Preconditioning in Immature Rabbit Hearts
Role of K<sub>ATP</sub> Channels

John E. Baker, PhD; Patricia Holman, BS; Garrett J. Gross, PhD

Background—The protective effects of ischemic preconditioning have been shown to occur in adult hearts of all species studied. We determined whether immature hearts normoxic or chronically hypoxic from birth could be preconditioned, the time window or memory of the cardioprotective effect, and the involvement of the K<sub>ATP</sub> channel.

Methods and Results—Isolated immature rabbit hearts (7 to 10 days old) were subjected to 0, 1, or 3 cycles of preconditioning consisting of 5 minutes of global ischemia plus 10 minutes of reperfusion. This was followed by 30 minutes of global ischemia and 35 minutes of reperfusion. Normoxic hearts (FIO<sub>2</sub> = 0.21) subjected to 1 cycle of preconditioning recovered 70±7% of left ventricular developed pressure compared with 43±8% recovery in nonpreconditioned controls. Three cycles of preconditioning did not result in additional recovery (63±8%). Hearts from rabbits raised from birth in hypoxic conditions (FIO<sub>2</sub> = 0.12) and subjected to 1 and 3 preconditioning cycles did not show increased recovery (68±8% and 65±5%) compared with nonpreconditioned hypoxic controls (63±9%), although the recovery was greater in chronically hypoxic hearts than in age-matched normoxic controls. Increasing the recovery period after the preconditioning stimulus from 10 to 30 minutes resulted in a loss of cardioprotection. Pretreatment of normoxic hearts for 30 minutes with the K<sub>ATP</sub> channel blocker 5-hydroxydecanoate (300 μmol/L) completely abolished preconditioning (70±7% to 35±9%) but had no effect on nonpreconditioned hearts (40±8%).

Conclusions—Immature hearts normoxic from birth can be preconditioned, whereas immature hearts hypoxic from birth cannot. Preconditioning in normoxic immature hearts is associated with activation of the K<sub>ATP</sub> channel.

Key Words: cardiovascular diseases ■ heart defects, congenital ■ hypoxia ■ ions ■ ischemia
Methods

Animals
Animals used in this study received humane care in compliance with the “Guide for the Care and Use of Laboratory Animals” formulated by the National Research Council, 1996.

Creation of Hypoxia From Birth
Pregnant New Zealand White rabbits were obtained from a commercial breeder. For the hypoxic studies, the kits were born in a normoxic environment and then transferred to a hypoxic environmental chamber (FIO₂ = 0.12) immediately after their first feeding. The oxygen level in the chamber was maintained at FIO₂ = 0.12 throughout the remainder of the study. For the normoxic studies, the kits were raised under identical conditions except that FIO₂ in the environmental chamber remained at 0.21 for the duration of the study.12

Perfusion System
Isolated rabbit hearts were instrumented as previously described.12 A 3-way tap, located immediately above the site of cannulation, allowed the entire perfusate to be diverted away from the heart to produce global, no-flow ischemia. Reperfusion was achieved by repositioning of the tap to allow perfusate to be delivered to the heart.

Perfusion Media
The standard perfusate was modified Krebs-Henseleit bicarbonate buffer13 (mmol/L): NaCl 118.5; NaHCO₃ 25.0; KCl 4.8; MgSO₄ 0.6; H₂O 1.2; KH₂PO₄ 1.2 (pH 7.4 when gassed with 95% O₂-5% CO₂), in which the calcium content was reduced to 1.8. Glucose (11.1 mmol/L) was added to the perfusate. Before use, all perfusion fluids were filtered through cellulose acetate membranes with a pore size of 5.0 µm to remove particulate matter. K ATP blockers were added to this perfusate as needed.

Assessment of Ventricular Function
Left and right ventricular function was monitored continuously throughout each experiment as previously described.12 End-diastolic pressure was initially set to 3 mm Hg for 2 minutes. The balloons were then progressively inflated with a microsyringe to set end-diastolic pressures to 8 mm Hg for the left ventricle and 4 mm Hg for the right ventricle, and developed pressure was recorded during steady-state conditions. Coronary-flow rate was measured throughout the experiment by timed collections of the coronary effluent from the right side of the heart into a graduated cylinder. Coronary flow rate was expressed as milliliters per minute.

