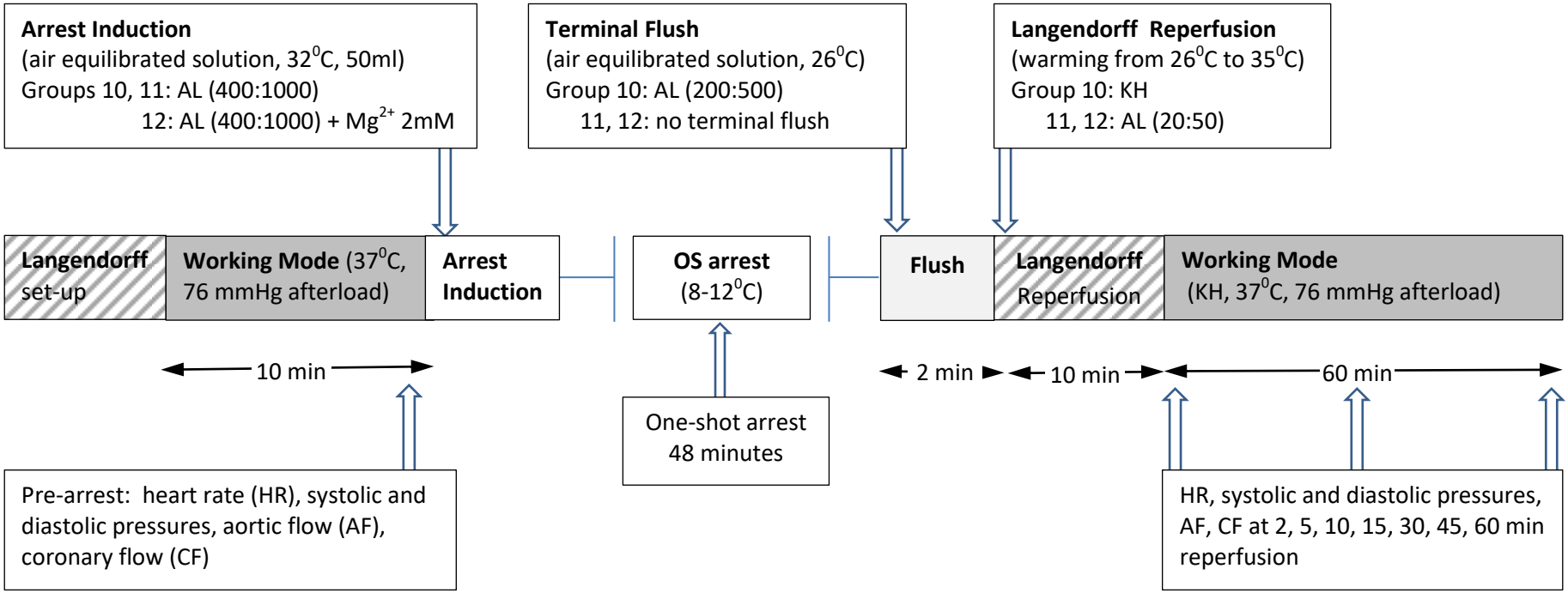


Figure 6.3: Timeline of experimental protocol for Study C: Developing a cold 50-minute one-shot AL arrest and reperfusion protocol

6.3 Results

6.3.1 Results of Study A: Effect of increased adenosine and lignocaine concentrations during warm AL arrest

This study compared the functional outcome of hearts arrested with AL (400:1000) or AL (600:1500) and a terminal flush with AL (400:1000) or AL (200:500) (Figure 6.1). Results are presented in Table 6.4 and Figure 6.4.

Additional hearts were also arrested with AL (200:500) or AL (600:1000) using the 50-minute arrest protocol with AL (200:500) terminal flush. Recovery in these hearts was more variable, with lower cardiac output and stroke volume, therefore these arresting concentrations of AL were not included in the study presented here.

Effect of AL concentration on coronary vascular resistance during 2-minute terminal flush, and time to first spontaneous beat and recovery during Langendorff mode reperfusion

The coronary vascular resistance for groups 1-3, calculated from coronary flow measured during the 2-minute terminal flush, is shown in Table 6.4. There were no significant differences in coronary vascular resistance which varied from 0.27 ± 0.01 to 0.33 ± 0.03 megadyne.sec.cm^{-5}.

The AL (400:1000) OS + AL (400:1000) Flush hearts (group 1) were not reperfused in Langendorff mode, in accordance with the protocol in Chapter 4. This group recorded the slowest time to first beat (9.7 ± 1.1 minutes). Groups 2 and 3 were initially reperfused in Langendorff mode before switching to working mode reperfusion. The time to first beat in Langendorff mode for the AL (400:1000) OS + AL (200:500) Flush group (group 2) was 1.2 ± 0.1 minutes, and for the AL (600:1500) OS + AL (200:500) Flush group (group 3) was 1.7 ± 0.3 minutes, both significantly faster than the group 1 time to first beat (p<0.05) (Figure 6.4).

Table 6.4: Study A: Functional parameters of hearts at terminal flush, 5 minutes and 60 minutes working mode reperfusion following 50-minute arrest at 32°C with one-shot delivery of AL (400:1000) or AL (600:1500) cardioplegia, and AL (400:1000) or AL (200:500) terminal flush.

		5 minutes reperfusion				60 minutes reperfusion			
Arrest Protocol	CVR at 2 minute terminal flush (megadyne.sec.cm^{-5})	% of pre-arrest AF	% of pre-arrest CF	Stroke Volume (ml/beat)	Rate Pressure Product (mmHg/min)	% of pre-arrest AF	% of pre-arrest CF	Stroke Volume (ml/beat)	Rate Pressure Product (mmHg/min)
Group 1 AL (400:1000) OS AL (400:1000)Flush	0.27 ± 0.01	† 0	† 0	† 0	† 0	74 ± 4	þ 105 ± 10	0.22 ± 0.02	38738 ±6863
Group 2 AL (400:1000) OS AL (200:500) Flush	0.33 ± 0.04	70 ± 7	90 ± 3	0.21 ± 0.01	32935 ± 1997	69 ± 6	91 ± 6	0.19 ± 0.01	33260± 924
Group 3 AL (600:1500) OS AL (200:500) Flush	0.33 ± 0.03	57 ± 14	87 ± 2	0.20 ± 0.02	32205 ±1438	42 ± 14	73 ± 4	0.15 ± 0.02	29100 ± 1502

† $p<0.05$: % aortic flow, % coronary flow, stroke volume, rate pressure product: Group 1 compared with groups 2 and 3 at 5 minutes reperfusion

þ $p<0.05$: % coronary flow: Group 1 compared with group 3 at 60 minutes reperfusion

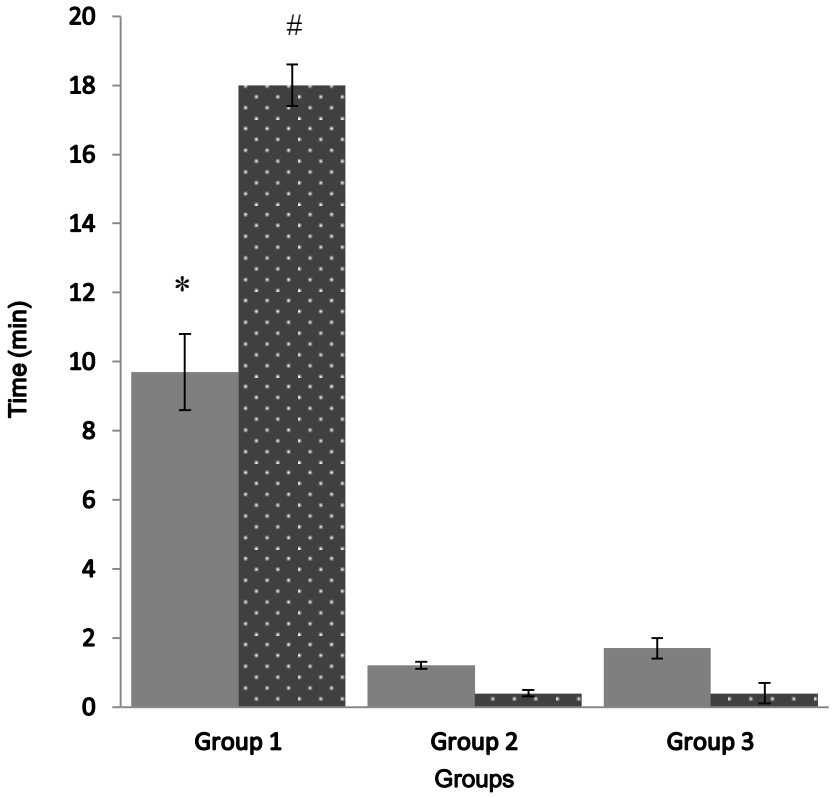


Figure 6.4: Time to first beat and time to aortic flow (minutes) in working mode reperfusion following 50-minute warm one-shot arrest

Time to first beat: ; time to aortic flow:

Group 1: AL (400:1000) OS arrest, AL (400:1000) terminal flush,
Group 2: AL (400:1000) OS arrest, AL (200:500) terminal flush,
Group 3: AL (600:1500) OS arrest, AL (200:500) terminal flush

* $p<0.05$: Time to first beat: Group 1 compared with groups 2 and 3
\# $p<0.05$: Time to aortic flow: Group 1 compared with groups 2 and 3

During Langendorff reperfusion the AL (400:1000) OS + AL (200:500) Flush group (group 2) and AL (600:1500) OS + AL (200:500) Flush group (group 3) recorded heart rates ranging from 71 ± 43 beats/min (group 2) and 19 ± 12 beats/min (group 3) at 2 minutes reperfusion, to 163 ± 19 beats/min (group 2) and 183 ± 20 beats/min (group 3) at 10 minutes reperfusion, which were not significantly different. Similarly, the coronary flow ranged from 12 ± 0.3 ml/min (group 2) and 11 ± 1.5 ml/min (group 3) at 2 minutes to 12 ± 1.2 ml/min (group 2) and 13 ± 0.7 ml/min (group 3) at 10 minutes reperfusion, which were not significantly different.

Effect of AL concentration on functional recovery during 60 minutes working mode reperfusion

The AL (400:1000) OS + AL (400:1000) Flush hearts (group 1) recorded the slowest time to aortic flow of 18.0 ± 0.6 minutes at reperfusion in working mode. Hearts in groups 2 and 3 were initially reperfused in Langendorff mode. The time to aortic flow for the AL (400:1000) OS + AL (200:500) Flush group (group 2) and AL (600:1500) OS + AL (200:500) Flush group (group 3) was 0.4 ± 0.1 minutes and 0.4 ± 0.3 minutes respectively, both significantly faster than the group 1 time (p<0.05) (Figure 6.4).

After achieving aortic flow, the hearts in group1 each recorded a period of tachycardia (450 beats/min) for 2-3 minutes of the first 5 minutes of aortic flow, which resolved spontaneously. Regular rhythm of 240-330 beats/min was established by 30 minutes of reperfusion in this group. Hearts in groups 2 and 3 maintained regular heart rate (263 – 321 beats/min) throughout the 60 minutes of working mode reperfusion.

Group 1 hearts (AL (400:1000) OS + AL (400:1000) Flush) recorded no recovery of aortic flow, coronary flow, stroke volume or rate pressure product at 5 minutes reperfusion. In contrast, at 5 minutes reperfusion, group 2 (AL (400:1000) OS + AL (200:500) Flush) and group 3 (AL (600:1500) OS + AL (200:500) Flush) had recovered 77 ± 4% and 57 ± 14% of pre-arrest aortic flow, respectively, and 100 ± 6% and 87 ± 2% of pre-arrest coronary flow, respectively, and stroke volume of 0.23 ± 0.01 ml/beat and 0.20 ± 0.02 ml/beat, respectively (Table 6.4).

By 30 minutes reperfusion the function of hearts in group 1 (AL (400:1000) OS + AL (400:1000) Flush) had improved, recording 85 ± 3% aortic flow, 94 ± 1% coronary flow and stroke volume of 0.26 ± 0.02 ml/beat. At 60 minutes reperfusion group 1 and group 2 (AL (400:1000) OS + AL (200:500) Flush) were not significantly different in percentage recovery of aortic flow (74 ± 4% and 72 ± 2%, respectively), or coronary flow (105 ± 10% and 94 ± 5%, respectively), or stroke volume (0.22 ± 0.02 and 0.19 ± 0.01 ml/beat, respectively. Functional parameters of group 3 (AL (600:1500) OS + AL (200:500) Flush) had declined at 60 minutes reperfusion, with 42 ± 14% of pre-arrest aortic flow, 73 ± 4% of pre-arrest coronary flow, stroke volume of 0.15 ± 0.02 ml/beat and rate pressure product of 29100 ± 1502 mmHg/min recorded at 60 minutes reperfusion, however only percentage recovery of coronary flow was significantly different from group 1 (p<0.05) (Table 6.4).

6.3.2 Results of Study B: Effect of magnesium and MPG adjuncts during warm AL arrest

a) Addition of magnesium to the terminal AL cardioplegia flush

In this study hearts were arrested for 48 minutes at 32^{o}C using AL (400:1000) followed by a 2-minute terminal flush of AL (200:500) with magnesium 4.0, 2.0, 1.0, or 0.075mM added. Functional recovery of these four groups was compared with hearts arrested with AL (400:1000) and a terminal flush of AL (200:500). The latter protocol, used for group 2 in Study A of this chapter, had resulted in excellent recovery of function. Results are presented in Table 6.5.

Effect of magnesium adjunct on coronary vascular resistance during 2-minute terminal flush, and time to first spontaneous reperfusion beat and time to aortic flow

The coronary vascular resistance for groups 4-7, calculated from coronary flow measured during the terminal flush, is shown in Table 6.5. Coronary vascular resistance varied from 0.25 ± 0.00 megadyne.sec.cm^{-5} in the AL (200:500) + Mg^{2+} 1mM flush group (group 6) to 0.38 ± 0.02 megadyne.sec.cm^{-5} in the AL (200:500) + Mg^{2+} 0.075mM flush group (group 7), with a significant difference between these two groups (p<0.05).

The time to first beat at reperfusion ranged from 1.1 ± 0.1 minutes for the AL (200:500) + Mg^{2+} 1mM flush group (group 6) and AL (200:500) + Mg^{2+} 2mM flush group (group 5), to 1.6 ± 0.2 minutes for the AL (200:500) + Mg^{2+} 0.075mM flush group (group 7). There were no significant differences in time to first beat between the groups.

The slowest time to aortic flow of 0.5 ± 0.1 minutes was recorded for the AL (200:500) + Mg^{2+} 4mM flush group (group 4) compared with the fastest time in group 5 (0.2 ± 0.1 minutes). There were no significant differences in time to aortic flow between the groups.

Table 6.5: Study B: Functional parameters of hearts at terminal flush, 5 minutes and 60 minutes working mode reperfusion following 50-minute one-shot arrest at 32°C with AL (400:1000) cardioplegia and AL (200:500) + magnesium (4, 2, 1 or 0.075mM) terminal flush.

