The Neonatal Heart: Developmental Differences, Response to Ischemia, and Protection during Cardiopulmonary Bypass

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Abstract

(J. Extra-Corpor. Tech.* 18[4] p. 208-218 Winter 1986) Accompanying the advent of open heart surgery was the need to better understand the relationship between ischemic damage and cell viability. Corrective cardiac surgical procedures necessitate a flacid, bloodless field to provide the surgeon optimal exposure. This interruption of blood flow, however, has many deleterious effects. A non-homogeneous cascade of events is produced that, if allowed to proceed unchecked, will ultimately lead to cell death. The time period of interrupted flow to the myocardium is referred to as ischemia, and results in certain cellular perturbations that are dependent on both temporal and temperature related factors. The effects of ischemia have been studied extensively, both clinically and in the laboratory, utilizing various techniques that almost exclusively employ the adult myocardium as a model. The resultant information has been broadly applied in formulating methods of myocardial protection across all ages. Cardiovascular physiology, however, is directly dependent upon the developmental state of the myocyte, showing changes in mechanical function, structure, and response to pharmacologic intervention throughout ontogeny. Therefore, a better understanding of the inherent differential characteristics must be considered in optimizing a plan for the ideal method of myocardial preservation in newborn hearts. This review will attempt to highlight some of the major developmental differences in the neonate, the effects of ischemia on the immature heart, and methods of myocardial protection during cardiopulmonary bypass.

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Developmental Differences

A host of physiological changes occur after birth which proceed throughout maturity and senescence. The neonatal period is included in the infancy stage which is arbitrarily defined as that time from birth to 12 months of age, and is represented by dramatic alterations in function. Anatomically this period is characterized by rapid increases in both height and weight which augment changes in body surface area. Body muscle mass as a percentage of total weight is much lower in the neonate compared to the adult. The neonate also possesses a greater percentage of body water composition than is present in the adult. Metabolism, body fluid compartmentalization, renal and hepatic function, circulation, and immune responses all represent major areas where developmental differences are evident. The perinatal period represents major changes in cardiovascular function as the heart shifts from fetal to extrauterine circulation with the initiation of respiration. In our ensuing discussion we will focus specifically on the biochemical and physiologic changes that occur within

the heart during early infancy.

Differential response to ischemic injury reflects the development stage of the myocyte. Histological examinations of mature and neonatal myocardium show that the neonate myocardium contains more water and less collagen per gram of tissue weight.[1] Compared to the adult the developing heart has a significantly higher proportion of non-contractile elements (nuclei, mitochondria, surface membranes) to the number of myofibrils.[2] This translates into a much greater force development per gram of tissue in the adult heart. The increased non-contractile portion of the fetal myocardium also give the ventricles a stiffer, non-distensible texture.[2]

In the human, the greatest increase in the rate of cardiac growth occurs during the first postnatal year, with the heart doubling in weight during the first six months, and tripling its weight by the end of the first year.[3] Besides having greater mass per unit body weight, newborn animals also have a greater capillary number. In humans, the capillary to fiber ratio equals 6 to 1 in the newborn and 1 to 1 in the adult.[4] In the rat heart it is estimated that half of all adult capillaries originate during the first three weeks of postnatal development.[5] This rapid proliferation corresponds to the high demands of the developing tissue by providing a means of rapid substrate procurement, necessary to meet the energy requirements imperative for tissue growth.[1]

Cardiac growth can be divided into three developmental stages: 1) early postnatal stage, 2) adult stage, and 3) senescent stage.[6]

The majority of changes occur during the early postnatal period. This stage is characterized by an increase in left ventricular mass compared to the right ventricle, increased number and volume of myocytes, rapid cell proliferation, and increased cardiac mass in proportion to body mass. Neonatal myocytes are characterized by being smaller and rounder than adult cells,[7] which provides for a less tightly packed myocardium. The change in left ventricular morphology, increasing both in volume and wall thickness, reflects the transition from placental to pulmonary gas exchange.[1] At the cellular level, mitochondrial number and activity increase rapidly, as does the amount of Deoxyribonucleic Acid. The adult stage reflects a decline in the rate of cardiac growth compared to body growth, with the heart weight remaining constant in the absence of pathological conditions. The senescent stage is characterized by a slight increase in cardiac mass as the myocytes increase in volume, while the number of capillaries decline.

