Delayed ischemic preconditioning is mediated by opening of ATP-sensitive potassium channels in the rabbit heart

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Bernardo, Nelson L., Michael D’Angelo, Shinji Okubo, Archi Joy, and Rakesh C. Kukreja. Delayed ischemic preconditioning is mediated by opening of ATP-sensitive potassium channels in the rabbit heart. Am. J. Physiol. 276 (Heart Circ. Physiol. 45): H1323–H1330, 1999.—Cardioprotection from preconditioning reappears 24 h after the initial stimulus. This phenomenon is called the second window of protection (SWOP). We hypothesized that opening of the ATP-sensitive potassium (KATP) channel mediates the protective effect of SWOP. Rabbits were preconditioned (PC) with four cycles of 5-min regional ischemia each followed by 10 min of reperfusion. Twenty-four hours later, the animals were subjected to sustained ischemia for 30 min followed by 180 min of reperfusion (I/R). Glibenclamide (Glib, 0.3 mg/kg ip) or 5-hydroxydecanoate (5-HD, 5 mg/kg iv) was used to block the KATP channel function. Infarct size was reduced from 41.2 ± 2.6% in sham-operated rabbits to 11.6 ± 1.0% in PC rabbits, a 71% reduction (n = 11, P < 0.01). Treatment with Glib or 5-HD before I/R increased the infarct size to 43.4 ± 2.6 and 37.8 ± 1.9%, respectively (P < 0.01 vs. PC group, n = 12/group). Sham animals treated with either Glib or 5-HD had an infarct size of 39.0 ± 3.4 and 37.8 ± 1.5%, respectively, which was not different from control (40.0 ± 3.8%) or sham (41.2 ± 2.6%) I/R hearts. Monophasic action potential duration (APD) at 50% repolarization significantly shortened by 28.7, 26.6, and 23.3% in sham animals during 10, 20, and 30 min of ischemia. However, no further augmentation in the shortening of APD was observed in PC hearts. Glib and 5-HD significantly suppressed ischemia-induced epicardial APD shortening, suggesting that 5-HD may not be a selective blocker of the mitochondrial KATP channel in vivo. We conclude that SWOP is mediated by a KATP channel-sensitive mechanism that may have occurred because of the opening of the sarcolemmal KATP channel in vivo.

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REPEATED BRIEF EPISODES of ischemia and reperfusion protect the heart against subsequent sustained ischemia and reperfusion (35). This phenomenon, known as ischemic preconditioning (PC), is an inherent capability of the myocardium to protect itself from ischemic damage. The initial protective effect of preconditioning is transient, disappearing within 2–3 h (50). Recent studies showed that cardioprotection from PC reappears 24 h after the initial stimulus. This phenomenon is known as “delayed preconditioning” or “second window of preconditioning” (27, 30). Delayed preconditioning can also be produced by heat shock (12) and free radicals (56) and with pharmacological agents including monophosphoryl lipid A (MLA) (54) or A1-adenosine agonist 2-chloro-N6-cyclopentyladenosine (4, 7). Understanding the mechanism(s) underlying the second window of preconditioning may provide a better insight into the nature of myocardial adaptation to ischemia and open new therapeutic avenues capable of modifying the outcome after myocardial ischemic episodes.

Opening of the ATP-sensitive potassium (KATP) channel has been shown to be one of the potential mechanisms in early preconditioning (17, 18, 43, 48) and delayed preconditioning induced by heat shock (20, 41) as well as pharmacological agents (15, 24, 32). However, it is not known whether opening of the KATP channel also mediates the delayed protective effect of ischemic preconditioning in rabbit. The protection afforded by this channel has been attributed to an increase in the outward potassium current followed by shortening of the action potential duration (APD). This, in turn, may spare ATP, thereby allowing less entry of calcium into the myocyte through the voltage-sensitive calcium channels. Decreased intracellular calcium overload then reduces the ischemic injury, and therefore enhances the preservation of myocytes. Within 1–3 min of acute coronary occlusion, there is a pronounced shortening in APD secondary to activation of the KATP channel (11) although the APD shortening is not related to the extent of cardiac protection. It has been proposed that the lack of such an association could be caused by the opening of the mitochondrial KATP channel rather than the sarcomemmal channel (16). In support of this hypothesis, a potent opener of the mitochondrial KATP channel, diazoxide, was shown to induce a significant cardioprotective effect in the isolated, perfused heart (16) and in ventricular myocytes (29, 47). The protective effect of diazoxide was blocked by 5-hydroxydecanoate (5-HD), a specific blocker of the mitochondrial KATP channel (16, 29, 47).

