Myocardial stunning in the neonate

P. D. Booker

Prolonged but reversible post-ischaemic ventricular dysfunction was first termed “myocardial stunning” in 1982 by Braunwald and Kloner. This condition typically occurs after cardiac surgery where the heart has sustained ischaemic–reperfusion damage during operation. Neonates are not immune from such problems and as early primary repair, rather than palliation, of congenital heart lesions is now commonplace, prevention and treatment of myocardial stunning is increasing in importance to the paediatric cardiac anaesthetist. It was thought previously that myocardial stunning occurred only after ischaemia–reperfusion, but it is now apparent that stunning can also be caused by hypoxia–reoxygenation. Moreover, irrespective of the aetiology, the nature and consequences of myocyte injury may be modified by post-natal age. Experimental studies indicate that the immature heart exhibits greater functional recovery than the adult heart after a short period of ischaemia (fig.1). Conversely, experimental models of normothermic ischaemia without reperfusion have shown that newborn hearts develop ischaemic contracture earlier than adult hearts. However, if reperfusion is started before the onset of contracture, newborn hearts are able to recover ventricular function and high-energy stores faster than adult hearts. Thus the explanation for this apparent paradox is not solely related to experimental end-points. Moreover, the response to ischaemia–reperfusion is significantly different between not only the newborn and the older neonate, but also between species, which makes study comparisons and interpretation difficult. Compared with adults requiring cardiac surgery for acquired lesions, operative management differs for hypoxia neonates in that it is initiation of cardiopulmonary bypass (CPB), rather than reversal of ischaemia, which may provide the first stimulus for myocyte injury. Myocardial stunning can develop in the absence of previous ischaemia and cyanotic neonates are at particular risk of “reoxygenation injury” and subsequent myocardial dysfunction when CPB or extracorporeal membrane oxygenation (ECMO) is initiated. Experimental studies have shown that hypoxia–reoxygenation may be more injurious than a similar period of ischaemia–reperfusion, although the hypoxic heart may have a greater tolerance of ischaemia than the normally oxygenated heart. Myocardial stunning is caused by myocyte dysfunction. Generation of free radicals, resulting in damage to cellular proteins and phospholipids, and disruption of intracellular calcium homeostasis, are the two, non-mutually exclusive mechanisms responsible for this post-injury dysfunction. Subsequently, disturbances in energy production or energy use, or both, by the contractile elements can be demonstrated. However, not only does the normal functioning of the immature myocyte differ from that of the adult, but the mechanism of the cellular response to injury is also different. The first part of this review examines the major physiological differences between myocytes from immature and adult hearts, and how those differences may affect the response to reversible injury. These age-related differences also dictate that the methods we use to prevent stunning, the subject of the second half of this review, may need to vary from those used in the adult.

Part 1. Developmental changes in cellular response to ischaemia–reperfusion or hypoxia–reoxygenation

