Delayed preconditioning with adenosine is mediated by opening of ATP-sensitive K⁺ channels in rabbit heart

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Bernardo, Nelson L., Shinji Okubo, Mohammed M. Maamieh, Mark A. Wood, and Rakesh C. Kukreja. Delayed preconditioning with adenosine is mediated by opening of ATP-sensitive K⁺ (KATP) channels in rabbit heart. Am. J. Physiol. 277 (Heart Circ. Physiol. 46): H128–H135, 1999.—The adenosine agonist 2-chloro-N⁶-cyclopentyladenosine (CCPA) induces delayed ischemic protection in vivo. We hypothesized that this protection is mediated by opening of ATP-sensitive K⁺ (KATP) channels and increased synthesis of 72-kDa heat shock protein (HSP 72). Six groups (n = 9–13 animals/group) of animals were studied: group I, control rabbits that received no treatment; group II, animals glibenclamide (0.3 mg/kg iv) 30 min before ischemia; group III, animals given 5-hydroxydecanoate (5-HD; 5 mg/kg iv) 15 min before ischemia; group IV, rabbits treated with CCPA (0.1 mg/kg iv) 24 h before ischemia; and groups V and VI, CCPA-treated animals that received the KATP-channel blockers glibenclamide or 5-HD, respectively, 30 or 15 min before ischemia. All animals were subjected to ischemia by 30 min of coronary artery occlusion followed by 3 h of reperfusion. Risk area was delineated by injection of 10% Evans blue dye, and infarct size was determined by triphenyltetrazolium staining. Action potential duration (APD) was measured with an epicardial electrode. HSP 72 was measured by Western blotting. CCPA caused a significant reduction in infarct size [12.02 ± 1.0 vs. 40.0 ± 3.8% (%area at risk) in controls, P < 0.01] that was blocked by glibenclamide (36.2 ± 3.1%, P < 0.01) and 5-HD (35.0 ± 2.9%, P < 0.01). Glibenclamide and 5-HD did not change infarct size in control rabbits. These blockers significantly suppressed ischemia-induced APD shortening in control and CCPA-treated animals. CCPA treatment did not induce HSP 72 in hearts. These data suggest that adenosine-initiated delayed protection is mediated via opening of KATP channels but does not involve the synthesis of HSP 72. After the initial insult (45). Studies in the rabbit and the dog have shown that a significant effect of preconditioning reappeared when sustained ischemia was initiated 24 h after repetitive short cycles of ischemia and reperfusion (24, 29). The phenomenon is referred to as the “second window of preconditioning” (SWOP) or “delayed preconditioning.” Adenosine has been proposed to be one of the important mediators of the early and delayed preconditioning. Liu et al. (26) blocked the early preconditioning effect when endogenous adenosine was pharmacologically antagonized with nonselective adenosine-receptor antagonists. In addition, they were able to significantly reduce the infarct size in rabbits that were given the adenosine A₁-selective agonist N⁶-(phenyl-2R-isopropyl)-adenosine (R-P1A). Tsuchida et al. (41) reproduced these findings using 2-chloro-N⁶-cyclopentyladenosine (CCPA), which is 10,000-fold more selective for the A₁ receptor. Baxter et al. (4) abolished SWOP by treating rabbits with 8-(p-sulphophenyl)-theophylline (8-SPT), a nonselective adenosine-receptor antagonist, given before and during ischemic preconditioning. In addition, the administration of CCPA in place of ischemic preconditioning by coronary artery occlusion also induced SWOP after 24 h in rabbit hearts (4). However, it is not known whether the downstream events following adenosine-induced delayed preconditioning have mechanisms similar to those in the acute window of protection. Considering that the half-life of adenosine is short, this would imply that the mere occupation of the A₁ receptors would not provide cardioprotection but, rather, that its “activation” starts a cascade of signal transduction events that significantly limits infarct size 24 h later.

Opening of the KATP channel as a potential mechanism in early preconditioning has been suggested by several studies (14, 37, 40). The activation of adenosine receptors is linked to the KATP channel via G protein (21). Gross and Auchampach (13) and Grover et al. (16) were able to block early preconditioning in dogs by pretreatment with the KATP-channel blocker glibenclamide. This is in consonance with the finding that the anti-ischemic effects induced by the KATP-channel activators pinacidil and cromakalim are reversed by glibenclamide. Previous studies from three independent laboratories have shown that delayed preconditioning with monophosphoryl lipid A (MLA), a nontoxic derivative of endotoxin, exerts its anti-ischemic protective effect via opening of the KATP channel (11, 20, 32). Furthermore, it was recently reported that heat-shock-induced delayed ischemic protection is also mediated by opening of the KATP channel (17, 36). However, it is not known whether the delayed preconditioning initiated by CCPA...
also exerts a protective effect via the opening of this channel.