		5 minutes reperfusion				60 minutes reperfusion			
Terminal Flush Solution	CVR during terminal flush (megadyne.sec.cm^{-5})	% of pre-arrest AF	% of pre-arrest CF	Stroke Volume (ml/beat)	Rate Pressure Product (mmHg/min)	% of pre-arrest AF	% of pre-arrest CF	Stroke Volume (ml/beat)	Rate Pressure Product (mmHg/min)
Group 4 AL (200:500) + Mg^{2+} 4 mM	0.30 ± 0.02	76 ± 1	106 ± 6 ƥ	0.23 ± 0.02	36400 ± 1400 ƥ	66 ± 7	97 ± 9	0.19 ± 0.01	36225 ±1150 ƥ
Group 5 AL (200:500) + Mg^{2+} 2 mM	0.32 ± 0.04	51 ± 16	85 ± 5	0.18 ± 0.04	30417 ± 5512	28 ± 12	74 ± 11	0.11 ± 0.03	29100 ± 2802
Group 6 AL (200:500) + Mg^{2+} 1 mM	0.25 ± 0.00 †	71 ± 6	105 ± 14	0.20 ± 0.01	36675 ± 3325	49 ± 22	82 ± 9	0.15 ± 0.05	32825 ± 2825
Group 7 AL (200:500) + Mg^{2+} 0.075 mM	0.38 ± 0.02	54 ± 7	64 ± 4	0.20 ± 0.01	27900 ± 1500	53 ± 13	61 ± 1	0.17 ± 0.03	27800 ± 800

† $p<0.05$ Coronary vascular resistance (CVR): Group 6 compared with group 7

ƥ $p<0.05$ % coronary flow and rate pressure product at 5 minutes, rate pressure product at 60 minutes: Group 4 compared with group 7

Effect of magnesium adjunct on functional recovery during 60 minutes working mode reperfusion

At 5 minutes reperfusion group 4 (AL (200:500) + Mg^{2+} 4mM flush) had recovered the highest percentage of pre-arrest aortic flow (76 ± 1%), and percentage of pre-arrest coronary flow (106 ± 6%), stroke volume (0.23 ± 0.02 ml/beat) and rate pressure product (36400 ± 1400 mmHg/min) compared with groups 5-7 (Table 6.5). These results were significantly different from the group 7 (AL (200:500) + Mg^{2+} 0.075mM flush) hearts in recovery of coronary flow, cardiac output and rate pressure product. Although group 4 (with 4mM Mg^{2+} adjunct) recorded the highest functional recovery in this study, comparison with results from group 2 (AL (400:1000) OS + AL (200:500) flush) in Study A of this chapter (77 ± 4% aortic flow, 100 ± 6% coronary flow, 0.23 ± 0.01 ml/beat stroke volume and 37790 ± 2166 mmHg/min rate pressure product (Table 6.2)), showed no significant difference in recovery of function at 5 minutes reperfusion.

At 60 minutes reperfusion group 4 (AL (200:500) + Mg^{2+} 4mM flush) continued to record the highest percentage recovery of aortic flow (66 ± 7%), coronary flow (97 ± 9%), and stroke volume (0.19 ± 0.01 ml/beat) compared with groups 5-7, however only group 4 and group 7 (AL (200:500) + Mg^{2+} 0.075mM flush) were significantly different in rate pressure product at 60 minutes reperfusion (p<0.05) (Table 6.5). In comparison, at 60 minutes reperfusion, the group 4 results in this study remained not significantly different from the group 2 (AL (400:1000) OS + AL (200:500) flush) hearts from Study A which recorded 72 ± 2% recovery of aortic flow, 94 ± 5% coronary flow, stroke volume of 0.19 ± 0.01 ml/beat and rate pressure product of 38154 ± 1341 mmHg/min (Table 6.2).

b) Addition of MPG to the terminal AL cardioplegia flush and Langendorff reperfusion buffer

This study compared functional outcomes between the AL+MPG group (group 8: AL (400:1000) OS, AL (200:500)+MPG 1mM terminal flush, KH + 1mM MPG Langendorff mode reperfusion) and the AL group (group 9: AL (400:1000) OS, AL (200:500) terminal flush). The results of the Chapter 4 study showed that MPG 1mM adjunct improved recovery of hearts arrested for 40 minutes with AL (200:500), therefore the AL+MPG group (group 8) was included in this study to assess whether MPG 1mM adjunct improved recovery of function when AL (400:1000) was used as the

cardioplegic solution. Results of this study are presented in Table 6.6a - c, and Figure 6.5a and b.

Effect of MPG adjunct on coronary vascular resistance during 2-minute terminal flush, and time to first spontaneous reperfusion beat and functional recovery during Langendorff reperfusion

The coronary vascular resistance during the 2-minute terminal flush for the AL+MPG group (group 8) was 0.32 ± 0.03 megadyne.sec.cm^{-5}, which was not significantly different from the coronary vascular resistance (0.28 ± 0.01 megadyne.sec.cm^{-5}) of the AL group (group 9).

There was no significant difference in time to first beat between the AL+MPG group and the AL group (0.9 ± 0.1 min and 1.2 ± 0.1 min, respectively). No significant differences in heart rate or coronary flow were recorded between the AL+MPG group and the AL group at 2, 5 and 10 minutes Langendorff reperfusion (Table 6.6a).

Effect of MPG adjunct on functional recovery during 60 min working mode reperfusion

There was no significant difference in time to aortic flow between the AL+MPG group (group 8) and the AL group (group 9) (1.0 ± 0.4 min and 0.4 ± 0.1 min, respectively). There was a significant difference between the AL group and the AL+MPG group in percentage recovery of aortic flow and cardiac output, and rate pressure product at 2, 5, 10, 15, 30, 45 and 60 minutes working mode reperfusion (p<0.05). The AL group also recovered significantly higher heart rate at 2 minutes reperfusion, systolic pressure at 5 and 45 minutes reperfusion, and aortic flow and cardiac output at 2, 45 and 60 minutes working mode reperfusion (p<0.05) (Figures 6.5a and b, Tables 6.6b and c).

Table 6.6 a: Study B: Functional parameters of hearts pre-arrest and during Langendorff reperfusion following 50-minute AL (400:1000) one-shot arrest at 32°C, AL (200:500) or AL (200:500) + MPG 1mM flush, and reperfusion with KH or KH + MPG 1mM (n=8).

Arrest Protocol	Group	Heart Rate (beats/min)	Systolic Pressure (mmHg)	Diastolic Pressure (mmHg)	Aortic Flow (ml/min)	Coronary Flow (ml/min)	% of pre-arrest CO	Stroke Volume (ml/beat)	Rate Pressure Product (mmHg/min)
Pre-Arrest	AL	304 ± 7	132 ± 3	60 ± 1	60 ± 2	20 ± 1	n/a	0.26 ± 0.01	40009 ± 1046
	AL + MPG	296 ± 7	130 ± 2	60 ± 1	62 ± 3	19 ± 1	n/a	0.28 ± 0.01	38475 ± 867
Langendorff Reperfusion 2 min	AL	107 ± 24	n/a	n/a	n/a	13 ± 1	n/a	n/a	n/a
	AL + MPG	59 ± 17	n/a	n/a	n/a	13 ± 1	n/a	n/a	n/a
Langendorff Reperfusion 5 min	AL	170 ± 8	n/a	n/a	n/a	16 ± 1	n/a	n/a	n/a
	AL + MPG	149 ± 23	n/a	n/a	n/a	14 ± 1	n/a	n/a	n/a
Langendorff Reperfusion 10 min	AL	183 ± 9	n/a	n/a	n/a	16 ± 1	n/a	n/a	n/a
	AL + MPG	166 ± 24	n/a	n/a	n/a	14 ± 1	n/a	n/a	n/a

Table 6.6 b: Functional parameters of hearts during working mode reperfusion (2-15 minutes) following 50-minute AL (400:1000) one-shot arrest at 32°C, AL (200:500) or AL (200:500) + MPG 1mM terminal flush, and Langendorff reperfusion with KH or KH + MPG 1mM (n=8).

Arrest Protocol	Group	Heart Rate (beats/min)	Systolic Pressure (mmHg)	Diastolic Pressure (mmHg)	Aortic Flow (ml/min)	Coronary Flow (ml/min)	% of pre-arrest CO	Stroke Volume (ml/beat)	Rate Pressure Product (mmHg/min)
		#			#		#		
Working Mode Reperfusion 2 min	AL	292 ± 8	127 ± 4	60 ± 1	46 ± 3	20 ± 2	81 ± 4	0.23 ± 0.01	39892 ± 3025
	AL + MPG	231 ± 25	114 ± 6	60 ± 4	28 ± 6	16 ± 1	55 ± 8	0.19 ± 0.02	27221 ± 3521
			#				#		#
Working Mode Reperfusion 5 min	AL	294 ± 10	126 ± 2	61 ± 1	46 ± 3	20 ± 1	83 ± 3	0.23 ± 0.01	37790 ± 2166
	AL + MPG	269 ± 7	119 ± 2	64 ± 1	39 ± 3	18 ± 2	70 ± 4	0.21 ± 0.01	32125 ± 753
							#		#
Working Mode Reperfusion 10 min	AL	297 ± 11	126 ± 3	61 ± 1	49 ± 3	19 ± 1	86 ± 3	0.23 ± 0.01	38125 ± 1816
	AL + MPG	278 ± 7	121 ± 2	64 ± 1	41 ± 3	18 ± 2	72 ± 4	0.21 ± 0.01	33541 ± 909
							#		#
Working Mode Reperfusion 15 min	AL	304 ± 13	125 ± 3	61 ± 1	50 ± 2	19 ± 1	87 ± 2	0.23 ± 0.01	37909 ± 1264
	AL + MPG	285 ± 5	121 ± 2	64 ± 1	43 ± 3	18 ± 2	75 ± 3	0.21 ± 0.01	34572 ± 856

# $p<0.05$ AL + MPG group (group 8) compared with AL group (group 9)

Table 6.6 c: Functional parameters of hearts during working mode reperfusion (30-60 minutes) following 50-minute AL (400:1000) one-shot arrest at 32°C, AL (200:500) or AL (200:500) + MPG 1mM terminal flush, and Langendorff reperfusion with KH or KH + MPG 1mM (n=8).

Arrest Protocol	Group	Heart Rate (beats/min)	Systolic Pressure (mmHg)	Diastolic Pressure (mmHg)	Aortic Flow (ml/min)	Coronary Flow (ml/min)	% of pre-arrest CO	Stroke Volume (ml/beat)	Rate Pressure Product (mmHg/min)
Working Mode Reperfusion 30 min	AL	308 ± 11	124 ± 2	61 ± 1	49 ± 3	19 ± 1	85 ± 2 #	0.22 ± 0.01	38256 ± 1301 #
	AL + MPG	292 ± 7	119 ± 2	64 ± 1	42 ± 3	17 ± 2	72 ± 4	0.20 ± 0.01	34662 ± 975
Working Mode Reperfusion 45 min	AL	316 ± 12	122 ± 2 #	63 ± 1	46 ± 2 #	19 ± 1	82 ± 2 #	0.21 ± 0.01	38497 ± 1416 #
	AL + MPG	296 ± 6	114 ± 2	66 ± 1	38 ± 3	17 ± 2	68 ± 4	0.19 ± 0.01	33616 ± 871
Working Mode Reperfusion 60 min	AL	321 ± 11	119 ± 3	64 ± 1	43 ± 2 #	19 ± 1	77 ± 3 #	0.19 ± 0.01	38154 ± 1341 #
	AL + MPG	297 ± 6	113 ± 2	66 ± 2	34 ± 3	17 ± 1	62 ± 5	0.17 ± 0.01	33514 ± 1009

# $p<0.05$ AL + MPG group (group 8) compared with AL group (group 9)

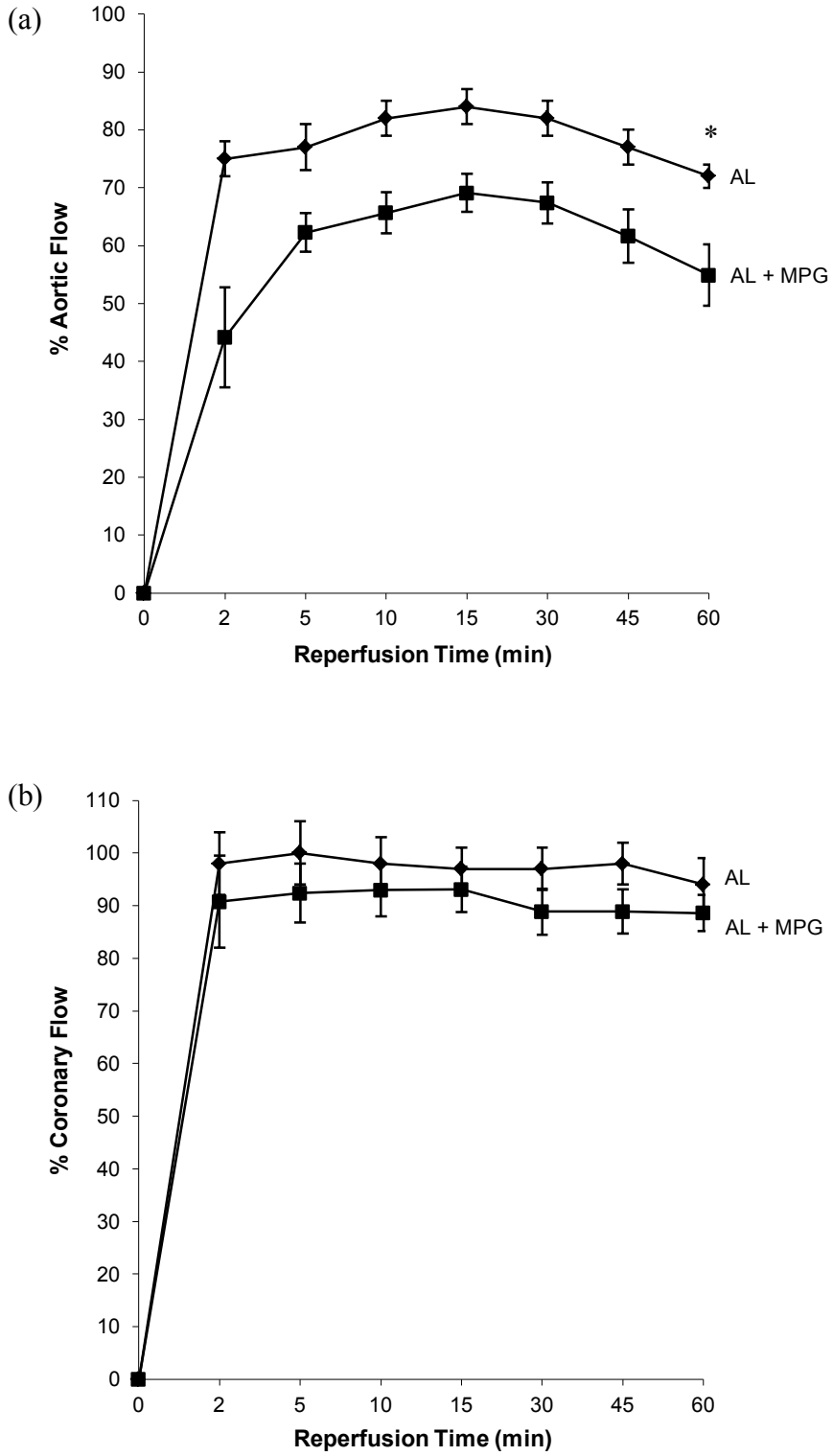


Figure 6.5: Percentage return of (a) aortic flow and (b) coronary flow during 60 minutes working mode reperfusion following 50-minute warm one-shot arrest with AL (400:1000) cardioplegia and reperfusion with AL (200:500) or AL (200:500) + MPG 1mM

* $p<0.05$ % aortic flow: AL group (group 9) compared with AL + MPG group (group 8)

6.3.3 Results of Study C: Effect of magnesium adjunct during cold AL arrest

This study compared the functional outcome of hearts arrested at cold temperatures using AL (400:1000) one-shot arrest + AL (200:500) terminal flush (group 10), AL (400:1000) one-shot arrest without a terminal flush (Group 11) or AL (400:1000) + magnesium 2mM one-shot arrest and no terminal flush (group 12). Results are presented in Table 6.7 and Figure 6.6.

Effect of cold arrest on time to first spontaneous reperfusion beat and recovery in Langendorff mode

There was no significant difference in time to first reperfusion beat between the groups, ranging from 0.4 ± 0.1 minutes to 0.6 ± 0.1 minutes (Figure 6.6).