DEVELOPMENTAL CHANGES IN SARCOLEMMAL IONIC FLUXES AND RESPONSE TO INJURY

Fluctuations in intracellular calcium ion (Ca\(^{++}\)) concentration necessary for excitation–contraction coupling are mediated by both the actions of channels in the sarclemma and sarcoplasmic reticulum (SR). In the adult rabbit, the SR provides 90% of the Ca\(^{++}\) used to activate myofilaments. The influx of Ca\(^{++}\) through voltage-dependent L-type calcium channels in the sarclemma triggers the opening of SR Ca\(^{++}\) channels, inducing the release of large amounts of Ca\(^{++}\) from the SR. In contrast, many studies using various immature mammalian heart preparations have shown that trans-sarcolemmal Ca\(^{++}\) flux is much more important than SR Ca\(^{++}\) release for the action potential-induced increases in cytosolic Ca\(^{++}\) concentration. However, human neonates may have relatively mature myocytes and SR Ca\(^{++}\) release, triggered by sarcolemmal Ca\(^{++}\) flux, may assume greater significance in early postnatal life in humans than in newborn animals. Nevertheless, the smaller density of calcium current in human neonates and young children compared with adults suggests that the density of L-type calcium channels increases with postnatal age. Furthermore, the rate of increase of intracellular Ca\(^{++}\) concentration,
relative to the maximum Ca\(^{++}\) concentration obtained after sarcolemmal depolarization, was found to be significantly smaller in neonates than in older children,\(^{37}\) suggesting relatively low functional efficiency of SR Ca\(^{++}\) release. In immature heart preparations, much of the Ca\(^{++}\) influx through the sarcoplemma is via the sodium–cation (Na\(^{+}–\)Ca\(^{++}\)) exchanger, rather than L-type calcium channels (fig. 2).\(^{7,11}\) The Na\(^{+}–\)Ca\(^{++}\) exchanger allows exchange of three Na\(^{+}\) ions for each Ca\(^{++}\) ion, the relative amount of Ca\(^{++}\) removal depending on intracellular Na\(^{+}\) and H\(^{+}\) ion concentrations. The adenosine triphosphate (ATP)-dependent sodium pump maintains the Na\(^{+}\) concentration gradient across the sarcoplemma.\(^{69}\) The density of Na\(^{+}–\)Ca\(^{++}\) exchangers in the sarcolemma of newborn mammals is species dependent, but is usually,\(^{11}\) although not always,\(^{2}\) greater in the newborn than in adults. This relative abundance of Na\(^{+}–\)Ca\(^{++}\) exchangers correlates with the greater importance not only of this method of Ca\(^{++}\) influx during systole, but also of Ca\(^{++}\) efflux during diastole.\(^{11,111}\) Factors favouring Ca\(^{++}\) influx via Na\(^{+}–\)Ca\(^{++}\) exchangers include prolonged sarcolemmal depolarization, decreased extracellular and increased intracellular Na\(^{+}\) concentration.\(^{111}\) Thus Na\(^{+}–\)Ca\(^{++}\) exchange may play an important role in determining Ca\(^{++}\) influx not only during cellular acidosis and ischaemia, but also during hyperkalaemic cardioplegic arrest.\(^{25}\) Sarcolemmal injury, secondary to ischaemia–reperfusion, can be caused not only by free radicals but also by intracellular ATP depletion, either by its effect on channel protein function or phospholipid topology, or both.\(^{89}\) Subsequent excessive inward Na\(^{+}\) flux through various sarcolemmal transport pathways, including the sodium fast channels, calcium channels and the Na\(^{+}–K\(^{+}\) pump, may result in intracellular Na\(^{+}\) accumulation.\(^{109}\) This increase in intracellular Na\(^{+}\) concentration can lead to increased sarcolemmal Ca\(^{++}\) influx during ischaemia and reperfusion, secondary to Na\(^{+}–\)Ca\(^{++}\) exchange. Na\(^{+}–\)Ca\(^{++}\) exchange activity during ischaemia–reperfusion may be greater in newborns than in adults.\(^{73}\) Although acidosis partially inhibits the Na\(^{+}–\)Ca\(^{++}\) exchanger,\(^{20}\) intracellular accumulation of Ca\(^{++}\) progressively increases if ischaemia is sustained. Experimental studies have indicated that ischaemia–reperfusion or acidosis, or both, inhibit Na\(^{+}–K\(^{+}\)–ATPase activity less in the newborn than in the adult.\(^{73}\) However, the beneficial decrease in intracellular Na\(^{+}\) and Ca\(^{++}\) accumulation this could produce is somewhat negated by a reduced basal level of activity of this enzyme in newborns.\(^{18}\) Although sarcolemmal channel activity during ischaemia–reperfusion may be greater in the newborn than in the adult, both Ca\(^{++}\) influx into the cell and resting intracellular Ca\(^{++}\) concentration are determined largely by the H\(^{+}\) gradient across the sarcoplemma. This is because another important source of Na\(^{+}\) influx is the Na\(^{+}–\)H\(^{+}\) exchanger, particularly when there is severe intracellular acidosis induced by prolonged ischaemia–reperfusion.\(^{116}\) Inhibition of Na\(^{+}–\)H\(^{+}\) exchange is protective against ischaemia–reperfusion injury, when the H\(^{+}\) ion gradient across the sarcoplemma is exacerbated; it inhibits the restoration of intracellular pH and hence the Ca\(^{++}\) overload mediated by Na\(^{+}–\)Ca\(^{++}\) exchange.\(^{31,38,116}\) Although extracellular acidosis partially inhibits the Na\(^{+}–\)H\(^{+}\) exchanger, intracellular retention of Ca\(^{++}\) increases progressively during sustained ischaemia because this Na\(^{+}–\)H\(^{+}\) exchange inhibition is over-ridden by the increasing intracellular acidosis.\(^{19}\) Experimental studies indicate that H\(^{+}\) ion extrusion from the cytosol via various sarcolemmal channels may be more efficient in the newborn than in the adult.\(^{72,84}\) The ATP-dependent Ca pump in the sarcoplemma does not normally transport significant amounts of Ca\(^{++}\) back out of the cell in either immature or adult hearts. Reactive oxygen intermediates, such as superoxide anion (O\(_{2}^{-}\)), may mediate both reperfusion and reoxygenation injury.\(^{19}\) Hypoxia and ischaemia deplete tissue concentrations of endogenous antioxidants, increasing vulnerability to reperfusion or reoxygenation injury, or both. Reoxygenation itself
Myocardial stunning in the neonate

In the adult, intracellular Ca\(^{++}\) concentration in the myocyte is restored to resting levels mainly as a result of SR Ca\(^{++}\)-ATPase, which binds Ca\(^{++}\) and ATP on its cytoplasmic side and translocates the cation into the SR lumen. The relative contributions of the SR pump and the Na\(^{+}\)-Ca\(^{++}\) exchanger in removing Ca\(^{++}\) from the cytosol during diastole varies among species, but immature mammal and human myocytes consistently show reduced SR Ca\(^{++}\) pump activity compared with those from adult hearts. The age-related changes in myocardial relaxation and in the relaxation response to various stimuli probably relate to these limitations in SR Ca\(^{++}\) pump activity or low level of phospholamban activity, or both. None the less, the SR probably makes a significant physiological contribution to intracellular Ca\(^{++}\) flux, even in newborns. Experimental studies have produced conflicting results as to whether or not the SR in the stunned myocardium can actively take up normal amounts of Ca\(^{++}\). Methodological differences may account for these disparities as the capacity of SR to actively transport Ca\(^{++}\) decreases time-dependently during ischaemia. However, although Ca\(^{++}\) leakage from the SR into the cytosol may occur subsequent to ischaemia–reperfusion injury in myocytes from both adult and immature hearts, excessive Ca\(^{++}\) flux across the sarcolemma is probably the predominant cause of intracellular Ca\(^{++}\) accumulation. Demonstration of a beneficial protective effect afforded by the addition of sarcolemmal calcium channel antagonists to cardiopлегic solutions offers indirect confirmation of this hypothesis.