One of the major components controlling cardiac function is the regulation of calcium (Ca^{++}) movement into and out of the cell (Figure 1). Disruption in normal

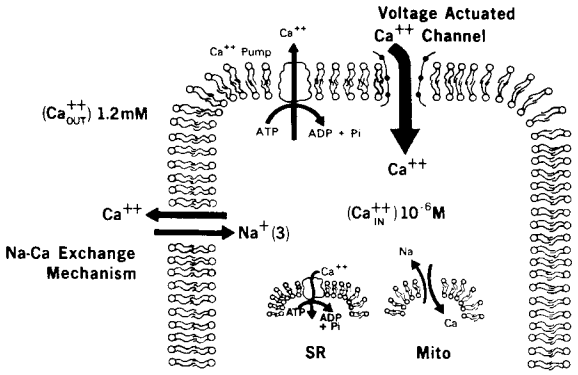

Figure 1: Calcium exchange mechanisms of neonatal cellular and subcellular membrane systems. The large arrows indicate the major control mechanisms involved in contraction/relaxation. SR, sarcoplasmic reticulum. MITO, mitochondria.

Ca^{++} homeostasis leads to major cellular dysfunction. Hypoxia followed by re-oxygenation depresses myocardial ATP concentration resulting in an increase in Ca^{++} influx. Re-oxygenation injury related to this calcium influx has been shown to occur in eight-week-old rabbit septal preparations, but was absent in 10-day-old rabbits.[8] This rapid change in sarcolemmal calcium permeability accentuates reperfusion related phenomena and may be correlated to age. Chizzonite and Zak[7] have reported that neonatal rat hearts are relatively insensitive to calcium induced cellular disruption before six days of age, but assume adult sensitivities by 15 days of age. They correlated these changes to the development of the surface membrane system and the sarcoplasmic reticulum. Developmental differences in excitation contraction coupling exist and can be related to Ca^{++}gradients across the cell membranes. Normal cardiac contraction is dependent upon both sarcolemmal and sarcoplasmic reticulum control of Ca^{++} movement. In the adult the major control of excitation-contraction coupling occurs at the sarcoplasmic reticulum.[10] In the neonate, cardiac contraction is more dependent upon the transsarcolemmal calcium influx which is mediated by voltage dependent Ca^{++} channels.[9] This is a function of the relatively insensitive sarcoplasmic reticulum which is functionally and structurally undeveloped during the early postnatal stage. Furthermore, the immature myocardium displays

greater sensitivity to the negative inotropic effects of calcium antagonists.[11,12] This sensitivity is a result of the dependency of the sarcolemmal controls of Ca^{++} movement, where the mechanisms of calcium channel blockers are thought to exert their effect.

A major distinction between developmental stages occurs with substrate usage (Figure 2). The two major

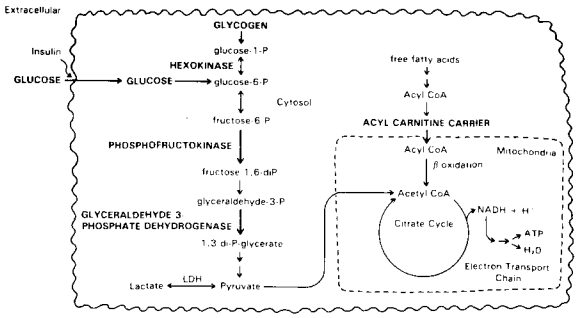

Figure 2: Metabolic pathways of the major substrates utilized within the myocyte. Increased substrate utilization by the neonate is indicated by highlighted intermediates and darkened arrows.

substrates or "fuels" of cardiac metabolism are carbohydrates and lipids. Substrate utilization is dependent upon many factors including: type and quantity of food consumed, blood nutrient composition, metabolic state, and pathologic conditions of the heart. Normally, free fatty acids are the major substrate for high energy phosphate production in the mature myocardium. Free fatty acids are activated in the cytosol to form Acyl CoA which enters the mitochondria. Beta oxidation produces two carbon fragments of acetyl CoA which enter the citric acid cycle (Figure 2). The reducing equivalents nicotinamide adenine dinucleotide ($NADH_2$) and flavin adenine dinucleotide ($FADH_2$) are generated during each rotation of the cycle. Both $NADH_2$ and $FADH_2$ enter the cytochrome pathway where they donate their protons, which flow along the cytochrome chain, synthesizing ATP from ADP via oxidative phosphorylation.