Perfusion Sequence
Preconditioning Studies
We performed the following experiments in a random order using 10 hearts from 6 groups to test the null hypothesis that immature hearts normoxic or chronically hypoxic from birth cannot be preconditioned. The 6 experimental groups were as follows: group 1, normoxic, nonpreconditioned; group 2, normoxic, 1 × 5 minutes of preconditioning; group 3, normoxic, 3 × 5 minutes of preconditioning; group 4, hypoxic, nonpreconditioned; group 5, hypoxic, 1 × 5 minutes of preconditioning; and group 6, hypoxic, 3 × 5 minutes of preconditioning. Figure 1 illustrates the experimental protocol. Immediately after aortic cannulation, hearts were perfused in the Langendorff mode14 at constant perfusion pressure of 42 mm Hg15 with balloons placed in the left and right ventricles. Biventricular function and coronary flow rate were recorded under steady-state conditions. Hearts were subjected to 0, 1, or 3 cycles of preconditioning, each consisting of 5 minutes of global, no-flow ischemia plus 10 minutes of reperfusion. In each instance, this was followed by 30 minutes of global, no-flow ischemia and 35 minutes of reperfusion. The rationale for investigating >1 preconditioning cycle was to determine if the threshold (the minimum stimulus necessary to produce preconditioning) was higher in chronically hypoxic hearts than in normoxic controls. During the reperfusion period, indexes of cardiac function were measured under steady-state conditions. In this way, each heart served as its own control.

Memory Studies
We assessed the effect of the period of time between the preconditioning stimulus and the prolonged ischemic insult on postischemic recovery in immature normoxic hearts. The duration of this period determines whether the myocardium retains the memory of the preconditioning stimulus that confers protection during subsequent sustained ischemia.16,17 We performed the following experiments in random order using 10 hearts from 4 groups. The 4 experimental groups were as follows: group 7, nonpreconditioned; group 8, 1 × 5 minutes of preconditioning with 10 minutes of reperfusion; group 9, 1 × 5 minutes of preconditioning with 20 minutes of reperfusion; and group 10, 1 × 5 minutes of preconditioning with 30 minutes of reperfusion.
hemodynamic data for immature hearts before and after the first preconditioning cycle, and after 35 minutes of reperfusion. Coronary flow rate in chronically hypoxic hearts was higher than in normoxic hearts as an adaptive response to increase oxygen delivery to the myocardium. Right ventricular developed pressure was higher in hypoxic ventricles than in normoxic ventricles as a consequence of right ventricular hypertrophy. The preconditioning stimulus depressed left and right ventricular developed pressures during the recovery period in all groups. The

**Results**

**Preconditioning in Immature Hearts**

The table also shows that hearts from chronically hypoxic rabbits were more tolerant of ischemia than normoxic controls, as shown by improved recovery of postischemic left ventricular developed pressure. Figure 2 shows that normoxic hearts subjected to 1 cycle of preconditioning (group 2) exhibited an increased recovery of left ventricular developed pressure, from $43 \pm 8\%$ in nonpreconditioned hearts (group 1) to $70 \pm 7\%$. Increasing the number of preconditioning cycles from 1 to 3 (group 3) did not result in additional increased recovery of developed pressure ($63 \pm 8\%$). In contrast, Figure 3 shows that chronically hypoxic hearts subjected to 1 cycle of preconditioning (group 5) did not show increased recovery of developed pressure compared with nonpreconditioned hearts (group 4) ($63 \pm 9\%$ vs $68 \pm 8\%$). Increasing the number of preconditioning cycles from 1 to 3 in chronically hypoxic hearts (group 6) did not result in additional protection ($65 \pm 5\%$). Thus, neither 1 or 3 cycles of preconditioning resulted in greater protection than that afforded by chronic hypoxia. Our data indicate that left ventricle from immature hearts normoxic from birth could be preconditioned, whereas left ventricle from immature hearts chronically hypoxic from birth could not be preconditioned. Recovery of developed pressure in the right ventricle was not increased by preconditioning in either normoxic or chronically hypoxic hearts.

**Mechanism Studies**

Preconditioning in mature hearts is mediated by activation of $K_{ATP}$ channels. We determined whether blockade of the $K_{ATP}$ channel before the preconditioning stimulus influences postischemic recovery in immature hearts. We performed the following experiments in random order using 10 hearts in 5 groups of normoxic rabbits. The 5 experimental groups were as follows: group 11, nonpreconditioned before ischemia; group 12, preconditioned, 30 minutes of perfusion with 5-hydroxydecanoate (300 $\mu$mol/L) before ischemia; group 13, preconditioned; group 14, 30 minutes of perfusion with 5-hydroxydecanoate (100 $\mu$mol/L) before preconditioning; and group 15, 30 minutes of perfusion with 5-hydroxydecanoate (300 $\mu$mol/L) before preconditioning. In each instance, the preconditioning stimulus consisted of 5 minutes of global, no-flow ischemia followed by 10 minutes of recovery. This was followed by 30 minutes of global, no-flow ischemia and 35 minutes of reperfusion.