DEVELOPMENTAL CHANGES IN SR FUNCTION AND RESPONSE TO INJURY

In the adult, intracellular Ca\(^{++}\) concentration in the myocyte is restored to resting levels mainly as a result of SR Ca\(^{++}\)-ATPase, which binds Ca\(^{++}\) and ATP on its cytoplasmic side and translocates the cation into the SR lumen. The relative contributions of the SR pump and the Na\(^{+}\)-Ca\(^{++}\) exchanger in removing Ca\(^{++}\) from the cytosol during diastole varies among species, but immature mammal and human myocytes consistently show reduced SR Ca\(^{++}\) pump activity compared with those from adult hearts. The age-related changes in myocardial relaxation and in the relaxation response to various stimuli probably relate to these limitations in SR Ca\(^{++}\) pump activity or low level of phospholamban activity, or both. None the less, the SR probably makes a significant physiological contribution to intracellular Ca\(^{++}\) flux, even in newborns. Experimental studies have produced conflicting results as to whether or not the SR in the stunned myocardium can actively take up normal amounts of Ca\(^{++}\). Methodological differences may account for these disparities as the capacity of SR to actively transport Ca\(^{++}\) decreases time-dependently during ischaemia. However, although Ca\(^{++}\) leakage from the SR into the cytosol may occur subsequent to ischaemia–reperfusion injury in myocytes from both adult and immature hearts, excessive Ca\(^{++}\) flux across the sarcolemma is probably the predominant cause of intracellular Ca\(^{++}\) accumulation. Demonstration of a beneficial protective effect afforded by the addition of sarcolemmal calcium channel antagonists to cardiopлегic solutions offers indirect confirmation of this hypothesis.

DEVELOPMENTAL CHANGES IN ENERGY SUBSTRATE UTILIZATION AND RESPONSE TO INJURY

Newborn mammalian myocardium consists of small myocytes containing small mitochondria that exhibit well-coupled oxidative phosphorylation and phosphorylating respiratory activity equal to that in adults. Normally, the substrate preference of the adult heart is determined by the relative concentrations of substrates in the blood. Thus when carbohydrate concentrations are high, carbohydrate oxidation is the main energy source but, in the fasted state, the myocardium relies primarily on \beta-oxidation of free fatty acids. In contrast, most of the energy requirements of the newborn heart are obtained from lactate oxidation and glycolysis. Experimental animal work suggests that the capacity to oxidize fatty acids and glucose is impaired in early postnatal life. However, over the first few days of life there is rapid...
maturation of the enzyme pathways involved in fatty acid oxidation, although glucose oxidation appears to mature at a slower rate.\textsuperscript{55,66} The stores of myocardial glycogen in the newborn decrease soon after birth, but the ability to metabolize amino acids anaerobically is retained throughout the neonatal period; both metabolic pathways may contribute to the neonate’s tolerance of hypoxia and ischaemic injury.\textsuperscript{55} However, experimental studies have been unable to demonstrate a consistent correlation between functional recovery and tissue ATP concentration.\textsuperscript{52,62} Furthermore, as contractile dysfunction can persist in the presence of near-normal ATP concentrations and normal mitochondrial function,\textsuperscript{35} it would appear that energy supply is not the limiting factor preventing return of normal myocardial contractility after ischaemia–reperfusion injury. Lactate production is enhanced in immature hearts compared with adult hearts during ischaemia, but it is not known if H\textsuperscript{+} ion production is also influenced by age. Although both anaerobic glycolysis and glycogen metabolism have lactate anions as end products, the relevant chemical reactions do not produce a net gain in H\textsuperscript{+} ions.\textsuperscript{28} During oxidative phosphorylation, most of the H\textsuperscript{+} ions produced by ATP hydrolysis are used in its re-synthesis. However, when ATP is formed by glycolysis rather than by mitochondrial metabolism or creatine phosphate breakdown, H\textsuperscript{+} ions arising from its hydrolysis are not consumed. Thus metabolic generation of protons during relatively short periods of ischaemia–reperfusion in both adult and immature hearts is primarily a result of the increased reliance on glycolysis for ATP re-synthesis. During prolonged ischaemia, however, H\textsuperscript{+} production secondary to nucleotide degradation becomes increasingly important and is subject to developmental influences (see below). Moreover, in adults there may be some additional contribution in H\textsuperscript{+} production related to lipolysis, membrane phosphoglyceride degradation, or both.\textsuperscript{26} Histidine residues provide some intracellular buffering of H\textsuperscript{+} ions, but the predominant intracellular buffer is inorganic phosphate, formed after transfer of phosphate from creatine phosphate to ADP. Unbuffered protons can leave the cell via the sarcolemmal Na\textsuperscript{+}–H\textsuperscript{+} exchanger but interstitial proton accumulation decreases the trans-sarcolemmal H\textsuperscript{+} ion gradient and results in a decrease in H\textsuperscript{+}, lactate ion efflux and Na\textsuperscript{+}–H\textsuperscript{+} exchange across the sarcolemma. While tissue remains ischaemic, however, a low pH may be of benefit as it depresses SR and myofilament ATPase activity and thereby slows the rate of ATP use.\textsuperscript{59} The rate of H\textsuperscript{+} ion removal from ischaemic tissue is determined by washout. Reperfusion of ischaemic tissue, for example by cardioplegia, leads to rapid efflux of accumulated intracellular protons, resulting in secondary influx of Ca\textsuperscript{2+} via the Na\textsuperscript{+}–Ca\textsuperscript{2+} exchanger. Contractile failure early during ischaemia is caused by a decrease in the sensitivity of the myofilaments to Ca\textsuperscript{2+}, together with reduced Ca\textsuperscript{2+} flux secondary to sarcoplasmal and SR calcium channel dysfunction. The decrease in myofilament sensitivity and calcium channel inhibition are caused by an increase in the intracellular concentration of inorganic phosphate and by a decrease in intracellular pH.\textsuperscript{20,58} However, inhibition of contractile function induced by acidosis is reduced in neonates, compared with adults (fig. 4).
breakdown by 5'-nucleotidase may be an important mechanism of high-energy phosphate loss. However, giving a 5'-nucleotidase inhibitor to neonatal rabbits does not improve either myocardial ATP concentrations or ventricular function after ischaemia–reperfusion (fig. 5). This is to be expected, as experimental and clinical studies suggest that immature hearts normally have decreased concentrations of 5'-nucleotidase compared with adult hearts. Deficiency of 5'-nucleotidase should ameliorate the loss of intracellular adenine nucleotides during reperfusion, so facilitating recovery after reperfusion by preserving the high-energy precursors able consequently to participate in salvage re-synthesis of ATP. Experimentally, 5'-nucleotidase activity may be related inversely to functional recovery after ischaemia–reperfusion, but this has not been confirmed in humans. This may be because of degradation of high-energy phosphates by alternative biosynthetic pathways not involving 5'-nucleotidase.

