Blockade of ATP-Sensitive Potassium Channels Prevents Myocardial Preconditioning in Dogs

Garrett J. Gross and John A. Auchampach

Single or multiple brief periods of ischemia (preconditioning) have been shown to protect the myocardium from infarction after a subsequent more prolonged ischemic insult. To test the hypothesis that preconditioning is the result of opening ATP-sensitive potassium (K\textsubscript{ATP}) channels, a selective K\textsubscript{ATP} channel antagonist, glibenclamide, was administered before or immediately after preconditioning in barbital-anesthetized open-chest dogs subjected to 60 minutes of left circumflex coronary artery (LCX) occlusion followed by 5 hours of reperfusion. Preconditioning was elicited by 5 minutes of LCX occlusion followed by 10 minutes of reperfusion before the 60-minute occlusion period. Glibenclamide (0.3 mg/kg i.v.) or vehicle was given 10 minutes before the initial ischemic insult in each of four groups. In a fifth group, glibenclamide was administered immediately after preconditioning. In a final series (group 6), a selective potassium channel opener, RP 52891 (10 µg/kg bolus and 0.1 µg/mg/min i.v.) was started 10 minutes before occlusion and continued throughout reperfusion. Transmural myocardial blood flow was measured at 30 minutes of occlusion, and infarct size was determined by triphenyltetrazolium staining and expressed as a percent of the area at risk. There were no significant differences in hemodynamics, collateral blood flow, or area at risk between groups. The ratio of infarct size to area at risk in the control group (28±6%) was not different from the group pretreated with glibenclamide in the absence of preconditioning (31±6%). Preconditioning produced a marked reduction (p<0.002) in infarct size (28±6% to 6±2%), whereas glibenclamide administered before or immediately after preconditioning completely abolished the protective effect (28±6% and 30±8%, respectively). RP 52891 also produced a significant (p<0.03) reduction (28±6% to 13±3%) in infarct size. These results suggest that myocardial preconditioning in the canine heart is mediated by activation of K\textsubscript{ATP} channels and that these channels may serve an endogenous myocardial protective role. (Circulation Research 1992;70:223–233)

A number of recent studies have been performed to determine the cellular mechanism of preconditioning. Murray and coworkers\textsuperscript{8} suggested that a reduction in ATP depletion or an increased washout of cellular catabolites may be partially responsible for preconditioning. Other investigators have suggested that a heat shock protein is expressed during a brief period of ischemia, which subsequently protects the myocardium by some unknown mechanism.\textsuperscript{9} However, Thornton et al\textsuperscript{14} found that inhibition of protein synthesis does not block preconditioning in rabbit hearts. More recent results of Liu et al\textsuperscript{10} suggest that preconditioning is mediated via activation of adenosine receptors since blocking these receptors prevents preconditioning. The cellular mechanism by which stimulation of adenosine receptors produces this protective effect is currently unknown; however, Kirsch et al\textsuperscript{11} have demonstrated that adenosine-1 (A\textsubscript{1}) receptors are coupled to ATP-sensitive potas-

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sium ($K_{\text{ATP}}$) channels in rat ventricular myocytes. A number of studies have suggested that opening of these $K_{\text{ATP}}$ channels by ischemia or hypoxia$^{12}$ or by selective potassium channel openers$^{13,14}$ exerts a protective effect on the ischemic-reperfused heart. Thus, the current study was designed to test the hypothesis that opening $K_{\text{ATP}}$ channels is an important part of the coupling mechanism by which preconditioning occurs in ischemic cardiac muscle. To test this hypothesis, we administered the selective $K_{\text{ATP}}$ channel antagonist, glibenclamide, before or immediately after a single 5-minute preconditioning stimulus in anesthetized dogs.

Materials and Methods

General Preparation

All experiments performed in this study conform to the guidelines of the American Physiological Society regarding the use and care of laboratory animals and those of the Animal Care Committee of the Medical College of Wisconsin. The Medical College of Wisconsin is accredited by the American Association of Laboratory Animal Care (AALAC).