**Table**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Heart Rate, bpm</th>
<th>Coronary Flow Rate, mL/min</th>
<th>LVDP, mm Hg</th>
<th>RVDP, mm Hg</th>
<th>Heart Rate, bpm</th>
<th>Coronary Flow Rate, mL/min</th>
<th>LVDP, mm Hg</th>
<th>RVDP, mm Hg</th>
<th>Heart Rate, bpm</th>
<th>Coronary Flow Rate, mL/min</th>
<th>LVDP, mm Hg</th>
<th>RVDP, mm Hg</th>
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<td>1. Control</td>
<td>249 ± 25</td>
<td>7 ± 1</td>
<td>102 ± 8</td>
<td>36 ± 7</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>222 ± 43</td>
<td>6 ± 1†</td>
<td>43 ± 9†</td>
<td>26 ± 6†</td>
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<td>2. 1×5’</td>
<td>237 ± 26</td>
<td>6 ± 2</td>
<td>97 ± 8</td>
<td>40 ± 6</td>
<td>228 ± 29</td>
<td>5 ± 1†</td>
<td>74 ± 10†</td>
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<td>231 ± 40</td>
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<td>7 ± 2</td>
<td>87 ± 9</td>
<td>45 ± 5</td>
<td>249 ± 15</td>
<td>6 ± 2</td>
<td>66 ± 10†</td>
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<td>8 ± 2</td>
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<tr>
<td>4. Control</td>
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<td>100 ± 9</td>
<td>47 ± 12*</td>
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<td>5. 1×5’</td>
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<td>90 ± 6</td>
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<td>7 ± 2</td>
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<tr>
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<td>102 ± 6</td>
<td>58 ± 11*</td>
<td>234 ± 34</td>
<td>9 ± 1</td>
<td>79 ± 7†</td>
<td>46 ± 10†</td>
<td>240 ± 42</td>
<td>10 ± 1†</td>
<td>66 ± 6*†</td>
<td>43 ± 9†</td>
</tr>
</tbody>
</table>

LVDP and RVDP indicate left and right ventricular developed pressure, respectively; 5’, 5-minute period of global, no-flow ischemia.

Values are mean ± SD from a minimum of 10 hearts per group.

*P<0.05, normoxic vs hypoxic; †P<0.05 before preconditioning vs after initial preconditioning and reperfusion.
Persistence of Memory

We then examined the relationship between the duration of the reperfusion period after a single 5-minute preconditioning stimulus before sustained ischemia and recovery of postischemic function. Figure 4 shows the results of an increase in the reperfusion period between the preconditioning stimulus and the prolonged ischemic insult from 10 to 30 minutes. Ten minutes of reperfusion after the preconditioning stimulus (group 8) resulted in a recovery of left ventricular developed pressure of 72±6%. The memory of preconditioning was retained after 20 minutes of reperfusion (group 9), with a recovery of 67±6%. However, after 30 minutes of reperfusion (group 10), the memory of preconditioning was lost, resulting in a recovery of developed pressure to 42±4%, which was no different than in nonpreconditioned hearts (42±6%; group 7). There were no differences in hemodynamics between groups before sustained ischemia.

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Figure 5 shows that in hearts nonpreconditioned before ischemia, recovery of developed pressure was 43±6% (group 11). Blockade of the K<sub>ATP</sub> channel for 30 minutes before ischemia in nonpreconditioned hearts with 300 μmol/L 5-hydroxydecanoate (group 12) had no effect on tolerance to ischemia, with recovery of 40±8%. Preconditioned hearts (group 13) recovered 70±7% of preischemic developed pressure compared with 43±6% in non preconditioned controls (group 11). Pretreatment of hearts for 30 minutes with 100 μmol/L 5-hydroxydecanoate (group 14) and 300 μmol/L 5-hydroxydecanoate (group 15) before preconditioning completely abolished preconditioning, with recoveries of 42±8% and 35±9%, respectively, which were no different than recoveries in nonpreconditioned hearts. 5-Hydroxydecanoate (100 and 300 μmol/L) depressed preischemic coronary flow rate and developed pressure in both the preconditioned and nonpreconditioned groups. Thus, 5-hydroxydecanoate was able to completely abolish preconditioning in immature normoxic hearts.