In contrast, catabolism of carbohydrates produce glucose molecules, which may enter the glycolytic pathway. The end product of glycolysis is the three carbon fragment pyruvate, formed at a rate of two moles per mole of glucose. Pyruvate is dehydrogenated within the mitochondria to form acetyl CoA which enters the citric acid cycle. Lactate is also utilized in the aerobic myocardium and is converted via lactate dehydrogenase to pyruvate, which is further metabolized. The net energy production varies between substrate consumed. When the free fatty acid palmitate is oxidized, 129 moles of ATP are generated per mole of palmitate.[13] This compares to 38 moles of ATP generated per fully oxidized mole of glucose, and 18 moles per mole of lactate.

The newborn heart has a greater dependence upon carbohydrates as its chief fuel source compared to that of the adult.[14] Since it relies heavily on glucose as its major substrate, it is more dependent upon glycolysis, and maintains only a limited capacity for fatty acid oxidation. This is evidenced by the low level of fatty acyl CoA carnitine transferase activity and the low concentration of its cofactor carnitine.[15] This, coupled with an increased hexokinase activity[16], facilitates the rapid conversion of glucose to glucose-6 phosphate, further stimulating glycolysis. The metabolic demands of the newborn, however, require continuous production of high energy phosphates, which could not be adequately achieved by glycolysis alone. Therefore, the neonate must also possess additional mechanisms to provide adequate substrate to meet the substantial needs of development. The increased perfusion provided by a high capillary to fiber ratio, along with the concomittant increase in respiration, provide the myocardium with greater quantities of molecular oxygen. Oxidative metabolism increases rapidly during the first few weeks of life,[16] leading to an increase in the energetic capacity of the heart. This increase in oxygen consumption is consistent with the increased respiratory rate of mitochondria, and the declining activity of enzymes involved in glycolysis.[15]

Early studies have shown that the ability of the newborn to withstand oxygen deprivation could be directly related to the presence of intrinsic glycogen deposits[17,18] which, through glycogenolysis, are able to provide the necessary substrate for anaerobic metabolism.[19] More recently, studies by Hoerter and associates in both rabbit and rat hearts,[14,20] have shown that neonatal myocytes possess increased rates of glycolytic flux when compared to adults. The shift from aerobic to anaerobic metabolism causes an immediate reduction in ATP by shifting from the oxidative production of 38 moles of ATP per mole of glucose, to the less efficient Embden-Meyerhoff pathway producing a net two moles of ATP, or three moles when glycogen is metabolized. ATP hydrolysis results in the production of certain purine nucleotides (ADP, AMP) which have a positive feedback effect on the glycolytic pathway by stimulating the activity of phosphofructokinase (PFK) and glyceraldehyde 3-phosphate dehydrogenase (G3PD). If anaerobic metabolism proceeds in the absence of

metabolite removal, an increase in secondary metabolites (lactic acid, protons, NADH) suppresses glycolysis by inhibiting the action of PFK.

A series of companion papers from the University of California Medical Center has compared the effects of ischemia and hypoxia in arterially perfused neonatal and adult rabbit hearts.[8,21,22,23] The authors have shown that myocardial postischemic ATP levels were well maintained in the neonate, but declined in the adult. Furthermore, reperfusion ATP levels were proportional to the recovery of mechanical function, and inversely related to intracellular calcium accumulation.[8] This is additional evidence which emphasizes the importance of enhanced anaerobic metabolism in the neonate.[22,23]

Work in our laboratory utilizing isolated rabbit hearts has shown that the neonate responds better to hypothermic global ischemia than either immature or mature animals.[24] A recent clinical study has looked at the effects of profound hypothermia, total circulatory arrest, and cardioplegia in protecting infants during surgery.[25] It was shown that the hearts protected by cardioplegia displayed better functional recovery, lower CK-MB isoenzyme levels, and fewer ultrastructural changes than those not protected. The authors, however, did note that hearts protected by cardioplegia had a higher incidence of intracellular and extracellular edema. Bull and associates have compared the effects of intermittent cross-clamping to cardioplegia in 400 consecutive pediatric patients.[26] They found that cardioplegia did not provide better myocardial protection, and perhaps created a feeling of false security that was evident when cross-clamp times exceeded 85 minutes.

