Blockade of ATP-Sensitive Potassium Channels Increases Infarct Size but Does Not Prevent Preconditioning in Rabbit Hearts

Jon D. Thornton, Christy S. Thornton, Diana L. Sterling, and James M. Downey

Ischemic preconditioning renders the heart resistant to infarction by an unknown mechanism. This study tests whether preconditioning may be working through activation of ATP-sensitive potassium channels. If that were the case, then blockade of the channels should eliminate preconditioning’s protection, and activation of these channels should mimic it. Thirty minutes of regional coronary ischemia followed by 3 hours of reperfusion caused 38.0±3.7% of the risk zone to become infarcted in control rabbits. Preconditioning with 5-minute ischemia followed by a 10-minute reperfusion before the 30-minute insult caused only 8.8±2.1% infarction, which was a reduction of 29.2% in infarct size by preconditioning (p<0.01 versus control value). Pretreatment with the potassium channel blocker glibenclamide at three different concentrations significantly elevated infarct size in the nonpreconditioned hearts at all doses. Preconditioning, however, continued to limit infarct size by an amount not different from that seen in the control group at all doses of glibenclamide. Pinacidil, a potassium channel agonist, given before a 30-minute ischemic insult resulted in infarct sizes no different from that seen in nonpreconditioned control rabbits. We conclude that ATP-sensitive potassium channels are not involved in preconditioning in the rabbit heart; however, blocking those channels does exacerbate ischemia. (Circulation Research 1993;72:44–49)

Key Words • ATP-sensitive K⁺ channels • glibenclamide • ischemic preconditioning • myocardial infarction • pinacidil

Preconditioning refers to a phenomenon in which the myocardium is made resistant to infarction by exposing it to a brief period of ischemia before a subsequent insult. The mechanism of this protection is currently unknown. The phenomenon has been demonstrated by various investigators in dogs,¹–³ pigs,⁴ and rabbits.⁵–⁷ The mechanism of preconditioning has been demonstrated not to involve opening collateral vessels⁸ or the synthesis of a protective protein.⁹ Recent work in our laboratory using the rabbit heart indicates that the buildup of adenosine during the first ischemic period triggers this effect by stimulating adenosine A₁ receptors. Adenosine receptor–blocking agents eliminated the protective effect of preconditioning, and substituting intracoronary adenosine⁶ or intravenous adenosine A₁–selective analogues for the brief ischemia conferred equal protection to the heart.⁹

Lasley et al¹⁰ have proposed that the anti-ischemic effects of A₁ agonists involve inhibitory G proteins, and we have recently demonstrated that preconditioning’s protection could be blocked at the G protein level.¹¹ The coupling of adenosine to ATP-sensitive potassium channels via G proteins has been demonstrated by Kirsch et al¹² in rat ventricular myocytes, making these channels prime candidates for this protection. Indeed, Gross and Auchampach¹³ reported that preconditioning in the dog could be blocked with glibenclamide, a selective inhibitor of the ATP-sensitive potassium channels. These investigators also reported that a promoter of this channel conferred a protection similar to preconditioning. The purpose of this experiment is to determine whether blockade of the ATP-sensitive potassium channels with glibenclamide could abolish the protection afforded by preconditioning in our rabbit model of myocardial infarction.

Materials and Methods

Surgical Preparation of Animals

New Zealand White rabbits of either sex, weighing between 1.55 and 2.93 kg, were anesthetized with intravenous sodium pentobarbital (30 mg/kg) administered via a marginal ear vein. The neck was opened with a ventral midline incision, and a tracheotomy was performed; the rabbits were ventilated with 100% oxygen via a positive-pressure respirator (MD Industries, Mobile, Ala.). Ventilation rate was 30–35 breaths per minute, and tidal volume was approximately 15 ml. The respiratory rate was adjusted to keep the blood pH in the physiological range. Catheters filled with heparinized saline (10 units/ml) were placed in the left carotid artery and jugular vein to monitor blood pressure and inject drugs, respectively. Additional anesthesia was...
also administered through the jugular vein as needed. A left thoracotomy was performed in the fourth intercostal space, and the pericardium was opened to expose the heart. A 2.0 silk suture with an RB taper needle was passed around a branch of the left coronary artery, and the ends of the silk were threaded through a small vinyl tube to form a snare. The coronary branch was occluded by pulling the snare, which was then fixed by clamping the tube with a mosquito hemostat. Myocardial isch- 