The purpose of the present investigation was to determine whether the KATP channel also plays a role in the development of delayed phase of ischemic preconditioning. A second goal was to test the hypothesis if the epicardial APD shortening, which occurs because of the opening of the sarcomemmal KATP channel, is suppressed by glibenclamide (the blocker of sarcomemmal and mitochondrial KATP channels) but not by 5-HD (29, 47). Our results show that the delayed effect of ischemic preconditioning as well as epicardial APD shortening was...
equally suppressed by glibenclamide and 5-HD. These data raise the question of the specificity of 5-HD in blocking the mitochondrial K\textsubscript{ATP} channel in vivo. To our knowledge, this is the first report investigating the K\textsubscript{ATP} channel as the mediator of delayed ischemic preconditioning in the rabbit myocardium.

**METHODS**

Animal care. New Zealand White rabbits (males; 2.5–3.3 kg) were used for this study. The care and use of the animals was conducted with the guidelines of the Committee on Animals of Virginia Commonwealth University and the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (DHHS Publication No. (NIH) 80–23, Revised 1985).

Drugs and chemicals. 5-HD was purchased from Research Biochemicals. Glibenclamide, Evans blue dye, and triphenyltetrazolium chloride (TTC) were obtained from Sigma Chemical (St. Louis, MO). The vehicle for glibenclamide was 40% propylene glycol and 10% ethanol in distilled water. All other chemicals were of analytical reagent quality.

Surgical preparation. The animals were anesthetized with an intramuscular injection of ketamine HCl (35 mg/kg) and xylazine (5 mg/kg). Further injections of ketamine-xylazine were given as needed throughout the surgical procedure. The animals were intubated orotracheally and ventilated on a positive-pressure ventilator. The tidal volume was set at 15 ml, and the respiratory rate was adjusted to 30–40 cycles/min. Ventilator setting and PO\textsubscript{2} were adjusted as needed to maintain the blood gas parameters within the physiological range. The prominent ear artery and a marginal ear vein were cannulated with a 20-gauge 24-gauge radiopaque intravenous catheter for blood sampling and measurement of arterial blood pressure. The cannulation of the vein allowed for continuous infusion of 0.9% NaCl solution. Electrocardiographic leads were attached to subcutaneous electrodes to monitor heart rhythm.

Day 1: Ischemic preconditioning protocol. All surgical procedures were performed under sterile conditions. The chest was opened by a left thoracotomy in the fourth intercostal space, and the pericardium was opened to expose the heart. A 5-0 silk suture with an atrumatic needle was then passed around the left anterior descending artery (LAD) midway between the atrioventricular groove and the apex of the heart. The animal was then heparinized with 500 U of heparin sodium to prevent thrombosis. The ends of the suture were then fixed in place. Instead, the chest remained open for 50 min of ischemia by pulling the snare. After 30 min of ischemia the ligature was released, and the heart was allowed to reperfuse for 180 min. The thoracic cavity was covered with a plastic film to minimize heat loss.

Infarct size assessment. At the end of the infarction protocol, the ligature around the coronary artery was retightened and 4–5 ml of 10% Evans blue dye was injected as a bolus into the jugular vein until the eyes turned blue. The animal was immediately killed, and the heart was removed and frozen. The frozen heart was then cut into six to eight transverse slices of equal thickness from the apex to the base. The area at risk was determined by negative staining with Evans blue. The slices were then incubated in a 1% TTC solution in isotonic pH 7.4 phosphate buffer at 37°C for 20 min. Tetrazolium reacts with NADH in the presence of dehydrogenase enzymes, causing viable tissue to stain a deep red color. The slices were subsequently fixed in a 10% Formalin solution. Red-stained viable tissue was easily distinguished from the infarcted pale, unstained necrotic tissue. The areas of infarcted tissue, the risk zone, and the whole left ventricle (LV) were determined by digital planimetry with computer morphometry using a Bioquant imaging software. The area for each region was averaged from the slices. Infarct size was expressed both as a percentage of the total LV and as a percentage of the ischemic risk area.