Response to Ischemia

Ischemia can be defined as a lack of blood flow to a region or organ and should not be confused with either hypoxia or anoxia which deal with the adequacy of oxygenation of a fluid or tissue. Mechanical function of the heart is compromised within seconds after flow is interrupted. This is evidenced by a decrease in contractility at a time when the level of electrical function remains unchanged.[27] The progression of events toward cellular necrosis proceeds very rapidly, and irreversible damage will occur after approximately 20 minutes of normothermic ischemia. A correlation probably exists between the depletion of the high energy phosphates, ATP and creatine phosphate (CP), and the decline of electromechanical activity.

Disruption of flow creates an imbalance between substrate supply and demand. The immediate effects of ischemia are related to the reduced availability of oxygen causing a disruption of oxidative phosphorylation within the mitochondria. As ischemia progresses free fatty acids are depleted resulting in a reduction in the flux through beta oxidation pathways of lipid metabolism, and a slowing of the tricarboxlic acid cycle.[28] Long chain Acyl CoA esters, the intermediates of free fatty acid metabolism, accumulate and profoundly affect energy production by inhibiting the transport of ADP and ATP across the inner mitochondrial membrane.[28] This inhibition of adenine nucleotide translocation limits the availability of precursors necessary for the regeneration of ATP. The depression of fatty acid utilization reflects a concomitant increase in carbohydrate utilization with a shift from aerobic to anaerobic metabolism (Pasteur effect).[21,30]

The disruption of aerobic metabolism causes an immediate decrease in the production of ATP by shifting from the normal 38 moles to only two moles produced anaerobically. CP stores are reduced in an effort to maintain the energetic demands of the failing myocyte. As ischemia continues, the sarcolemma is affected and ionic imbalances begin to occur. The action potential becomes compromised as intracellular potassium (K^+) begins leaking out of the cell, resulting in interrupted sodium (Na^+)-K^+ exchange.[31] Simultaneously there is an increase in cellular permeability facilitating the movement of Na^+ and Chloride (Cl^-) ions into the cell by simple diffusion. The reduced ATP causes increased levels of ADP, AMP, and Pi, which stimulate anaerobic metabolism by increasing glycogenolysis. Glycogen is rapidly converted to glucose with a rapid depletion of glycogen stores.

Up to this point most of the changes occurring within the myocardium are a result of a shift in metabolism from aerobic to anaerobic pathways. There are important ionic imbalances that also result from ischemia that exacerbate the cellular damage. Specifically, calcium has been shown to play an integral role in the development of ischemic damage which can be related to its important function in the maintenance and regulation of cardiac metabolism, and its involvement in the sequence of events of contraction-relaxation.[32,33] During myocardial ischemia there is an immediate loss of calcium uptake at the sarcoplasmic reticulum during diastole, resulting in increased cytosolic levels. Calcium influx at the sarcolemma increases as a result of permeability changes of the membrane and the disruption of normal efflux mechanisms. Both conditions lead to the intracellular accumulation of calcium.[32]

Intracellular hypercalcemia activates the actinomyosin ATPase enzyme system causing excessive hydrolysis of ATP. The further depletion of ATP causes a disruption in the exchange of Na^+ and Ca^{++}. Under these conditions, certain lipases and proteases are activated that undermine the integrity of the membrane lipid bilayer. At the same time the mitochondria become overloaded with calcium which greatly impedes their ability to function.[34] The overall effect of increasing calcium levels is an increase in the diastolic resting tension which may lead to contracture. Although the cellular changes up to this point are detrimental, they are generally reversible. Thus, if reperfusion were to occur, the cell could "recover" some or all of its preischemic function. Extending the ischemic period, however, would evoke irreversible changes in the myocardium.