**Measurement of Blood Glucose**

To determine if blockade of potassium channels was affected, we measured the blood glucose level of control and glibenclamide-treated animals. Blockade of potassium channels results in an increased release of insulin from the pancreas, thus causing blood glucose levels to fall. Blood glucose levels were determined using the Ames Glucometer 3 (Miles Inc., Diagnostics Division, Mishawaka, Ind.). Test strips and control strips were Ames Glucofilim.

**Measurement of Infarct and Risk Area**

At the end of each experiment, the heart was quickly removed and mounted on a Langendorff apparatus and flushed with room temperature saline for 60 seconds. The silk suture under the coronary branch was then tightly tied to occlude the artery, and a 0.5% suspension of fluorescent particles (1–10-μm diameter from Duke Scientific Corp., Palo Alto, Calif.) were infused into the perfusate to differentiate the risk zone as the tissue with no fluorescence. The heart was removed from the Langendorff apparatus, weighed, and then frozen. When frozen, the heart was cut into transverse slices 2 mm thick. The slices were thawed and stained by incubation for 20 minutes in 1% triphenyltetrazolium chloride in pH 7.4 buffer. Tetrazolium reacts with NADH and dehydrogenase enzymes and causes all tissue still having the enzymes and cofactors to stain a deep red color. The infarcted area of the heart loses these constituents and does not cause formation of the pigment. The slices were then soaked in 10% formalin to enhance the contrast of the stain. After staining, the slices were sandwiched between two glass plates to a uniform 2 mm thickness, and the region of infarcted tissue and the risk zone were traced through the glass. The area of infarct and the risk zone were determined by planimetry of the tracings. The volume of infarcted myocardium and myocardium at risk was calculated by multiplying the planimetered areas by the slice thickness.

**Chemicals**

The potassium channel blocker glibenclamide was purchased from Sigma Chemical Co., St. Louis, Mo., and was dissolved in a vehicle consisting of equal parts of 1N sodium hydroxide, ethanol, and polyethylene glycol (molecular weight, 200). Once in solution, the drug:vehicle solution was mixed 1:4 with 0.9% saline. The potassium channel agonist pinacidil was a gift from Lilly Research Laboratories, Indianapolis, Ind., and was dissolved in 1.5% of 2N hydrochloric acid.

**Experiment Protocols**

This experiment involved 102 rabbits divided into 10 groups. All animals were subjected to a 30-minute coronary occlusion followed by 180 minutes of reperfusion. All preconditioned animals experienced an additional 5-minute coronary occlusion followed by a 10-minute reperfusion before the 30-minute occlusion. Three animals were used as vehicle controls and were only admin- 

**Statistics**

We tested for differences between the 10 groups by one-way analysis of variance using a Newman-Keuls post hoc test. A value of $p<0.05$ was considered to be significant. Confidence limits in the text are standard error of the mean.

**Results**

The control group included 25 rabbits. Fourteen of these animals were historical controls from previous studies and 11 were contemporary. Infarct sizes in the two control groups were not different (37.6±5.4% and 38.3±4.8% of the risk zone infarcted for the two groups, respectively); therefore, the groups were combined and represent the control group in all statistical analyses of infarct size. The combining of groups was done to increase the power of the analysis. Only the contemporary control rabbits are included in the hemodynamic analysis.

