A Comparison of Adenosine-Induced Cardioprotection and Ischemic Preconditioning in Dogs

Efficacy, Time Course, and Role of $K_{ATP}$ Channels

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**Background** Adenosine has been proposed to be an important mediator of ischemic preconditioning. Intracoronary administration of adenosine has recently been shown to mimic the effects of preconditioning in isolated rabbit hearts. However, it is not known whether this agent can duplicate the effects of preconditioning in vivo or in other species. Thus, the first objective of the present study was to determine whether adenosine can limit myocardial necrosis to the same extent as preconditioning in anesthetized dogs. A second objective was to determine whether the duration of the adenosine-induced cardioprotection persisted as long as that of ischemic preconditioning. Finally, a third aim was to determine whether adenosine mediates its cardioprotection via the $K_{ATP}$ channel, which has been shown to be an important mediator of preconditioning in several animal species, including dogs.

**Methods and Results** Barbital-anesthetized open-chest dogs were subjected to 60 minutes of left anterior descending coronary artery (LAD) occlusion followed by 4 hours of reperfusion. Preconditioning was elicited by 10 minutes of LAD occlusion followed by 10 or 60 minutes of reperfusion before the 60-minute occlusion period. Adenosine (400 μg/min) or an equivalent volume of saline was infused into the LAD for 10 minutes, followed by a 10- or 60-minute drug-free period before the 60-minute ischemic insult. Glibenclamide (0.3 mg/kg IV), a selective $K_{ATP}$ channel blocker, was given 15 minutes before adenosine administration, and another selective $K_{ATP}$ channel blocker, 5-hydroxydecanoate (5-HD, 3 mg/min IC) was infused concomitantly with adenosine into the LAD for 10 minutes. Transmural myocardial blood flow was measured at 5 minutes of occlusion, and infarct size was determined by triphenyltetrazolium staining and expressed as a percent of the area at risk (AAR). There were no significant differences in hemodynamics, collateral blood flow, or AAR between groups. Preconditioning with either 10 or 60 minutes of reperfusion produced a marked reduction ($P<.05$) in infarct size (6.7±2.5% and 8.7±2.6%, respectively, versus 26.9±4.3% in controls). Administration of adenosine with a 10-minute drug-free period before 60 minutes of occlusion resulted in a marked decrease in infarct size similar to that seen with preconditioning (9.6±1.7% versus 26.9±4.3% in controls); however, the protection disappeared when a 60-minute drug-free period was allowed after adenosine administration (23.0±2.4% versus 26.9±4.3% in controls). In addition, treatment with either glibenclamide or 5-HD completely abolished the protective effects of adenosine (26.4±6.8 and 25.0±4.4%, respectively, versus 26.9±4.3% in controls).

**Conclusions** These results clearly reveal that (1) a 10-minute intracoronary infusion of adenosine exhibits the same efficacy as ischemic preconditioning in reducing myocardial necrosis in dogs; (2) similar to preconditioning, adenosine mediates its cardioprotection via a cardiac $K_{ATP}$ channel-linked mechanism; and (3) adenosine-induced cardioprotection is transient (disappearing within 60 minutes), whereas ischemic preconditioning persists for at least 60 minutes. These data support the hypothesis that endogenous adenosine released during ischemia is an important mediator of ischemic preconditioning; however, important differences exist between the time course of effects of exogenously administered adenosine and preconditioning, which suggests that other factors may also be involved. (Circulation. 1994;89:1229-1236.)

**Key Words** ischemia • adenosine • potassium

Ischemic preconditioning is an endogenous protective mechanism in which brief periods of myocardial ischemia and reperfusion render the myocardium resistant to a subsequent more sustained ischemic insult. This phenomenon has been shown to occur in a variety of animal species. Recent clinical evidence suggests that ischemic preconditioning may occur in humans as well. Since Murry et al described this phenomenon in 1986, numerous studies have been performed to elucidate the mechanism of preconditioning; however, its cellular basis is still not fully understood.

