Global Myocardial Ischemia in the Newborn, Juvenile, and Adult Isolated Isovolumic Rabbit Heart

Age-Related Differences in Systolic Function, Diastolic Stiffness, Coronary Resistance, Myocardial Oxygen Consumption, and Extracellular pH

Mark D. Parrish, Aena Payne, and David E. Fixler

Controversy persists over the relative tolerance of the immature myocardium to global ischemia. Thus, we evaluated the physiologic effects of 30, 60, and 180 minutes of global ischemia in an isolated, isovolumic rabbit heart model, at 3 different ages: newborns (less than 1 week of age) (n = 36), juveniles (4 to 6 weeks old) (n = 36), and adults (5 to 7 months old) (n = 36). Following 30 and 60 minutes of ischemia, respectively, adults recovered 87±4% (mean ±SEM) and 90±7% of baseline systolic function, and juveniles recovered 91 ±10% and 85±8%. In contrast, newborns recovered only 27 ±6% and 28 ±4% of baseline systolic function (p<0.05 compared to adults and juveniles). During ischemia, newborn hearts became stiff more rapidly, reaching 361 ±46% of baseline stiffness by 60 minutes, whereas adults and juveniles were at 122 ±33% and 92 ±18% of baseline stiffness (p<0.05 newborns compared to adults and juveniles). With reperfusion after 60 minutes of ischemia, the work efficiency of the newborn heart deteriorated to 39±7% of baseline, compared with 95 ±7% and 91 ±7% of baseline efficiency in the adult and juvenile hearts (p<0.05, newborns compared to adults and juveniles). The ratio of tissue wet-to-dry weights were similar in all age groups after ischemia. However, tissue pH was significantly higher in newborns during ischemia (6.54 ± 0.06, 6.69 ± 0.07, and 6.85 ± 0.09 in adults, juveniles, and newborns, after 60 minutes of ischemia) (p<0.05 newborns versus adults). We conclude that the newborn rabbit hearts are more susceptible to ischemic injury than the juvenile and adult hearts. (Circulation Research 1987;61:609-615)

Clinical experience suggests that the neonatal heart is more vulnerable to injury from global ischemia than is the mature heart in patients undergoing open heart surgery. However, laboratory studies have not supported this clinical impression. Work from one group of investigators demonstrated that immature (1-week-old) rabbit myocardium was more resistant to ischemic injury than the adult myocardium. They correlated this resistance to ischemic injury with better preservation of tissue adenosine triphosphate (ATP) levels in the immature heart. However, they did note that ischemic contracture occurred at a higher ATP level in the immature heart. In an isolated dog heart model, puppies (2 to 6 months of age) had less deterioration of diastolic function following one hour of global ischemia than did adult dogs. Other workers found no differences in myocardial function between puppies and adult dogs following global ischemia, or between newborn and adult rabbit papillary muscles during a short period of hypoxia.

Several important factors may account for the disparate results from these various studies, such as 1) differing levels of maturity of the experimental models and 2) differing durations of ischemia. Therefore, the purpose of this study was to examine the age-related effects of global myocardial ischemia on systolic and diastolic cardiac performance using a wider age range and longer periods of ischemia. We used in vivo, isolated, isovolumic contracting hearts from rabbits less than 1 week of age (newborns), 4 to 6 weeks old (juveniles), and adults exposed to ischemia for 30, 60, and 180 minutes.

Materials and Methods

Methods

Standard techniques for an isolated isovolumic contracting rabbit heart preparation were modified primarily to isolate and maintain the heart within the chest cavity. This was done to minimize both ischemic time and mechanical trauma to the heart. In brief, we used New Zealand white rabbits from three age groups: newborns (less than 1 week old, mean age 3.5±0.03 days), juveniles (4 to 6 weeks old), and adults (5 to 7 months old). Rabbits were sedated with pentobarbital (50 mg/kg), and ventilated. A midline thoracotomy was performed, and the hearts were rapidly isolated and instrumented within the chest cavity. Retrograde coronary perfusion was begun with a warmed, oxygenated...
Krebs-Henseleit solution. Coronary perfusion was started without any intervening ischemic period. The perfusate had the following constituents: glucose 4.9 mM, NaCl 110 mM, NaHCO3 22 mM, KCl 3.4 mM, MgSO4 0.5 mM, CaCl2 1.8 mM, and KH2PO4 1.1 mM.