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TABLE 1. Hemodynamic Data in Rabbits

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Group</th>
<th>Preischemia</th>
<th>Ischemia</th>
<th>Reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HR (bpm)</td>
<td>BP (mm Hg)</td>
<td>HR (bpm)</td>
</tr>
<tr>
<td>Untreated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1</td>
<td>260±8</td>
<td>76±3</td>
<td>250±7</td>
</tr>
<tr>
<td>PC</td>
<td>2</td>
<td>228±7</td>
<td>67±6</td>
<td>225±11</td>
</tr>
<tr>
<td>0.15 mg/kg Glib</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3</td>
<td>257±11</td>
<td>70±7</td>
<td>260±9</td>
</tr>
<tr>
<td>PC</td>
<td>4</td>
<td>276±7</td>
<td>75±9</td>
<td>240±11</td>
</tr>
<tr>
<td>0.30 mg/kg Glib</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>267±9</td>
<td>76±5</td>
<td>241±7</td>
</tr>
<tr>
<td>PC</td>
<td>6</td>
<td>262±7</td>
<td>67±7</td>
<td>246±6</td>
</tr>
<tr>
<td>3.0 mg/kg Glib</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7</td>
<td>283±15</td>
<td>71±6</td>
<td>274±11</td>
</tr>
<tr>
<td>PC</td>
<td>8</td>
<td>275±12</td>
<td>81±3</td>
<td>251±9</td>
</tr>
<tr>
<td>0.5 mg/kg Pin</td>
<td>9</td>
<td>254±10</td>
<td>61±5</td>
<td>261±10</td>
</tr>
<tr>
<td>1.0 mg/kg Pin</td>
<td>10</td>
<td>272±11</td>
<td>75±5</td>
<td>259±17</td>
</tr>
</tbody>
</table>

HR, heart rate; bpm, beats per minute; BP, mean arterial blood pressure; PC, preconditioned; Glib, glibenclamide; Pin, pinacidil. Values are mean±SEM.

*p<0.02 vs. the untreated control value.

ment with 0.3 mg/kg glibenclamide completely blocked the hypotensive effect of pinacidil (change in mean arterial pressure was +0.5±2.19 mm Hg, n=4).

Infarct Size Data

Table 2 presents the body weights and numbers of the animals in the study as well as the infarct size data. Infarct sizes for all of the glibenclamide groups are also plotted in Figure 1. Infarct size in the untreated control group averaged 38.0±3.7% of the risk zone. Preconditioning provided significant protection from infarction in the untreated group (8.8±2.1% infarction). Glibenclamide treatment before the 30-minute ischemia in nonpreconditioned animals resulted in a significant increase in infarct size for groups 3, 5, and 7 (62.0±7.5% [p<0.05], 58.3±4.5% [p<0.05], and 76.8±5.1% risk zone infarction [p<0.02] for the untreated control group versus the 0.15, 0.30, and 3.0 mg/kg dose groups, respectively). The preconditioned animals in each of the glibenclamide-treated groups had significantly smaller infarct to risk area ratios than their drug-treated control groups (p<0.02 versus the respective control group). When glibenclamide was administered 5 minutes before preconditioning, infarct sizes in the three dosage groups were 15.9±5.7%, 21.2±5.9%, and 19.5±5.0% risk zone infarction, respectively. None of these was significantly different from the nontreated preconditioned infarct size. The limitation of infarct size for each drug dose can be calculated by subtracting the mean infarct size in the absence of preconditioning from that with preconditioning. Those differences were 46.2%, 37.1%, and 57.3% for the 0.15, 0.30, and 3.0