The increase in sarcolemmal permeability occurs not only by an interruption in the morphology of the lipid bilayer, but also by a disruption of certain enzyme systems within the membrane. These membrane ATPases that are involved in the regulation of transmembrane ionic gradients malfunction, resulting in a massive influx of both Na^+ and Cl^- ions.[35] The extrusion of Na^+ can no longer keep pace with the inflow, resulting in a decrease in the net negative intracellular charge, and facilitating both the inward movement of Cl^- as well as K^+ efflux.[36] The intracellular accumulation of solutes results in increased osmolarity which draws water into the cell, resulting in cellular edema.

Besides the increase in sarcolemmal permeability, the membranes of certain intracellular organelles are also affected by ischemia. The membranes of the sarcoplasmic reticulum, lysosomes, and mitochondria react in a similar fashion to the plasma membrane. The major damaging pathway involves the degradation of phospholipids which cleave the fatty acids by the actions of phospholipases.[35] This gives rise to lysophospholipids and unesterfied fatty acids that proceed in disrupting the fluid portion of the membrane. Within the mitochondria certain degradative processes occur simultaneously with the irreversible phase of ischemia exacerbating cellular dysfunction. Mitochondria become engorged evidenced by a massive increase in matrix space and by the presence of dense matrix deposits.[31] The cristae become enlarged, disoriented, and lose their dense preischemic appearance. Continuing cytosolic calcium accumulation causes granular deposits of calcium phosphate to accumulate rapidly during reperfusion. Simultaneously, the damaged mitochondria sequester calcium in preference to performing oxidative phosphorylation[33] adversely affecting the ability of the cell to produce ATP necessary for reparative processes and electromechanical function.

Thus, some of the predisposing factors that contribute to irreversible cellular injury may be summarized as: glycogen granule depletion;[17,18] complete utilization of high energy phosphates;[37] disruption of membrane bound proteins by the release of proteolytic enzymes; [38] and the inability of the cell to maintain calcium homeostasis with the accumulation of the calcium-phosphate salts in the inner-mitochondrial matrix.[34].

One of the major combatants used to control and suppress the effects of ischemia is the use of hypothermia, which is almost universally employed. Work in our laboratory utilizing the isolated heart preparation has shown age related differences in response to myocardial hypothermic ischemia in the rabbit.[24] Three groups of rabbits, neonates (eight days), immature (33 days), and mature (115 days), were subjected to 90 minutes of hypothermic (20 °C) ischemia. Following ischemia, the neonatal group demonstrated significantly better overall hemodynamic recovery of preischemic baseline values (Figure 3). In the mature group,

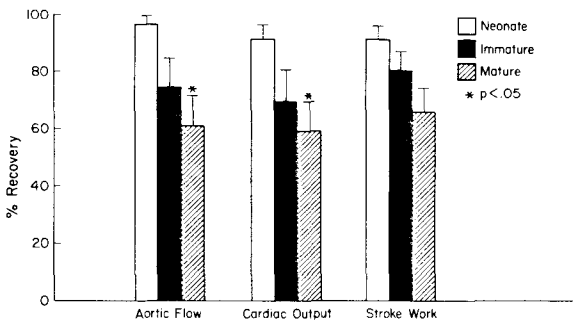

Figure 3: Postischemic percent recovery of aortic flow, cardiac output, and stroke work across several age groups of rabbits following 90 minutes of global ischemia at 20° C. Values are mean plus standard error of the mean. * $p < .05$ vs neonatal group.

all postischemic values fell below control demonstrating impaired left ventricular performance. Myocardial water content was significantly higher in the older age groups. The coronary sinus effluent was sampled for creatine kinase (CK) just prior to the ischemia and then again at 10 and 30 minutes postischemia. Only the mature group demonstrated a significant rise in CK leakage following ischemia. With protection provided only by deep hypothermia, the isolated neonatal rabbit heart demonstrated a remarkable ability to recover from a prolonged period of ischemia.