Through a left atrial incision, a compliant, fluid-filled latex balloon was passed into the left ventricular cavity. A 27-gauge thermistor needle was inserted superficially into the left ventricular muscle to monitor temperature. Two 21-gauge needle pH probes were also inserted into the left ventricular muscle and positioned superficially so that the needle tip could be seen through a thin muscle layer. The glass electrodes were turned to face inwards.

After instrumentation, hearts were allowed to stabilize for 30 to 60 minutes, allowing myocardial tissue pH and left ventricular developing pressure to stabilize. During this stabilization period, the pacing rate was set to at least 120 beats/min, or 10% above the intrinsic heart rate. The temperature of the myocardium was adjusted to 28°C by warming the perfusate. Fluid was added to the left ventricular balloon to adjust the ventricular diastolic pressure to approximately 8 mm Hg. Coronary perfusion rate was set at 1–3 ml/min (newborn), 15–25 ml/min (juvenile), and 40–60 ml/min (adult). These flow rates were chosen to give equivalent flow per gram of tissue to all three age groups.

**Measurements and Calculations**

After stabilization, the following measurements were obtained for all animals: 1) mean aortic pressure, 2) left ventricular diastolic and developed pressure, 3) coronary flow rate (measured effluent from right ventricle), 4) myocardial temperature, 5) myocardial pH (readings from 2 probes were averaged), 6) coronary arterial and venous blood gases, and 7) left ventricular stiffness. The left ventricular stiffness was estimated by measuring left ventricular diastolic pressure at 5 increments of balloon volume between 100 and 250% of the baseline volume. Left ventricular stiffness was taken as the slope of this pressure-volume relation.

Arterial and venous oxygen contents were estimated from the oxygen tension:

\[
\text{Oxygen content (in ml/ml of perfusate) = } P_{O_2} \text{ (in torr)} \times 0.00003
\]

Myocardial oxygen consumption (MV\textsubscript{O\textsubscript{2}}) was then calculated:

\[
\text{MV}_{O_2} = [\text{arterial } O_2 \text{ content} - \text{venous } O_2 \text{ content}] / [\text{coronary flow}]
\]

Coronary resistance was also estimated:

\[
\text{Coronary resistance (units) = } \frac{[\text{mean aortic pressure}] / [\text{coronary flow/g}]}{[\text{coronary flow/g}]}
\]

Mean left ventricular pressure was assessed by measuring the area under the left ventricular pressure curve. Since no stroke work was performed by the hearts, we used the mean left ventricular pressure per minute as an index of active ventricular work. The relative work efficiency of the hearts was estimated from the mean pressure per minute: myocardial oxygen consumption ratio.\textsuperscript{8,9}

**Experimental Protocols**

1) **CONTROL.** After baseline measurements, hearts were maintained continuously for 2 hours. Repeat measurements were performed every 30 minutes. At the end of the experiment, the hearts were dissected free from the chest, and all atrial tissue and great vessels were trimmed from the hearts. The right ventricular free wall was removed from the left ventricle and each tissue sample weighed separately. The septum was included with the left ventricle. The tissues were then dried and reweighed to obtain right ventricular free wall and left ventricular weights. Eight hearts for each age group were studied by this protocol.

2) **30 MINUTES ISCHEMIA.** After baseline measurements, flow to the heart was discontinued, and the pacemaker was turned off. The hearts were allowed to remain ischemic for 30 minutes at 28°C. After 30 minutes, reperfusion of the heart was begun for 90 minutes at the preischemic flow rate, and pacing was resumed. At the end of the experiment, tissue weights were obtained as for the control group. During the protocol, myocardial pH and temperature were monitored continuously. Myocardial stiffness was assessed at the beginning and end of ischemia and intermittently during reperfusion. Systolic performance, coronary perfusion pressure, and myocardial oxygen consumption were recorded at 10, 30, 60, and 90 minutes of reperfusion. Eight hearts for each age group were studied by this protocol.