TABLE 2. Body Weight and Infarct Size in Rabbits

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Group</th>
<th>n</th>
<th>Body wt (kg)</th>
<th>Risk area (cm²)</th>
<th>Infarct area (cm²)</th>
<th>I/R (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1</td>
<td>25</td>
<td>2.1±0.0</td>
<td>0.89±0.09</td>
<td>0.34±0.05</td>
<td>38.0±3.7</td>
</tr>
<tr>
<td>PC</td>
<td>2</td>
<td>9</td>
<td>2.1±0.1</td>
<td>0.64±0.08</td>
<td>0.06±0.02*†</td>
<td>8.8±2.1*†</td>
</tr>
<tr>
<td>0.15 mg/kg Glib</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3</td>
<td>7</td>
<td>2.3±0.1</td>
<td>0.80±0.09</td>
<td>0.53±0.13</td>
<td>62.0±7.5‡</td>
</tr>
<tr>
<td>PC</td>
<td>4</td>
<td>5</td>
<td>2.1±0.1</td>
<td>0.65±0.07</td>
<td>0.10±0.04†</td>
<td>15.9±5.7†</td>
</tr>
<tr>
<td>0.30 mg/kg Glib</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>7</td>
<td>2.3±0.1</td>
<td>1.1±0.19</td>
<td>0.58±0.08</td>
<td>58.3±4.5‡</td>
</tr>
<tr>
<td>PC</td>
<td>6</td>
<td>10</td>
<td>2.1±0.1</td>
<td>0.82±0.10</td>
<td>0.20±0.08†</td>
<td>21.2±5.9†</td>
</tr>
<tr>
<td>3.0 mg/kg Glib</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7</td>
<td>8</td>
<td>2.2±0.1</td>
<td>1.1±0.11</td>
<td>0.85±0.10*</td>
<td>76.8±5.1*</td>
</tr>
<tr>
<td>PC</td>
<td>8</td>
<td>8</td>
<td>2.4±0.1</td>
<td>0.76±0.03</td>
<td>0.15±0.04†</td>
<td>19.5±5.0†</td>
</tr>
<tr>
<td>0.5 mg/kg Pin</td>
<td>9</td>
<td>9</td>
<td>2.3±0.1</td>
<td>0.99±0.10</td>
<td>0.30±0.05</td>
<td>32.0±4.1</td>
</tr>
<tr>
<td>1.0 mg/kg Pin</td>
<td>10</td>
<td>6</td>
<td>2.2±0.2</td>
<td>0.80±0.09</td>
<td>0.31±0.07</td>
<td>37.4±6.0</td>
</tr>
</tbody>
</table>

I/R, percentage of the risk zone infarcted, calculated as (infarct area/risk area)×100%; PC, preconditioned; Glib, glibenclamide; Pin, pinacidil. Values are mean±SEM.

*p<0.02 vs. untreated control group.

†p<0.02 vs. corresponding control group for same treatment.

‡p<0.05 vs. untreated control group.
mg/kg groups, respectively. At no dose was the shift in infarct size caused by preconditioning less than the 29.2% reduction that was seen in the untreated groups. Not shown in Figure 1 are the three animals in the vehicle-only group. Infarct size in the vehicle group was 34.2±5%, which was not different from the untreated control group.

The final two groups in the study were treated with the potassium channel agonist pinacidil at doses of 0.5 and 1.0 mg/kg. This treatment resulted in infarction ratios that were not significantly different from the untreated control ratios, which were 32.0±4.1% and 37.4±6.0%, respectively (see Figure 2).

**Blood Glucose Data**

Treatment with the potassium channel blocker glibenclamide significantly lowered the blood glucose in all six groups receiving the drug ($p<0.02$ versus initial blood glucose measurement). There were no significant differences between the groups at either time point during the study. The percent change in blood glucose ranged from $-23.2±4.5\%$ to $-38.0±4.9\%$, and there were no significant differences between the groups.

**Discussion**

This study does not support the hypothesis that preconditioning in the rabbit is mediated by activation of ATP-sensitive potassium channels. No dose of glibenclamide, a potent blocker of this channel, was found that could block the protection afforded by preconditioning. Along those same lines, neither dose of pinacidil, a promoter of those channels, offered any protection against infarction. An interesting finding of this study was that glibenclamide pretreatment actually increased infarct size in all of the nonpreconditioned groups over that seen in the untreated control group. The explanation most compatible with the present data is that opening of the ATP-sensitive potassium channels is beneficial to the ischemic cell but that they open naturally during ischemia, perhaps because of falling cytosolic ATP levels. The fact that pinacidil was unable to protect the myocardium from ischemia also argues against a role for the ATP-sensitive potassium channel in preconditioning in the rabbit.