DEVELOPMENTAL CHANGES IN CONTRACTILE PROTEINS AND RESPONSE TO INJURY

Clinical studies have demonstrated significant postnatal increases in myocardial contractility. This is related to an increase in the relative amount of contractile protein and delivery of Ca\(^{2+}\) to the myofilaments. In addition, there are differences in kinase, myosin, actin and tropomyosin isoforms between the newborn and adult human, although the functional significance of this developmental change is unknown. There do not appear to be any developmental variations in C-protein in the heart. However, developmental changes in troponin isoforms have been shown to be of functional importance. There are two troponin I isoforms, the relative proportion of the immature skeletal isoform decreasing with maturation. The switch to the adult cardiac isoform appears to be complete postnatally in humans. There is considerable functional difference in the two troponin I isoforms such that the myofibrillar ATPase of the fetus and newborn has an increased sensitivity to Ca\(^{2+}\) and desensitivity to acidosis. In addition, expression of the four different troponin T isoforms differs with age, another reason why newborn myofibrils have enhanced Ca\(^{2+}\) sensitivity compared with adults. Although some experimental work suggests that the decrease in contractile force in stunned myocardium is a result of a decrease in maximum calcium-activated force without desensitization of the myofilaments to Ca\(^{2+}\), other studies have demonstrated the converse. It is probable that both mechanisms are involved to a greater or lesser degree. The reduction in sensitivity for Ca\(^{2+}\), secondary to myofilament injury occurring during reperfusion–reoxygenation, is probably a result of a reduced affinity of troponin C for Ca\(^{2+}\). This suggests that age-related changes may not affect the reduced affinity of troponin C for Ca\(^{2+}\) induced by stunning, as troponin C is invariant throughout development. However, the altered Ca\(^{2+}\) binding of troponin C may either be a direct result of reperfusion–reoxygenation injury or an indirect result of degradation of other regulatory thin filament proteins, such as troponin I and T. The latter possibility increases potential developmental influences. The time course of recovery suggests that normal function returns only when irreversibly damaged protein is replaced. This hypothesis is consistent with observations that inotropic drugs that increase cytosolic Ca\(^{2+}\) concentration during systole can enhance function in stunned myocardium to near normal levels. The finding that maximal tension-generating capabilities of isolated myocytes can be unchanged while injured suggests that stunning has no significant effects on myosin, actin or crossbridge states.