One of the hallmarks of delayed protection by ischemia and heat shock is the increased expression of stress proteins, particularly the inducible isofom of the 72-kDa heat shock protein (HSP 72). The stress proteins are synthesized in the heart in response to a short elevation in whole body temperature as well as a variety of stressful stimuli, including ischemia, exposure to transition metals, pressure overload, and oxygen radicals (10, 18, 23, 28). Very often, these proteins are associated with improved recovery of contractile function and reduction of infarct size after subsequent myocardial ischemia and reperfusion (19, 22, 35, 38). Because CCPA also induces delayed protection similar to that induced by either heat shock or ischemic preconditioning, we were interested in determining whether the delayed pharmacological preconditioning with this drug is associated with the increased synthesis of HSP 72 in the heart. A preliminary report of this investigation was presented at the 70th sessions of the American Heart Association (8).

**MATERIALS AND METHODS**

Animals. Male New Zealand White rabbits (2.8–3.3 kg) were used for the studies. The care and use of animals were conducted in accordance with the guidelines of the Committee on Animals of Virginia Commonwealth University and the National Institutes of Health Guide for the Care and Use of Laboratory Animals [DHHS Publication No. (NIH) 85–23, Revised 1985, Office of Science and Health Reports, Bethesda, MD 20892].

Materials. CCPA and 5-hydroxydecanoate (5-HD) were purchased from Research Biochemicals (Natick, MA). Glibenclamide, Evans blue dye, and triphenyltetrazolium chloride (TTC) were obtained from Sigma (St. Louis, MO). CCPA was dissolved in 0.9% saline and injected in conscious rabbits. Various chemicals were dissolved in 0.9% saline and injected intravenously (TTC) were obtained from Sigma (St. Louis, MO). Glibenclamide, Evans blue dye, and triphenyltetrazolium (TTC) were obtained from Sigma (St. Louis, MO). CCPA was dissolved in 0.9% saline and injected in conscious rabbits. Various chemicals were dissolved in 0.9% saline and injected intravenously. The following were used in the study: 5-hydroxydecanoate (5-HD); 5 mg/kg iv) 15 min before sustained ischemia and reperfusion; CCPA, CCPA-pretreated animals treated with 5-hydroxydecanoate (5-HD; 5 mg/kg iv) 15 min before sustained ischemia and reperfusion; and 5-HD + CCPA, CCPA-pretreated animals treated with 5-HD (5 mg/kg iv) 15 min before sustained ischemia and reperfusion.

**Study protocol.** The study protocols are summarized in Fig. 1. Six groups (n = 9–13 animals/group) of animals were studied. Group I (control) rabbits received no drug treatment but received 0.5 ml of saline. Group II rabbits received glibenclamide 0.3 mg/kg iv 15 min before coronary occlusion. Group III rabbits were given 5-HD (5 mg/kg iv) 15 min before coronary occlusion. Group IV rabbits were treated with CCPA (0.1 mg/kg iv) administered 24 h before the infarction protocol. Animals in groups V and VI were treated with CCPA as in group IV and then received glibenclamide or 5-HD, respectively, 30 min or 15 min before the infarction protocol.

All animals underwent 30 min of coronary artery occlusion followed by 3 h of reperfusion. Risk area was delineated by injection of 10% Evans blue dye, and infarct size was determined using computer morphometry of TTC-stained sections.

**Surgical procedure infarction protocol.** The rabbits were anesthetized with an intramuscular injection of ketamine hydrochloride (35 mg/kg) and xylazine (5 mg/kg). Subsequent doses of ketamine-xylazine (10 mg/kg and 2 mg/kg, respectively) were administered during the experiment as needed to maintain surgical anesthesia. The neck was opened with a ventral midline incision, and a tracheostomy was performed, followed by intubation. The animal was then mechanically ventilated on a positive-pressure ventilator (MD Industries, Mobile, AL) using compressed room air at 30–35 cycles/min with a tidal volume of ~15 ml. Ventilator settings and P<sub>O</sub><sub>2</sub> were adjusted as needed to maintain the arterial blood gas parameters within the physiological range. The jugular vein was cannulated with a polyethylene (PE) catheter for continuous infusion of 0.9% NaCl solution. The carotid artery likewise was dissected and cannulated with a PE catheter for blood sampling and continuous arterial pressure monitoring. Electrocardiographic leads were attached to subcutaneous electrodes to monitor either limb lead II or lead III.