The ATP-sensitive potassium channel has been linked to numerous effectors of myocardial metabolism and function. Cook et al.\textsuperscript{19} have reported that the vasodilatory effect of the potassium channel agonist pinacidil may be due to its stimulation of potassium channels in vascular smooth muscle. Some studies even report protection against ischemia with potassium channel promoters. Fish et al.\textsuperscript{16} reported that potassium channel activators suppress arrhythmias that are due to delayed repolarization. Grover et al.\textsuperscript{17} demonstrated that the potassium channel activator cromakalim protected dog hearts from infarction and improved postischemic function in isolated rat hearts. The same group has also reported improved postischemic function with the potassium channel agonists nicorandil and pinacidil.\textsuperscript{18,19} They also demonstrated that the protection could be blocked by the potassium channel blocker glibenclamide. Similar protective results were obtained by Cole et al.\textsuperscript{10} with pinacidil in the guinea pig. Of course, we only examined two doses of pinacidil, and we cannot rule out the possibility that we may have missed a critical dose.

We found that glibenclamide extends myocardial infarction in the rabbit at all doses tested. Gross et al.\textsuperscript{21} have shown that stunning in the dog is exacerbated with glibenclamide at a dose of 1 mg/kg. Gross and Auchampach\textsuperscript{13} reported that glibenclamide blocked the protective effect of preconditioning in their dog model, but no effect was seen in nonpreconditioned dogs treated with glibenclamide at the 0.3 mg/kg dose. However, those investigators did see an increased infarct size when glibenclamide was given to dogs at higher doses.\textsuperscript{22} Interestingly, some investigators have reported that the sulfonylureas, a drug class that includes glibenclamide,
Table 3. Blood Glucose Levels in Rabbits

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Group</th>
<th>n/N</th>
<th>Blood glucose (mg/dl)</th>
<th>Change in blood glucose (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Initial</td>
<td>Reperfusion</td>
</tr>
<tr>
<td>0.15 mg/kg Glib</td>
<td>3</td>
<td>5/7</td>
<td>133.4±7.2</td>
<td>96.4±2.4*</td>
</tr>
<tr>
<td>PC</td>
<td>4</td>
<td>5/5</td>
<td>167.8±13.7</td>
<td>103.8±12.1*</td>
</tr>
<tr>
<td>0.30 mg/kg Glib</td>
<td>5</td>
<td>7/7</td>
<td>185.9±22.5</td>
<td>114.3±15.5*</td>
</tr>
<tr>
<td>PC</td>
<td>6</td>
<td>4/10</td>
<td>213.0±5.7</td>
<td>141.5±12.4*</td>
</tr>
<tr>
<td>3.0 mg/kg Glib</td>
<td>7</td>
<td>2/8</td>
<td>136.0±9.5</td>
<td>104.5±7.6*</td>
</tr>
<tr>
<td>PC</td>
<td>8</td>
<td>2/8</td>
<td>133.5±15.2</td>
<td>94.5±15.9*</td>
</tr>
</tbody>
</table>

n, Number of animals in which blood glucose was measured; N, total number in group; Glib, glibenclamide; PC, preconditioned. Values are mean±SEM.

*p<0.02 vs. corresponding value for initial blood glucose.

actually protect the rat myocardium from ischemia, presumably via stimulation of glucose utilization and glycolytic flux.23 If glibenclamide actually does extend infarction, then the use of this drug as a hypoglycemic agent in patients with coronary artery disease might be contraindicated. Although the detrimental effects of glibenclamide during acute myocardial infarction may be unique to the rabbit heart, this effect probably warrants further study, considering the large population of diabetic patients currently taking this drug, many of whom have coronary artery disease.