Epicardial APD. The activity of the ventricular K\textsubscript{ATP} channel was assessed during ischemia by placing an epicardial probe (MAP electrode, EP Technologies, Sunnyvale, CA) in the center of the ischemic region. The probe was placed with a constant pressure to the perceived center of the ischemic zone. The APD at 50% and 90% repolarization (APD\textsubscript{50} and APD\textsubscript{90}, respectively) was determined during preischemia and after every 10 min of LAD occlusion. The APD was accepted only if it fulfilled the following criteria: 1) constant configuration and stable resting membrane potential and 2) stable amplitude of phase 2 > 10 mV during control recording. The percent APD change was defined as

\[
\text{APD} (\%) = \frac{\text{APD}_{50} \text{ or } 90 \text{ after reperfusion} - \text{APD}_{50} \text{ or } 90 \text{ before occlusion}}{\text{APD}_{50} \text{ or } 90 \text{ before occlusion}} \times 100
\]
Study protocol. Rabbits were randomly assigned to one of the seven groups, and all animals were subjected to 30 min of sustained ischemia followed by 180 min of reperfusion. In addition, five to eight animals from each group were used for measurement of APD. The experimental protocol is illustrated in Fig. 1. In group I (n = 13), rabbits received no treatment on day 1 of the experiment. In group II (n = 11), nonpreconditioned control rabbits were treated with glibenclamide (0.3 mg/kg ip) 30 min before sustained ischemia and reperfusion on day 2. In group III (n = 11), nonpreconditioned control rabbits were treated with 5-HD (5 mg/kg iv) 15 min before sustained ischemia and reperfusion on day 2. In group IV (sham operated, n = 9), the chest was opened and a suture was placed around the coronary artery on day 1 of the experiment. However, the artery was not occluded and thus the animals were not preconditioned. In group V (n = 11), the animals received preconditioning with four 5-min coronary artery occlusions each followed by a 10-min reperfusion period on day 1 of the experiment. In group VI (n = 12), preconditioned animals received glibenclamide (0.3 mg/kg ip) 30 min before sustained ischemia and reperfusion on day 2. In group VII (n = 12), preconditioned animals were treated with 5-HD (5 mg/kg iv) 15 min before sustained ischemia and reperfusion on day 2.

Blood pH and gases. Arterial blood gases and pH were measured seven times for groups IV–VII during the day 1 protocol and seven times for all groups during ischemia-reperfusion. These measurements were taken to ensure proper physiological respiration during the experiment.

Measurement of hemodynamics. Hemodynamic measurements included heart rate, systolic arterial pressure, and mean arterial pressure (MAP). The rate-pressure product was determined as the product of the heart rate and peak arterial pressure. These parameters were continuously measured throughout the duration of the experimental protocol with a strip-chart recorder.

Statistics. All measurements of infarct size, risk areas, and APD are expressed as group means ± SE. Changes in hemodynamics, APD, and infarct size variables were analyzed by a one-way repeated-measures ANOVA to determine the effect of time, group, and time by group interaction. If the global tests showed major interactions, post hoc contrasts between different time points within the same group or between different groups were performed using a t-test. Statistical differences with a P value of <0.05 were considered significant.

RESULTS

Exclusions and mortality. A total of 96 rabbits were entered into the present study. A summary of the number of animals in each group and the reasons for exclusion is shown in Table 1.

Blood pH and gases. The pH was maintained between 7.20 and 7.50. The PCO2 was maintained between 20 and 50 and the PO2 between 65 and 150. The HCO3 was sustained between 15.0 and 28.0, and the O2 saturation was consistently kept above 95%. An exception to these ranges was in one animal of group V that was respirated with 100% O2 during the reperfusion.

Systemic hemodynamics. Heart rate and MAP remained reasonably stable throughout the reperfusion period, although these parameters dropped gradually at most of the data points in all groups (Table 2). Except for a few time points, the mean values were not significantly different between the groups and at any time point within the groups.