DEVELOPMENTAL CHANGES IN CORONARY ENDOTHELIAL FUNCTION AND RESPONSE TO INJURY

Impairment of coronary vascular endothelial function induced by ischaemia–reperfusion is characterized by a reduced vasodilator response to endothelial stimulation of nitric oxide. Although hypothermia impairs release of nitric oxide from coronary endothelium, this appears to be a rapidly reversible phenomenon on rewarming. Experimental work has suggested that immature hearts have better recovery of endothelial function compared with adult hearts after hypothermic ischaemia–reperfusion (fig. 6). Ischaemic hearts reperfused with leukocyte-depleted blood or given monoclonal antibodies to leucocyte adhesion molecules demonstrate significantly reduced endothelial reperfusion injury, suggesting that neutrophil activation is involved. Coronary vascular endothelial cells differ from myocytes in that their response to ischaemia–reperfusion is in complete contrast with their response to hypoxia–reoxygenation. The addition of a nitric oxide donor or L-arginine to cardioplegia, or post-CPB administration of nitroglycerin has been shown to ameliorate reperfusion injury induced by coronary endothelial dysfunction. This is because during ischaemia, blood flow is stagnant and neutrophils become adherent and then “activated” during reperfusion. Nitric oxide inhibits neutrophil adherence to vascular endothelium and inhibits subsequent activation and oxygen free radical generation. Conversely, nitric oxide may accelerate reoxygenation damage when blood flow is high and neutrophil adherence unlikely.
Constitutive nitric oxide synthase activity is related to oxygen tension, and uncontrolled reoxygenation using a high arterial partial pressure of oxygen leads to excessive nitric oxide production. This excess of nitric oxide reacts with superoxide anion to generate highly toxic reactive oxygen intermediates that can cause lipid peroxidation and subsequent myocardial dysfunction. Experimental studies have shown that suppression of nitric oxide production during controlled reoxygenation can reduce endothelial and myocardial dysfunction.

**Part 2. Principles of prevention of myocardial stunning in the neonate**

Determination of the optimal method of myocardial protection in the neonate has been the subject of much experimental research. However, the use of diverse mammalian species, each with different rates of development, may appear an unreliable basis to provide appropriate data. Moreover, many studies have relied on isolated heart preparations that fail to model non-coronary collateral flow, neurohormonal influences and the effects of cardiopulmonary bypass (CPB). Nevertheless, this experimental evidence, together with accumulating clinical experience, has provided useful guiding principles that are helping clinicians find safe, effective methods of enhancing myocardial protection for the neonate at risk of stunning. Most techniques of intraoperative myocardial protection rely on reducing myocardial oxygen demand which is determined primarily by electro-mechanical activity and secondarily by metabolic rate. Electromechanical work is minimized by potassium-induced diastolic arrest, and basal metabolic rate is reduced with hypothermia. However, the effects of hypothermia and cardioplegic arrest on myocardial oxygen consumption may differ in the neonate compared with the adult, and it would appear that for neonates, cardioplegic arrest may add little extra myocardial protection to that offered by hypothermia alone (fig. 7). Thus profound hypothermic circulatory arrest, without the use of cardioplegia, remains a widely used method of combining myocardial protection with a bloodless and motionless surgical field. Non-neurological complications and haemodynamic profile are similar if low-flow CPB or circulatory arrest is used. However, the higher incidence of neurological complications associated with circulatory arrest has revived interest in strategies that allow perfusion to be maintained throughout surgery in even the smallest neonate. This has led to the development of specific cardioprotective techniques for neonates. In addition to the main preventative strategies of hypothermia and cardioplegia, other adjuvant techniques are under investigation.

**HYPOTHERMIA**

Myocardial temperature may be decreased using perfusion cooling or topical cooling, or both. Topical cooling can be achieved by bathing the heart surface with ice slush, although this method is ineffective at cooling the most vulnerable sub-endocardial regions and most paediatric cardiac surgeons use topical cooling only as an adjunct to perfusion-induced cooling. Cold cardioplegic solutions or CPB, or both, are very effective at cooling the heart and can be used in combination. It is comparatively easy in neonates to ensure that CPB-induced hypothermia produces temperature equilibration throughout all tissues of the body, as neonates have a higher proportion of vessel-rich tissues than adults. Whole body perfusion cooling allows low CPB flow rates or circulatory arrest, as tissue energy requirements are substantially reduced at low temperature. However, the temperature dependency of biochemical reactions is variable and hypothermia, therefore, can lead to derangement of intracellular homeostasis. In particular, the variable effect of low temperature on sarcoplasmic and SR channels results in increased intracellular Ca++ concentration. Thus although hypothermia is believed to improve tolerance to ischaemia, intracellular Ca++ accumulation induced by hypothermia may lead to exacerbation of ischaemia–reperfusion injury. Experimental studies have indicated that hypothermic perfusion before ischaemia may attenuate functional recovery after reperfusion, but that this detrimental effect can be ameliorated by hypocalcaemic perfusion before or during cardioplegic arrest. One clinical study suggested that rapid myocardial...
cooling could result in significant damage and recommended “slow” cooling before induction of cardioplegic arrest.132 However, more recent experimental studies have been unable to confirm that rapid cooling is more detrimental than slow cooling to the immature myocardium.455 Furthermore, prolonged hypothermic CPB perfusion may itself be deleterious to the immature heart,44 particularly if electromechanical activity is sustained.107 In addition to intracellular Ca \(^{2+}\) accumulation, prolonged hypothermic perfusion may be associated with hyperglycaemia and inhibition of mitochondrial function, which leads to metabolic derangement and decreased ATP production.107 In clinical practice, perfusion cooling at a rate of 1 °C min\(^{-1}\) is used as a compromise between restricting the duration of pre-arrest hypothermic perfusion and optimizing tissue temperature equilibration. Inter-species differences and use of different study end-points have complicated the identification of the optimal temperature for prolonged myocardial protection. A study of ATP metabolism and contractility in canine hearts subjected to either normothermic (37°C) or hypothermic (15°C) cardioplegic arrest found similar reductions in tissue ATP concentrations in each group.46 However, hypothermic perfusion resulted in a three-fold increase in adenosine and AMP concentrations compared with normothermic hearts, suggesting that hypothermia to 15°C may produce beneficial inhibition of nucleotide breakdown. Furthermore, myocardial function was not significantly different between the two groups, suggesting that hypothermia to 15°C is not detrimental to myocardial function. It must be emphasized, however, that these relatively innocuous effects of hypothermia relate to arrested hearts; when electromechanical activity is maintained, hypothermic perfusion may cause myocardial injury.107 In rabbits, there appears to be a biphasic relationship between the extent of protection and degree of hypothermia (fig. 8). Decreasing myocardial temperatures to less than approximately 20 °C did not confer additional protection, but higher temperatures provoked rapid loss in protection.57 In contrast, a study involving neonatal pigs found that time to ischaemic contracture and decline in ATP concentrations were significantly less in hearts maintained at 12°C compared with hearts at 19°C. Moreover, the protective effects of hypothermia were significantly reduced if pre-ischaemic ATP concentrations were low.113 One clinical study has suggested that myocardial temperatures less than approximately 15 °C may inhibit energy substrate production by anaerobic glycolysis to a greater degree than energy consumption.59 In keeping with these findings, most centres using circulatory arrest techniques aim to maintain myocardial temperatures between 15 and 20°C throughout surgery. The period immediately after corrective surgery is undoubtedly crucial to ultimate recovery, as reperfusion–reoxygenation injury may be occurring at the same time as oxygen consumption is increasing, secondary to the increase in metabolic rate and initiation of reparative and electromechanical activity. Inevitably, it has been difficult to identify the relative importance of the effects of reperfusion, reoxygenation and rewarming on the immature heart. Experimental studies have shown that hypothermia-induced depression of myocardial function and depression of metabolic activity may persist during rewarming.106107 Moreover, rewarming from profound hypothermia can cause structural derangement of the sarcolemma, possibly caused by lipid peroxidation.67 The standard rewarming rate of 1 °C min\(^{-1}\) is a compromise between the time required for optimizing tissue temperature equilibration and keeping reperfusion of the non-working heart to a minimum.59 None the less, clinical experience indicates that a significant increase in contractility occurs during the later stages of rewarming. Thus some prolongation of normothermic perfusion to the working heart may allow better myocardial recovery before the workload of the heart increases as a result of the increase in systemic vascular resistance after CPB.