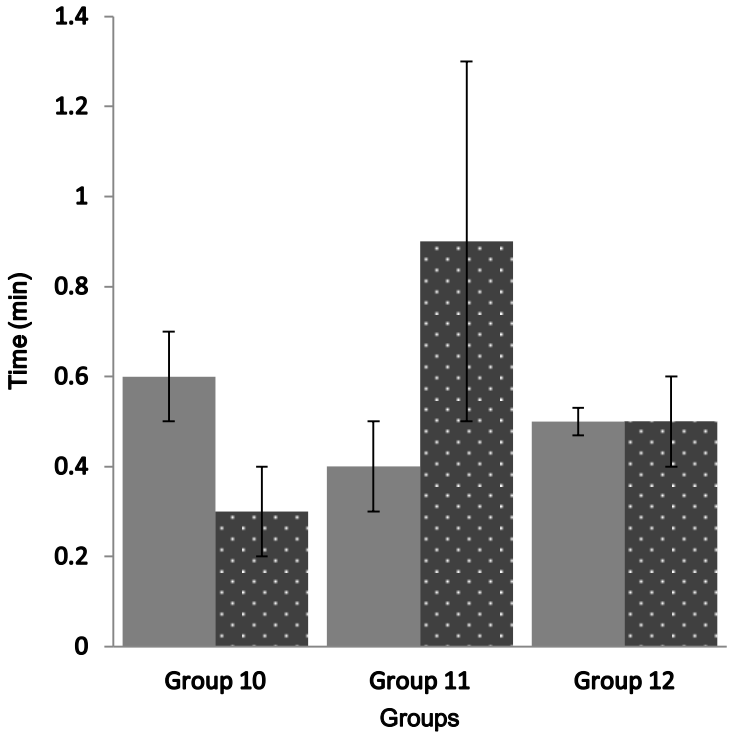


Figure 6.6: Time to first beat and time to aortic flow (minutes) in working mode reperfusion following 50-minute cold one-shot arrest

Time to first beat ; time to aortic flow

Group 10: AL (400:1000) one-shot arrest, AL (200:500) terminal flush
Group 11: AL (400:1000) one-shot arrest, no terminal flush,
Group 12: AL (400:1000) + Mg^{2+} 2mM one-shot arrest, no terminal flush

Table 6.7: Study C: Functional parameters of hearts at terminal flush, 5 minutes and 60 minutes working mode reperfusion following 50-minute one-shot cold arrest (8-12°C) with AL (400:1000) cardioplegia, or AL (400:1000) + Mg^{2+} cardioplegia.

		5 minutes reperfusion				60 minutes reperfusion			
Arrest Protocol	CVR at 10 minutes Langendorff reperfusion (megadyne.sec.cm^{-5})	% of pre-arrest AF	% of pre-arrest CF	Stroke Volume (ml/beat)	Rate Pressure Product (mmHg/min)	% of pre-arrest AF	% of pre-arrest CF	Stroke Volume (ml/beat)	Rate Pressure Product (mmHg/min)
Group 10 AL (400:1000) OS AL (200:500) flush	0.30 ± 0.00	85 ± 9	98 ± 2 †	0.23 ± 0.02	35605 ± 3474	55 ± 14	99 ± 5 †	0.16 ± 0.02	35525 ± 3103
Group 11 AL (400:1000) OS No terminal flush	0.34 ± 0.07	63 ± 26	100 ± 11	0.22 ± 0.05	28900 ± 4900 þ	74 ± 14	103 ± 3 þ	0.21 ± 0.02	34325 ± 1925 þ
Group 12 AL (400:1000) + Mg^{2+} 2mM OS No terminal flush	0.30 ± 0.02	82 ± 1	122 ± 2	0.25 ± 0.01	34438 ± 654	75 ± 4	127 ± 6	0.20 ± 0.01	40038 ± 902

† $p<0.05$ % coronary flow at 5 minutes and 60 minutes, group 10 compared with group 12

þ $p<0.05$ Rate pressure product at 5 minutes and % coronary flow and rate pressure product at 60 minutes, group 11 compared with group 12

During Langendorff reperfusion the AL (400:1000) OS + AL(200:500) terminal flush group (group 10) recorded heart rates ranging from 65 ± 15 beats/min at 2 minutes to 167 ± 7 beats/min at 10 minutes reperfusion with coronary flows of 16 ± 0.2 ml/min. The AL(400:1000) OS + AL (20:50) Langendorff (group 11), recorded heart rate of 45 ± 5 beats/min and coronary flow of 14 ± 3 ml/min at 2 minutes, and heart rate of 126 ± 6 beats/min and coronary flow of 15 ± 3 ml/min at 10 minutes reperfusion, with a significant difference in heart rate between groups 10 and 11 at 10 minutes reperfusion (p<0.05). The heart rate of the AL (400:1000) + Mg^{2+} group (group 12) ranged from 68 ± 21 beats/min at 2 minutes to 135 ± 1.6 beats/min at 10 minutes, with coronary flow of 16 ± 1 ml/min. There were no significant differences in heart rate between groups 10 and 12 or groups 11 and 12, and no significant differences in coronary flow or coronary vascular resistance between the 3 groups during Langendorff reperfusion (Table 6.7).

Effect of cold arrest on functional recovery during 60 min working mode reperfusion

The shortest time to aortic flow of 0.30 ± 0.1 minutes was recorded in the AL (400:1000) OS + AL (200:500) terminal flush group (group 10), while group 11 (AL(400:1000) OS + AL (20:50) Langendorff) recorded the longest time to aortic flow of 0.9 ± 0.4 minutes, with no significant differences between groups (Figure 6.6).
There were no significant differences in functional recovery between groups 10 and 11 during working mode reperfusion (Table 6.7). At 5 minutes of working mode reperfusion there was a significant difference between the AL (400:1000) + Mg^{2+} group (group 12) and the AL (400:1000) OS + AL(200:500) terminal flush group (group 10) in percentage recovery of coronary flow and cardiac output, and at 60 minutes reperfusion in percentage recovery of coronary flow (p<0.05). Similarly, there was a significant difference between group 12 and group 11 (AL(400:1000) OS + AL (20:50) Langendorff) in percentage recovery of cardiac output and rate pressure product at 5 minutes, and in percentage recovery of coronary flow and rate pressure product at 60 minutes working mode reperfusion (p<0..05) (Table 6.7). Group 12 had the most rapid and consistent return of function, with the lowest intra-group variation in functional measurements, which was maintained throughout the 60 minutes of working mode reperfusion.

6.4 Discussion

The previous chapter showed that the addition of 1mM MPG to the terminal flush and reperfusion buffer after 40-minute one-shot arrest with AL(200:500) led to improved recovery of function at reperfusion, and reversal of stunning. In this chapter the effect of varying the concentrations of adenosine and lignocaine in AL cardioplegia on recovery was assessed, and the arrest and reperfusion protocols were modified to improve functional outcome at reperfusion. Doubling the concentrations of adenosine and lignocaine to AL(400:1000) led to functional superiority following 50-minute one-shot arrest at 32^{o}C. Addition of MPG or magnesium did not significantly improve functional recovery following warm arrest, however addition of 2mM magnesium to AL (400:1000) did improve functional recovery following cold arrest. These results are now discussed.

6.4.1 Doubling adenosine and lignocaine levels during AL warm one-shot arrest led to significantly improved outcomes that were not further improved by MPG or magnesium

The present study showed that doubling the adenosine and lignocaine concentrations in AL cardioplegia (AL(400:1000)) during warm one-shot arrest with a terminal flush of AL(200:500) led to improved functional recovery during 60 minutes reperfusion compared to recovery following AL(200:500) one-shot arrest and terminal flush reported in Chapter 5. AL(400:1000) hearts recovered 77% aortic flow and 100% coronary flow at 5 minutes working mode reperfusion, and 72% aortic flow and 94% coronary flow at 60 minutes reperfusion (Table 6.4). In contrast, the AL(200:500) hearts recorded no aortic flow and 3% coronary flow at 5 minutes reperfusion, and recovered 75% aortic flow and 80% coronary flow at 60 minutes reperfusion (Figures 5.4 and 5.6).

Addition of 4mM Mg^{2+} to the terminal flush following warm AL(400:1000) arrest resulted in the highest recovery of reperfusion parameters compared with lower magnesium concentrations (Table 6.5), but did not improve return of function compared with the AL(400:1000) hearts without added magnesium (Table 6.6c). At 5 minutes reperfusion, recovery of aortic flow in the AL with added magnesium group was 76% and coronary flow recovery 106%, and at 60 minutes aortic flow was 66% and coronary flow was 97%, which was not significantly different from recovery in the AL(400:1000) group.

Addition of MPG to the AL(400:1000) protocol reduced recovery in most functional parameters compared with AL(400:1000) arrest without MPG (Figure 6.5). For example, the AL(400:1000)+MPG group recorded return of 62% aortic flow and 70% cardiac output at 5 minutes reperfusion, and 55% aortic flow and 62% cardiac output at 60 minutes reperfusion. In comparison, the AL(400:1000) group recovered significantly higher returns with 77% aortic flow and 83% cardiac output at 5 minutes and 72% aortic flow and 77% cardiac output at 60 minutes reperfusion.

The underlying mechanisms for improved protection with AL(400:1000) at warm (32^{o}C) temperatures are not known. It was shown in Chapter 5 that the functional loss in warm arrested AL (200:500) hearts was reversed with MPG, suggesting involvement of ischemia-induced generation of hydroxyl radicals [83]. In this chapter, the doubling of AL concentrations resulted in adenosine, lignocaine, or the combination reducing ischaemia-reperfusion injury, and possibly reducing the formation of hydroxyl ions or other reactive oxygen or nitrogen species.

Adenosine and lignocaine have each been shown to inhibit generation of reactive oxygen species [391,403], individually in a dose-dependent manner and also in a synergistic manner [454]. As mentioned previously, hydroxyl radicals are formed by redox-reduced mitochondrial electron transport chain carriers during reoxygenation of ischaemic tissue at reperfusion [501] and by interactions between other reactive oxygen and nitrogen species [502-504]. Hydroxyl ions are considered amongst the most reactive and toxic, and least selective, of all radicals, and therefore the most damaging [505,506]. In 1992 Das and Misra reported that lignocaine inhibited DMPO-hydroxyl adduct formation in a dose-dependent manner, with elimination of DMPO-OH adduct formation at a lignocaine concentration of 300μM [407]. Additionally, Mikawa and colleagues [507] and Shi and colleagues [454] showed that lignocaine significantly decreased the production of reactive oxygen species by neutrophils. It is therefore possible that the added protection derived from doubling the adenosine and lignocaine was the result of the increased lignocaine concentration and possible blunting of hydroxyl ion formation and downstream reactions.

Adenosine inhibits production of oxygen-derived free radicals by stimulating A_2 receptors [211,508,509] and possibly by inhibiting mitochondrial metabolism [222,475] during ischaemia reperfusion [510]. However, it is unclear at present if adenosine directly inhibits hydroxyl ion formation. In rat brain striatum, for example, adenosine receptor agonists and antagonists have paradoxical effects in reducing hydroxyl radical generation by L-DOPA [511]. Adenosine, via A_1 receptor activation, also has K_{ATP} channel opening properties and Obata has shown that opening this channel can cause hydroxyl radical generation during ischemia [512]. Obata further showed that hydroxyl ion production can result from membrane depolarisation, which would be unlikely in the present study because normokalaemic AL cardioplegia arrests at polarised membrane potentials, as shown in Chapter 4. Further studies are required to investigate the different contributions of adenosine, lignocaine and AL to improved cardioprotection in the rat heart model.

Addition of MPG to the warm AL(400:1000) protocol significantly decreased functional recovery during reperfusion. MPG reduces hydroxyl radical generation, scavenges hydroxyl radicals and inhibits hydroxyl radical reactions during ischaemia [83,486] and at reperfusion [486]. MPG also scavenges the peroxynitrite anion formed from the reaction between nitric oxide and superoxide [485] (Figure 6.7). Paradoxically, reactive nitrogen species such as peroxynitrite have been implicated in cellular damage and antioxidant depletion [513] and protective effects on myocardium and endothelium [514-516]. Reactive oxygen species produced within the mitochondria have also been shown to act as signalling molecules to stimulate protective pathways that improve cardioprotection during ischaemia [517-519]. Additionally, the cardioprotective effect involving generation of reactive oxygen species can be abolished by MPG [487,517,520]. Electron paramagnetic resonance studies may be required to demonstrate the effects of adenosine, lignocaine and MPG on levels of reactive oxygen and nitrogen species during warm AL arrest and reperfusion, and the link to increased or reduced cardioprotection.

Doubling the concentrations of adenosine and lignocaine in AL cardioplegia resulted in higher return of aortic flow and coronary flow after 50-minute one-shot arrest at 32°C. The addition of MPG or raised Mg^{2+} levels did not improve recovery during reperfusion. Further studies are required to show the sites and complex underlying mechanisms of AL cardioprotection.

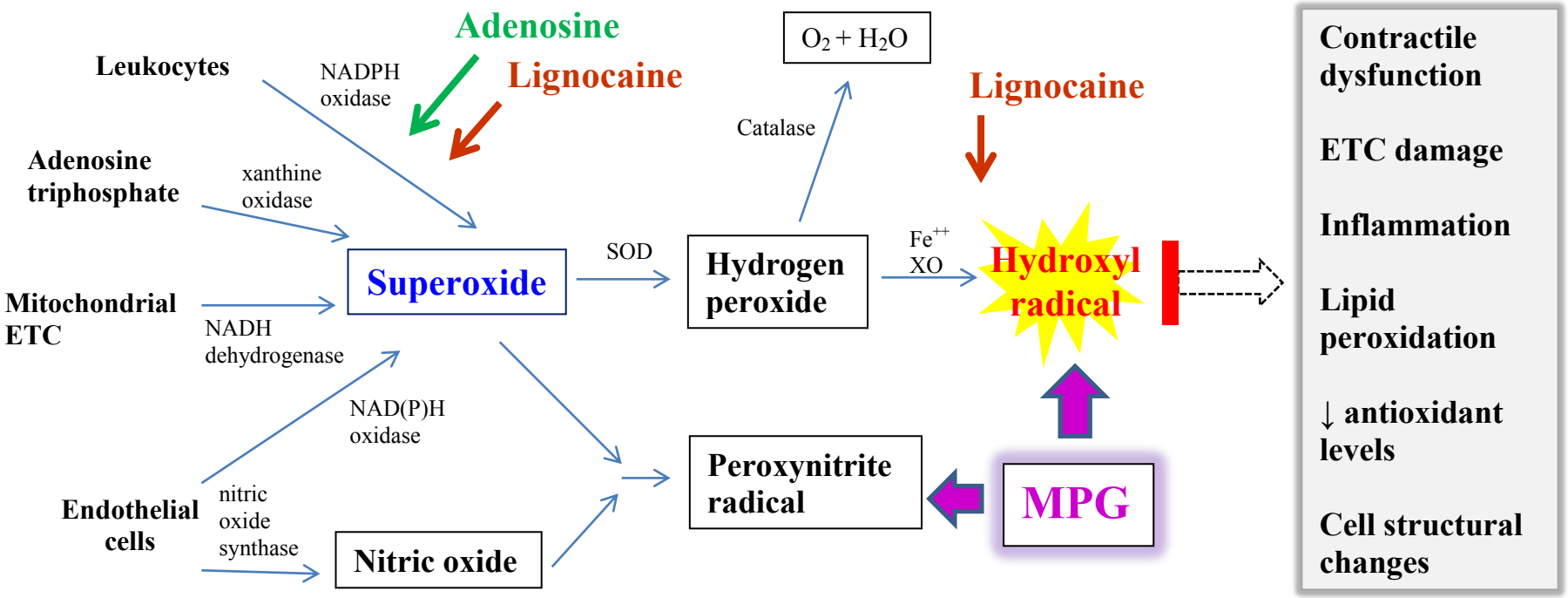


Figure 6.7 : Diagram illustrating pathways of superoxide and hydroxyl radical generation during ischaemia and at reperfusion, and the actions of adenosine, lignocaine and MPG to reduce radical-induced reperfusion injury.