Infarct size. Infarct size (in % of risk area) was not significantly different between the control and sham-operated animals (40.0 ± 3.8% vs. 41.2 ± 2.6%, P > 0.05, n = 9–11/group; Fig. 2). Preconditioning resulted in a significant decrease in infarct size from 41.2 ± 2.6% of the area at risk in the sham-operated hearts to 11.6 ± 2.6% of the area at risk, a 71% reduction from sham-operated hearts (P < 0.01, n = 11). Treatment of preconditioned rabbits with glibenclamide or 5-HD before ischemia-reperfusion resulted in a significant increase in the infarct size to 43.4 ± 2.6 and 37.8 ± 1.9%, respectively (P < 0.01 from preconditioned group, n = 12/group). Also, nonpreconditioned control rabbits treated with either glibenclamide or 5-HD had an
infarct size of 39.0 ± 3.4 and 37.8 ± 1.5%, respectively. These values were not significantly different compared with control (40.0 ± 3.8%) or sham (41.2 ± 2.6%) ischemic-reperfusion hearts. A similar trend in infarct size was observed when it was expressed as a percentage of the LV. The mean infarct size values in control (21.5 ± 2.2%) and sham-operated (21.7 ± 2.6%) hearts were not significantly different (P > 0.05). Preconditioning significantly reduced the infarct size to 6.5 ± 1.0% (P < 0.01 vs. control and sham). Both glibenclamide and 5-HD blocked preconditioning-induced protection without inducing significant effects in the nonpreconditioned rabbits. Moreover, these drugs did not alter the infarct size in the nonpreconditioned control hearts. The risk areas ranged from 50 to 56% with no significant differences among all groups (P > 0.05). These data suggest that changes in the size of infarct observed among various groups were not related to the percentage of area of LV occluded by our technique.

APD. The APD 50 significantly shortened by 28.7, 26.6, and 23.3% in sham animals during 10, 20, and 30 min after ischemia, respectively (Fig. 3). A similar degree of APD 50 shortening was observed in the control animals (not shown). By 30 min of reperfusion, the APD

### Table 1. Mortality and exclusions

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Animals</th>
<th>No. Excluded</th>
<th>Survival, %</th>
<th>Reason for Exclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (group I)</td>
<td>14</td>
<td>1</td>
<td>93</td>
<td>Animal died of ventricular tachycardia/fibrillation during infarction protocol.</td>
</tr>
<tr>
<td>Glib (group II)</td>
<td>13</td>
<td>2</td>
<td>85</td>
<td>One rabbit died right after induction of anesthesia; the other animal died of congestive heart failure during reperfusion stage.</td>
</tr>
<tr>
<td>5-HD (group III)</td>
<td>13</td>
<td>2</td>
<td>85</td>
<td>Hearts from the two animals did not have any risk zone as ligature came off during injection of Evans blue dye.</td>
</tr>
<tr>
<td>Sham (group IV)</td>
<td>12</td>
<td>3</td>
<td>75</td>
<td>One animal died during the surgical preparation before ischemia; one animal's heart was all stained with Evans blue dye; third rabbit was in cardiogenic shock after 30-min sustained ischemia.</td>
</tr>
<tr>
<td>PC (group V)</td>
<td>16</td>
<td>4</td>
<td>75</td>
<td>Two rabbits died during the day 1 preconditioning protocol of respiratory cause (one animal was in congestive heart failure after closure of chest, and other rabbit died because of ventilator failure); one animal died of cardiogenic shock during reperfusion period on 2nd day; fourth rabbit's heart was all blue, precluding any measurements.</td>
</tr>
<tr>
<td>PC + Glib (group VI)</td>
<td>14</td>
<td>2</td>
<td>86</td>
<td>Hearts of both animals were all blue from Evans blue dye.</td>
</tr>
<tr>
<td>PC + 5-HD (group VII)</td>
<td>13</td>
<td>1</td>
<td>92</td>
<td>Heart was all blue because suture came off during injection of Evans blue dye.</td>
</tr>
</tbody>
</table>

**Total** 95 15 84

Glib, glibenclamide; 5-HD, 5-hydroxydecanoate; sham, sham operated; PC, preconditioned.