**CARDIOPLEGIA**

Although there is widespread use of cardioplegia in paediatric cardiac surgery, optimal temperature, composition and delivery pressure of the solution remain subjects of controversy. The main reason for using cardioplegia is to maintain the heart in diastolic arrest while the aorta is clamped and there is no coronary blood flow. In addition, the cardioplegic solution can be used to assist in the uniform cooling or warming of the arrested heart. However, if profound hypothermia is induced by CPB, the additional cooling effect of cardioplegia infusion on the myocardium may be relatively small. Moreover, experimental evidence suggests that solutions at temperatures <4°C may have detrimental effects, probably secondary to endothelial injury.4 Clinical studies in adults suggest that use of warm cardioplegia to induce diastolic arrest or infusion again, or both, immediately before unclamping the aorta may reduce reperfusion–reoxygenation damage,18 although this has not been confirmed in neonates. The cardioplegic solution usually contains a high concentration of K\(^+\) (15–20 mmol litre\(^{-1}\)) that produces electromechanical arrest by reducing the resting membrane potential and inactivating the fast sodium channel in the sarcolemma. Return of electromechanical activity while the aorta is clamped, which increases the possibility of myocardial injury, is more likely to occur when myocardial temperatures are
myocyte to hyperkalaemia. In adults, hyperkalaemia produces a significant increase in cell volume and reduction in contractility that is not seen in myocytes from immature rabbits. This developmental difference may relate to calcium-induced myocyte injury, as hyperkalaemia-induced depolarization of immature myocytes does not result in as great an increase in intracellular Ca$^{2+}$ concentration as has been demonstrated in adults. The mechanism for this Ca$^{2+}$ influx during hyperkalaemia varies with age. In adults the primary entry route for the increase in cytosolic Ca$^{2+}$ is the sarcolemmal calcium channel, whereas in the immature myocyte it is the Na$^+$/Ca$^{2+}$ exchanger. The SR has no influence on cytosolic Ca$^{2+}$ during hyperkalaemia at any age. The potential for hypothermia and hyperkalaemia to cause calcium influx has made the optimal Ca$^{2+}$ concentration of cardioplegia for the neonate a controversial topic.

Immature rabbit hearts receive maximum myocardial protection when the cardioplegic solution contains only Ca$^{2+}$ 0.3–0.4 mmol litre$^{-1}$, although neonatal piglet hearts appear to benefit from normocalcaemic solutions. More recent work may have helped resolve this issue. Myocardial protection appears to be independent of cardioplegic Ca$^{2+}$ concentration in normal neonatal piglet hearts. However, when a comparable group of piglets subjected to hypoxia–reoxygenation injury before cardioplegic arrest are given normocalcaemic blood cardioplegia, they demonstrated significantly reduced functional recovery and ATP concentrations after CPB compared with those given hypocalcaemic blood cardioplegia (fig. 9). In addition, the concentration of magnesium (Mg$^{2+}$) in the cardioplegic solution has a significant influence on the extent of Ca$^{2+}$ influx induced by hyperkalaemia. Increasing concentrations of extracellular Mg$^{2+}$ can displace Ca$^{2+}$ from the glycocalyx, thus reducing the amount of extracellular Ca$^{2+}$ available for trans-sarcolemmal entry, whether by calcium channel or Na$^+$/Ca$^{2+}$ exchanger. It follows that the optimum Ca$^{2+}$ concentration of cardioplegia for the neonate varies depending on the complex interaction between pre-injury hypoxia, duration of ischaemia, Mg$^{2+}$ concentration of the solution and myocardial temperature. Although the relative merits and demerits of blood and crystalloid cardioplegic solutions remain subjects of controversy, it appears that blood is becoming the preferred vehicle.