Adenosine and lignocaine inhibit production of reactive oxygen species, and N-(2-mercaptopropionyl)-glycine (MPG) scavenges hydroxyl radicals (intracellular and extracellular) and peroxynitrite radicals (adapted from Zweier and Talukder, 2006; Vinten-Johansen *et al*, 1999).

6.4.2 Added magnesium during cold arrest improved functional recovery

Addition of magnesium to the group 11 protocol (AL(400:1000) arrest, AL(20:50) Langendorff reperfusion) further improved recovery following cold arrest, with more rapid and consistent reanimation and improved functional parameters maintained during the working mode reperfusion period. For example, AL with added magnesium returned 122 ± 2% coronary flow at 5 minutes reperfusion, and 127 ± 6% coronary flow and 75 ± 4% aortic flow at 60 minutes reperfusion. In contrast, AL without magnesium resulted in recovery of 98 ± 2% coronary flow at 5 minutes, and 99 ± 5% coronary flow and 55 ± 14% aortic flow at 60 minutes reperfusion.

As previously mentioned, magnesium is a naturally occurring calcium antagonist which reduces calcium loading associated with ischaemia-reperfusion injury. Magnesium's ability to limit intracellular calcium accumulation during ischaemia [476,521-523] and reduce potassium-induced vasoconstriction [182,524] have led to its inclusion as a component in hyperkalaemic cardioplegia. A recent clinical study by Onorati and colleagues, using cold AL microplegia supplemented with magnesium, reported reduced markers of myocardial damage and improved post-operative outcome compared with cold hyperkalaemic cardioplegia [413]

Additionally, magnesium prevents ATP depletion [525] and also preserves adenosine triphosphatase activity [526] which is reduced by up to 70% when mitochondria are exposed to 30 to 40 minutes of hypothermia (4^{o}C) [481]. In contrast, maintaining warm temperatures during nondepolarising cardioplegia has been shown to assist in preserving polarised arresting potentials, reducing calcium loading and improving supply of ATP [443]. In the present study, the addition of magnesium to AL cardioplegia may have afforded extra cardioprotection or protected against deleterious effects of hypothermia during cold AL one-shot arrest in the isolated rat heart model.

6.5 Conclusion

The first study in this chapter showed that 50-minute warm (32^{o}C) one-shot arrest using AL(400:1000) resulted in excellent functional recovery at reperfusion, and superior function compared with recovery following 40-minute warm one-shot arrest with AL(200:500) in Chapter 5. MPG did not add any further protection, indicating that the higher AL levels reduced ischemia-reperfusion injury possibly due to hydroxyl radicals. Increasing magnesium levels in the terminal flush to 4.5mM also did not improve recovery following warm AL(400:1000) arrest (Study B). However in Study C, 2.5mM magnesium in the arresting solution significantly improved recovery following cold AL(400:1000) arrest, which highlights the effect of hypothermia on post-arrest functional recovery. The results of this chapter, showing comparable recovery following warm or cold arrest with AL cardioplegia, may be useful to support changing current practices since many surgeons are reluctant to change from cold to warm cardioplegia delivery.

Chapter 7

Comparing normokalaemic AL cardioplegia with del Nido solution at warm and cold temperatures

7.1 Introduction

Hyperkalemic del Nido solution, designed in the 1990s as a paediatric cardioplegia solution [371,372], contains 24mM potassium and a very low calcium concentration (0.24mM), magnesium as a calcium competitor (~7mM), and lignocaine (0.36mM) to stabilise the myocyte membrane. More recent formulations of a Plasma-lyte A based del Nido cardioplegia solution are mixed with whole blood (20%) to improve buffering, promote aerobic and anaerobic metabolism, and reduce oxygen radical production and ischaemic stress [371].

In the previous chapter, the modified arrest and reperfusion protocol using normokalaemic AL (400:1000) solution resulted in significantly higher functional recovery following 50-minute one-shot arrest at 32^{o}C. No additional benefit was demonstrated by augmenting the cardioplegia terminal flush or Langendorff reperfusion solutions with magnesium or the antioxidant MPG during the warm arrest protocol, however the addition of magnesium to cold AL cardioplegia (ALM) improved functional recovery to comparable to warm AL arrest. In this chapter normokalaemic AL solution was compared with hyperkalaemic del Nido solution (Table 7.1) at warm and cold arresting temperatures.

7.1.1 Aim

The aim of this chapter was to assess and compare functional recovery of hearts after 50-minute one-shot arrest with AL (400:1000), ALM or del Nido solution at warm (32^{o}C) or cold (8-12^{o}C) temperatures.

7.1.2 Hypothesis

The hypothesis for this study was that 50-minute one-shot arrest with AL(400:1000) would result in greater functional recovery at reperfusion than arrest with del Nido solution, at warm and cold arresting temperatures

7.3 Methods

7.3.1 Cardioplegia and reperfusion solutions

1) Adenosine 400μm + Lignocaine 1000μm (AL (400:1000))
2) Adenosine 200μm + Lignocaine 500μm (AL (200:500))
3) Adenosine 20μm + Lignocaine 50μm (AL (20:50))
4) Adenosine 400μm + Lignocaine 1000μm + Mg^{2+} (2.5mM total) (ALM)
5) del Nido solution
6) Krebs-Henseleit buffer (KH)

Table 7.1 Composition, and concentration of components (mM), of AL (400:1000), ALM and del Nido cardioplegia solutions.

Solution components	Adenosine-Lignocaine cardioplegia	del Nido cardioplegia
Adenosine	0.4	
Lignocaine	1.0	0.36
NaCl	117.0	91.66
KCl	5.9	24.3
NaH_2PO_4	1.2	0.18
$NaHCO_3$	25.0	13.79
$CaCl_2$	1.12	0.24
$MgCl_2$	0.512	1.13
Glucose	10.0	1.1
Na gluconate		17.33
Na acetate		20.34
Mannitol		13.72
Mg SO_4		6.18
Mg SO_4 (in ALM)	2.0	

7.3.2 Experimental groups

Rats were randomly assigned to 4 experimental groups.

Table 7.2: Experimental groups, and arrest and reperfusion protocols

Group	Arrest protocol	Reperfusion protocol
1 (**AL warm**) (n=8)	AL (400:1000) induction (32^{o}C), 48 minutes arrest (32^{o}C), AL (200:500) 2 min terminal flush (32^{o}C),	Langendorff mode, 10 min, KH, 37^{o}C, Working mode, 60 min, KH, 37^{o}C
2 (**del Nido warm**) (n=7)	del Nido induction (32^{o}C), 48 minutes arrest, (32^{o}C), del Nido 2 min terminal flush (32^{o}C),	Langendorff mode, 10 min, KH, 37^{o}C, Working mode, 60 min, KH, 37^{o}C
3 (**ALM cold**) (n=6)	AL (400:1000) + Mg^{2+} (ALM) induction (32^{o}C), 48 minutes arrest (8-12^{o}C) , no terminal flush	Langendorff mode, 10 min, AL (20:50) warming from 26^{o}C to 35^{o}C, Working mode, 60 min, KH, 37^{o}C
4 (**del Nido cold**) (n=6)	del Nido induction (8^{o}C), 48 minutes arrest (8-12^{o}C), no terminal flush	Langendorff mode, 10 min, KH warming from 26^{o}C to 35^{o}C, Working mode, 60 min, KH, 37^{o}C

7.3.3 Arrest and reperfusion protocols

As in the previous studies, hearts were prepared and perfused in working mode as detailed in Chapter 2: Materials and Methods Section 2.5.
The following arrest and reperfusion protocols were used (Figure 7.1, Table 7.2).

Groups 1 and 2: Warm arrest protocol

Hearts were arrested with 50ml of warm (32^{o}C) cardioplegic solution, maintained at 32^{o}C in a warming chamber, and the temperature monitored at 10, 20, 30, and 40 minutes arrest. After 48 minutes arrest the colour of the external heart tissue was noted, a 2-minute flush of warm cardioplegic solution (32^{o}C) was administered and the coronary flow recorded for calculation of cardiovascular resistance. Hearts were reperfused in Langendorff mode for 10 minutes with KH buffer at 36-37^{o}C. The time to first beat was recorded, and heart rate and coronary flow were measured at 2, 5 and 10 minutes of reperfusion. The hearts were then perfused in working mode with KH buffer at 37^{o}C for 60 minutes, and the time to achieve aortic flow noted. Functional parameters (heart rate, systolic and diastolic pressures, aortic and coronary flow) were recorded at 2, 5, 10, 15, 30, 45, and 60 minutes of working mode perfusion.

Group 3: ALM cold arrest protocol

Hearts were arrested with 50ml of warm (32^{o}C) ALM cardioplegic solution (containing Mg^{2+} 2.5mM), heart temperature was maintained at 8-12^{o}C in a cold chamber, and the temperature monitored at 10, 20, 30, and 40 minutes arrest. The cold chamber was removed after 43 minutes arrest to allow the hearts to start warming towards room temperature. No terminal flush was administered, and after 48 minutes arrest heart colour was noted and hearts reperfused in Langendorff mode for 10 minutes with tepid AL (20:50) which was slowly warmed from 26^{o}C to 35^{o}C. The time to first beat was recorded, and heart rate and coronary flow noted at 2, 5 and 10 minutes of Langendorff reperfusion. Hearts were then reperfused and monitored in working mode as for groups 1 and 2 above.

Group 4: del Nido cold arrest protocol

Hearts were arrested with 7ml of cold (8°C) del Nido solution. The volume of cardioplegic solution was calculated according to the published protocol of 20ml per kilogram body weight delivered in 1-2 minutes at 8-12°C in a single dose [371]. The rate of delivery of arrest solution was slowed to 4-5 ml/min with an adjustable clamp on the delivery tubing. Hearts were maintained at 8-12°C in a cold chamber, and the heart temperature monitored at 10, 20, 30, and 40 minutes arrest. The cold chamber was removed after 43 minutes arrest to allow the hearts to start warming towards room temperature. After 48 minutes arrest the colour of the external heart tissue was noted, and the hearts were reperfused in Langendorff mode for 10 minutes with tepid KH buffer which was slowly warmed from 26°C to 35°C. The time to first beat was recorded, and heart rate and coronary flow noted at 2, 5 and 10 minutes of Langendorff reperfusion. Hearts were then reperfused and monitored in working mode as for groups 1 and 2.

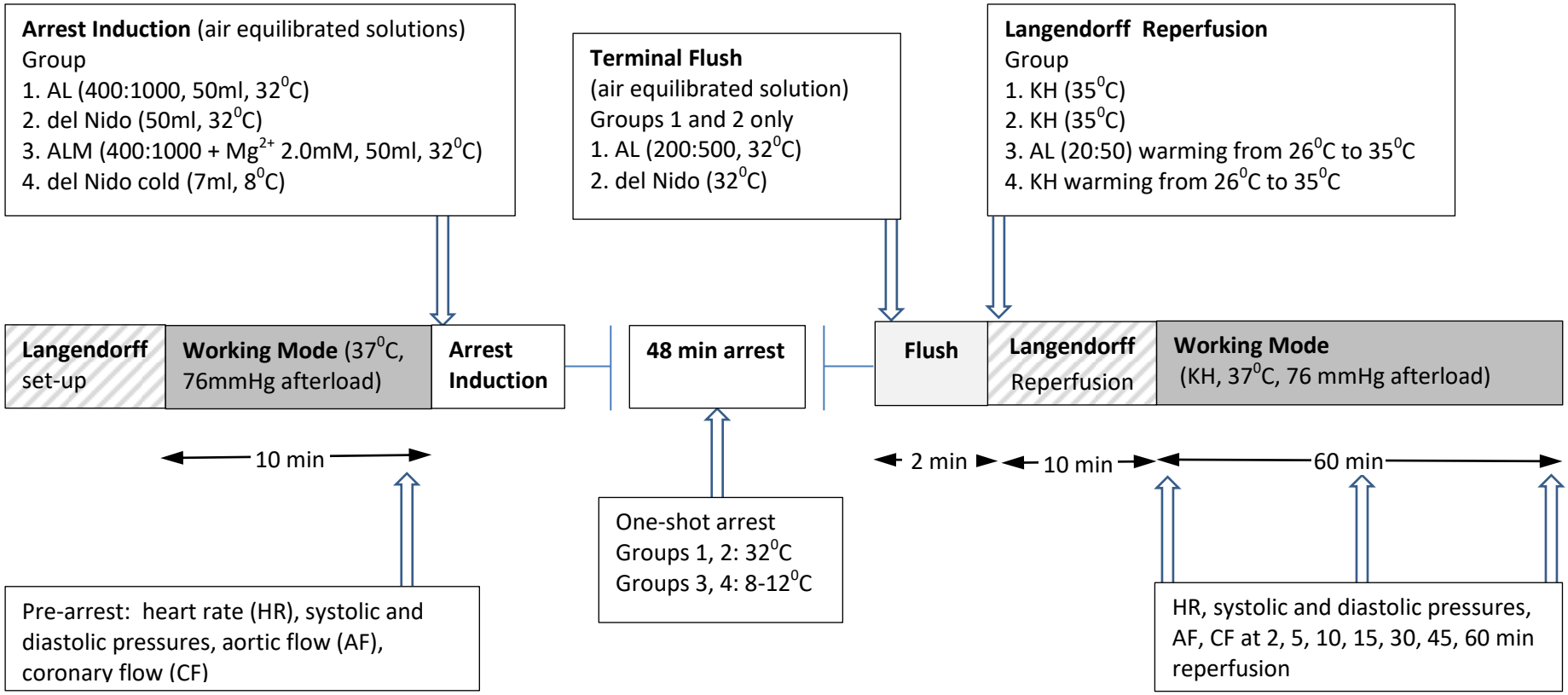


Figure 7.1: Timeline of experimental protocol comparing AL and del Nido cardioplegia for warm or cold one-shot arrest

7.4 Results

There were no significant differences between the groups in stabilised functional parameters pre-arrest (heart rate, systolic and diastolic pressures, aortic and coronary flows, cardiac output, stroke volume, rate pressure product) (Table 7.3a).

7.4.1 Effect of cardioplegia solution on coronary flow following warm arrest

Coronary flow was not measured after 48 minutes arrest in ALM cold and del Nido cold groups as no terminal cardioplegia flush was administered in these protocols. Coronary flow during the terminal flush was significantly higher, and coronary vascular resistance significantly lower, in the AL warm group (18 ± 1 ml/min and 0.27 ± 0.01 megadyne.sec.cm^{-5} respectively) compared with the del Nido warm group (13 ± 1 ml/min and 0.38 ± 0.02 megadyne.sec.cm^{-5} respectively) (p<0.05).

7.4.2 Effect of temperature and cardioplegia solution on reperfusion recovery in Langendorff mode

Time to first spontaneous reperfusion beat

The shortest time to first beat was recorded in the ALM cold group (0.54 ± 0.04 min), which was not significantly different from the del Nido cold group (0.58 ± 0.06 min). The AL warm group had a significantly longer time to first beat (1.05 ± 0.13 min) than the ALM cold and del Nido cold groups (p<0.05). Time to first beat was longest and most variable in the del Nido warm group (1.35 ± 0.47 min), which was significantly different from the ALM cold and del Nido cold groups (p<0.05).

Change in physical appearance of hearts

The appearance of five of the seven hearts in the del Nido warm group had changed to a dark purple colour with visible contraction bands by 48 minutes arrest, compared with the natural pink colour and smooth contours maintained in AL warm and ALM cold hearts. The del Nido warm hearts returned to approximately the original size and shape during Langendorff reperfusion and their colour improved to a dark pink, however the colour of these hearts was not restored to the natural pink colour seen before arrest.

Recovery of heart rate, coronary flow and coronary vascular resistance during Langendorff reperfusion

Heart rate and coronary flow were measured at 2, 5 and 10 minutes reperfusion in Langendorff mode (Figures 7.2a, b and c).