### Table 2. Hemodynamic data

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline</th>
<th>Preischemia</th>
<th>30-min Isch</th>
<th>60-min Rep</th>
<th>120-min Rep</th>
<th>180-min Rep</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (group I)</td>
<td>HR 170 ± 7</td>
<td>184 ± 8</td>
<td>202 ± 12</td>
<td>189 ± 9</td>
<td>186 ± 9</td>
<td>182 ± 11</td>
</tr>
<tr>
<td></td>
<td>MAP 91 ± 2</td>
<td>89 ± 4</td>
<td>67 ± 5</td>
<td>77 ± 4</td>
<td>64 ± 5</td>
<td>62 ± 4</td>
</tr>
<tr>
<td></td>
<td>RPP 17,827 ± 845†</td>
<td>18,625 ± 1,049</td>
<td>16,345 ± 1,579</td>
<td>16,655 ± 1,073</td>
<td>13,899 ± 1,311</td>
<td>13,591 ± 894</td>
</tr>
<tr>
<td>Glib (group II)</td>
<td>HR 178 ± 10</td>
<td>199 ± 11</td>
<td>195 ± 8</td>
<td>209 ± 13</td>
<td>216 ± 14</td>
<td>206 ± 16</td>
</tr>
<tr>
<td></td>
<td>MAP 89 ± 4</td>
<td>87 ± 6</td>
<td>65 ± 4</td>
<td>67 ± 7</td>
<td>57 ± 5</td>
<td>54 ± 4</td>
</tr>
<tr>
<td></td>
<td>RPP 18,072 ± 1,425†</td>
<td>20,259 ± 2,066</td>
<td>15,329 ± 1,127</td>
<td>17,136 ± 1,731</td>
<td>15,175 ± 1,482</td>
<td>14,722 ± 1,480</td>
</tr>
<tr>
<td>5-HD (group III)</td>
<td>HR 180 ± 8</td>
<td>197 ± 9</td>
<td>180 ± 12</td>
<td>192 ± 9</td>
<td>187 ± 11</td>
<td>184 ± 13</td>
</tr>
<tr>
<td></td>
<td>MAP 80 ± 4*</td>
<td>76 ± 4</td>
<td>63 ± 4</td>
<td>66 ± 5</td>
<td>64 ± 4</td>
<td>55 ± 2</td>
</tr>
<tr>
<td></td>
<td>RPP 16,756 ± 1,094‡</td>
<td>17,498 ± 1,181</td>
<td>13,708 ± 1,544</td>
<td>15,389 ± 1,469</td>
<td>14,518 ± 1,388</td>
<td>12,401 ± 1,126</td>
</tr>
<tr>
<td>Sham (group IV)</td>
<td>HR 161 ± 8</td>
<td>176 ± 9</td>
<td>187 ± 7</td>
<td>187 ± 7</td>
<td>199 ± 12</td>
<td>186 ± 12</td>
</tr>
<tr>
<td></td>
<td>MAP 74 ± 3‡</td>
<td>71 ± 3‡</td>
<td>63 ± 4</td>
<td>64 ± 4</td>
<td>60 ± 4</td>
<td>58 ± 5</td>
</tr>
<tr>
<td></td>
<td>RPP 14,160 ± 801</td>
<td>15,273 ± 999</td>
<td>14,963 ± 1,315</td>
<td>14,689 ± 1,032</td>
<td>15,427 ± 1,318</td>
<td>13,722 ± 879</td>
</tr>
<tr>
<td>PC (group V)</td>
<td>HR 198 ± 9†</td>
<td>194 ± 6</td>
<td>206 ± 5</td>
<td>203 ± 8</td>
<td>196 ± 7</td>
<td>194 ± 7</td>
</tr>
<tr>
<td></td>
<td>MAP 77 ± 4†</td>
<td>73 ± 4†</td>
<td>66 ± 4</td>
<td>64 ± 4</td>
<td>64 ± 4</td>
<td>59 ± 4</td>
</tr>
<tr>
<td></td>
<td>RPP 18,092 ± 908‡</td>
<td>17,325 ± 766</td>
<td>16,860 ± 934</td>
<td>16,406 ± 936</td>
<td>15,820 ± 916</td>
<td>14,539 ± 804</td>
</tr>
<tr>
<td>PC + Glib (group VI)</td>
<td>HR 199 ± 7†</td>
<td>200 ± 8</td>
<td>203 ± 8</td>
<td>193 ± 10</td>
<td>186 ± 11</td>
<td>188 ± 11</td>
</tr>
<tr>
<td></td>
<td>MAP 75 ± 4‡</td>
<td>72 ± 3‡</td>
<td>61 ± 4</td>
<td>64 ± 3*</td>
<td>63 ± 4</td>
<td>60 ± 4</td>
</tr>
<tr>
<td></td>
<td>RPP 17,598 ± 813†</td>
<td>17,256 ± 750</td>
<td>15,222 ± 1,001</td>
<td>15,157 ± 982</td>
<td>14,529 ± 1,066</td>
<td>14,172 ± 1,125</td>
</tr>
<tr>
<td>PC + 5-HD (group VII)</td>
<td>HR 200 ± 8*</td>
<td>192 ± 8</td>
<td>197 ± 9</td>
<td>190 ± 9</td>
<td>192 ± 9</td>
<td>191 ± 15</td>
</tr>
<tr>
<td></td>
<td>MAP 65 ± 2*</td>
<td>72 ± 2*</td>
<td>58 ± 5</td>
<td>56 ± 3*</td>
<td>54 ± 4</td>
<td>51 ± 5</td>
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<tr>
<td></td>
<td>RPP 16,857 ± 500‡</td>
<td>16,552 ± 826</td>
<td>14,968 ± 826</td>
<td>13,741 ± 856</td>
<td>13,463 ± 843§</td>
<td>12,663 ± 1,077</td>
</tr>
</tbody>
</table>