Adenosine is a metabolite of adenine nucleotides; it accumulates quickly in large amounts in the ischemic myocardium. A large body of evidence suggests that adenosine can protect the heart against ischemia/reperfusion injury. Numerous recent studies have shown that activation of adenosine A$_1$ receptors mimicked and blocked some of these receptors abolished the effect of ischemic preconditioning to reduce infarct size. Liu and coworkers have further demonstrated that in isolated blood-perfused rabbit hearts, intracoronary infusion of adenosine for 5 minutes followed by 10 minutes of recovery is as effective as preconditioning to reduce infarct size. In contrast, Li and Kloner have recently shown that adenosine fails to mediate the cardioprotective effects of ischemic preconditioning in rats. Data obtained from our laboratory showed that intracoro-
nary infusion of either a high dose of adenosine or a low dose of dipyridamole separately over a 5-minute period before a prolonged 60-minute occlusion period did not mimic preconditioning; however, intracoronary infusion of a combination of the two produced a significant reduction in infarct size. Thus, it is not clear whether local administration of adenosine has the ability by itself to mimic preconditioning in dogs. Therefore, the first major objective of the present study was to determine whether intracoronary administration of adenosine for a longer period (10 minutes) could duplicate the effects of preconditioning in dogs. Since preconditioning has been shown to persist for approximately 2 hours in dogs, a second aim was to determine whether the adenosine-induced protection could last as long as that of preconditioning.

Gross and Auchampach have recently shown that activation of K\textsubscript{ATP} channels is an important mechanism by which preconditioning occurs in dogs. Furthermore, Grover et al. showed that a selective adenosine A\textsubscript{1} receptor agonist, R-PIA, reduced infarct size in dogs and this effect was blocked by glibenclamide, which suggests an important link between adenosine A\textsubscript{1} receptor activation and the K\textsubscript{ATP} channel. Therefore, a final objective was to test whether the K\textsubscript{ATP} channel is involved in mediating the cardioprotective effect of adenosine. For this purpose, we administered either of two selective K\textsubscript{ATP} channel blockers, glibenclamide\textsuperscript{12} and 5-hydroxydecanoate (5-HD),\textsuperscript{13, 14} in the presence of adenosine.

**Methods**

**General Preparation of Dogs**

Adult mongrel dogs of either sex weighing 19 to 25 kg were fasted overnight, anesthetized with a combination of sodium barbital (200 mg/kg IV) and sodium pentobarbital (15 mg/kg IV), and ventilated by a respirator (model 607, Harvard Apparatus, South Natick, Mass) with room air supplemented with 100% oxygen. Atelactasis was prevented by maintaining an expiratory pressure of 5 to 7 cm H\textsubscript{2}O with a trapezoidal expiratory waveform. Arterial blood pH, P\textsubscript{O\textsubscript{2}} and P\textsubscript{CO\textsubscript{2}} were monitored at selected intervals by an automatic blood gas system (AVL 995, AVL Scientific Corp, Roesswein, Germany) and maintained within a normal physiologic range (pH 7.35 to 7.45, P\textsubscript{O\textsubscript{2}} 80 to 120, P\textsubscript{CO\textsubscript{2}} 25 to 40) by adjusting the respiratory rate and oxygen flow and by intravenous infusion of 1.5% sodium bicarbonate when necessary. Body temperature was maintained at 38±1°C by a heating pad.

Aortic blood pressure and left ventricular systolic and end-diastolic pressures were measured with a double pressure transducer–tipped catheter (Millar PC 771) inserted into the aorta and left ventricle via the left carotid artery. Left ventricular dp/dt was determined by electronic differentiation of the left ventricular pressure pulse. The right femoral vein and artery were cannulated for drug administration and for withdrawal of a reference blood flow sample used in the determination of myocardial tissue blood flow.