The highest heart rate at 2, 5 and 10 minutes reperfusion was recorded in the del Nido cold group (128 ± 10 to 198 ± 4 bpm) and the lowest heart rate in the del Nido warm group (48 ± 20 to 155 ± 23 bpm), with significant difference at 2 and 5 minutes reperfusion (p<0.05). Hearts in the del Nido warm group displayed weak contractility with variable heart rate and irregular rhythm during the Langendorff reperfusion period. The heart rate of the ALM cold group (64 ± 10 to 136 ± 8 bpm) was significantly lower than the AL warm group at 5 and 10 minutes reperfusion, and also significantly lower than the del Nido cold group at each time point (p<0.05).

There were no significant differences in coronary flow or coronary vascular resistance between the ALM cold group and the AL warm group during the Langendorff reperfusion period. At 2 minutes reperfusion, the ALM cold group had the highest coronary flow and lowest coronary vascular resistance (15 ± 1 ml/min and 0.33 ± 0.02 megadyne.sec.cm^{-5} respectively), and the del Nido warm group had the lowest coronary flow and highest coronary vascular resistance (12 ± 1 ml/min and 0.4 ± 0.02 megadyne.sec.cm^{-5} respectively), however these differences did not reach significance due to within group variability in the data.

At both 5 and 10 minutes reperfusion the coronary flow was significantly higher and coronary vascular resistance significantly lower in the AL groups (AL warm group:17 ± 1 ml/min and 0.29 ± 0.02 megadyne.sec.cm^{-5} respectively; ALM cold group:16 ± 1 ml/min and 0.31 ± 0.02 megadyne.sec.cm^{-5}) compared with the del Nido warm group (13 ± 1 ml/min and 0.38 ± 0.01 megadyne.sec.cm^{-5}), and the del Nido cold group at 10 minutes reperfusion (13 ± 1 ml/min and 0.37 ± 0.02 megadyne.sec.cm^{-5} respectively) (p<0.05 at every time point). Of note is the lack of significant difference in coronary flow and coronary vascular resistance between the del Nido warm and del Nido cold groups at every time point during Langendorff reperfusion.

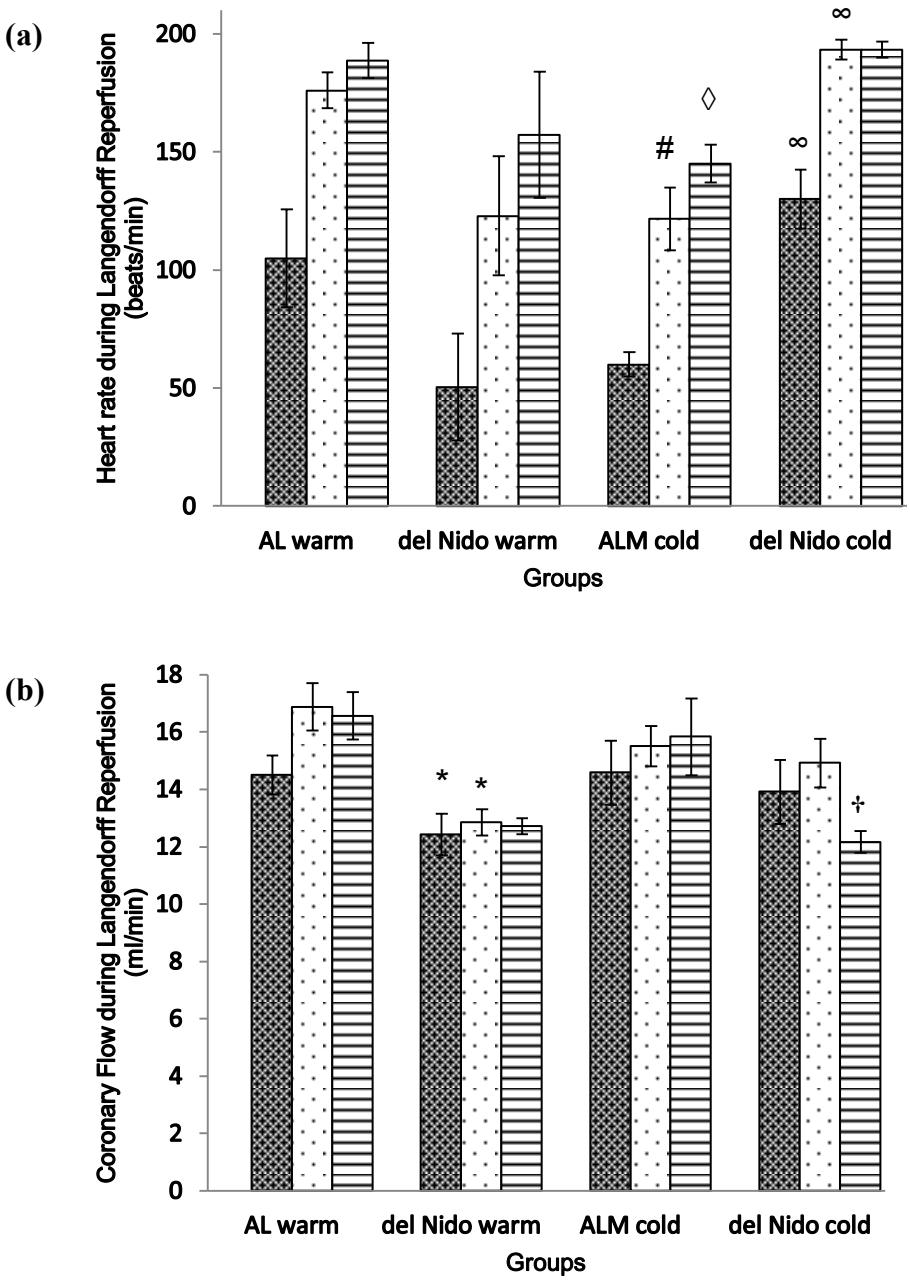


Figure 7.2: (a) Heart rate and (b) Coronary Flow recorded at 2 minutes ▩, 5 minutes ▨, and 10 minutes ▤ Langendorff reperfusion following arrest with AL or del Nido cardioplegia at warm or cold temperatures

(a) Heart rate
∞ p<0.05 del Nido cold compared with ALM cold and del Nido warm
# p<0.05 ALM cold compared with AL warm
◊ p<0.05 ALM cold compared with AL warm and del Nido cold

(b) Coronary flow
* p<0.05 del Nido warm compared with ALM cold and AL warm
† p<0.05 del Nido cold compared with ALM cold and AL warm

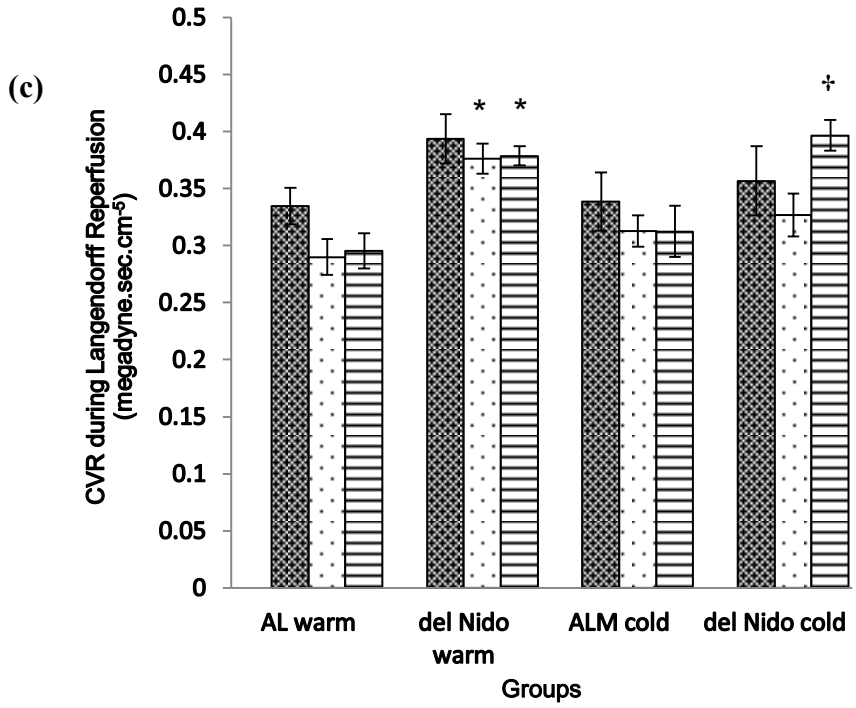


Figure 7.2: (c) Coronary vascular resistance recorded at 2 minutes , 5 minutes , and 10 minutes Langendorff reperfusion following arrest with AL or del Nido cardioplegia at warm or cold temperatures

* $p<0.05$ del Nido warm compared with ALM cold and AL warm
† $p<0.05$ del Nido cold compared with ALM cold and AL warm

7.4.3 Effect of temperature and cardioplegia solution on functional recovery during 60 minutes working mode reperfusion

Functional data, recorded at 2, 5, 10, 15, 30, 45 and 60 minutes reperfusion in working mode, are presented in Table 7.3 a - d.

Time to aortic flow in working mode

The shortest time to aortic flow in working mode occurred in the del Nido cold group (0.14 ± 0.02 min), which was significantly shorter than the del Nido warm group (1.43 ± 0.6 min), the ALM cold group (0.4 ± 0.05 min) and the AL warm group (0.43 ± 0.12 min) ($p<0.05$ for each group).

Table 7.3a: Functional parameters of hearts pre-arrest and during working mode reperfusion (2 minutes) following one shot delivery of adenosine-lignocaine (AL) or del Nido cardioplegia for 50-minute arrest at warm (32°C) or cold (8-12°C) temperatures.

Arrest Protocol	Cardioplegia delivery regime n	Heart Rate (beats/min)	Systolic Pressure (mmHg)	Diastolic Pressure (mmHg)	Aortic Flow (ml/min)	Coronary Flow (ml/min)	% of pre-arrest CO	Stroke Volume (ml/beat)	Rate Pressure Product (mmHg/min)
Pre-arrest	AL warm	304 ± 7	136 ± 3	59 ± 1	63 ± 3	20 ± 1	n/a	0.27 ± 0.01	41300 ± 1433
	del Nido warm	309 ± 12	29 ± 1	60 ± 1	64 ± 1	18 ± 1	n/a	0.27 ± 0.01	39693 ± 1303
	ALM cold	305 ± 14	132 ± 3	60 ± 1	57 ± 1	20 ± 1	n/a	0.25 ± 0.01	40050 ± 1578
	del Nido cold	323 ± 8	139 ± 2	60 ± 1	62 ± 2	21 ± 1	n/a	0.26 ± 0.01	44863 ± 1080
Working Mode Reperfusion 2 min	AL warm	281 ± 11 #	129 ± 4 #	60 ± 1 #	46 ± 4 #	20 ± 2 δ †	76 ± 5 #	0.23 ± 0.02 #	36301 ± 2109 þ
	del Nido warm	223 ± 45	112 ± 1	70 ± 1	19 ± 5 †	15 ± 1	46 ± 3	0.14 ± 0.01	30026 ± 1052 †
	ALM cold	261 ± 10	138 ± 2	60 ± 1	44 ± 2	25 ± 1 ◊	90 ± 2	0.27 ± 0.01	36017 ± 1105 ◊
	del Nido cold	308 ± 12	136 ± 2	60 ± 1	52 ± 2	19 ± 1	85 ± 3	0.23 ± 0 01	41783 ± 1251

# $p<0.05$ del Nido warm compared with AL warm, ALM cold and del Nido cold
◊ $p<0.05$ del Nido cold compared with ALM cold
† $p<0.05$ ALM cold compared with del Nido warm
þ $p<0.05$ del Nido warm compared with AL warm and del Nido cold
δ $p<0.05$ AL warm compared with ALM cold

Table 7.3b: Functional parameters of hearts during working mode reperfusion (5-10 minutes) following one shot delivery of adenosine-lignocaine (AL) or del Nido cardioplegia for 50-minute arrest at warm (32°C) or cold (8-12°C) temperatures.

Arrest Protocol	50 min cardioplegia delivery regimen	Heart Rate (beats/min)	Systolic Pressure (mmHg)	Diastolic Pressure (mmHg)	Aortic Flow (ml/min)	Coronary Flow (ml/min)	% of pre-arrest CO	Stroke Volume (ml/beat)	Rate Pressure Product (mmHg/min)
Working Mode Reperfusion 5 min	AL warm	283 ± 11 #	127 ± 2 #	61 ± 1 #	46 ± 3 #	19 ± 1 †	80 ± 4 #	0.23 ± 0.01 #	35881 ± 1622 #
	del Nido warm	256 ± 13	105 ± 5	69 ± 2	0 ± 6	14 ± 1 δ	42 ± 7	0.13 ± 0.02	27182 ± 2376
	ALM cold	282 ± 9	131 ± 2	60 ± 1	44 ± 2	23 ± 1 ◊	88 ± 3	0.24 ± 0.01	36850 ± 1208
	del Nido cold	308 ± 7	131 ± 2	60 ± 1	48 ± 1	19 ± 2	80 ± 3	0.22 ± 0.01	40200 ± 806
Working Mode Reperfusion 10 min	AL warm	287 ± 12 #	125 ± 2 #	61 ± 1 #	51 ± 3 #	19 ± 1 †	84 ± 3 #	0.24 ± 0.01 #	35871 ± 1567 #
	del Nido warm	261 ± 13	108 ± 3	69 ± 1	23 ± 6	15 ± 1	46 ± 7	0.14 ± 0.02	28196 ± 1830
	ALM cold	291 ± 9	127 ± 2	61 ± 1	44 ± 2	21 ± 1	85 ± 2	0.23 ± 0.01	36829 ± 1234
	del Nido cold	315 ± 7	130 ± 2	60 ± 1	47 ± 2	20 ± 2	80 ± 3	0.21 ± 0.01	40916 ± 875

# $p<0.05$ del Nido warm compared with AL warm, ALM cold and del Nido cold
◊ $p<0.05$ del Nido cold compared with ALM cold
† $p<0.05$ del Nido warm compared with ALM cold
δ $p<0.05$ ALM cold warm compared with AL warm

Table 7.3c: Functional parameters of hearts during working mode reperfusion (15-30 minutes) following one shot delivery of adenosine-lignocaine (AL) or del Nido cardioplegia for 50-minute arrest at warm (32°C) or cold (8-12°C) temperatures.

Arrest Protocol	50 min cardioplegia delivery regimen	Heart Rate (beats/min)	Systolic Pressure (mmHg)	Diastolic Pressure (mmHg)	Aortic Flow (ml/min)	Coronary Flow (ml/min)	% of pre-arrest CO	Stroke Volume (ml/beat)	Rate Pressure Product (mmHg/min)
Working Mode Reperfusion 15 min	AL warm	296 ± 13 #	125 ± 2 #	61 ± 1 #	52 ± 3 #	18 ± 1 †	85 ± 3 #	0.23 ± 0.01 #	36938 ± 1431 #
	del Nido warm	264 ± 14	112 ± 2	68 ± 2	26 ± 5	15 ± 1	50 ± 6	0.15 ± 0.02	29609 ± 1904
	ALM cold	295 ± 8	126 ± 2	60 ± 1	45 ± 1	21 ± 1	87 ± 2	0.22 ± 0.01	37200 ± 1068
	del Nido cold	315 ± 8	132 ± 2	60 ± 1	48 ± 2	21 ± 2 þ	83 ± 2	0.22 ± 0.01	41454 ± 1129
Working Mode Reperfusion 30 min	AL warm	302 ± 11 #	124 ± 2 #	61 ± 1 #	52 ± 3 #	19 ± 1 †	85 ± 3 #	0.23 ± 0.01 #	37513 ± 1399 #
	del Nido warm	279 ± 13	110 ± 2	71 ± 1	27 ± 4	14 ± 1 δ	50 ± 5	0.15 ± 0.01	30632 ± 1452
	ALM cold	308 ± 9	127 ± 1 ◊	60 ± 1	45 ± 1	23 ± 1	89 ± 2	0.22 ± 0.01	39017 ± 848
	del Nido cold	318 ± 9	133 ± 3	60 ± 1	47 ± 2	23 ± 1	84 ± 2	0.22 ± 0.01	42320 ± 1314

# $p<0.05$ del Nido warm compared with AL warm, ALM cold and del Nido cold
◊ $p<0.05$ del Nido cold compared with AL warm
† $p<0.05$ del Nido warm compared with ALM cold
δ $p<0.05$ ALM cold compared with AL warm
þ $p<0.05$ AL warm compared with del Nido warm

Table 7.3d: Functional parameters of hearts during working mode reperfusion (45-60 minutes) following one shot delivery of adenosine-lignocaine (AL) or del Nido cardioplegia for 50-minute arrest at warm (32°C) or cold (8-12°C) temperatures.