Values are means ± SE. Isch, ischemia; Rep, reperfusion; HR, heart rate (in beats/min); MAP, mean arterial pressure (in mmHg); RPP, rate-pressure product (in mmHg/min). *P < 0.05 vs. control; †P < 0.05 vs. sham; §P < 0.05 vs. Glib; $P < 0.05 vs. PC.
returned to nearly baseline preischemic levels. In the preconditioned hearts, the APD$_{50}$ also shortened but was not significantly different compared with the sham-operated group. A similar trend in the changes in the APD$_{90}$ was observed during ischemia-reperfusion (Fig. 3B). Pretreatment with glibenclamide and 5-HD did not produce significant changes in baseline APD$_{50}$ or APD$_{90}$. However, these drugs significantly suppressed the ischemia-induced shortening of APD$_{50}$ and APD$_{90}$ in the sham-operated and preconditioned hearts.

DISCUSSION

The major findings are summarized as follows. 1) Ischemic preconditioning, 24 h before ischemia-reperfusion, resulted in a significant protection of the heart as indicated by reduced infarct size. 2) A significant shortening of APD was observed during 10–30 min of sustained ischemia in the control and sham-preconditioned hearts; the preconditioned hearts did not demonstrate further augmentation in APD shortening during this period. 3) The $K_{ATP}$ blockers glibenclamide and 5-HD abrogated the protective effect of preconditioning. The epicardial APD shortening was equally suppressed by glibenclamide and 5-HD, suggesting that these drugs did not discriminate between the sarcolemmal versus the mitochondrial $K_{ATP}$ channel in vivo. These results suggest that opening of $K_{ATP}$ channels plays an important role in the second window of preconditioning in the rabbit heart. It is not clear whether this protection results from the opening of the sarcolemmal or the mitochondrial $K_{ATP}$ channel.

The phenomenon of delayed preconditioning in the myocardium is an area of investigation that has branched from the study of early or classical preconditioning. The early phase of preconditioning has been consistently demonstrated in every species studied (28). On the other hand, delayed preconditioning appears to be a species-specific phenomenon. It has been consistently observed in rabbits (6, 30, 44) and dogs (27) but remains controversial in rats (23, 42, 52) and appears to be absent in pigs (45). Delayed preconditioning has also been observed in cultured myocytes (36, 37). Early ischemic preconditioning appears to engage a final common pathway involving the activation of G-coupled receptors such as adenosine, $\alpha$-adrenergic, bradykinin, and opioids (39). These receptors activate protein kinase C (PKC), which appears to play a central role in conferring ischemic tolerance via a variety of kinases (14). PKC and tyrosine kinase inhibition has been shown to abolish phenylephrine-induced functional preconditioning in rat and infarct size reduction in vivo in rabbit hearts (33, 38). Delayed preconditioning against infarction with ischemic and heat shock preconditioning was abolished by chelerythrine, a specific inhibitor of PKC (2, 26). Conversely, administration of 1,2-dioctanoyl-sn-glycerol, the physiological activator of PKC, induced delayed preconditioning against subsequent ischemia-reperfusion (3). Activation of the $K_{ATP}$ channel appears to be an endogenous adaptive mechanism that protects the myocardium against ischemia-reperfusion damage (11). PKC is a potential candidate that links the receptors stimulated by mediators released during ischemia to the activation of the $K_{ATP}$ channel. De Weille et al. (13) reported that stimulation of PKC with a phorbol ester resulted in the activation of the $K_{ATP}$ channel in rat insulinoma cells. Hu et al. (21) showed that PKC-activating phorbol ester induced currents with properties of a $K_{ATP}$-channel current at a reduced intracellular ATP concentration in rabbit and human ventricular myocytes. Similarly,
Activation of the $K_{ATP}$ channel is at least partially responsible for the increase in outward $K^+$ currents, shortening of APD, and increase in extracellular $K^+$ concentration during anoxic or globally ischemic conditions (5); it also modulates arrhythmogenesis in a variety of experimental conditions (10, 25). Miyoshi et al. (34) showed that ischemia-induced APD shortening was observed at both the endocardial and epicardial layers in response to $K_{ATP}$-channel modulators during regional ischemia, although greater shortening was observed at the epicardium. 5-HD suppressed the shortening preferentially at the epicardial layer, suggesting that the drug was blocking the sarcosommal $K_{ATP}$ channel in vivo. Sakamoto et al. (46) showed that 5-HD reduced early accumulation of $K^+$ during ischemia, an effect that was similar to that of glibenclamide. In the present investigation also, epicardial APD$_{50}$ and APD$_{90}$ were significantly shortened during ischemia, which effect was suppressed not only by glibenclamide but also by 5-HD. These data raise the possibility that 5-HD may not be the selective blocker of mitochondrial $K_{ATP}$ Channel in vivo.