A left thoracotomy was performed through the fourth intercostal space, and the pericardium opened to expose the heart. A 5-0 silk suture with a traumatic needle was then passed around the left anterior descending artery (LAD)
midway between the atrioventricular groove and the apex. The ends of the tie were then threaded through a small vinyl tube to form a snare. To induce infarction, the LAD was occluded for 30 min by pulling the snare and then fixing it in place by clamping the vinyl tube with a hemostat. A bolus of heparin sodium (500 IU) was given immediately before coronary occlusion for prophylaxis against thrombus formation around the snare. Myocardial ischemia was confirmed visually in situ by regional cyanosis, S-T elevation/depression or T wave inversion on electrocardiogram, hypokinetic/hypokinesia or akinesia/paradoxical wall motion, or regional wall thickening. After 30 min of ischemia, the snare was released and the heart was allowed to reperfuse for 180 min. This was readily confirmed by hyperemia over the surface of the previously ischemic-cyanoctic segment.

Measurement of infarct size. At the end of each experiment (180-min reperfusion), the ligature around the LAD was retightened and ~4 ml of 10% Evans blue dye was injected as a bolus into the jugular vein until the rabbit’s eyes turned blue. The rabbit was killed immediately, and the heart was removed and frozen. The heart was then cut into 8–10 transverse slices from apex to base of equal thickness (1 mm). The area at risk was determined by negative staining with Evans blue. The slices were then incubated in 1% TTC solution in isotonic pH 7.4 phosphate buffer at 37°C for 20 min. The slices were subsequently fixed in 10% Formalin solution for 6 h. 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 computer morphometry using Bioquant imaging software. Infarct size was expressed as both a percentage of the ischemic risk area and a percentage of the LV.

Epicardial action potential duration. The activity of the ventricular $K_{ATP}$ channel was assessed during ischemia and early reperfusion (at 5 and 30 min) by placing an epicardial probe (MAP electrode, EP Technologies, Sunnyvale, CA) in the center of the ischemic area as reported previously (7). Action potential duration (APD) was recorded with a handheld placement electrode and recorded at a chart speed of 100 mms⁻¹. The electrode was placed with a constant pressure to the necrotic area of the ischemic zone. The APD at 50 (APD$_{50}$) and 90% repolarization (APD$_{90}$) were determined during pres ischemia and after every 10 min of LAD occlusion. The APD was accepted only if it fulfilled the following criteria: 1) constant configuration and stable baseline membrane potential, and 2) stable amplitude of phase 2 >10 mV during control recording. The percent APD change was defined as

$$\text{APD} (%) = \frac{\text{APD}_{50/90} \text{ (before occlusion)} - \text{APD}_{50/90} \text{ (before occlusion)}}{\text{APD}_{50/90} \text{ (before occlusion)}} \times 100$$

Measurement of HSP 72. Two animals from group I (control) and three rabbits from group IV (CCPA) were used for measurement of HSP 72. The animals underwent the same protocol preparation as described earlier except that they were killed without undergoing the coronary occlusion and reperfusion. For positive control, three additional animals were subjected to whole body hyperthermia by raising the temperature to 42°C for 15 min 24 h before death. After adequate anesthesia was administered, a median sternotomy was performed and the heart was quickly removed. The left ventricle was then separated and kept frozen in liquid nitrogen until analyzed by Western blot as described previously (17).

Measurement of experimental parameters. Hemodynamic measurements included heart rate and mean arterial pressure (MAP). Rate-pressure product (RPP) was calculated as the product of heart rate and peak arterial pressure. Arterial blood gases were measured at preset periods during the experiment and as needed to guide in the ventilatory support of the animal. In addition, blood glucose was determined on the same schedule using a commercially available colorimetrically based blood glucose kit and the corresponding strip reader (Accuchek, Boehringer Mannheim). This was done to ensure that animals receiving glibenclamide would not have profound hypoglycemia that might adversely affect the experiment.

RESULTS
Exclusions and mortality. A total of 89 rabbits were used in this study. A summary of the number of animals in each group and the reasons for exclusion is provided in Table 1.