A left thoracotomy was performed at the fifth intercostal space, the lung retracted, the pericardium incised, and the heart suspended in a cradle. A 1.0- to 1.5-cm segment of the left anterior descending coronary artery (LAD) was dissected from surrounding tissue distal to the first diagonal branch, and a calibrated electromagnetic flow probe (Statham SP 7515) was placed around the vessel. A flowmeter (Statham 2202) was used to measure coronary blood flow, and a micrometer-driven mechanical occluder was placed distal to the flow probe. The occluder was used to zero the flow probe (LAD was occluded for 10 seconds) 20 minutes before the initial coronary occlusion and later to occlude the artery. Myocardial oxygen demand and cardiac workload are known to be affected by heart rate; thus, heart rate was kept at approximately 150 beats per minute in all animals. If the basal heart rate was <150 beats per minute, the heart was paced at that rate with rectangular pulses of 4-millisecond duration and a voltage twice threshold via bipolar electrodes sutured to the left atrial appendage. Paxing was not used in the few animals with initial rates >150 beats per minute, and if the rate exceeded 160 beats per minute, the animals were excluded. In this way, we attempted to control the oxygen demand at approximately the same level in all animals. Heart rate, hemodynamics, and LAD blood flow were monitored and recorded by a polygraph (model 7, Grass Instrument Co, Quincy, Mass) throughout the experiment. The left atrial appendage was cannulated for radioactive microsphere injection and the right femoral artery cannulated for withdrawal of a reference blood flow sample used for measurement of myocardial tissue blood flow. The right femoral vein was cannulated for drug administration. A small catheter with a 30-gauge needle bent at 90° was inserted into the LAD distal to the flow probe and occluded for intracoronary drug infusions.

**Chemicals**

Adenosine and glibenclamide were purchased from Sigma, and 5-HD was generously supplied by Dr Iciilio Cavero from Rhone-Poulenc Rorer, France. Adenosine and 5-HD were freshly dissolved in saline, and the dose of glibenclamide for each dog was dissolved in 0.5 mL propylene glycol, 0.5 mL ethanol, and 0.5 mL 1N NaOH before administration.

**Experimental Design**

Seven groups of animals were studied to determine whether adenosine can limit myocardial necrosis to an extent similar to preconditioning, whether the adenosine-induced cardioprotection persists as long as that of ischemic preconditioning, and whether adenosine mediates its cardioprotection via activating K\textsubscript{ATP} channels (Fig 1). Dogs in nonpreconditioned groups (series 1, 3, 4, 5, 7) were subjected to 60 minutes of LAD occlusion followed by 4 hours of reperfusion, whereas preconditioned dogs (series 2 and 6) received a single 10-minute occlusion followed by 10 or 60 minutes of reperfusion before being subjected to the same protocol as used in nonpreconditioned groups. In nonpreconditioned dogs (series 1, 3, 4, 5, 7), saline (series 1), adenosine (400 µg/min, series 3 and 7), 5-HD (3 mg/min) + adenosine (series 5), and glibenclamide (0.3 mg/kg) + adenosine (series 6) were infused into the LAD at a rate of 1 mL/min for 10 minutes instead of the 10-minute occlusion period used in preconditioned dogs with the exception of glibenclamide, which was given intravenously 15 minutes before adenosine administration. Doses of adenosine, glibenclamide, and 5-HD were chosen on the basis of previous studies from this laboratory.\textsuperscript{8}

**Infarct Size Determination**

After 4 hours of reperfusion, the LAD was reocluded and cannulated just distal to the occlusion site. Subsequently, 10 mL of saline and 10 mL of patent blue dye were injected at equal pressures into the LAD and left atrium, respectively, to determine the anatomic area at risk (AAR) and the nonischemic area. The heart was then immediately fibrillated, removed, and sliced into serial transverse sections 6 to 7 mm in width. The nonstained ischemic area was separated from the blue-stained normal area, and the two regions were incubated at 37°C for 20 to 30 minutes in 1% 2,3,5-triphenyltetrazolium chloride (TTC) in 0.1 mol/L phosphate buffer adjusted to pH 7.4. The TTC stains the noninfarcted myocardium a brick red color, indicating the presence of a formazin precipitate that results from the reduction of TTC by dehydrogenase enzymes present in viable tissue. After storage overnight in 10% formaldehyde, infarcted and noninfarcted tissues within the
AAR were carefully separated and weighed. Infarct size (IS) was expressed as a percent of the area at risk (IS/AAR).