The main advantages of blood cardioplegia are its greater buffering and oxygen carrying capacities compared with crystalloid cardioplegia. Although there is a shift in the oxyhaemoglobin dissociation curve to the left secondary to hypothermia, oxygen unloading from cold blood cardioplegia is greater than that expected because of a compensating rightward shift induced by myocardial tissue acidosis, hypercapnia and rewarming. The buffering capacity of blood cardioplegia is usually much greater than that of crystalloid cardioplegia because of the presence of the amino acid histidine, which has an imidazole group that can accept protons. The other potential advantages of blood cardioplegia, which include its beneficial oxygen free radical scavenging, rheological and oncotic properties, do not appear to be subject to developmental influences. The few experimental studies comparing multi-dose blood and crystalloid cardioplegic solutions in hypothermic, immature hearts have shown that the blood vehicle offers better myocardial protection. A single dose of crystalloid cardioplegic solution given at the onset of ischaemia may add to the protection offered by profound hypothermia, but repeated doses appear to be of little further benefit or may even inhibit subsequent recovery of the immature heart. This detrimental effect of multi-dose cardioplegia may be a result of the repeated alteration in H$^+$ ion gradient between the inside and outside of the cell, causing ions to move through the Na$^+$/H$^+$ exchanger during every infusion. There has been concern that hyperkalaemic cardioplegia may induce endothelial cell injury, although high pressure delivery of the solution may be more to blame. High infusion pressure can induce endothelial injury and although control of infusion pressure does not guarantee normal shear stress in the epicardial coronary artery, it is minimized at aortic root pressures of 90–120 mm Hg in the adult dog. Similarly, a study using mature rats has shown that cardioplegia delivered at pressures of 75–105 mm Hg produced better functional and metabolic myocardial recovery than when cardioplegia delivery was at either higher or lower pressures (fig. 10). Thus although there has been no confirmatory clinical evidence, it would seem advisable that in the human neonate, cardioplegia is infused at normal aortic root pressures, that is 60–80 mm Hg. Similarly, reducing coronary artery pressure during initial reperfusion may improve endothelial function by limiting excessive nitric oxide production induced by shear stress. Experimental studies have shown that amino acid enrichment of cardioplegic solutions improves both meta-

![Figure 9](image-url)

Neonatal piglets were placed on hyperoxaemic cardiopulmonary bypass (CPB) and subjected to either multi-dose normocalcaemic or hypocalcaemic blood cardioplegic arrest for 70 min. Half the piglets had previously undergone ventilation for 60 min with an inspired oxygen concentration of 8% to render them hypoxic. Myocardial function was assessed 30 min after CPB and expressed as mean (SD) percentage of pre-CPB values. Both hypocalcaemic and normocalcaemic multi-dose blood cardioplegic solutions allowed complete recovery in the non-hypoxic group. In contrast, the group who had been exposed to acute hypoxia–reoxyegenation injury before cardioplegic arrest demonstrated significantly reduced recovery when normocalcaemic blood cardioplegia was used (*P < 0.05). Adapted from Bolling and colleagues, with permission.
bolic and functional recovery of ischaemic immature myocardium.86 108 In addition to replenishing myocardial energy substrates, glutamate and aspartate inhibit l-arginine transport and subsequent production of reactive oxygen intermediates, hence reducing reoxygenation injury.46 Experimental studies have shown that fumarate may offer metabolic support without causing the systemic vasodilatation that characterizes glutamate administration.86 Fumarate, a Krebs’ cycle intermediate, can be reduced to succinate with the concomitant oxidation of NADH, resulting in ATP production, even under hypothermic, ischaemic or hypoxic conditions. Furthermore, in addition to its metabolic effects, fumarate stimulates mitochondrial uptake of Ca++ under anoxic conditions and may also act as a free radical scavenger. Other cardioplegic additives that have produced encouraging amelioration of stunning include nitric oxide donors, L-arginine, adenosine and potassium channel blockers. It is important to differentiate between reperfusion injury and reoxygenation injury when reviewing the use of agents that affect nitric oxide production (see also DEVELOPMENTAL CHANGES IN CORONARY ENDOTHELIAL FUNCTION AND RESPONSE TO INJURY above). Reperfusion injury may be reduced by using agents that enhance nitric oxide production,10 whereas reoxygenation injury can be reduced by using agents that suppress nitric oxide production.46 Alternatively, reoxygenation injury can be minimized by delaying and controlling reoxygenation (see CONTROLLED REOXYGENATION below). Adenosine and ATP-sensitive potassium channel blockers inhibit hyperkalaemic-induced Ca++ influx into the myocyte,50 and therefore may not be particularly beneficial in neonates. However, both adenosine and ATP-sensitive potassium channels have been implicated in the mechanism of preconditioning (see PRECONDITIONING next).