Arrest Protocol	50 min cardioplegia delivery regimen	Heart Rate (beats/min)	Systolic Pressure (mmHg)	Diastolic Pressure (mmHg)	Aortic Flow (ml/min)	Coronary Flow (ml/min)	% of pre-arrest CO	Stroke Volume (ml/beat)	Rate Pressure Product (mmHg/min)
Working Mode Reperfusion 45 min	AL warm	314 ± 11 #	123 ± 2 #	62 ± 1 #	49 ± 3 #	19 ± 1 ϸ †	83 ± 2 #	0.22 ± 0.01 #	38672 ± 1313 #
	del Nido warm	285 ± 12	105 ± 1	72 ± 1	24 ± 3	13 ± 1 δ	45 ± 4	0.13 ± 0.01	30038 ± 1477
	ALM cold	313 ± 7	127 ± 1	61 ± 1	44 ± 1	24 ± 1	88 ± 2	0.22 ± 0.01	39554 ± 716
	del Nido cold	319 ± 9	132 ± 3	60 ± 1	45 ± 2	23 ± 1	81 ± 2	0.21 ± 0.01	42148 ± 1237
Working Mode Reperfusion 60 min	AL warm	321 ± 13 #	120 ± 2 #	64 ± 1 #	48 ± 2 #	18 ± 1 ϸ †	80 ± 2 #	0.21 ± 0.01 #	38418 ± 1429 #
	del Nido warm	286 ± 12	103 ± 2	73 ± 1	18 ± 2	12 ± 1 δ	37 ± 2	0.11 ± 0.01	29334 ± 1075
	ALM cold	311 ± 7	126 ± 1	61 ± 1	42 ± 1	24 ± 1	86 ± 2	0.21 ± 0.01	39007 ± 878
	del Nido cold	318 ± 9	129 ± 4	60 ± 1	43 ± 2	23 ± 1	79 ± 2	0.21 ± 0.01	41067 ± 1311

\# $p<0.05$ del Nido warm compared with AL warm, ALM cold and del Nido cold
† $p<0.05$ del Nido warm compared with ALM cold
δ $p<0.05$ ALM cold compared with AL warm
ϸ $p<0.05$ AL warm compared with del Nido warm

Recovery of heart rate, pressures and rate pressure product

There were no significant differences in heart rate between the ALM cold, AL warm and del Nido cold groups at any time point during working mode reperfusion. The del Nido warm group had a significantly lower heart rate than the AL warm, ALM cold and del Nido cold groups at every time point during working mode reperfusion (*p*<0.05 at 2, 30, 45 and 60 min; Dunnet's C positive at 5, 10 and 15 min). All hearts in the del Nido warm group recorded periods of arrhythmia lasting from a few seconds to several minutes at variable times during working mode reperfusion.

There were no significant differences in systolic or diastolic pressures between the ALM cold, AL warm and del Nido cold groups except at 30 minutes reperfusion, when the systolic pressure of the AL warm group (124 ± 2 mmHg) was significantly lower than the del Nido cold group (133 ± 3 mmHg) (*p*<0.05). The del Nido warm group had a significantly lower systolic pressure than the AL warm, ALM cold and del Nido cold groups at every time point during working mode reperfusion (*p*<0.05 at 2, 10, 30, 45 and 60 min, Dunnet's C positive at 5 and 15 min). The diastolic pressure of the del Nido warm group was significantly higher than the AL warm, ALM cold and del Nido cold groups at every time point during working mode reperfusion (*p*<0.05).

The rate pressure product of the del Nido warm group was significantly lower than the AL warm and del Nido cold groups at 2 minutes reperfusion, and the AL warm, ALM cold and del Nido cold groups from 5 to 60 minutes of working mode reperfusion (*p*<0.05 at every time point). The ALM cold group (36017 ± 1105 mmHg/min) had a significantly lower rate pressure product than the del Nido cold group (41783 ± 1251 mmHg/min) at 2 minutes reperfusion only (*p*<0.05).

Recovery of aortic flow

There were no significant differences in percentage recovery of aortic flow between the AL warm, ALM cold and del Nido cold groups at any time point during reperfusion (Figure 7.3a, Table 7.3).
Recovery of aortic flow at 2 minutes reperfusion ranged from 80 ± 4% for the del Nido cold group to 28 ± 7% for the del Nido warm group. The AL warm, del Nido warm, ALM cold and del Nido cold groups each recorded their maximum percentage return

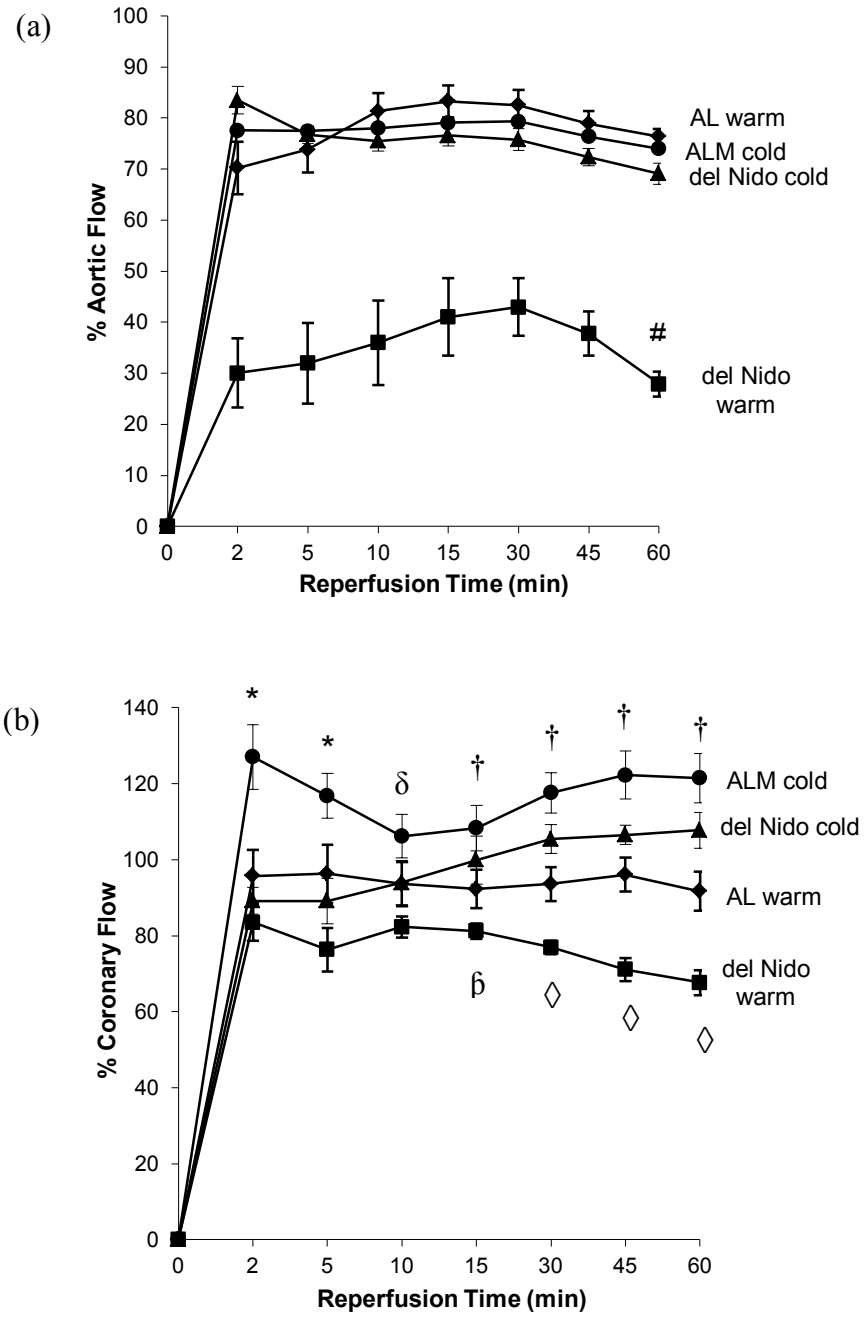


Figure 7.3: Percentage recovery of (a) aortic flow and (b) coronary flow during 60 minutes of working mode reperfusion following arrest with AL or del Nido cardioplegia at warm or cold temperatures

(a) % aortic flow
# $p<0.05$ del Nido warm compared with AL warm, ALM cold and del Nido cold

(b) % coronary flow
* $p<0.05$ ALM cold compared with del Nido cold, AL warm and del Nido warm
δ $p<0.05$ ALM cold compared with del Nido warm
† $p<0.05$ ALM cold compared with del Nido warm and AL warm
þ $p<0.05$ del Nido warm compared with del Nido cold
◊ $p<0.05$ del Nido warm compared with del Nido cold and AL warm

of aortic flow at 30 minutes of reperfusion, achieving 83 ± 3%, 41 ± 6%, 79 ± 2% and 75 ± 2% of flow respectively. The del Nido warm group recovered a significantly lower percentage aortic flow than the AL warm, ALM cold and del Nido cold groups at every time point. (p<0.05 at 2, 45 and 60 minutes; Dunnet's C positive at 5, 10, 15 and 30 minutes).

Recovery of coronary flow

The highest recovery of coronary flow was recorded in the ALM cold group, with 128 ± 6% of pre-arrest flow at 2 minutes reperfusion and 110–124% for the remainder of the reperfusion period. This recovery was significantly higher than the del Nido warm group throughout reperfusion, and the del Nido cold group at 2 and 5 minutes reperfusion, and the AL warm group at 2, 5, and 30 to 60 minutes reperfusion (p<0.05 at every time point) (Figure 7.3b, Table 7.3).

The AL warm group had recovered 96 ± 7% of coronary flow at 2 minutes reperfusion, and maintained 92–96% of flow for the remaining reperfusion time, which was significantly higher than the del Nido warm group at 30, 45 and 60 minutes reperfusion (p<0.05 at every time point).

The del Nido cold group recovered 91 ± 3% of coronary flow at 2 minutes reperfusion, and then recorded 90-105% for the remaining reperfusion period. There was no significant difference in percentage recovery of coronary flow between the del Nido warm and del Nido cold groups during the first 10 minutes of working mode reperfusion, however recovery of coronary flow in the del Nido cold group was significantly higher than in the del Nido warm group from 15 to 60 minutes reperfusion (p<0.05 at every time point). The del Nido warm group recorded 84 ± 4% recovery of coronary flow at 2 minutes reperfusion, decreasing to 68 ± 3% by 60 minutes of reperfusion.

Recovery of cardiac output and stroke volume

The ALM cold group consistently maintained the highest percentage recovery of cardiac output for the 60 minutes of working mode reperfusion, however there was no significant difference in recovery of cardiac output between the ALM cold, AL warm and del Nido cold groups during the reperfusion period. At 2 minutes reperfusion

percentage recovery of cardiac output ranged from 92 ± 2% for the ALM cold group to 43 ± 4% for the del Nido warm group, and recovery of cardiac output for the del Nido warm group remained significantly lower than all other groups throughout reperfusion (p<0.05 at every time point) (Table 7.3).

The recovery of stroke volume profiles of the 4 groups were similar to the cardiac output profiles, with the del Nido warm group recording a significantly lower stroke volume than the other groups throughout reperfusion (p<0.05 at every time point) (Table 7.3).

7.5 Discussion

Depolarising potassium cardioplegia continues to be the choice of the majority of cardiac surgeons [377,438,445]. As discussed in the introductory chapter, membrane depolarisation and ischaemia [126,158,162] during cardiopulmonary bypass, and the cold-to-warm transition at reperfusion, may lead to less than optimal recoveries. Single-dose hyperkalemic del Nido solution (24mM K^+) appears to be gaining popularity in the United States, largely as a result of surgeons and perfusionists seeking improved myocardial protection for the older diseased heart and the very young heart undergoing complex corrective surgery [371,527,528].

In the previous chapter, the addition of magnesium to AL (400:1000) cardioplegia for one-shot warm (32^{o}C) arrest did not further improve post-arrest recovery, however addition of 2mM magnesium (ALM) during cold arrest did improve recovery to comparable to warm AL arrest. In this chapter, one-shot nomokalemic AL(M) cardioplegia was compared with one-shot depolarizing del Nido solution at warm (32^{o}C) and cold (8-12^{o}C) temperatures. One-shot arrest with del Nido solution at warm temperatures significantly reduced post-arrest left ventricular function compared with del Nido arrest at cold temperatures. In contrast, the 'temperature versatility' of polarising AL(M) solution was again demonstrated with significantly higher aortic and coronary flows than del Nido solution following arrest at warm temperatures, and significantly higher coronary flow in early reperfusion and equivalent aortic flow compared to del Nido arrest at cold temperatures.

7.5.1 Comparison of polarising ALM and depolarising del Nido cardioplegia for cold one-shot arrest

Increased magnesium benefits AL hearts at cold arresting temperatures

Results in Chapter 6 showed that raised magnesium concentrations in AL cardioplegia improved functional recovery following arrest at cold temperatures (Table 6.7). The current chapter further demonstrated that addition of 2mM magnesium to AL (400:1000) cardioplegia (ALM) for arrest at cold temperatures resulted in excellent functional recovery, which may have been related to magnesium's ability to modulate calcium transport and preserve membrane stability [529], improve coronary flow [182,523,524] and maintain ATP reserves at cold temperatures [481,526]. Raised magnesium levels also reduce intracellular calcium loading [476,521-523] which may have improved cardioprotection from calcium accumulation during hypothermia and potential altered excitation-contraction coupling in cardiomyocytes after rewarming [530].

Reperfusion with AL solution results in lower heart rate during Langendorff reperfusion after cold arrest

In the present chapter, the ALM group recorded a more gradual increase in contractility and lower heart rate (64 ± 10 to 136 ± 8 bpm) than the del Nido cold group (128 ± 10 to 198 ± 4 bpm) during Langendorff reperfusion (Figure 7.2a). This may have been due to improved "polarised" protection during reperfusion of ALM hearts with AL (20:50) solution, whereas del Nido hearts were reperfused with KH buffer. In support of this proposal, Belardinelli and colleagues have shown that adenosine 50μM hyperpolarises the membranes of the sino-atrial node pacemaker cells by approximately 12mV [218], while Pankucsi and colleagues reported that lignocaine reduces calcium influx and depresses contractile force [531] which may have impacted on return of contractile activity in the present study.

ALM cardioplegia leads to significantly higher coronary flow but comparable HR and LV function after cold arrest

During early working mode reperfusion the ALM group had a significantly higher coronary flow (23 ± 1 to 25 ± 1 ml/min) than the del Nido cold group (19 ± 1 to 19 ± 2 ml/min), however after 15 minutes reperfusion there was no significant difference in

coronary flow between these groups (Table 7.3). Similarly, during working mode reperfusion there were no significant differences between the ALM and del Nido cold groups in heart rate, systolic and diastolic pressures, aortic flow, cardiac output or stroke volume (Table 7.3). Menasche [532] reported a similar level of recovery in isolated working rat hearts arrested at 15-18^{o}C with intermittent delivery (30 min intervals, 3 min flush) of normokalaemic (4mM K^{+}) cardioplegia with raised magnesium (13mM) followed by reperfusion with buffer containing 1mM calcium. Those hearts achieved 83% recovery of aortic flow, compared with 79% recovery of aortic flow for hearts in this study arrested with cold one-shot ALM (5.9mM K^{+}) and reperfused with 1.2mM calcium. Cold temperatures appear to offer myocardial protection for the left ventricle when using cold ALM or del Nido solutions, which may relate to comparable calcium handling and mitochondrial function. Further studies are required to examine this hypothesis.