Regional Myocardial Blood Flow Measurement

Regional myocardial tissue blood flow of the normal and ischemic regions was measured by the radioactive microsphere technique as previously described in this laboratory.10

Criteria for Exclusion

To ensure that all animals included in data analysis were healthy and exposed to a similar extent of ischemia, the following criteria were used to exclude unsatisfactory dogs: (1) subendocardial collateral flow >0.15 mL·min⁻¹·g⁻¹; (2) heart rate >160 beats per minute; and (3) more than three consecutive attempts required to convert ventricular fibrillation with low-energy DC pulses applied directly to the heart.

Statistical Analysis

All values are expressed as mean±SEM. Differences between groups in hemodynamics, blood gases and pH, myocardial blood flow, and infract size were compared by a two-factor ANOVA with repeated measures and Fisher’s least significant difference test. Linear regression analysis was also performed to determine the relation between transmural collateral blood flow and infract size expressed as a percent of the AAR. Differences in regression lines between groups were compared by ANCOVA. Differences between groups were considered significant at P<.05.

Results

Mortality and Exclusions

A total of 58 dogs were randomly assigned to one of seven groups: control (group 1), preconditioned with 10 minutes or 60 minutes of reperfusion (groups 2 and 6), adenosine-treated with 10 minutes or 60 minutes drug free (groups 3 and 7), 5-HD + adenosine (group 4), and glibenclamide + adenosine (group 5). Five dogs were excluded because subendocardial collateral blood flow was >0.15 mL·min⁻¹·g⁻¹ (1 each in groups 1, 2, and 4; 2 in group 3). Thus, 53 dogs completed the protocol satisfactorily and were used in data analysis (n=12 for the control group, n=9 for the preconditioned group with 10 minutes of reperfusion, n=8 for adenosine-treated with a 10-minute drug-free period, and n=6 for the other four groups).

Hemodynamics

Heart rate, mean aortic blood pressure, the rate-pressure product, left ventricular dp/dt, and LAD coronary arterial blood flow were monitored throughout the experiment; the baseline values for all series are summarized in Table 1. Hemodynamics were not significantly different among groups throughout the experiment except that LAD blood flow during the 10 minutes of drug infusion was markedly higher in groups treated with either adenosine (400 μg/min, 115±16 mL/min), 5-HD + adenosine (115±17 mL/min), or glibenclamide + adenosine (108±12 mL/min) than in the nonpreconditioned saline-treated controls (36±5 mL/min). The increase in LAD coronary blood flow produced by 400 μg/min of adenosine infusion (77±12 mL/min) was not

| Table 1. Baseline Values of Systemic Hemodynamics |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                | Heart Rate, bpm | MAP, mm Hg     | RPP, mm Hg · min⁻¹ · 1000⁻¹ | LV dp/dt, mm Hg/s | LAD CBF, mL/min |
| Control (n=12) | 151±3           | 94±9            | 15.9±1.8                  | 1987±256         | 36±5           |
| PC (n=9)       | 153±2           | 81±5            | 14.8±0.8                  | 1656±99          | 32±5           |
| ADO (n=8)      | 151±2           | 90±4            | 15.4±0.7                  | 1824±124         | 33±6           |
| 5-HD + ADO (n=6)| 151±2           | 95±10           | 16.3±2.0                  | 1770±312         | 28±4           |
| GLIB + ADO (n=6)| 152±4           | 91±8            | 15.4±1.6                  | 1750±200         | 29±3           |
| PC-60 min (n=6)| 147±3           | 90±5            | 14.9±0.8                  | 1625±249         | 28±4           |
| ADO-60 min (n=6)| 154±3           | 95±6            | 16.3±1.2                  | 1925±183         | 27±9           |