Protection during Cardiopulmonary Bypass

The major component of myocardial preservation is cardioplegia. Literally translated as heart (cardio) stopping (plegia), it is routinely used in most cardiac surgical centers throughout the world.[39] The function of cardioplegia is to arrest the heart as rapidly as possible during diastole, facilitating the conservation of high energy phosphates. Secondary effects of cardioplegia are to provide substrate for both aerobic and anaerobic metabolism and to maintain intracellular pH by the use of buffering agents.[39] Additionally, the cardioplegic solution may be used as a vehicle to help distribute the desired level of hypothermia throughout myocardial layers. The majority of cardioplegic solutions in clinical use today are based on the composition of extracellular fluid and use high dose K^+ as the principle arresting agent—thus minimizing abrupt deviations from the composition of fluids to which the tissue is normally exposed.[40] In general, the solution should be balanced with the addition of electrolytes and solutes to approximate that of the extracellular environment. The high K^+ dose results in depolarizing the membrane by altering the transsarcolemmal ionic gradient, and therefore, the transmission of the action potential. This has the desired effect of decreasing energy consumption by ceasing electromechanical function. The elevated K^+ also aids in reducing the quantity of cytosolic ionized calcium below the level that initiates contraction, assuring diastolic arrest. Both hyperkalemia and hypothermia succeed in arresting the heart during diastole and creating an environment that decreases the detrimental effects of ischemia.[41,42]

The level of hypothermia is an important component of myocardial protection. The efficacy of hypothermia in cardioplegic solutions has been demonstrated by Lange and associates.[43] Additional benefits of hypothermic, cardioplegic solutions are ensuring post-ischemia mitochondrial respiratory function,[44] improving left ventricular postischemic function,[45] and protecting the myocardium from acute transient increases of certain ions.[46]

Many other components have been studied as possible additives to the basic cardioplegic solution. Magnesium (Mg^{++}) is an essential ion necessary for cellular function and serves as a cofactor for certain enzymes involved in energy transfer reactions and active transport.[47] Increased extracellular concentrations of Mg^{++} are known to displace Ca^{++} from its sarcolemmal binding site.[46] Although Mg^{++} has a weaker cardioplegic effect than K^+, it is known to shorten the action potential and decrease cardiac conduction.[27] Recently, experiments in the isolated rat heart have shown that Mg^{++}, when given just prior to reperfusion, inhibited Ca^{++} mediated functional damage.[47]

Certain membrane stabilizing agents are used to decrease Na^+ permeability and inhibit depolarization, causing an immediate reduction of cardiac electromechanical activity. The local anesthetic agents lidocaine and procaine have been utilized in cardioplegic solutions for this purpose.[49,50] Hyperosmolar agents such as mannitol, increase the osmolarity of the extracellular fluid slowing the intracellular influx of water, which counteracts the effect of Na^+ accumulation within the cell.[51] This hypertonicity has been shown to prevent the formation of intracellular edema during ischemia. Mannitol may also play a role in preventing the damaging effects of free hydroxyl radicals produced during reoxygenation.

The addition of certain amino acids has been shown to replenish depleted citric cycle intermediate (aspartate, oxaloacetate, and succinate).[52,53,54] Glutamate has been given just prior to reperfusion to help maintain the redox balance and enhance high energy phosphate production. It is thought that these high energy molecules could then be channeled into reparative processes within the plasma membranes, rather than initiating mechanical activity.[55]

The addition of glucose, alone[56,57] or with insulin,[58] to cardioplegic solutions has been shown to decrease the rate of intrinsic glycogen utilization during ischemia, and to stabilize the energy dependent ATPase system.[58] Insulin has been added prophylactically to augment high energy phosphate supply by stimulating glycogenesis, increasing preischemic glycogen reserves.[59]

Recently, a certain class of pharmaceuticals, the slow calcium channel blockers or calcium antagonists, have received much attention as myocardial protective agents. These compounds have been shown to restrict transmembrane calcium ion flux during ischemia, reducing intracellular accumulation of Ca^{++}.[60,61,62] Other cytoprotective mechanisms attributed to these agents include lysozomal stabilization,[63] as well as the preservation of left ventricular compliance and contractility.[64] There are concerns, however, regarding the

efficacy of these additives when employed with hypothermia.[62,65,66,67] Hearse and associates have stated that the beneficial effects of calcium channel inhibition may be rendered redundant with hypothermia, possibly indicating a common pathway of protection.[61,65] Hicks and associates[68] have recently shown that although verapamil enhanced the protective effects of potassium cardioplegia, there was an associated increase of transient intraoperative dysfunction of the AV node.