7.5.2 Comparison of polarising AL and depolarising del Nido cardioplegia for warm one-shot arrest

AL cardioplegia maintains higher coronary flow than del Nido solution after 50-minute warm one-shot arrest

During the terminal cardioplegia flush following 50-minute warm (32^{o}C) one-shot arrest, the AL group recorded a significantly higher coronary flow (1.4 times higher) and significantly lower (29% lower) coronary vascular resistance (18 ± 1 ml/min and 0.27 ± 0.01 megadyne.sec.cm^{-5} respectively) than the del Nido group (13 ± 1 ml/min and 0.38 ± 0.02 megadyne.sec.cm^{-5} respectively). Similar significant differences in coronary flow between the AL and del Nido groups were recorded during the period of Langendorff reperfusion (Figure 7.2b and c). The significant reduction in coronary flow in the del Nido warm group may have been due to the effect of high potassium concentrations vasoconstricting the coronary arteries [145,286], however other vasoconstrictive mediators may have been involved.

AL cardioplegia leads to significantly higher recovery of function at reperfusion after warm one-shot arrest

Larger, and potentially clinically important, functional differences were found between the AL and del Nido cardioplegia strategies during 60 minute reperfusion following arrest at 32^{o}C. During working mode reperfusion the AL group recorded significantly

higher return of all functional parameters compared with the del Nido group. For example, at 2 minutes reperfusion the AL group had recovered 70 ± 5% aortic flow and 96 ± 7% coronary flow, and at 60 minutes reperfusion recorded 76 ± 2% aortic flow and 92 ± 5% coronary flow (Figure 7.3a and b, Table 7.3). The del Nido group recorded 28 ± 7% aortic flow and 84 ± 4% coronary flow at 2 minutes reperfusion, and 27 ± 2% and 68 ± 3% respectively at 60 minutes reperfusion, which contrasted with recovery after normokalaemic, normothermic AL arrest. The underlying mechanisms involved in the significantly lower functional recovery with del Nido cardioplegia compared to AL cardioplegia at warm arrest temperatures were not investigated in this thesis. However, despite the del Nido warm group recovering 86-93% of heart rate during 60 minutes reperfusion, recovery of aortic flow was significantly lower, suggesting impaired left ventricular (LV) pump function. A higher diastolic pressure in the del Nido warm arrested hearts also indicates decreased compliance and impaired relaxation. Torrance and colleagues reported that impaired diastolic relaxation is a sign of myocardial stiffness linked to acidosis, depleted ATP levels and impaired cellular regulation of calcium transport [533] which are factors in ischaemia-reperfusion injury.

Hearts in the del Nido warm group also developed conspicuous discolouration and contraction bands during arrest. The possible onset of rigor during ischaemia has been identified as a major factor in determining the outcome of reperfusion injury [94]. Therefore the poor return of function in del Nido hearts may be linked to greater calcium loading during ischaemic arrest in a high potassium environment, which led to early ischaemia-reperfusion injury, and had flow-on effects to reduce post-arrest functional recovery over the 60 minute reperfusion period. Makazan and colleagues also reported that reversion from low calcium concentrations to physiological calcium levels, as was the case in del Nido hearts at reperfusion, has been linked to the calcium paradox defined by calcium overload, myocardial cell damage and left ventricular dysfunction [534]. Further work is required to test this del Nido “calcium loading” hypothesis, and the reasons for greater cardioprotection provided by AL cardioplegia at the cellular level.

7.6 Conclusion

The present chapter has demonstrated the efficacy and versatility of adenosine and lignocaine cardiopegia at both cold and warm temperatures. During early reperfusion, hearts in ALM cold and AL warm groups recorded significantly better coronary flow than hearts in the del Nido cold group. Throughout working mode reperfusion the ALM cold and AL warm groups recorded equivalent functional recovery to the del Nido cold group in all other parameters. In contrast, hearts arrested with del Nido solution at warm temperatures recorded significantly reduced recovery of all functional parameters compared with hearts arrested at warm or cold temperatures with AL or ALM respectively. These results demonstrate the advantages, at warm and cold temperatures, of polarised arrest and physiological calcium and potassium levels throughout the arrest and reperfusion intervals. The cardioprotective properties of AL cardioplegia may allow development of warm polarised arrest protocols which avoid the adverse effects of hypothermic hyperkalaemic arrest.

Chapter 8

General Discussion

8.1 Study background

While mortality rates in cardiac surgery remain low at around 1-2% for coronary artery bypass grafting (CABG) and up to 7% for combined CABG and valve surgery [535], post-operative complications appear to be rising due to an aging population, failed angioplasties, and an increased number of repeat surgeries and complex paediatric corrective procedures [107,272,355,536,537]. Despite low mortality rates, 10% of CABG patients will experience reduced left ventricular function and output lasting days to weeks [538], 25 to 40% of patients will have post-operative atrial fibrillation [539], up to 40% will have some form of acute renal injury [3], up to 20% develop acute respiratory distress syndrome [4], 30 to 40% experience transient cognitive dysfunction and delirium [540] and 2 to 3% of patients will suffer a stroke [541].

Reasons for these post-operative complications are not fully understood. Some have been related to age, number of comorbidities, length on bypass time, the trauma of surgery, reduced physiological reserve and sub-optimal cardioprotective strategies [272,332,542]. The early warm reperfusion period is a vulnerable time after hypothermic surgical arrest [8,11,310,322,439], and this may be exacerbated by the high concentrations of depolarising potassium in cardioplegia, which has been linked to ischaemia-reperfusion injury [9,10,290,306,543], activation of vascular endothelium to become leaky and pro-inflammatory [292,544] and oxidant injury [127,129].

The main goal of this thesis was to examine the effect of polarising adenosine and lignocaine (AL) cardioplegia on functional recovery after warm (32^{o}C) and cold (8-12^{o}C) arrest compared to more conventional hyperkalaemic cardioplegia.

8.2 Hypothesis

The main hypothesis for this thesis was that normokalaemic AL cardioplegia would provide cardioprotection at warm or cold temperatures, using intermittent or single dose cardioplegia delivery. In addition, it was hypothesised that one-shot AL cardioplegia would lead to improved post-arrest functional recovery compared with hyperkalaemic del Nido cardioplegia delivered at warm or cold temperatures.

8.3 Summary of findings

The main findings of this thesis were:

1) Comparison of continuous or intermittent delivery of AL cardioplegia for 40-minute or 60-minute arrest at 32°C (Chapter 3) resulted in no significant difference in post-arrest functional recovery, demonstrating equivalent cardioprotection at this temperature (Table 3.3a and b). Lignocaine only cardioplegia was not as effective as AL cardioplegia in maintaining coronary vascular resistance or functional recovery during reperfusion.

2) Chapter 4 results showed that warm (32^{o}C) normokalaemic (5.9mM K^{+}) AL cardioplegia induced arrest at membrane potentials close to physiological resting state, providing optimal arrest conditions for functional recovery during reperfusion. Hypokalaemic solutions (1mM, 3mM K^{+}) hyperpolarised the membranes and higher potassium concentrations (16mM, 25mM K^{+}) caused membrane depolarisation, and reduced function. The hypokalaemic and hyperkalaemic groups recorded significantly higher coronary vascular resistance during arrest (Figures 4.2a-c), and slower and significantly lower recovery of functional parameters during reperfusion (Figures 4.3a-d, 4.4a-c) compared with recovery following polarised arrest induced by normokalaemic AL cardioplegia.

3) Administration of a single dose of AL cardioplegia for induction of 40-minute one-shot arrest at 32^{o}C (Chapter 5) resulted in myocardial stunning at reperfusion (Figures 5.4, 5.6) with reduced recovery of function when compared with the warm intermittent (20 minute re-dosing interval) and continuous AL arrest protocols used in Chapters 3 and 4. Addition of the antioxidant MPG 1mM to the terminal flush and reperfusion buffer of the one-shot arrest protocol provided optimal protection from myocardial stunning compared with administration during arrest or during reperfusion only (Figures 5.5, 5.7), and improved recovery of function to equivalent to the warm intermittent arrest protocol.

4) In Chapter 6, doubling the concentrations of adenosine and lignocaine to AL(400:1000) for 50-minute warm (32^{o}C) one-shot arrest prevented myocardial stunning and resulted in superior functional recovery compared with warm one-shot arrest with AL(200:500). Addition of MPG or increased magnesium concentrations did not further improve recovery. In contrast, raising the magnesium concentration to 2.5mM during cold AL(400:1000) arrest did improve functional outcome, resulting in comparable reperfusion recovery following arrest with AL(M) cardioplegia at warm or cold temperatures.

5) Comparison of cardioprotection with AL(M) or del Nido solutions at warm (32^{o}C) or cold (8-12^{o}C) temperatures (Chapter 7) confirmed the 'temperature versatility' of AL solution. At cold temperatures, 50-minute one-shot arrest with polarising ALM solution resulted in significantly higher coronary flow in early reperfusion and equivalent aortic flow compared with del Nido arrest. Warm one-shot arrest with hyperkalaemic del Nido solution resulted in the lowest functional recovery, with significantly reduced recovery at reanimation and decreased left ventricular function during reperfusion, compared with del Nido arrest at cold temperatures and AL arrest at warm or cold temperatures.

8.4 Discussion

8.4.1 Setting the Stage: The importance of maintaining the heart's cell membrane potential close to resting levels for optimal arrest and recovery

A major finding in this thesis was the profound influence that varying the heart's membrane potential had on post-arrest recovery [545]. Both membrane depolarisation (16 and 25mM K^+) and hyperpolarisation (0.1 and 3mM K^+) had adverse outcomes on post-arrest recovery in the isolated rat heart at warm temperatures (32°C) (Chapter 4). In contrast, when AL maintained membrane potential at or around the heart's resting cell voltage (5.9mM K^+) post-arrest recovery was optimal, as discussed below. Membrane potentials varied from -39mV in the 25mM K^+ only cardioplegia group to -183mV for the AL 0.1mM K^+ cardioplegia group, and was -75mV in the AL normokalaemic hearts (Chapter 4). The other important finding of this work was the close agreement between estimating the heart's membrane potential from the Nernst equation, and direct microelectrode studies in the literature, supporting Gette's proposal [546] that the heart's membrane potential is predominately a potassium potential. Gettes wrote in 1976:

> In (heart) fibers which do not have pacemaker characteristics the diastolic potential behaves as a K^+ electrode and varies as expected by the Nernst relationship when the extracellular K^+ concentration is above 3mM [546]

In the present thesis, maintaining membrane potential around the diastolic (resting) value compared to depolarisation with conventional high potassium cardioplegia reinforces the view proposed by Chambers and Hearse in 1999 that cell membrane potential is a fundamental property of the heart, and a potential target for improved myocardial protection [273].

As reported above, AL cardioplegia with 5.9mM K^+ provided optimal recovery of function following two-hour warm intermittent arrest with 81% recovery of aortic flow and 113% coronary flow at 60 minutes reperfusion (Figures 4.2b, 4.3b and d, 4.4a-c), and in Chapter 7, 76% recovery of aortic flow and 92% coronary flow after 50-minute warm one-shot arrest (Figures 7.4a-c, 7.5a and b). At low and high non-physiological potassium concentrations, 40% of hearts in the AL 0.1mM K^+ or 25mM K^+ alone groups

failed to recover heart rate, developed pressures or cardiac output after 1-hour arrest. In addition, AL with intermediate potassium levels (10mM) was found to be detrimental and resulted in slower recovery of aortic flow, and delayed and lower recovery of cardiac output and stroke volume compared with normokalaemic AL. These results suggest that depolarisation or hyperpolarisation of membrane potential at arrest is a major factor influencing cardioprotection and post-arrest recovery of function in the isolated rat heart arrested at warm temperatures (32°C).

Jin and colleagues [412] reported similar variations in membrane potentials to those obtained in this thesis. In a paediatric trial they compared traditional crystalloid potassium cardioplegia (20mM K^+) with two hyperkalaemic solutions (20mM K^+ and 10mM K^+) containing AL and physiological calcium levels. While 10mM potassium in AL solution depolarised the membrane potential to -67mV, 20mM K^+ cardioplegia or 20mM K^+ combined with AL caused depolarisation to -46mV. These clinical results are in close agreement with the membrane potentials reported in Chapter 4, where 10mM K^+ and 16mM K^+ in AL(200:500) cardioplegia depolarised membrane potentials to -65mV and -49mV respectively in isolated rat hearts. In support of the Chapter 4 findings, Jin and colleagues [412] also found that depolarising potassium (20mM K^+) led to reduced perioperative haemodynamics, and higher post-operative cardiac troponin I (cTnI) levels, than moderately raised potassium concentrations (10mM K^+).

The polarised concept was also supported by the 2008 case study of O'Rullian and colleagues who reported excellent functional recovery following prolonged complex 're-do' valve surgery using near-continuous adenocaine microplegia [547]. Post-operatively the patient exhibited normal systemic potassium levels and haematocrit, and no arrhythmias, and made an uneventful recovery, demonstrating the cardioprotective properties of polarised adenosine–lignocaine arrest. More recently, two prospective, randomised trials using variants of AL cardioplegia have been completed in Verona. Results in high risk patients receiving cold potassium-based cardioplegia enriched with ALM and insulin showed superior myocardial protection with lower troponin-I and lactate levels during early reperfusion, improved haemodynamics and shorter hospital stay compared to high potassium depolarisjng Buckberg solution [413]. In the second trial using normokalaemic ALM cold microplegia on lower risk patients, similar results were reported with lower coronary sinus troponin, lactate and potassium levels at reperfusion and one day less of intensive care compared with patients receiving hyperkalaemic cardioplegia [415].

The isolated rat heart data demonstrated that maintenance of cell membrane potential with physiological levels of potassium in the AL arrested heart (polarised state) improved post-cardioplegia performance. Polarised arrest at warm (32^{o}C) temperatures may avoid the deleterious effects of hyperkalaemic arrest with hypothermia [8,9,182,291,294,298,308,310], and the added injury caused by rewarming from hypothermic temperatures [11,326]. However, further studies are required to examine the underlying mechanisms of 'polarised' protection, and its importance in adult and paediatric cardiac patients.

8.4.2 Towards a New Warm AL Cardioplegia Paradigm

Intermittent versus continuous cardioplegia delivery

For over 50 years the field of cardioplegia has been dominated by high potassium solutions and the use of hypothermic temperatures for added protection [62,311]. Continuous delivery of hyperkalaemic cardioplegia at warm temperatures was shown to be cardioprotective [181,329-331,548], however the constant flow obstructed the surgeon's field of view, and intermittent warm delivery produced conflicting reports of the level of myocardial protection [339-345].

In Chapter 3 the efficacy of AL cardioplegia was assessed during warm (32^{o}C) arrest using either an intermittent delivery protocol (18-minute no flow: 2-minute flush) or continuous cardioplegia. Equivalent protection occurred if AL was infused continuously or intermittently (Figure 3.3a and b) [549]. This data supports the earlier canine study of Corvera and colleagues [409] who showed that intermittent delivery of warm (37°C) AL cardioplegia was equivalent to AL or high potassium cardioplegia delivered at hypothermic temperatures. Unfortunately, the Corvera group did not include a warm high potassium group in their study for comparison with AL at 37°C. The Corvera study and the present thesis indicate that AL has the potential to be used as an arresting agent in warm intermittent cardioplegia delivery protocols. The author of this thesis is unaware of any clinical use of AL at warmer temperatures.