bpm indicates beats per minute; MAP, mean aortic pressure; RPP, rate-pressure product; LV, left ventricular; LAD, left anterior descending coronary artery; CBF, coronary blood flow; PC, preconditioning; ADO, adenosine; 5-HD, 5-hydroxydecanoate; and GLIB, glibenclamide. Values are mean±SEM. There were no significant differences among groups.
3. FIG was ventricle control, 32.3±2.3%; reperfusion, drug-free period, 31.6±2.3%; 5-HD, 34.0±2.1%; and adenosine with 60 minutes of reperfusion, 32.0±1.3%; 5-HD plus adenosine, 34.8±2.7%; glibenclamide + adenosine, 34.0±2.1%; preconditioning with 60 minutes of reperfusion, 29.6±2.9%. Infarct size expressed as a percent of the AAR was 26.9±4.3% in control animals and was markedly reduced by preconditioning with 10 minutes of reperfusion and pretreatment with adenosine with a 10-minute drug-free period (6.7±2.5% and 9.6±1.7%, respectively, P<.05 versus controls). Treatment with 5-HD (3 mg/min IC) or glibenclamide (0.3 mg/kg IV) at doses we have previously shown to have no effect on infarct size completely blocked the protective effects of adenosine (25.0±4.4% and 26.4±6.8%, respectively, P>.05 versus controls; Fig 2).

Fig 3 demonstrates the relation between infarct size (IS/AAR) and transmural collateral blood flow to the ischemic region at 5 minutes of occlusion. An inverse relation was found between these two parameters in all groups. The regression lines in the preconditioned group and the group treated with adenosine were shifted downward and had a flatter slope (Fig 3). The lines for the groups treated with 5-HD plus adenosine or glibenclamide + adenosine (Fig 3C and 3D) were shifted back like those observed in the nonpreconditioned saline-treated control group. These results indicate that at any given transmural collateral flow, infarct size would be expected to be smaller in the preconditioned group and the adenosine-treated group.

**Infarct Size and Area at Risk**

The anatomic AAR expressed as a percent of the left ventricle was not significantly different between groups: control, 32.3±2.3%; preconditioning with 10 minutes of reperfusion, 33.7±1.9%; adenosine with a 10-minute drug-free period, 31.6±2.3%; 5-HD + adenosine, 34.8±2.7%; glibenclamide + adenosine, 34.0±2.1%; preconditioning with 60 minutes of reperfusion, 32.0±1.3%; and adenosine with a 60-minute drug-free period, 29.6±2.9%. Infarct size expressed as a percent of the AAR was 26.9±4.3% in control animals and was markedly reduced by preconditioning with 10 minutes of reperfusion and pretreatment with adenosine with a 10-minute drug-free period (6.7±2.5% and 9.6±1.7%, respectively, P<.05 versus controls). Treatment with 5-HD (3 mg/min IC) or glibenclamide (0.3 mg/kg IV) at doses we have previously shown to have no effect on infarct size completely blocked the protective effects of adenosine (25.0±4.4% and 26.4±6.8%, respectively, P>.05 versus controls; Fig 2).

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Fig 4. Bar graph illustrating the effects of different protocols on infarct size expressed as a percent of the area at risk (AAR). Preconditioning with 60 minutes of reperfusion (PRE, n=6) produced a significant reduction in infarct size, whereas adenosine with a 60-minute drug-free period (ADO, n=6) did not affect the infarct size compared with the nonpreconditioned control group (CONT, n=12). These results indicate that the protection afforded by preconditioning persisted for at least 1 hour; however, adenosine-induced protection totally disappeared within 1 hour.

Fig 4 shows that preconditioning with 60 minutes of reperfusion, like that with 10 minutes of reperfusion (6.7±2.5%), resulted in a marked reduction in infarct size (8.7±2.6%); however, adenosine with a 60-minute drug-free period had no effect on infarct size (23.0±2.4% versus 26.9±4.3% in controls). Fig 5A reveals that the regression line in the preconditioned group with 60 minutes of reperfusion, like that with 10 minutes of reperfusion, was shifted downward and had a flatter slope. In contrast, the line in the group treated with adenosine followed by a 60-minute drug-free period was shifted back to that in the nonpreconditioned saline-treated control group (Fig 5B). These results indicate that the protection afforded by preconditioning persisted at least for 1 hour; however, adenosine-induced protection totally disappeared within 1 hour.