A wide variety of buffering agents have been utilized to counteract the intracellular acidosis produced by proton and lactic acid accumulation during ischemia. Bicarbonate buffers tend to be the most widely used and rely on exchange mechanisms of bicarbonate and carbon dioxide. Other buffering agents such as TRIS (hydroxymethyl aminomethane), phosphate, and histidine (Imidazole), possess acid base characteristics not dependent upon the bicarbonate-CO_2 exchange.[69]

Steroids, such as methylprednisolone, have been included in cardioplegic solutions because of their stabilizing effects on membranes, lysozomes, and capillary endothelium.[58] They are also known to be effective vasodilators.

The efficacy of cardioplegia may also be enhanced by the addition of blood. Proponents of its use in cardioplegia argue that blood is a superior vehicle for delivering oxygen to the myocardium allowing for continued aerobic metabolism and more efficient production of ATP.[31,70,71,72,73,74] Additional benefits of these solutions are attributed to the rheologic properties of whole blood, namely: the inherent buffering capacity of red blood cells; plasma oncotic properties; and, the delivery of metabolic substrates and electrolytes.[75] When the temperature of blood-containing solutions is lowered, however, physical alterations of the fluid dynamics result in an increase in viscosity. These changes include rouleaux formation and sludging and become evident when the temperature of whole blood is reduced below 17° C. Additionally, it is known that hemoglobin loses its ability to dissociate oxygen when blood temperature is decreased.[76] The use of synthetic agents with high oxygen solubilities in cardioplegic solutions have been investigated. Perfluorocarbon emulsions, such as Fluosol-DA and Fluosol-43, have been shown to be superior in the delivery of oxygen when compared to both blood and crystalliod cardioplegia.[77,78]

Administration of a bolus dose of cardioiplegic solution just prior to reperfusion has been investigated by a number of groups.[147,55,79,80] Yano and associates[47] have termed this "terminal cardioplegia" and have shown that in the rat late administration of magnesium cardioplegia retards the reperfusion related influx of calcium in myocardial cells that are near the state of irreversible injury. Follette and colleagues have demonstrated that the maintenance of cardioplegia during initial reperfusion lessens postischemic edema and improves compliance in the dog.[80] The use of reperfusate cardioplegia prevents electromechanical energy consumption during initial reperfusion by keeping the heart asystolic, allowing high energy phosphates produced by anaerobic metabolism to be channeled into reparative processes rather than wasted on mechanical work.[55]

The heterogeneous nature of both hypoxic and ischemic injury make the task of formulating the ideal cardioplegia solution difficult. The majority of work thus far completed has almost exclusively employed the adult or mature myocardium as a reference. Whether these results also apply to the developing myocardium remains to be established. This question has been investigated in our laboratory.[24,81] The recovery of left ventricular function after hypothermic (28° C) ischemia in neonates, with the hearts being intermittently perfused with either cardioplegic (hyperkalemic) or non-cardioplegic solutions, was examined in isolated seven-day-old rabbit hearts.[81] The cardioplegia treated hearts displayed the best overall recovery of baseline

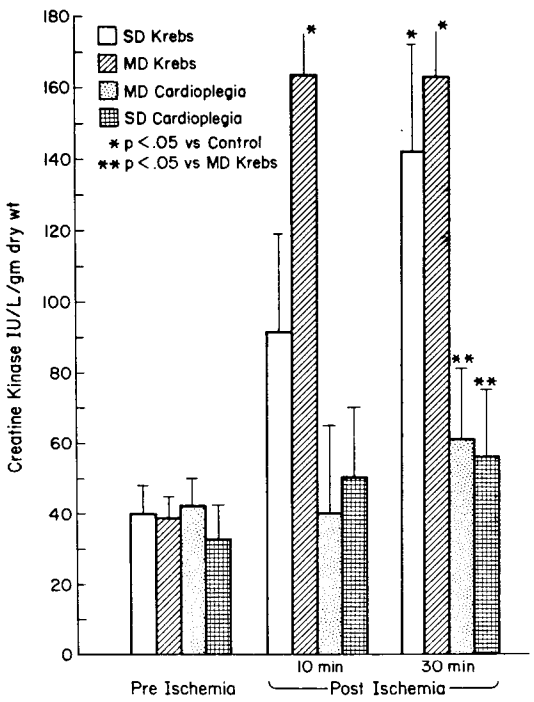

Figure 4: Creatine kinase release in neonatal hearts protected with cardioplegic or non-cardioplegic solutions during 120 minutes of 28° C global ischemia. SD Krebs, singledose Krebs-Henseleit solution. MD, multidose.

function. Creatine kinase release in the coronary sinus effluent did not increase following ischemia in the cardioplegia treated hearts, but was elevated in the non-cardioplegia treated animals (Figure 4). This study supports the use of cardioplegia for myocardial preservation during open heart surgery in infants as well as adults.