Adult mongrel dogs of either sex, weighing 15–25 kg, were fasted overnight, anesthetized with sodium barbitral (200 mg/kg i.v.) and sodium pentobarbital (15 mg/kg i.v.), and ventilated by a respirator (model 607, Harvard Apparatus, South Natick, Mass.) with room air supplemented with 100% oxygen. Atelectasis was prevented by maintaining an expiratory pressure of 5–7 cm H$_2$O with a trap. Arterial blood pH was maintained between 7.35 and 7.45 by adjusting the respiratory rate and by intravenous infusion of 1.5% sodium bicarbonate when necessary. Arterial $P_O_2$ and $P_CO_2$ were monitored at selected intervals by means of a blood gas analyzer. Body temperature was maintained at 38±1°C by a heating pad. Arterial blood samples were also obtained at various times for the measurement of blood glucose levels by using a glucometer (Tracer II, Boehringer Mannheim, Indianapolis, Ind.).

Aortic blood pressure and left ventricular systolic and end-diastolic pressures were measured by using a double pressure transducer-tipped catheter (PC 771, Millar Instruments, Houston, Tex.) that was 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 lungs were retracted, the pericardium was incised, and the heart was suspended in a cradle. A 1.0–1.5-cm segment of the left circumflex coronary artery (LCX) was dissected from surrounding tissue proximal to the first marginal branch, and a calibrated electromagnetic flow probe (Statham SP 7515) was placed around the vessel. A flowmeter

![Figure 1](https://example.com/fig1.png)

**Figure 1.** Schematic diagram of the experimental protocol used to determine the role of ATP-sensitive potassium channels in myocardial preconditioning. Non preconditioned animals (groups 1, 2, and 6) were subjected to 60 minutes of left circumflex coronary artery occlusion (Occ) followed by 5 hours of reperfusion (Rep). Preconditioned animals (groups 3, 4, and 5) were subjected to one 5-minute episode of coronary occlusion followed by 10 minutes of reperfusion before the 60-minute occlusion period. Glibenclamide (GLIB, 0.3 mg/kg) was administered 10 minutes before the initial occlusion in groups 2 and 4 and immediately after preconditioning in group 5. Vehicle and RP 52891 (10 μg/kg bolus and 0.1 μg/kg/min infusion) were administered 10 minutes before the initial occlusion, and RP 52891 infusion was continued throughout the entire experimental period. Radioactive microspheres were administered at 3 and 30 minutes after occlusion in the non preconditioned groups (1, 2, and 6) and at 3 minutes of the initial 5-minute preconditioning period and at 30 minutes after the initiation of the sustained 60-minute occlusion period in the preconditioned groups (3, 4, and 5).

(Statham 2202) was used to measure coronary blood flow. A micrometer-driven mechanical occluder was placed distal to the flow probe so there were no branches between the probe and occluder. The occluder was used to zero the flow probe and later occlude the artery.

A catheter was placed in the left atrium via the atrial appendage for the injection of tracer microspheres. The heart was paced at 150 beats per minute with rectangular pulses of 4 msec duration and a voltage twice threshold via bipolar electrodes sutured to the left atrial appendage. Heart rate was determined from the electrocardiogram (limb lead II). The incidence of ventricular fibrillation during occlusion and after reperfusion was also assessed. The electrocardiogram, hemodynamics, and LCX blood flow were monitored and recorded by a polygraph (model 7, Grass Instrument Co., Quincy, Mass.) throughout the experiment.

Experimental Design

The experimental design is illustrated in Figure 1. Six groups of animals were studied to determine the role of the $K_{\text{ATP}}$ channel in myocardial preconditioning. Non preconditioned dogs (groups 1, 2, and 6) were subjected to 60 minutes of LCX occlusion followed by 5 hours of reperfusion, whereas preconditioned dogs (groups 3, 4, and 5) received a single 5-minute occlusion followed by 10 minutes of reperfusion before being subjected to the same protocol.
used in the nonpreconditioned groups. In nonpreconditioned dogs, vehicle (1.0N NaOH, propylene glycol, ethanol [1:1:1; total=1.5 ml], and saline [8.5 ml], group 1), glibenclamide (0.3 mg/kg i.v., group 2), or the potassium channel opener RP 52891 (10 μg/kg bolus i.v. and 0.1 μg/kg/min infusion, group 6) was administered 10 minutes before LCX occlusion. In the preconditioned dogs, vehicle (group 3) or glibenclamide (0.3 mg/kg i.v., group 4) was administered 10 minutes before preconditioning, whereas in group 5, glibenclamide (0.3 mg/kg i.v.) was administered immediately following preconditioning after reperfusion. This dose of glibenclamide has been previously shown to significantly attenuate the coronary vasodilator effect of the potassium channel opener EMD 56431 in anesthetized dogs.15 Finally, after the 5-hour reperfusion period in all groups, the hearts were electrically fibrillated, removed, and prepared for infarct size determination, myeloperoxidase content, and regional myocardial blood flow.