Discussion

We demonstrated that hearts from immature rabbits normoxic from birth can be preconditioned. In contrast, hearts from immature rabbits hypoxic from birth were resistant to preconditioning. Chronically hypoxic hearts were also more resistant to sustained ischemia than were normoxic hearts, consistent with our previous results.11,12 The memory of preconditioning in immature normoxic hearts was lost 30 minutes after the preconditioning stimulus. The K<sub>ATP</sub> channel blocker 5-hydroxydecanoate completely abolished the cardio-protective effects of preconditioning in immature normoxic hearts, whereas 5-hydroxydecanoate did not affect recovery of postischemic function in nonpreconditioned normoxic hearts.

Preconditioning of Immature Hearts

Adaptation to chronic hypoxia from birth confers tolerance to subsequent ischemia compared with age-matched normoxic controls.11 We previously suggested that there are similarities between adaptation to chronic hypoxia and preconditioning regarding the ability of these 2 processes to protect the heart against a subsequent ischemic insult.12 In the present study, we demonstrate for the first time that left ventricle of
immature rabbit hearts normoxic from birth could be preconditioned, whereas immature hearts chronically hypoxic from birth could not be preconditioned. Our data suggest that chronically hypoxic immature hearts are already protected by adaptation to hypoxia and that additional cardioprotection by ischemic preconditioning is not possible. In our previous study, in which hearts were subjected to 18 minutes of global no-flow ischemia, recovery of right ventricular function was greater in chronically hypoxic hearts than in normoxic controls. In the present study, in which hearts were subjected to 30 minutes of global no-flow ischemia, recovery of right ventricular function in chronically hypoxic hearts was no different from normoxic controls. We attribute the observed differences between the present and previous studies to the increased duration of ischemia. The right ventricle was resistant to preconditioning in our study. This raises the possibility that the right ventricle may already be preconditioned or that the preconditioning stimulus was insufficient to protect the right ventricle against the subsequent period of prolonged ischemia.

The ability of ischemic preconditioning to provide additional protection in chronically hypoxic hearts during subsequent postnatal development is unknown. Evidence to support this possibility, however, is based on the observation by Tajima et al. that hearts from chronically hypoxic adult rats could be preconditioned, although no mechanisms were uncovered to explain the additive protective effect of preconditioning on adaptation to chronic hypoxia. Additional studies are needed to define the relationship between age, adaptation to hypoxia, and ischemic preconditioning, as well as the mechanisms involved.

We considered the possibility that the threshold for preconditioning was higher in chronically hypoxic hearts than in normoxic controls. However, increasing the number of preconditioning cycles from 1 to 3 did not result in additional cardioprotection in either normoxic or chronically hypoxic hearts. There was a trend toward a slight reduction in cardioprotection with multiple preconditioning cycles in both normoxic and chronically hypoxic hearts, although the effect was not significant. Our finding is in agreement with previous studies that showed 5-hydroxydecanoate had no effect on recovery from ischemia in non preconditioned hearts. This finding is in agreement with previous studies that showed 5-hydroxydecanoate had no effect on injury during the cycle of ischemia and reperfusion.

Adaptation of immature rabbits to chronic hypoxia from birth increases tolerance of the heart to subsequent ischemia. The K<sub>ATP</sub> channel blocker glibenclamide abolished this cardioprotective effect. Ischemic preconditioning in immature rabbit hearts also increased tolerance to ischemia. 5-Hydroxydecanoate abolished this cardioprotective effect. Thus, ischemic preconditioning and adaptation to chronic hypoxia in immature hearts appear to share a final common effector, the K<sub>ATP</sub> channel, although the signal transduction pathway in the immature heart that results in increased activation of the K<sub>ATP</sub> channel is unknown.