Summary

The processes of ischemic injury can be described as a non-homogeneous cascade of events that alter biochemical mechanisms responsible for providing the necessary energy to maintain cellular homeostasis. Clinical methods of myocardial protection are based on the concomitant use of hypothermia and cardioplegia. Hypothermia is utilized to depress metabolism by decreasing enzymatic driven reactions and stabilizing the lipid bilayer component of membrane systems. Cardioplegic solutions succeed in arresting the heart in diastole, and with cardiopulmonary bypass, provide a bloodless flacid field. Both hypothermia and cardioplegia act synergistically to: 1) impede the depletion of high energy phosphates; 2) maintain cellular integrity by stabilizing plasma membranes; 3) restrict fluctuations in acid base status; 4) retard the influx of certain divalent cations upon reperfusion.

Biochemical and physiological differences exist throughout the development of the heart, with the major changes occurring during early postnatal development. The developing myocardium: 1) has more non-contractile mass than mature myocardium; 2) is less compliant; 3) has a marked increase in cardiac mass during the first postnatal year; 4) has an increased capillary to fiber ratio, and 5) contains greater numbers

Table 1

Age Related Changes in Myocardial Development

Parameter	Neonate	Adult	Species	Reference
Morphology				
Rate of Heart Growth	↑↑	↓	Man	3
Heartweight : Bodyweight	↑	↓	Rat	6
LV Mass : RV Mass	↑	=	Man	2, 6
Myocyte Number : Volume	↑	NA	Man	6
Structural				
Collagen Content	↓	↑	Sheep	1
H_2O Content	↑	↓	Sheep	1
Non-contractile Proteins	↑	=	Sheep	1
Circulation				
Capillary Number	↑	↓	Rat	5
Capillary : Fiber	↑↑	↓	Man	4
Myocardial Blood Flow	↑	↓	Man	16
Subcellular				
Mitochondria (activity and number)	↑	=	Man, Rat	6, 9
Ca^{++} Homeostasis ; Transsarcolemmal	↑	=	Rat	9
Sarcoplasmic Reticulum	↓	↑	Rat, Rabbit	9, 10
Mitochondria	↓	↑	Rat	9
Ca^{++} Antagonist Sensitivity	↑	=	Rabbit	11, 12
Metabolism				
Glycolysis	↑↑	↓	Rabbit, Rat	14, 20, 22, 23
Lipolysis	↓	↑	Rat	15
Glycogenolysis	↑	↓	Sheep, Rabbit	17, 18, 19

↑, Increased. ↑↑, Greatly increased. ↓, Decreased. =, Maintained. NA, Not available.

and volume of myocytes. The lack of fully developed membrane systems in the neonate, coupled with loosely packed myocytes, results in a more flexible myocardium than the adult. This period also represents a shift from anaerobic metabolism (glycolysis) to aerobic metabolism through oxidative phosphorylation. Substrate usage shifts from carbohydrates to free fatty acids. Calcium regulation varies among myocardial age groups. Calcium homeostasis is primarily controlled at the sarcolemma in the neonate, while the adult control is exerted at the sarcolemma, sarcoplasmic reticulum, and mitochondria. Perhaps the greatest distinguishing factor related to cellular preservation is the enhanced capability for glycolytic flux in the neonate, enabling the cell to produce adequate levels of high energy phosphates necessary to withstand brief periods of ischemia. The transitional period from anaerobic to aerobic metabolism proceeds with metabolic overlap where both glycolysis and mitochondrial respiration act concurrently. This provides the neonate with both a means of meeting the demands of rapidly developing tissue and may help explain the enhanced tolerance to hypoxic stress.

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