**Infarct Size Determination**

After 5 hours of reperfusion, the LCX was reocluded and cannulated just distal to the occlusion site. Subsequently, 10 ml saline and 10 ml Patent blue dye were injected at equal pressures into the LCX and left atrium, respectively, to determine the anatomic area at risk and the nonischemic area. The heart was then immediately fibrillated, removed, and sliced into serial transverse sections 6–7 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 20–30 minutes in 1% 2,3,5-triphenyltetrazolium chloride (TTC) in 0.1 M 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 area at risk were carefully separated and weighed. Infarct size was expressed as a percent of the area at risk and as a percent of the total left ventricle.

**Regional Myocardial Blood Flow**

Regional myocardial blood flow was determined by the radioactive microsphere technique as previously described in this laboratory.16 Microspheres were administered at 3 minutes into the 5-minute preconditioning period and at 30 minutes of the 60-minute occlusion period. In dogs without preconditioning, microspheres were administered at 3 and 30 minutes of occlusion. In all animals, the final microsphere was given at 5 hours of reperfusion. Carbonized plastic microspheres (15 μm diameter, New England Nuclear, Boston, Mass.) labeled with 141Ce, 51Cr, 186Ru, or 52Nb were suspended in isotonic saline with 0.01% Tween 80 added to prevent aggregation. The microspheres were ultrasonicated for 5 minutes followed by 5 minutes of vortexing immediately before injection. Approximately 1 ml of the microsphere suspension (2–4×10^6 spheres) was injected into the left atrium followed by several warm (37°C) saline flushes (5 ml). Just before sphere administration, a reference blood flow sample was withdrawn from the femoral artery at a constant rate of 6.5 ml/min for 3 minutes.

On the next day, the tissue slices were sectioned into subepicardium, midmyocardium, and subendocardium of nonischemic (three pieces) and ischemic noninfarcted and infarcted regions (five pieces), and the tissue samples were weighed (0.5–1.0 g). Transmural pieces were obtained from the center of several transverse sections used to determine infarct size and were at least 1 cm from the perfusion boundaries as indicated by the Patent blue dye. All samples were counted in a gamma counter (Tracer Analytic 1195, TM Analytic, Elk Grove, Ill.) to determine the activity of each isotope in each sample. The activity of each isotope was also determined in the reference blood flow samples. Myocardial blood flow was calculated by using a preprogrammed computer (Apple IIe) to obtain the true activity of each isotope in individual samples and tissue blood flow determined by using the equation

\[
Q_m = Q_r C_m / C_r
\]

where \(Q_m\) is myocardial blood flow (milliliters per minute per gram of tissue), \(Q_r\) is the rate of withdrawal of the reference blood flow sample (6.5 ml/min), \(C_r\) is the activity of the reference blood flow sample (counts per minute), and \(C_m\) is the activity of the tissue sample (counts per minute per gram). Transmural blood flow was calculated as the weighted average of the three layers in each region.

**Myeloperoxidase Determination**

Myeloperoxidase (MPO) activity, an index of neutrophil infiltration, was determined in myocardial tissue samples (100–400 mg) obtained from three areas within the area at risk, the infarct area, the area directly adjacent to the infarct area (border zone), and the distant noninfarcted area.17 The samples were immediately frozen in liquid nitrogen and stored at −70°C.