The cardioprotective effect of K<sub>ATP</sub> channel openers, used at concentrations that do not shorten action potential duration, is abolished by the K<sub>ATP</sub> channel blocker 5-hydroxydecanoate. This suggests that 5-hydroxydecanoate may not act on the sarcolemmal K<sub>ATP</sub> channel. Potassium channels are also found in the inner mitochondrial membrane, where they control mitochondrial volume and energetics. Diazoxide, a K<sub>ATP</sub> channel opener, is 1000 times more selective for opening mitochondrial K<sub>ATP</sub> channels than sarcolemmal K<sub>ATP</sub> channels. The cardioprotective effect of diazoxide during ischemia is completely abolished by 5-hydroxydecanoate, which indicates a role for the mitochondrial K<sub>ATP</sub> channel in protection of ischemic myocardium. 5-Hydroxydecanoate completely abolished the cardioprotective effects of preconditioning in immature hearts, which suggests a cardioprotective role for mitochondrial K<sub>ATP</sub> channels in immature hearts.

**Memory of Preconditioning**

In adult rabbit hearts preconditioned by a single 5-minute period of occlusion, a time delay of 15 to 30 minutes between the preconditioning stimulus and the prolonged ischemic insult results in a loss of cardioprotection. Similarly, we have shown that in immature rabbit hearts preconditioned by a single 5-minute period of occlusion, the memory of preconditioning is also lost after a time delay of 30 minutes between the preconditioning stimulus and the prolonged ischemic insult. Our data suggest there is no age-related difference in the memory of preconditioning between immature and mature rabbit hearts. The protective effects of ischemic preconditioning elicited by a single 5-minute period of occlusion reappear 24 to 72 hours after the preconditioning stimulus in adult rabbits. Additional studies are needed to determine whether this “second window of protection” is present in immature rabbit hearts.

**Involvement of K<sub>ATP</sub> Channel**

In adult rabbit hearts, blockade of the K<sub>ATP</sub> channel with glibenclamide abolished the protective effect of preconditioning. Our present study shows that pretreatment of immature hearts with 100 and 300 μmol/L of the ischemia-selective K<sub>ATP</sub> channel blocker 5-hydroxydecanoate completely abolishes the protective effect of preconditioning. Our study is the first to demonstrate preconditioning in immature rabbit hearts and the involvement of the K<sub>ATP</sub> channel. 5-Hydroxydecanoate alone had no effect on recovery from ischemia in non preconditioned hearts. This finding is in agreement with previous studies that showed 5-hydroxydecanoate had no effect on injury during the cycle of ischemia and reperfusion.

**Clinical Relevance**

Clinically, adult human myocardium can be preconditioned by brief periods of planned or unplanned ischemia, with protection mediated by K<sub>ATP</sub> channel activation. During coronary angioplasty, the severity of ST-segment depression is diminished during a second balloon inflation compared with the first. Administration of glibenclamide, a K<sub>ATP</sub> channel blocker, 90 minutes before angioplasty eliminated this cardioprotective effect. Angina that precedes a myocardial infarction within 48 hours confers endogenous cardioprotection. Preconditioning also preserves high-energy phos-
phates in patients undergoing coronary artery bypass surgery. 2

Cardiopulmonary bypass operations in children to correct congenital heart defects represent a planned ischemic insult for which ischemic preconditioning may be beneficial. In children with some forms of congenital heart disease, adequate access to all regions of the heart may be denied to cardiopulmonary solutions. Incomplete cardioprotection with cardioplegia in infants and children after surgical repair of congenital heart defects has been demonstrated by a deterioration in systolic function. 15 We have shown that protection of ischemic immature rabbit myocardium with traditional cardioplegia may be inadequate. 15 Thus, the potential exists of ischemic immature rabbit myocardium with traditional cardioplegia in infants and children after surgical repair of congenital heart defects has been demonstrated by a deterioration in systolic function. 31 We have shown that protection of ischemic immature rabbit myocardium with traditional cardioplegia may be inadequate. 15 The mechanism of preconditioning in immature, whereas immature hearts chronically hypoxic from birth can be preconditioned, whereas immature hearts normoxic from birth can be preconditioned before cardiac surgery in children with congenital heart disease. Another potential area of application for ischemic preconditioning in children would be in the setting of cardiac transplantation.

In conclusion, we have shown that isolated, crystalloid-perfused, immature hearts normoxic from birth can be preconditioned, whereas immature hearts chronically hypoxic from birth cannot be preconditioned. The response of blood-perfused immature hearts to a preconditioning stimulus remains unknown. The mechanism of preconditioning in immature hearts is associated with activation of the K-ATP channel. Additional studies are needed to define the relative contributions of the sarcocemmal and mitochondrial K-ATP channels as well as the signal transduction mechanism responsible for K-ATP channel activation.

Acknowledgments

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References


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