PRECONDITIONING

Repeated ischaemic episodes would be expected to have a cumulative adverse effect on myocardial function, but instead of becoming sensitized, the heart exhibits a marked resistance to myocardial injury after subsequent prolonged ischaemia. This capacity for endogenous protection, termed preconditioning, can also be initiated by other stimuli such as hypoxia.22 When preconditioned myocardium is subjected to sustained ischaemia, ATP use and anaerobic glycolysis occur at much slower rates than normal, and the duration of ischaemia that can be tolerated before irreversible injury to the myocyte occurs is greatly extended. The mechanism for preconditioning is unknown, but must involve the release of endogenous substances from the ischaemic myocardium because regional preconditioning can protect remote non-preconditioned tissue. Current research has implicated adenosine A1 and α, receptors, inhibitory G proteins, ATP-sensitive potassium channels and protein kinase C in this phenomenon.22 97 Preconditioning requires a minimum duration of initiating insult and its protective effects last only a few hours. Early studies established that preconditioning could limit infarct size and reduce reperfusion injury-induced ventricular arrhythmias. More recently, experimental studies have demonstrated that preconditioning can enhance the recovery of stunned human myocardium21 and animal work has demonstrated that preconditioning preserves normal mitochondrial function and facilitation of glycolysis during reperfusion.115 Preconditioning has been demonstrated in adults undergoing coronary artery surgery,117 although the efficacy of this phenomenon in humans remains a subject of debate.88 More clinical studies in adults undergoing cardiac surgery are required before the therapeutic potentials for paediatric practice can be explored.

CONTROLLED REOXYGENATION

Experimental studies have demonstrated that hyperoxic reoxygenation of previously hypoxic immature piglet hearts causes oxidant injury, reduction in anti-oxidant reserve capacity and a decrease in myocardial contractility.78 The extent of oxidant injury secondary to reoxygenation appears to correlate with nitric oxide production. Suppression of excessive nitric oxide production by controlling reoxygenation and supplementing blood cardioplegia with glutamate-aspartate increases functional recovery.46 Experimental studies have shown that reducing oxygen tension to approximately 100 mm Hg during reoxygenation avoids oxidant injury, retains normal anti-oxidant reserve and improves myocardial functional recovery (fig. 11). These data suggest that reoxygenation injury can be minimized by initiating CPB at the ambient oxygen tension of the hypoxic neonate and delaying controlled reoxygenation until after cardioplegic arrest. Immature hearts subjected to reoxygenation injury before cardioplegic arrest have increased reperfusion injury and decreased functional recovery compared with hearts not subjected to reoxygenation before arrest, even if blood cardioplegia is used. Supplementation of the cardioplegic solution or the CPB prime with antioxidants,77 or strategies designed to reduce nitric oxide production may reverse this increase in reperfusion injury.46 A further increase in recovery can be produced if reoxygenation injury can be minimized by delaying reoxygenation until cardioplegic arrest is initiated.75
OTHER ADJUVANT THERAPY

An infusion of glucose–potassium–insulin given before stunning may promote glucose uptake by the myocardium and increase intracellular energy substrate concentrations. In addition, insulin reduces the concentration of free fatty acids and promotes myocardial glycogen synthesis. Moreover, Krebs’ cycle carbohydrate metabolism after reperfusion is improved, as insulin stimulates the activity of pyruvate dehydrogenase, which is normally inactivated during ischemia. Clinical studies in adults have shown that the haemodynamic effects of catecholamines may be increased by using high-dose insulin to shift myocardial metabolism from free fatty acid oxidation to carbohydrate metabolism. However, the practical difficulties of maintaining normoglycemia while giving insulin throughout the perioperative period continue to make this therapy relatively unpopular. Adults given infusions of glutamate during stunning have demonstrated improved haemodynamic performance and metabolic profile. Glutamate and aspartate enhance glycolysis, improve lactate clearance, replenish Krebs’ cycle intermediates and stimulate alternative anaerobic pathways for regeneration of ATP during ischemia–reperfusion. Although these potential benefits explain why preoperative infusions of certain amino acids have been advocated, glutamate causes pronounced dose-related systemic vasodilatation, and it is usually given only as an additive to the cardioplegic solution or CPB prime.

Conclusions

As primary corrective surgery in the neonate becomes more common, more complex and more time consuming, the search continues for therapeutic strategies that will extend the safe duration of induced myocardial ischemia. Although the use of animal models to investigate the influence of age on myocardial stunning is understandable and necessary, it also implies that such work should be subject to cautious interpretation before extrapolation of their conclusions to the human neonate. Nevertheless, the information engendered from many of these studies has suggested modifications in our management of neonates undergoing cardiac surgery that have subsequently proved useful and efficacious. Now that specific biochemical markers of myocardial damage that can be sampled from coronary sinus outflow have been identified, clinical studies should be able to help further refine and optimize our currently available methods of myocardial protection for this challenging group of patients.

References

Myocardial stunning in the neonate


51. Kajser L, Jansson E, Schmidt W, Bomfin V. Myocardial


Myocardial stunning in the neonate