Hemodynamic parameters. Except for the indicated significant differences in Table 2, the heart rate and MAP were comparable among the six groups at baseline, during the period of coronary occlusion, and in the early part of the reperfusion period. RPP, an index of myocardial oxygen demand, was comparable among groups at all time points. All groups had a similar decline in blood pressure after coronary occlusion with no tendency toward recovery during the reperfusion period.

Blood glucose. Blood glucose levels in all the groups were comparable with no documented hypoglycemia in the animals given glibenclamide. Values were in the hyperglycemic order, ranging from 152 to 288 mg/dl.

Infarct size. Pretreatment of rabbits with CCPA resulted in a significant decrease in infarct size from 40.0 ± 3.8% in controls to 12.0 ± 1.0% (area at risk) 24 h later, which represented a 70% reduction (mean ± SE, $P < 0.05$) (Fig. 2). Treatment of “CCPA-preconditioned” rabbits with glibenclamide or 5-HD before ischemia-reperfusion resulted in a significant increase in the infarct size to 36.2 ± 3.1 and 35.0 ± 2.9%, respectively ($P < 0.05$ vs. CCPA only). Glibenclamide or 5-HD did not cause significant changes in the infarct size in rabbits compared with that of control animals that were not treated with CCPA. A similar trend was observed when the infarct size was expressed as a percentage of the LV (Fig. 2). The risk areas ranged from 50 to 62% of the LV with no significant differences among the groups ($P > 0.05$). These data suggest that changes in the infarct sizes observed among various groups were not related to the percentage of area of LV occluded by our technique.
APD50 returned to preischemic baseline levels after 30 min of reperfusion. A similar trend in the shortening of APD90 in the control and CCPA-treated rabbits. The extent of APD50 shortening between the control and CCPA-treated rabbits was not significantly different (Fig. 3B). The APD50 returned to preischemic baseline levels after 30 min of reperfusion. A similar trend in the shortening of APD90 was observed (Fig. 3B). The extent of APD50 shortening between the control and CCPA-treated rabbits was not significantly different (P > 0.05). Pretreatment with glibenclamide or 5-HD significantly suppressed the ischemia-induced shortening of APD50 and APD90 in the control and CCPA-treated rabbits.

**DISCUSSION**

Salient findings. The primary goal of this study was to determine whether the delayed phase of "pharmacological preconditioning" induced by adenosine agonist Action potential duration. Figure 3 shows changes in APD50 during ischemia and reperfusion. There was a significant shortening of APD50 (expressed as %preischemic baseline) by 24.4 ± 5.3, 25.5 ± 6.6, and 18.3 ± 3.3% in control and 26.4 ± 4.4, 21.9 ± 5.7, and 20.0 ± 5.4% in CCPA-treated rabbits at 10, 20, and 30 min after ischemia (means ± SE, P < 0.05) (Fig. 3A). The APD90 was observed (Fig. 3B). The extent of APD50 shortening between the control and CCPA-treated rabbits was not significantly different (P > 0.05). Pretreatment with glibenclamide or 5-HD significantly suppressed the ischemia-induced shortening of APD50 and APD90 in the control and CCPA-treated rabbits.

Expression of HSP 72. In the positive controls (heat-stressed heart), there was increased expression of HSP 72 (Fig. 4). The animals that were pharmacologically preconditioned with CCPA demonstrated no increase in HSP 72 compared with the nontreated controls.

### 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</td>
<td>14</td>
<td>1</td>
<td>93</td>
<td>Animal died of intractable ventricular fibrillation during the ischemia protocol</td>
</tr>
<tr>
<td>Glib</td>
<td>13</td>
<td>2</td>
<td>85</td>
<td>One rabbit died of cardiogenic shock during the reperfusion period; the other animal died right after induction of anesthesia</td>
</tr>
<tr>
<td>5-HD</td>
<td>13</td>
<td>2</td>
<td>85</td>
<td>Hearts from the 2 excluded rabbits were not used because the ligature came off during injection of Evans blue dye and the risk zone was absent</td>
</tr>
<tr>
<td>CCPA</td>
<td>14</td>
<td>2</td>
<td>86</td>
<td>One animal died of cardiogenic shock right after the ischemia protocol; the second rabbit did not have any risk zone, precluding its use in data analysis</td>
</tr>
<tr>
<td>Glib + CCPA</td>
<td>10</td>
<td>1</td>
<td>90</td>
<td>Rabbit died of refractory hypotension and severe metabolic acidosis going into the 2nd h of the reperfusion period</td>
</tr>
<tr>
<td>5-HD + CCPA</td>
<td>11</td>
<td>0</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>75</td>
<td>8</td>
<td>89</td>
<td></td>
</tr>
</tbody>
</table>