In the present investigation, we observed no further augmentation in APD shortening in the preconditioned hearts, suggesting two possibilities. First, the additional channels may not have been opened in the preconditioned hearts during ischemia. Second, the cardioprotection caused by opening of the $K_{ATP}$ channel is independent of APD shortening. These data are in accord with the reports suggesting a lack of correlation between the APD shortening and cardioprotection with bimakalim and cromakalim, openers of the $K_{ATP}$ channel (19, 53). Similarly, pyranoyl cyanoguanidine analogs have been shown to retain the glibenclamide-reversible cardioprotective effects but lack the APD shortening effect. Garlid et al. (16) first proposed that mitochondrial $K_{ATP}$ channels could be involved in the cardioprotective effect of preconditioning. Using the mitochondrial $K_{ATP}$ channel opener diazoxide, they demonstrated a significant cardioprotective effect of the drug in the isolated, perfused heart (16). A similar protective effect of diazoxide has been shown in ventricular myocytes (29, 47) and in rabbit heart in vivo (1). The cardioprotective effect of diazoxide was blocked by 5-HD, thereby further confirming the role of the mitochondrial $K_{ATP}$ channel.

We used glibenclamide as one of the inhibitors of the $K_{ATP}$ Channel. Recently, glibenclamide has been demonstrated to block the cAMP-activated chloride conductance ($I_{Cl,cAMP}$; 49). The Hill coefficients for the effect of glibenclamide on $I_{Cl,cAMP}$ and $K_{ATP}$ channels are roughly similar, but the effective concentration range is considerably higher (half-maximal inhibition of $I_{Cl,cAMP}$ ~30 µM compared with 5–10 µM for half-maximal inhibition of $K_{ATP}$ channels). We as well as several other investigators have used 0.3 mg/kg glibenclamide to block the channel function. Although we have not directly measured levels of glibenclamide in the plasma, Bekheit et al. (5) reported 0.4 ± 0.03 µM in dog plasma after 30 min of intravenous injection with 0.15 mg/kg glibenclamide. We used a double concentration of gliben-
clamamide, i.e., 0.3 mg/kg, which would roughly equal 0.8 μM in plasma. The concentration of the drug in the cell membrane could be even lower, which is far below that required to inhibit the I_{Cl,Clamp} channel. Moreover, we have confirmed our results using 5-HD, which is an ischemia-selective K_{ATP} channel blocker. Furthermore, neither glibenclamide nor 5-HD, when present during the 30-min ischemic period, had any effect in exacerbation of ischemia-induced injury.

In summary, we have demonstrated the role of the K_{ATP} channel in mediating delayed ischemic preconditioning by blocking it with glibenclamide and 5-HD. The ability of these blockers to suppress APD shortening during ischemia further confirms that the drugs were acting at the K_{ATP} channel. It is not clear whether the sarcolemmal or mitochondrial K_{ATP} or both of these channels contribute to the delayed cardioprotective effect of ischemic preconditioning. Future studies will be necessary to unravel the specific signal transduction mechanism(s) by which delayed preconditioning leads to the opening of these channels and differentiating the specific role played by each of the sarcolemmal and mitochondrial K_{ATP} channels in mediating delayed cardioprotective effect in vivo.

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