The increase in infarct size in the glibenclamidetreated groups may have resulted from a decrease in glycolytic substrate via insulin release from the pancreas; however, the decrease was modest. Furthermore, glucose is a minor substrate for the heart, because it primarily metabolizes lactate and free fatty acids. In fact, we have found that complete elimination of glucose from the perfusate has no effect on infarct size in either control or preconditioned hearts as long as a mitochondrial substrate (pyruvate) is present.24 Glibenclamide has been reported to abolish reactive hyperemia in the coronary arteries. Glibenclamide may have increased infarct size by interfering with coronary blood flow regulation such that reperfusion was inadequate. The effect could not have involved reduced collateral flow, because rabbits lack preformed collateral vessels. The final possibility is a direct effect of potassium channel blockade on the myocyte, making it less tolerant of ischemia by some unknown mechanism. The increase in infarct size and the hypoglycemia were independent of the glibenclamide dose in these studies, suggesting that all doses fully blocked the channels.

The possibility that ATP-sensitive potassium channels might mediate preconditioning seemed a logical one in light of our belief that adenosine A1 receptors trigger preconditioning.8 We have recently found that preconditioning in the rabbit model can be blocked with pertussis toxin treatment, indicating that preconditioning is linked by the G_{i} protein.11 Kirsch et al12 demonstrated that adenosine A1 receptors stimulate the ATP-sensitive potassium channels in rat ventricular myocytes and that a pertussis toxin-sensitive G protein links those receptors. Fosset et al13 also showed the same channels to be pertussis toxin sensitive in insulinoma cells. Therefore, it is tempting to hypothesize that the ATP-sensitive potassium channel could also be the effector for preconditioning in the rabbit. However, the current data do not support this hypothesis.

We have no ready explanation for the discrepancy between the present data and the previously mentioned dog studies of Gross and Auchampach.13 Those differences may be due to species variability in the mechanism of preconditioning’s protection, but that seems highly unlikely. The number of dogs in each of the groups in the Gross and Auchampach study was quite small, and a significant drug-induced shift of the collateral flow versus infarct size plot could not be demonstrated unless several groups were pooled. Nevertheless, their data are very suggestive of a blockade of protection with glibenclamide in the dog model. However, in the present study, the data are also clear. Preconditioning, if anything, had an enhanced protection in the presence of glibenclamide, since the difference in infarct sizes between the control and preconditioned animals in each treatment group was greater than the difference between the nontreated control and preconditioned groups. There was a trend to increased infarct size in the preconditioned groups, but none of these differences achieved significance and, therefore, may or may not have been real. It is possible that the toxic effect of glibenclamide could have simply negated the protection afforded by preconditioning in the Gross and Auchampach study, giving the impression that preconditioning was blocked; that explanation seems unlikely, however, since no effect was seen in their nonpreconditioned hearts.

Another possibility is that ATP-sensitive potassium channels in the rabbit heart may simply not be sensitive to glibenclamide. Another possible species difference may be in the amount of drug that is protein bound. However, the release of insulin from the pancreas would indicate that a large amount of the drug is free and able to bind receptors. One obvious shortcoming of this study is that we have no way of verifying that either glibenclamide or pinacidil actually altered potassium channel function as expected. The doses of glibenclamide chosen for our study were 0.5, 1, and 10 times that shown to be effective in the dog. All of the doses produced significant hypoglycemia, as demonstrated in Table 3, indicating that at least potassium channels in the rabbit’s pancreas were blocked, thereby increasing
insulin release. Glibenclamide has been shown to block potassium channels in rat ventricular myocytes.\textsuperscript{12} We recently examined preconditioning in the open-chest rat and found that glibenclamide had exactly the same effect as that seen in the rabbit. At the 0.3 mg/kg dose, infarct size was increased in the nonpreconditioned hearts, but protection was unaltered in those that were preconditioned.\textsuperscript{26}

In conclusion, we find that 1) blockade of ATP-sensitive potassium channels fails to block the protective effect of preconditioning in the rabbit heart, which argues against the hypothesis that the mechanism of preconditioning involves opening of these channels; 2) pinacidil, a promoter of this channel, did not confer protection against infarction; and 3) glibenclamide causes significant infarct extension in the nonpreconditioned animals, suggesting that this drug may not be appropriate for patients with coronary artery disease.

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Blockade of ATP-sensitive potassium channels increases infarct size but does not prevent preconditioning in rabbit hearts.

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