Can the delivery interval be extended with warm AL arrest?

The next hypothesis addressed in this thesis was that the AL cardioplegia dosing interval could be extended and still afford protection at warmer temperatures. Twenty minutes is a common cardioplegia dosing interval used by surgeons [332], and was the interval duration used in Chapters 3 and 4 discussed above. In Chapters 5 and 6 a warm 'one-shot' protocol with a single dose of AL cardioplegia for induction and maintenance of arrest for a 40-minute or 50-minute interval at 32^{o}C was investigated. Results showed that 40-minute warm one-shot arrest with AL(200:500) did not provide comparable cardioprotection to warm intermittent arrest, with slower post-arrest recovery (Figure 5.3) and significantly reduced left ventricular flows (Figure 5.4). However, when the hydroxyl radical scavenger MPG was added to the terminal AL cardioplegia flush and reperfusion buffer following 40-minute warm one-shot AL arrest functional recovery was significantly improved (Figures 5.4 and 5.6). These results implied the involvement of hydroxyl radicals [83,407] and possible attenuation of stunning with the MPG reperfusion therapy. Therefore functional outcome of warm (32^{o}C) 40-minute one-shot AL(200:500) arrest may have been adversely affected by radical species produced during ischaemia and at reperfusion which resulted in reperfusion-induced damage and myocardial stunning.

Extending the AL dosing interval to 50 minutes at warm and cold temperatures

Due to the loss of functional recovery following 40-minute warm one-shot AL arrest, the effect of increasing the concentrations of adenosine and lignocaine in AL cardioplegia was then investigated. Doubling the concentrations to AL(400:1000) was found to significantly improve functional recovery after 50-minute warm one-shot arrest, demonstrating that loss of protection after 40-minute arrest was associated with the lower AL(200:500) concentrations. The data from the AL(400:1000) study further demonstrated that the higher AL concentrations may have protected against hydroxyl radical production [407,508], which contrasted with the AL(200:500) study (Chapter 5) where the addition of MPG resulted in improved post-arrest recovery. Increased magnesium did not improve functional recovery and therefore protection in the isolated rat heart at warm temperatures (Table 6.5). However magnesium supplementation was required during cold 50-minute one-shot AL (400:1000) arrest for optimal recovery at reperfusion (Table 6.7), indicating the involvement of a magnesium-dependent pathway such as impaired intracellular calcium control at hypothermic temperatures [411].

Further studies comparing different concentrations and ratios of adenosine and lignocaine in crystalloid and blood-based cardioplegia at varying temperatures may improve the efficacy of AL cardioplegia under different clinical conditions.

To summarise, this group of studies has shown that polarising AL(200:500) cardioplegia administered intermittently every 20 minutes produced equivalent functional recovery compared to continuous delivery at 32°C [549]. Doubling the adenosine and lignocaine concentrations to AL (400:1000) produced rapid and stable arrest, and excellent functional recoveries following 50-minute one-shot arrest at 32^{O}C with normal magnesium levels, and after cold arrest (8-12^{o}C) with AL supplemented with increased magnesium.

8.4.3 Comparison of AL and depolarising del Nido cardioplegia for 50-minute one-shot arrest

Finally, AL one-shot cardioplegia was compared with high potassium del Nido cardioplegia which is popular in the United States of America. It was found that warm AL arrested hearts recorded significantly better functional parameters throughout arrest and reperfusion compared with warm del Nido arrested hearts. For example, at 2 minutes reperfusion the AL group had recovered 76% cardiac output with a stroke volume of 0.23ml/beat compared to 46% cardiac output and 0.14ml/beat stroke volume for the del Nido group. At 60 minutes reperfusion, the difference in the cardiac output was 80% compared to 37%, and the stroke volume was 0.21ml/beat compared to 0.11ml/beat for the AL and del Nido groups, respectively.

Although the mechanisms involved in decreased functional recovery in warm del Nido hearts were not studied, the reduced recovery may have been due to a combination of arrest ischaemia and reperfusion injury conditions. Injury during ischaemic arrest [68,354] may have been compounded by intracellular calcium overload induced by potassium depolarisation, and by the change from low to physiological calcium levels at reperfusion [534]. Both of these factors may have impacted on ischaemia-reperfusion injury and restoration of contractile activity at reanimation [65,374-376].

While it is acknowledged that del Nido cardioplegia was not designed for warm arrest, the comparison emphasises the versatility of polarising AL cardioplegia. In addition to the benefits of polarised arrest for recovery of myocardial and vascular function, the

avoidance of hypothermia during warm AL arrest may be a significant factor [273,550]. In 2016 Schaible and colleagues showed that hypothermia/rewarming alters excitation-contraction coupling in cardiomyocytes, possibly due to decreased myofilament calcium sensitivity in conjunction with increased cardiac troponin I phosphorylation [530]. This may contribute to hypothermia/rewarming dysfunction in the intact heart following cardiac surgery.

The significantly higher functional recovery following warm AL arrest also suggests that AL cardioplegia may maintain improved energy reserves and limit oxygen demand in hearts during arrest at 32^{o}C. Adenosine-lignocaine cardioplegia has previously demonstrated preservation of ATP and phosphocreatine (PCr) levels in an *in vivo* rat model of regional myocardial ischaemia [458], and improved aerobic ATP utilisation in clinical practice [415].

Hearts arrested at cold temperatures with ALM recovered higher coronary flow during Langendorff and early working mode reperfusion compared with cold del Nido arrested hearts, and equivalent return of function in other parameters. Importantly, in the clinical situation, del Nido is administered cold and in a 4:1 crystalloid to blood ratio to maintain low calcium levels and improve cardioprotection [382,416].

Current clinical single-dose arrest strategies use hyperkalaemic solutions at hypothermic temperatures, primarily in paediatric patients with congenital heart defects [371,379,551]. However, the growing interest in single-dose arrest strategies for paediatric and adult surgery has been tempered by concerns regarding the level of cardioprotection offered by a single dose of hyperkalaemic cardioplegia during complex and prolonged bypass times [332,416]. Polarising AL cardioplegia may offer superior cardioprotection with an alternative single-dose warm arrest strategy. Translational safety trials are required involving different one-shot crystalloid to whole blood ratios or microplegia strategies using the AL polarising arrest and reanimation concept.

8.5 Clinical significance

This thesis has shown that arrest with AL solution at 32°C provided numerous advantages and benefits which may be relevant in a clinical setting:

1) AL induces polarised arrest which may prevent the adverse clinical effects of depolarising hyperkalaemic arrest [462] including sodium and calcium loading [276,277,306] coronary vasoconstriction [182,267,287,290,291,552] coronary vessel spasm [10,284,285] arrhythmias and conduction disturbances [9] and stunning [304,306].

2) AL arrest lowers coronary vascular resistance which may provide greater uniformity of cardioplegia delivery and protection of the myocardium and coronary endothelium from ischaemic injury [458,553,554].

3) AL cardioplegia contains physiological potassium levels. AL normokalaemic arrest may be useful in patients with renal disease or metabolic disturbances where meticulous control of electrolytes is essential. An important finding of the Chapter 4 study was that a potassium concentration below 3mM resulted in poor recovery of post-cardioplegia function, which showed the importance of checking and maintaining the patient's plasma potassium level within the physiological range prior to administration of AL cardioplegia.

4) Based on other properties of AL [555], warm AL arrest (with heart and systemic temperature maintained at 32°C) may potentially improve protection of the brain [184,347], decrease the inflammatory reaction to cardiopulmonary bypass [157], and avoid the adverse effects on energy metabolism and contractile function [11,326] which can occur when the heart is rewarmed following hypothermic arrest.

5) Intermittent AL cardioplegia may offer the surgeon greater versatility over the surgical visual field at warmer temperatures while maintaining cardioprotection during the no-flow interval.

6) One-shot AL arrest, in addition to decreasing total cardioplegia volume, may reduce haemodilution and expedite surgery to reduce cross-clamp time and the duration of cardiopulmonary bypass [416].

7) AL cardioplegia may offer surgeons an alternative strategy of polarised arrest with a warm normokalaemic and normocalcaemic environment for the heart and body throughout arrest, reanimation and reperfusion.

8.6 Limitations

One of the advantages of the isolated heart technique is that it allows assessment of cardiac function without the confounding effects present in an *in vivo* model. However, this may also be a limitation as it does not evaluate the intact body response to treatment interventions along with resulting physiological interactions. Additionally, isolated hearts perfused with crystalloid buffers remain viable for a limited time. Use of a larger *in vivo* animal model would allow assessment of the whole body response and also extension of study time points for up to 24 hours of post-arrest recovery.

Importantly, the present study uses healthy rat hearts which may not be applicable clinically. Patients undergoing cardiac surgery have hearts with variable disease states. The key findings of this thesis need to be replicated in an aging animal model to more closely represent the current clinical situation involving older co-morbid individuals. Clinical trials would then be required to demonstrate the efficacy and versatility of AL cardioplegia in humans.

Lastly, direct comparison of recovery of function at reperfusion cannot be made between the isolated heart model with reperfusion in working mode using pre-set preload and afterload pressures, and the clinical situation using crystalloid or blood-based cardioplegia and dynamic transition from cardiopulmonary bypass.

8.7 Future Studies

The studies in this thesis were conducted on healthy mature rats, therefore further studies with specific functional, physiological and morphological endpoints are required to assess the level of protection afforded by AL cardioplegia for immature and aged diseased hearts in small and larger animal models.

Potential future studies include investigating

1) the duration of polarised arrest in sarcolemmal and mitochondrial membranes with different concentrations of AL
2) the effect of AL cardioplegia on mitochondrial membrane potential and mitochondrial pore regulation [495]
3) production of reactive oxygen species during warm and cold AL arrest and at reperfusion using electron spin resonance techniques
4) changes in cellular metabolism, using ^{31}P NMR (ATP, PCr, pH, free Mg^{2+}), during warm AL arrest and at reperfusion
5) AL cardioprotection during intermittent delivery and single dose delivery for extended cross-clamp times.

The results of these studies may lead to improvement of AL solution as a cardioplegic agent:

The studies in this thesis could be extended by translation to a pig model of cardiopulmonary bypass for detailed investigation of the *in vivo* response to warm AL one-shot cardioplegia, and the effects of temperature on adenosine [238] and lignocaine activity [410,411] and reperfusion functional recovery. Low dose systemic adenosine-lignocaine (adenocaine) treatment has potent anti-inflammatory effects [454]. Trials using AL cardioplegia in conjunction with low dose systemic AL could assess reduction in the severity of the inflammatory reaction to cardiopulmonary bypass compared with hyperkalaemic arrest [157]. The resulting development and modification of AL cardioplegia and arrest protocols may increase clinical applications.

Another focus of future studies would be to assess the level of morbidity associated with warm AL arrest compared to del Nido arrest in human trials. As previously discussed, a wide range of post-operative complications occur with current hyperkalaemic cardioplegia protocols. Long term sequelae such as myocardial

fibrosis [536] may also result from hypothermic hyperkalaemic cardioplegia. The potential ability of AL(M) to reduce or prevent these complications warrants further investigation.

8.8 General conclusion

In conclusion, this thesis has shown that adenosine and lignocaine cardioplegia is superior to hyperkalaemic arresting solutions at warm temperatures and can be administered as continuous, intermittent or single-dose cardioplegia. Cardioprotective mechanisms afforded by warm (32^{o}C) AL cardioplegia may involve polarised arresting membrane potentials, maintenance of normothermia and normocalcaemia during arrest and reperfusion, and possibly protection from radical-induced reperfusion injury. Further studies are required to investigate the mechanisms of AL cardiprotection at warm temperatures. The warm and cold AL(M) arrest protocols developed in this thesis may have clinical potential as alternative cardioplegia strategies to hypothermic, hyperkalaemic cardioplegia.

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APPENDIX A

Composition of perfusion buffers and cardioplegic solutions

Table A1: Modified Krebs-Henseleit solution (containing glucose)

Compound	Concentration (mM)
Sodium	145
Potassium	5.9
Chloride	124.5
Magnesium	0.512 (free Mg^{2+} = 0.5mM)
Calcium	1.12 (free Ca^{2+} = 1.07mM)
Glucose	10
Phosphate	1.2
pH	7.4

Table A2: Modified Krebs-Henseleit solution (containing glucose) with MPG

Compound	Concentration (mM)
Sodium	145
Potassium	5.9
Chloride	124.5
Magnesium	0.512 (free Mg^{2+} = 0.5mM)
Calcium	1.12 (free Ca^{2+} = 1.07mM)
Glucose	10
Phosphate	1.2
MPG (N-(2-mercaptopropionyl)-glycine)	1.0
pH	7.4

Table A3: Adenosine and Lignocaine solutions

1) AL (200:500)

Compound	Concentration (mM)
Sodium	145
Potassium	5.9
Chloride	124.5
Magnesium	0.512 (free Mg^{2+} = 0.5mM)
Calcium	1.12 (free Ca^{2+} = 1.07mM)
Glucose	10
Phosphate	1.2
Adenosine	0.2
Lignocaine	0.5
pH	7.7

2) AL (400:1000):
   Adenosine 0.4mM and Lignocaine 1.0mM added to KH solution as in Table A3.

3) AL (600:1500)
   Adenosine 0.6mM and Lignocaine 1.5mM added to KH solution as in Table A3.

Table A4: Adenosine and Lignocaine solutions with MPG

1) AL (200:500) with MPG

Compound	Concentration (mM)
Sodium	145
Potassium	5.9
Chloride	124.5
Magnesium	0.512 (free Mg^{2+} = 0.5mM)
Calcium	1.12 (free Ca^{2+} = 1.07mM)
Glucose	10
Phosphate	1.2
Adenosine	0.2
Lignocaine	0.5
MPG (N-(2-mercaptopropionyl)-glycine)	1.0
pH	7.7

2) AL (400:1000) with MPG
MPG 1.0mM added to AL (400:1000) as in Table A4.

Table A5: Adenosine and Lignocaine solution with high Mg^{2+}

Compound	Concentration (mM)
Sodium	145
Potassium	5.9
Chloride	124.5
Magnesium	2.56 (free Mg^{2+} = 2.5mM)
Calcium	1.12 (free Ca^{2+} = 1.07mM)
Glucose	10
Phosphate	1.2
Adenosine	0.4
Lignocaine	1.0
pH	7.7

Table A6: Lignocaine solution

Compound	Concentration (mM)
Sodium	145
Potassium	5.9
Chloride	124.5
Magnesium	0.512 (free Mg^{2+} = 0.5mM)
Calcium	1.12 (free Ca^{2+} = 1.07mM)
Glucose	10
Phosphate	1.2
Lignocaine	0.5
pH	7.7

Table A7: High Potassium solutions

1) 16mM K^+ in Krebs Henseleit solution

Compound	Concentration (mM)
Sodium	145
Potassium	16
Chloride	124.5
Magnesium	0.512 (free Mg^{2+} = 0.5mM)
Calcium	1.12 (free Ca^{2+} = 1.07mM)
Glucose	10
Phosphate	1.2
pH	7.7

2) 25mM K^+ in Krebs Henseleit solution

Final concentration of 25mM K^+ in KH as in Table A7.

Table A8: Adenosine and Lignocaine solutions with various K^+ concentrations

1) AL (200:500) with 0.1mM K^+

Compound	Concentration (mM)
Sodium	145
Potassium	0.1
Chloride	124.5
Magnesium	0.512 (free Mg^{2+} = 0.5mM)
Calcium	1.12 (free Ca^{2+} = 1.07mM)
Glucose	10
Phosphate	1.2
Adenosine	0.2
Lignocaine	0.5
pH	7.7

2) AL (200:500) with 3mM K^+
Final concentration of 3mM K^+ in AL solution as in Table A8.

3) AL(200:500) with 10mM K^+
Final concentration of 10mM K^+ in AL solution as in Table A8.

4) AL(200:500) with 16mM K^+
Final concentration of 16mM K^+ in AL solution as in Table A8.