Control, rabbits subjected to 30 min of ischemia followed by 3 h of reperfusion; Glib, control rabbits treated with glibenclamide (0.3 mg/kg) 30 min before sustained ischemia and reperfusion; 5-HD, control rabbits treated with 5-hydroxydecanoate (5 mg/kg) 15 min before sustained ischemia and reperfusion; CCPA, control rabbits treated with 2-chloro-N6-cyclopentyladenosine; Glib + CCPA, CCPA rabbits treated with glibenclamide (0.3 mg/kg) 30 min before sustained ischemia and reperfusion; 5-HD + CCPA, CCPA rabbits treated with 5-hydroxydecanoate (5 mg/kg) 15 min before sustained ischemia and reperfusion.

### Table 2. Hemodynamic variables during ischemia and reperfusion

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline</th>
<th>Preischemia</th>
<th>30-min Ischemia</th>
<th>60-min Reperfusion</th>
<th>120-min Reperfusion</th>
<th>180-min Reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>HR</td>
<td>170 ± 7</td>
<td>184 ± 8</td>
<td>198 ± 9</td>
<td>189 ± 9</td>
<td>187 ± 9</td>
</tr>
<tr>
<td></td>
<td>MAP</td>
<td>91 ± 2</td>
<td>89 ± 4</td>
<td>75 ± 4</td>
<td>77 ± 4</td>
<td>64 ± 5</td>
</tr>
<tr>
<td></td>
<td>RPP</td>
<td>17,827 ± 845</td>
<td>18,629 ± 1,049</td>
<td>17,058 ± 1,062</td>
<td>16,653 ± 1,073</td>
<td>13,899 ± 1,311</td>
</tr>
<tr>
<td>Glib</td>
<td>HR</td>
<td>178 ± 10</td>
<td>199 ± 11</td>
<td>210 ± 11</td>
<td>209 ± 13*</td>
<td>216 ± 14*</td>
</tr>
<tr>
<td></td>
<td>MAP</td>
<td>89 ± 4</td>
<td>87 ± 6</td>
<td>73 ± 6</td>
<td>67 ± 7</td>
<td>57 ± 11</td>
</tr>
<tr>
<td></td>
<td>RPP</td>
<td>18,072 ± 1,425</td>
<td>20,259 ± 2,066</td>
<td>17,884 ± 1,566</td>
<td>17,136 ± 1,731</td>
<td>15,175 ± 1,482</td>
</tr>
<tr>
<td>5-HD</td>
<td>HR</td>
<td>180 ± 8</td>
<td>197 ± 9</td>
<td>201 ± 10</td>
<td>192 ± 9</td>
<td>187 ± 11</td>
</tr>
<tr>
<td></td>
<td>MAP</td>
<td>80 ± 4</td>
<td>76 ± 4</td>
<td>67 ± 4</td>
<td>66 ± 5</td>
<td>64 ± 4</td>
</tr>
<tr>
<td></td>
<td>RPP</td>
<td>16,756 ± 1,094</td>
<td>17,498 ± 1,181</td>
<td>16,097 ± 1,399</td>
<td>15,389 ± 1,469</td>
<td>14,518 ± 1,388</td>
</tr>
<tr>
<td>CCPA</td>
<td>HR</td>
<td>194 ± 6</td>
<td>197 ± 8</td>
<td>187 ± 7</td>
<td>182 ± 9</td>
<td>173 ± 9</td>
</tr>
<tr>
<td></td>
<td>MAP</td>
<td>84 ± 2</td>
<td>75 ± 2</td>
<td>68 ± 2</td>
<td>67 ± 2</td>
<td>64 ± 3</td>
</tr>
<tr>
<td></td>
<td>RPP</td>
<td>18,430 ± 746</td>
<td>17,272 ± 650</td>
<td>14,857 ± 722</td>
<td>14,102 ± 774</td>
<td>12,806 ± 499</td>
</tr>
<tr>
<td>Glib + CCPA</td>
<td>HR</td>
<td>187 ± 6</td>
<td>218 ± 11</td>
<td>206 ± 15</td>
<td>212 ± 13</td>
<td>208 ± 10</td>
</tr>
<tr>
<td></td>
<td>MAP</td>
<td>84 ± 2</td>
<td>72 ± 3</td>
<td>68 ± 4</td>
<td>67 ± 4</td>
<td>61 ± 3</td>
</tr>
<tr>
<td></td>
<td>RPP</td>
<td>17,972 ± 523</td>
<td>18,620 ± 11,062</td>
<td>16,771 ± 741</td>
<td>17,337 ± 1,051</td>
<td>16,126 ± 813</td>
</tr>
<tr>
<td>5-HD + CCPA</td>
<td>HR</td>
<td>181 ± 6</td>
<td>214 ± 9</td>
<td>213 ± 6</td>
<td>207 ± 8</td>
<td>207 ± 8*</td>
</tr>
<tr>
<td></td>
<td>MAP</td>
<td>88 ± 4</td>
<td>81 ± 5</td>
<td>69 ± 3</td>
<td>63 ± 3</td>
<td>57 ± 2</td>
</tr>
<tr>
<td></td>
<td>RPP</td>
<td>18,628 ± 1,254</td>
<td>20,389 ± 965</td>
<td>17,845 ± 923</td>
<td>16,249 ± 903</td>
<td>15,288 ± 479</td>
</tr>
</tbody>
</table>