3) **60 MINUTES ISCHEMIA.** This protocol was similar to protocol 2, except that the ischemic period was extended to 60 minutes. Measurements were obtained at baseline, and during ischemia and reperfusion as in protocol 2. Eight hearts for each age group were studied by this protocol.

4) **180 MINUTES ISCHEMIA.** After baseline measurements, the hearts were made ischemic as in protocol 2. However, ischemia was extended to 180 minutes, at which point the experiment was concluded and no reperfusion was attempted. Myocardial tissue pH was monitored continuously, and left ventricular stiffness was assessed frequently. Twelve hearts for each age group were evaluated with this protocol.

5) **NORMAL TISSUE WEIGHTS.** For comparison purposes, an additional 5 hearts for each age group were excised from the chests of anesthetized and ventilated rabbits. These hearts did not undergo the isolation and perfusion procedure previously described. These hearts were obtained to measure wet and dry weights for the left ventricle and right ventricular free wall in the intact rabbit.

**Statistical Analysis**

Comparisons between independent groups (each age group) were performed using Bartlett's test for equality of variances, one-way analysis of variance, and Welch's approximation to the one-way analysis of variance for groups with unequal variances. In addition, Newman-Keuls multiple comparisons test was performed. For repeated measures, parametric repeated measures analysis of variance and multiple pairwise comparisons tests were performed. Linear
regression analysis was performed for comparison of balloon volumes and resting pressures. The level of statistical significance for all comparisons was chosen as p<0.05.10

Results
Baseline and Control Data
Juvenile hearts were approximately 10 times larger than newborn hearts, and adult hearts approximately 25 times larger than newborn hearts (Table 1). As expected (due to fetal circulatory physiology), newborn hearts were also characterized by a relatively right ventricular hypertrophy.

By experimental design, left ventricular diastolic pressure and coronary flow per gram were not significantly different among the age groups. Although heart rates were significantly higher in newborn hearts than in the other age groups, this difference is minimal. Baseline coronary perfusion pressure was significantly lower in newborns and juveniles compared to adults, reflecting a relatively decreased coronary resistance.

Absolute left ventricular developed pressure was decreased in newborns compared to juveniles and adults. Although absolute MVo2 was decreased in newborns and juveniles, MVo2 per gram of tissue was not different among the age groups. The efficiency of pressure work ([mean pressure per minute × heart rate]/MVo2) was significantly greater in newborns than in juveniles or adults. We found no age-related differences in left ventricular stiffness.

Table 2 shows the relative changes with time in left ventricular pressure work, MVo2, left ventricular efficiency, coronary resistance, and left ventricular stiffness for the control groups. The control preparations were quite stable for two hours. After 120 minutes of perfusion, there were no significant changes from baseline, and no significant differences among groups.

Response to Ischemia
Syntotic function. With reperfusion, both adults and juvenile hearts achieved 85 to 90% of baseline left ventricular systolic function (Figure 1). Recovery was nearly complete after both 30 and 60 minutes of global ischemia. In contrast, newborn hearts recovered poorly, ultimately achieving only 15 to 30% of baseline performance. Furthermore, newborns tended to deteriorate during the reperfusion period, whereas systolic performance gradually improved in juveniles and adults during reperfusion.

Left ventricular efficiency. Figure 2 shows the relative changes in cardiac efficiency (pressure work/MVo2) after ischemia. For all age groups, there was an initial decrease in the amount of work performed per milliliter of oxygen consumed. However, juveniles and adults tended to return toward baseline efficiency after 10 minutes of reperfusion. In contrast, newborns continued to use oxygen at preischemic levels while systolic function deteriorated, producing a significant decrease in efficiency of the reperfused newborn heart compared to baseline values.

Myocardial stiffness. Newborn hearts lost compliance very early during ischemia, reaching nearly maximal stiffness by 60 minutes of ischemia (Figure 3). In contrast, after 60 minutes of ischemic time, stiffness of the adult and juvenile hearts was not significantly different from baseline stiffness.

Age-related differences in myocardial stiffness were also observed during reperfusion of the heart (Figure 4). Newborn hearts continued to stiffen during reperfusion, whereas there was little change in the stiffness of the juvenile and adult hearts during reperfusion.