Regional Myocardial Blood Flow

Transmural myocardial blood flow data in the ischemic (LAD) region are shown in Table 2. There were no significant differences between groups in blood flow to any layer of the ischemic myocardium at 5 and 30 minutes of occlusion and at the end of 4 hours of reperfusion. These data indicate that all groups of animals were subjected to equivalent degrees of ischemia. No significant differences were observed among groups in the nonischemic region.

Discussion

Efficacy of Adenosine and Preconditioning in Reducing Infarct Size

The results of the present study are the first to clearly demonstrate that adenosine can duplicate the effects of ischemic preconditioning in canine hearts. Initially, we found that 10 minutes of coronary artery occlusion before a 60-minute occlusion period resulted in a marked reduction in infarct size compared with dogs that were not preconditioned (6.7±2.5% versus 26.9±4.3%). These results, which demonstrate the remarkable protective effect of ischemic preconditioning, closely agree with previous results from our laboratory10 as well as those of Li et al,15 in which a significant reduction in infarct size was observed in a canine model of ischemic preconditioning similar to that used in the present study. Subsequently, we observed that intracoronary infusion of adenosine for 10 minutes followed by 10 minutes of recovery markedly reduced infarct size (8.6±1.9%), to the same extent as preconditioning (6.7±2.5%). In agreement, Liu et al indicated that in isolated blood-perfused rabbit hearts, adenosine reduced myocardial infarct size to the same extent as did ischemic preconditioning. Miura et al showed that dihydropyridine, an adenosine uptake inhibitor, decreased the threshold for preconditioning in rabbit hearts. A recent study performed by Auchampach and Gross showed that in canine hearts, intracoronary infusion of a low or high dose of adenosine or a low dose of dihydropyridine over a 5-minute period before a prolonged 60-minute occlusion period did not mimic preconditioning; however, intracoronary infusion of a combination of adenosine and dihydropyridine produced a significant reduction in infarct size. Based on the results of that previous study with adenosine in our laboratory and the present results, it appears that 5 minutes of 400 µg/min adenosine is insufficient to reach a high enough interstitial concentration to mimic ischemic preconditioning, whereas a 10-minute infusion is sufficient in the canine heart. The present results together with those of
TABLE 2. Transmural Myocardial Blood Flow

<table>
<thead>
<tr>
<th>Ischemic Region (LAD)</th>
<th>5 Minutes of Occlusion</th>
<th>30 Minutes of Occlusion</th>
<th>4 Hours of Reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=12)</td>
<td>0.08±0.01</td>
<td>0.10±0.02</td>
<td>1.00±0.19</td>
</tr>
<tr>
<td>PC (n=9)</td>
<td>0.09±0.01</td>
<td>0.13±0.02</td>
<td>0.86±0.15</td>
</tr>
<tr>
<td>ADO (n=8)</td>
<td>0.10±0.02</td>
<td>0.14±0.03</td>
<td>0.84±0.12</td>
</tr>
<tr>
<td>5-HD+ADO (n=6)</td>
<td>0.09±0.02</td>
<td>0.13±0.02</td>
<td>1.03±0.11</td>
</tr>
<tr>
<td>GLIB+ADO (n=6)</td>
<td>0.07±0.03</td>
<td>0.08±0.02</td>
<td>0.79±0.15</td>
</tr>
<tr>
<td>PC-60 min (n=6)</td>
<td>0.09±0.03</td>
<td>0.10±0.03</td>
<td>0.65±0.16</td>
</tr>
<tr>
<td>ADO-60 min (n=6)</td>
<td>0.08±0.02</td>
<td>0.09±0.02</td>
<td>1.08±0.18</td>
</tr>
</tbody>
</table>

LAD indicates left anterior descending coronary artery; PC, preconditioning; ADO, adenosine; 5-HD, 5-hydroxy-decanoate; and GLIB, glibenclamide. Values are mean±SEM. There were no significant differences between the control and other groups.

others also provide direct evidence that adenosine is capable of mimicking preconditioning. However, the fact that only 5 minutes of ischemia is necessary to precondition the heart versus 10 minutes of adenosine suggests that important differences exist between these two interventions.