Values are means ± SE. Glib, glibenclamide; 5-HD, 5-hydroxydecanoate; CCPA, 2-chloro-N6-cyclopentyladenosine; HR, heart rate; MAP, mean arterial pressure; RPP, rate-pressure product. *P < 0.05 vs. control.
is abrogated by glibenclamide and 5-HD, blockers of K\textsubscript{ATP} channels. A second goal was to demonstrate whether the delayed protective effect is associated with the enhanced synthesis of HSP 72, a protein believed to have an anti-infarct effect in the ischemic heart (19). The present study shows that glibenclamide and 5-HD significantly reversed the CCPA-induced delayed cardioprotection and suppressed ischemia-induced APD shortening. No major differences in heart rate, MAP, or RPP were observed among the groups during the infarction protocol, suggesting that the changes in myocardial infarcts were independent of the systemic hemodynamics. CCPA pretreatment did not increase the synthesis of HSP 72, ruling out the role of this protein in drug-induced delayed preconditioning. Taken together, these data suggest that K\textsubscript{ATP} channels mediate the delayed phase of ischemic protection induced by adenosine, which is independent of the synthesis of HSP 72.

Adenosine and cardioprotection. After the onset of myocardial ischemia, ATP depletion occurs rapidly (34) and adenosine is released in large amounts (43) in the interstitial space, where it can interact with its receptors. The A\textsubscript{1} receptor, which is located on the cardiac myocytes (6), is involved in the cardioprotective effect of ischemic preconditioning. Administration of the nonse-
ective adenosine receptor antagonist 8-SPT, before the ischemic preconditioning protocol, abolished early preconditioning in rabbits (25). Conversely, the intracor-
nary infusion of adenosine in isolated rabbit hearts or intravenous administration of the A1-selective agonist R-PIA before the sustained period of ischemia and reperfusion resulted in significant reduction of infarct size acutely (26). Additionally, overexpression of myocardial A1 receptor protects the heart from ischemic dam-
age in transgenic mice (31). Baxter et al. (4) showed that transient activation of the A1 receptor CCPA induced delayed protection in the rabbit heart. Such a transient activation of A1 receptor has also been in-
volved in the mechanism of the delayed cardioprotection induced by ischemic preconditioning. Dana et al. (9) were able to maintain the rabbits in a protected state against myocardial infarction by repeated activation of A1 receptor with intermittent dosing of CCPA over a 10-day period. However, continuous activation of A1 receptor with a high-dose chronic infusion of CCPA led to the downregulation of the signaling mechanism and loss of protection (42). These results suggest that there is tachyphylaxis to the benefits of adenosine due to a desensitization of the A1 receptor following high-
dose chronic infusion of CCPA.