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<th>Table 1. Baseline Measurements for Newborn, Juvenile, and Adult Rabbit Hearts</th>
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<td>LV weight (grams)</td>
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<td>LV/RV weight ratio</td>
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<td>Heart rate (beats/min)</td>
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<td>LV diastolic pressure (mm Hg)</td>
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<td>Coronary flow/g of LV (ml/min/g)</td>
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<td>Coronary perfusion pressure (mm Hg)</td>
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<td>Coronary resistance × LV weight (units)</td>
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<td>LV developed pressure (mm Hg)</td>
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<tr>
<td>MVo2/g of LV</td>
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<td>Efficiency (pressure × rate/MVo2) (mm Hg/ml O2)</td>
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<td>Stiffness (∆mm Hg/∆LV balloon volume%)</td>
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Results are mean±SEM. *Significantly different from adult group, p<0.05; †significantly different from juvenile group, p<0.05.

<table>
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<th>Table 2. Control (Nonischemic) Group at 120 Minutes of Perfusion</th>
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<td>LV work (pressure × heart rate)</td>
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<td>Efficiency (work/MVo2)</td>
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<td>Coronary resistance</td>
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<td>LV stiffness</td>
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Results are mean±SEM.
Coronary resistance. There were no significant changes in coronary resistance in any age group after 30 or 60 minutes of ischemia (Figure 5). There was a very mild and transient decrease in coronary resistance in juveniles and adults following 60 minutes of ischemia, but this change was not significant compared to baseline values. Newborn hearts did show a significant increase in coronary resistance during reperfusion, compared to adults and juveniles.

Tissue pH. During prolonged ischemia, newborn hearts showed an earlier plateau in tissue pH compared with juvenile and adult hearts (Figure 6). This earlier plateau resulted in a significantly higher tissue pH at 40, 50, and 60 minutes of ischemia. With reperfusion of the heart after 30 or 60 minutes of ischemia, tissue pH rapidly returned to baseline levels within 5 minutes. There were no significant age-related differences in tissue pH during reperfusion (data not shown).

Wet/dry weight ratio. Table 3 shows tissue wet/dry weight ratios for the three age groups and various ischemic protocols. In normal hearts, not exposed to Krebs perfusion or ischemia, the newborn hearts have a significantly higher water content than adult or juvenile hearts. With Krebs perfusion (control group), the age groups are no longer different. Thus, adults and juveniles accumulate significant amounts of water during Krebs perfusion, whereas newborns do not accumulate additional water. Ischemia and reperfusion produced no additional important changes in tissue water content.

Discussion

The results of this study suggest that newborn hearts are more vulnerable to global ischemic injury than juvenile or adult hearts. This vulnerability in characterized in part by an early and rapid increase in diastolic stiffness during ischemia. With reperfusion, stiffness continues to increase. Although left ventricular stiffness appears to increase to a greater extent in newborns exposed to 30 minutes of ischemia than in the 60-minute ischemic group (Figure 4), this measurement had a large variability, and there was no statistically significant difference between the two newborn groups. Coronary resistance also increases rapidly during reperfusion, perhaps reflecting the increase in myocardial tissue pressures.

Another characteristic of the newborn vulnerability to ischemia is a severe loss of systolic performance during reperfusion. Interestingly, there is very little difference between 30 and 60 minutes of ischemia in recovery of systolic function. This indicates that the injury in the newborn either occurs very early during ischemia or during reperfusion. Corresponding to this loss of systolic function is a decrease in work efficiency in the newborn hearts during reperfusion. This decrease in work efficiency with ischemia has previously been described for mature hearts exposed to cardioplegic arrest.11