Numerous studies suggest that adenosine mediates its cardioprotective effects via activation of adenosine A1 receptors. In a similar canine model, Grover et al11 have demonstrated that intracoronary infusion of R-PIA, a selective A1 receptor agonist, mimicked ischemic preconditioning. Downey et al12 showed that in rabbit hearts in situ, intravenous administration of adenosine A1-selective agonists protected the heart to a degree similar to that of preconditioning. Additionally, blockade of adenosine receptors has been shown to abolish the protection afforded by preconditioning. Tsuchida et al13 and Liu et al14 demonstrated that preconditioning in anesthetized rabbits is abolished by blockade of adenosine A1 receptors. Similar results have been obtained by Schwarz et al19 in pigs. Recent results obtained in our laboratory15 also showed that pretreatment with DFCPX, a selective adenosine A1 receptor antagonist,20 blocked the beneficial effect of ischemic preconditioning in dogs. Taken together, the present results and those obtained from various other laboratories strongly suggest that adenosine triggers or mediates the effects of preconditioning in several species and protects ischemic hearts, probably via stimulating adenosine A1 receptors. Results obtained by Liu and Downey21 in rats, however, suggest that adenosine is not a universal mediator of preconditioning in all species.

Adenosine and Preconditioning Act via KATP Channel–Related Mechanisms

Activation of adenosine A1 receptors has been shown to lead to an increase in KATP channel activity.22 Ito et al23 and Kirsch et al24 found that adenosine or the selective A1 receptor agonist cyclopentyladenosine (CPA) activated the KATP channel in neonatal rat or guinea pig ventricular myocytes via G proteins. Therefore, the second objective of this study was to determine whether adenosine protected the ischemic myocardium by activating the KATP channel. In the present experiments, we found that treatment with either glibenclamide or 5-HD, two selective KATP channel antagonists,13,14 at doses we have shown previously to have no effects on infarct size,8,10 completely abolished the protective effects of adenosine (26.4±6.8% and 25.0±4.4%, respectively, versus 26.1±5.7% in controls). Recent results of Auchampach and Gross8 also demonstrated that glibenclamide antagonized the cardioprotection afforded by the combined treatment with adenosine and diprydamole. These data suggest that adenosine mediates cardioprotection via stimulating an adenosine A1 receptor–G protein–KATP channel–related mechanism.

That activation of adenosine A1 receptors leads to an increase in KATP channel activity via G proteins has been suggested to be one important mechanism by which preconditioning occurs. In support of this hypothesis, Gross and Auchampach10 and Auchampach et al14 have shown that glibenclamide or sodium 5-HD blocked the effects of preconditioning when administered either before or after the preconditioning period. These studies were the first to suggest that the KATP channel is involved in mediating the effects of preconditioning. Subsequently, this finding has been confirmed by various investigators in pigs,24 rabbits,25 and dogs.11 Grover et al11 recently found that a selective adenosine A1 receptor agonist, R-PIA, produced a reduction in infarct size similar to that produced by ischemic preconditioning in dogs and that this beneficial effect was abolished by glibenclamide. Preliminary data obtained in pigs by Van Winkle et al26 showed that the antiinfarct effect of R-PIA was also blocked by sodium 5-HD. Taken together, these results suggest that the KATP channel is an important mediator of the cardioprotective effects of adenosine and preconditioning. However, since these results are all based on the specificity of glibenclamide and sodium 5-HD to block only the KATP channel, a note of caution should be interjected in this interpretation. Alternatively, it may be possible that these two antagonists may be blocking the effects of adenosine and preconditioning at some other site separate from the KATP channel, i.e., the adenosine receptor, G protein, etc. Future experiments are needed to test this possibility.

Although the present study suggests that both adenosine and preconditioning mediate their cardioprotection via activating the KATP channel, the mechanism by which KATP channel activation protects the myocardium during a subsequent ischemic period is currently not fully understood. Activation of the KATP channel has been shown to result in a shortening of action potential
duration, a decrease in calcium influx, a rapid loss of contractile function, and an energy preservation that would slow down intracellular ATP depletion. Numerous studies demonstrating that activation of the $K_{\text{ATP}}$ channel accelerates the ischemia-induced shortening of action potential duration, slows down cellular metabolism, preserves intracellular ATP content, and limits reperfusion injury support this hypothesis. Previous studies in dogs and pigs recently demonstrated that metabolism is retarded in preconditioned hearts, with a resultant decrease in ATP degradation and accumulation of toxic cellular catabolites that may play a part in the mechanism of preconditioning. Whether a more rapid or greater activation of the $K_{\text{ATP}}$ channel by adenosine and/or preconditioning during the prolonged ischemic period produces these beneficial metabolic changes remains to be determined.