Adenosine and K_{ATP} channel. Considering that the half-life of adenosine is short, this would imply that mere occupation of A1 receptors would not be protective but, rather, that its “activation” may have initiated a cascade of signaling events. It is known that stimula-
tion of A1 receptor results in the activation of the pertussis toxin-sensitive G_i protein (21). This in turn may open the K_{ATP} channel via phosphorylation medi-
ated by protein kinase C. Opening of the K_{ATP} channel has been shown to be protective due to the increase in the outward K^+ current (5) resulting in the shortening of action potential, which in turn may spare ATP, thereby allowing less entry of Ca^{2+} into the myocyte. Decreased intracellular Ca^{2+} overload then results in less ischemic injury and better myocyte preservation. In the present studies, we observed significant APD shortening after ischemia in control as well as CCPA groups, although the degree of shortening was not significantly different between the groups. However, one must recognize that the epicardial APD shortening does not necessarily reflect the entire myocardium. Furthermore, the magnitude of APD shortening is relatively small and may not be sensitive enough to detect a significant difference between the control and the CCPA-pretreated animals. Despite these method-
ological limitations, the data clearly suggest that epicar-
dial APD shortening is not related to cardioprotection, because the infarct size was significantly reduced in the CCPA-treated group compared with that in the control. Furthermore, glibenclamide and 5-HD suppressed the epicardial APD shortening during ischemia and also abrogated CCPA-induced delayed protection. These results are in accord with the recent reports suggesting a lack of correlation between the APD shortening and cardioprotection with bimakalim and cromakalim, open-
ers of the K_{ATP} channel (15, 46). Furthermore, K_{ATP}-
channel openers and ischemic preconditioning are pro-
tective in nonstimulated cardiac myocytes in which action potential is not the factor (25). The reason for dissociation of APD shortening with cardioprotection was recently linked to the opening of the mitochondrial K_{ATP} channels (12). Cardiac myocytes possess K_{ATP} channels located in the inner mitochondrial membrane that also respond to the openers of the sarcolemmal channels. Diazoxide has been reported to specifically activate mitochondrial K_{ATP} channels with little effect on APD (12). A significant cardioprotective effect of this drug was reported in the isolated perfused rat heart (12) and in vivo in the rabbit (2). Similarly, Liu et al. (27) showed that diazoxide significantly reduced the rate of cell death following simulated ischemia in adult ventricular cardiac myocytes. The protective effect of diazoxide was blocked by 5-HD, which has been pro-
posed to be the inhibitor of mitochondrial K_{ATP} channel (27). Because 5-HD blocked the CCPA-induced limitation in infarct size, a role of the mitochondrial K_{ATP} channel may be possible in the cardioprotective effect. However, the suppression of ischemia-induced shorten-
ing of sarcolemmal APD with 5-HD argues against this hypothesis and raises questions about the selectivity of the drug in blocking mitochondrial K_{ATP} channel in vivo. Further studies are required to discriminate the specific role of sarcolemmal or mitochondrial K_{ATP} channel in mediating the delayed cardioprotective ef-
fect of CCPA in vivo.

Role of HSP 72. The accumulation of HSP 72 is associated with a delayed anti-ischemic effect in the heat-shocked animals as well as genetically engineered mice (17, 30). In the present study, we found no increase in the synthesis of the HSP 72 in CCPA-treated ani-
mals. Similarly delayed pharmacological preconditioning with MLA was not associated with the enhanced synthesis of HSP 72 (3, 47). Recently, we reported that, despite the abrogation of heat-shock preconditioning by glibenclamide and 5-HD, there was no reduction in the expression of HSP 72 (17). Similarly, the time course of HSP 72 accumulation following heat shock was not completely correlated with the anti-ischemic effect of heat-shock stimulus (38, 44). Therefore, it appears that increased expression of HSP 72 might just be a marker of the response of the myocyte to stress, and activation of other unknown mediators may also be important in enhancing the cellular resistance to ischemia. Nevertheless, our failure to observe the increase in expression of HSP 72 rules out the role of this protein in CCPA-
induced delayed ischemic protection.

In conclusion, since the first description of the role of K_{ATP} channel in ischemic preconditioning by Gross and co-workers (1), it is now widely believed that this channel acts as the “end effector” of preconditioning induced by endogenous stresses as well as pharmacologi-
cal agents including CCPA as reported in the present study. However, it is not clear whether the sarcolemmal or mitochondrial K_{ATP} channels, or both of these chan-
nels, contribute to the delayed cardioprotective effect of adenosine. Future studies will be necessary to unravel the mechanism(s) by which the activation of A1 receptor
plays a role in the opening of sarcolemmal or mitochondrial \(K_{\text{ATP}}\) channel, which ultimately leads to the delayed cardioprotection.

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