Numerous mechanisms have been implicated in myocardial ischemic injury, any of which might explain age-related differences in vulnerability to ischemia.
One important mechanism of ischemic injury has been described as the "no-reflow" phenomenon.\textsuperscript{12} This phenomenon becomes manifest during reperfusion of the ischemic heart, when portions of the myocardium (particularly the subendocardium) may receive inadequate reflow.\textsuperscript{13-15} The effect of decreasing age and size on this phenomenon has not been studied. In our model, coronary venous $P_O_2$ increased slightly in newborns following 30 and 60 minutes of ischemia. This reflected a modest decrease in oxygen consumption in the newborns (62 ± 6\% and 76 ± 4\% of baseline, after 30 and 60 minutes of ischemia, respectively). Oxygen consumption in adults and juveniles did not change significantly following ischemia. Although the decrease in oxygen consumption in the newborns could be due to a maldistribution of flow following ischemia, the ventricular function decreased to a much greater degree than did oxygen consumption. Thus, it seems unlikely that inadequate oxygen delivery to the working myocardium (no-reflow) would entirely explain the loss of systolic function in newborns. In addition, this mechanism would not explain the early changes in myocardial stiffness in the newborn hearts that occurs before reperfusion.

A second important mechanism of myocardial injury with ischemia is tissue ATP depletion. Accumulation of rigor complexes and consequent myocardial contracture have been strongly correlated with tissue ATP levels.\textsuperscript{16-18} However, this is an unlikely mechanism to explain our observations since other workers have found that ATP is relatively preserved during ischemia in the immature heart.\textsuperscript{2} In addition, our findings of a higher tissue pH during ischemia in the newborn suggest that metabolic activity is inhibited earlier in the newborn heart than in the adult.

A third mechanism that may contribute to age-related differences during ischemia is differences in tissue pH. We found that the newborn myocardium has a higher tissue pH during the early portion of ischemia than does the adult. Intracellular acidosis has been implicated as an important mechanism for the deactivation of the adenosine triphosphatase activity of cardiac myofilaments.\textsuperscript{19} Acidosis appears to reduce the affinity of myofibrillar troponin C for calcium, thus inhibiting
myofilament contraction. Others have observed that newborn hearts have a relatively greater intracellular buffering capacity than the adult. Thus, we speculate that increased intracellular hydrogen ion concentration may protect the adult heart from ischemic contracture by inhibiting contractile activity and inhibiting the formation of rigor complexes.

Impaired processing of calcium by the sarcoplasmic reticulum following ischemia may play a very important role in cellular injury. Certainly there are important functional and structural age-related differences in myocardial sarcoplasmic reticulum. The newborn sarcoplasmic reticulum appears to have a decreased ability to store intracellular calcium. In addition, there is quantitatively less sarcoplasmic reticulum in newborns than in adults. Thus, we speculate that the immaturity of the sarcoplasmic reticulum in our newborn hearts could account for the rapid increase in stiffness of the myocardium during ischemia.

Another important mechanism of ischemic cellular damage is free radical injury. Oxygen radicals are important mediators of ischemic injury, particularly during reperfusion. Immature tissues, including heart and lung, are relatively deficient in the antioxidant enzymes needed to detoxify free radicals. Furthermore, immature sarcolemma has a particularly high content of polyunsaturated fatty acids, which may increase the susceptibility of this membrane to free radical injury. Since our newborn hearts showed considerable deterioration during reperfusion, we speculate that free radical damage may have contributed to the injury in this age group.

Although theoretical considerations may lead to the prediction of an increased vulnerability of newborn myocardium to ischemic injury, previous workers have found either less vulnerability or no important age-related differences. Reconciling these published results with our own observations is difficult. We do note that most of these investigators used either older rabbits (greater than 1 week of age) or puppies. Other workers have used isolated muscle strips, in which coronary microcirculation is not a consideration. In the isolated rabbit septal preparation, coronary flow is generally one third the flow in our model and potentially important geometric factors are not operative. However, it remains unclear which of these differences between experimental models accounts for the contradictory observations.

We conclude that the newborn rabbit heart is more susceptible to injury from global ischemia than the adult. We speculate on several mechanisms for this increased vulnerability including age-related differences in 1) coronary microcirculation, 2) tissue pH and ATP, 3) sarcoplasmic reticulum calcium transport, and 4) free radical detoxification.

References


Global myocardial ischemia in the newborn, juvenile, and adult isolated isovolumic rabbit heart. Age-related differences in systolic function, diastolic stiffness, coronary resistance, myocardial oxygen consumption, and extracellular pH.

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