How preconditioned hearts maintain $K_{\text{ATP}}$ channel activation or memorize the preconditioning effect for 1 hour or even longer is still a mystery. A potential candidate is protein kinase C. DeWeille and coworkers recently demonstrated that stimulation of protein kinase C by somatostatin or the phorbol ester phorbol myristate acetate activates the $K_{\text{ATP}}$ channel. Strasser et al have shown that 5 minutes of ischemia is sufficient to activate protein kinase C, and Ytrehus et al showed that activation of protein kinase C may be involved in the secondary or memory phase of ischemic preconditioning in rabbit hearts. Therefore, it is reasonable to speculate that protein kinase C activation might phosphorylate the membrane $K_{\text{ATP}}$ channel, hence maintaining channel opening or decreasing the threshold for channel activation during the subsequent sustained ischemic period.

Alternatively, adenosine may exert its beneficial effects via other cellular mechanisms. Adenosine has been shown to attenuate norepinephrine release from sympathetic nerves during early ischemia and to antagonize the positive inotropic effect of catecholamines mediated via the adenosine cyclase system. These effects would compromise oxygen demand of the heart during ischemia and decrease the rate of ATP degradation. Adenosine has also been shown to increase glucose transport during ischemia and by this mechanism may increase myocardial energy production. Adenosine has also been shown to inhibit superoxide anion production from neutrophils, inhibit platelet aggregation and microthrombi formation in the capillaries, and possibly reduce the no-reflow phenomenon. Which of these actions of adenosine, if any, are involved in ischemic preconditioning is unknown; however, recent preliminary studies of Vander Heide et al suggest that adenosine acting via an $A_3$ receptor slows ischemic metabolism and mimics preconditioning via an inhibition of norepinephrine release from sympathetic nerves during ischemia in dog hearts. Obviously, further studies are needed to determine which of these actions of adenosine are more important in producing ischemic preconditioning and the role of the $K_{\text{ATP}}$ channel in this phenomenon.

**Time Course Discrepancy of Adenosine- and Preconditioning-Induced Protection**

Despite the efficacy of adenosine in mimicking the effects of preconditioning, its protective effects did not persist as long as those of preconditioning. In the present experiments, it was found that the adenosine-induced cardioprotection totally disappeared 60 minutes after drug administration (23.0±2.4% versus 26.9±4.3% in controls), whereas ischemic preconditioning persisted more than 1 hour (8.7±2.6% versus 26.9±4.3% in controls). This observation suggests that the dose of adenosine administered exogenously in the present study may not be high enough to achieve an interstitial concentration comparable to that obtained during 10 minutes of preconditioning ischemia. Previous results obtained by Auchampach and Gross demonstrating that adenosine mimicked ischemic preconditioning only in the presence of dipyridamole, an adenosine uptake inhibitor, agree with the present findings. Alternatively, the present results suggest that other cellular mechanisms or factors in addition to adenosine may also be involved in mediating the phenomenon of preconditioning.

**Conclusions**

The results of the present study clearly reveal that (1) a 10-minute intracoronary infusion of adenosine is as effective as ischemic preconditioning in reducing myocardial necrosis in dogs; (2) adenosine, like preconditioning, may produce its protection via an adenosine $A_3$ receptor–$K_{\text{ATP}}$ channel–linked mechanism; and (3) adenosine-induced cardioprotection is transient, whereas ischemic preconditioning persists for at least an hour. These data support the hypothesis that endogenous adenosine released during ischemia is an important trigger or mediator of ischemic preconditioning and also suggest that multiple complex mechanisms must be involved in producing this endogenous cardioprotective phenomenon.

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