MPO was extracted from the tissue samples by two separation procedures.18,19 Initially, the samples were homogenized in 50 mM potassium phosphate buffer (pH 6) containing 5 mM EDTA and centrifuged at 40,000g for 15 minutes. The supernatant, which contains the water-soluble heme-containing proteins hemoglobin and myoglobin, was decanted, and the pellet was resuspended in 50 mM potassium phosphate buffer (pH 6) containing 5 mM EDTA and 0.5% hexadecylammonium bromide to solubilize the MPO. Subsequently, the suspension was rehomogenized four times for 10 seconds each, sonicated three times for 10 seconds each, and centrifuged at 40,000g for 15 minutes. The supernatant was then heated in a water bath at 60°C for 2 hours, a procedure previously shown to increase the recovery of MPO.19
MPO activity was assayed in triplicate using o-dianisidine as the substrate. A 0.1-ml aliquot of the supernatant was mixed with 2.9 ml of 50 mM potassium phosphate buffer (pH 6) containing 0.167 mg/ml o-dianisidine and 0.0005% hydrogen peroxide. The change in absorbance was measured spectrophotometrically at 460 μm and recorded on a chart recorder for approximately 3 minutes. Results were expressed as units of MPO activity per gram wet tissue weight, where 1 unit is defined as that quantity of enzyme that degrades 1 μmol hydrogen peroxide per minute at 25°C.

Criteria for Exclusion

Strict criteria were used to ensure that the animals included in data analysis were healthy and exposed to similar degrees of ischemia. Dogs were excluded if 1) heartworms were found after the animals were killed, 2) subendocardial collateral flow was greater than 0.15 ml/min/g, or 3) more than three consecutive attempts were required to convert ventricular fibrillation with low-energy DC pulses applied directly to the heart.

Statistical Analysis

All values were expressed as mean±SEM. Differences between groups in hemodynamics, collateral blood flow, and blood glucose were compared by using a two-way analysis of variance (ANOVA) for repeated measures and Fisher’s least significant difference. An unpaired t test (two tailed) was used to determine differences between groups regarding infarct size and MPO. Linear regression analysis was used to determine the relation between collateral blood flow in the inner two thirds of the ventricular wall in the ischemic area and myocardial infarct size expressed as a percent of the area at risk. Analysis of covariance, with collateral flow as the covariate, was used to determine if differences in this relation were observed between treatment groups. Differences between groups were considered significant if p<0.05.

Results

Mortality and Exclusions

Forty-six dogs were randomly assigned to one of six groups: control (group 1, n = 7), glibenclamide without preconditioning (group 2, n = 8), preconditioning (group 3, n = 8), glibenclamide+preconditioning (group 4, n = 7) where glibenclamide was administered 10 minutes before preconditioning, preconditioning+glibenclamide (group 5, n = 8) where glibenclamide was administered immediately after preconditioning and the potassium channel opener RP 52891 (group 6, n = 8). Three dogs were excluded because subendocardial collateral blood flow was greater than 0.15 ml/min/g (one each in groups 1, 2, and 4), one was excluded because heartworms were found after the dog was killed (group 5), and two dogs were excluded because of intractable ventricular fibrillation after reperfusion (one each in groups 3 and 6). Thus, 40 dogs completed the protocol satisfactorily and were used in data analysis.

Hemodynamics

The hemodynamic data are summarized in Table 1. There were no significant differences in heart rate, mean arterial blood pressure, the rate/pressure product, or LCX coronary blood flow between groups throughout the experiment with the exception of an increase in LCX blood flow in the RP 52891–treated group at 1 and 5 hours of reperfusion. There were small decreases in mean arterial pressure and the rate/pressure product as compared with baseline within several groups during preconditioning or during the sustained occlusion period, but most of these changes had returned to baseline at 5 hours of reperfusion.

Regional Myocardial Blood Flow

Regional myocardial blood flow data in the nonischemic (left anterior descending coronary artery) and ischemic-reperfused (LCX) regions are summarized in Table 2. In the nonischemic region, transmural and midmyocardial blood flow were significantly lower during preconditioning or 3 minutes into the sustained 60-minute occlusion period in the preconditioned, glibenclamide+preconditioning, or preconditioning+glibenclamide groups as compared with the control group. In the ischemic-reperfused region, there were no significant differences between groups in any layer during preconditioning or at 3 or 30 minutes of occlusion, which suggests that all groups were subjected to equivalent degrees of ischemia. At 5 hours of reperfusion, transmural blood flow was significantly higher in the RP 52891–treated animals as compared with the control group.

Infarct Size

Infarct size and area at risk data are summarized in Figure 2. The anatomic area at risk expressed as a percent of the left ventricle was not significantly different between groups: control, 40.5±1.9%; glibenclamide, 39.0±1.0%; preconditioning, 36.6±2.8%; glibenclamide+preconditioning, 39.4±1.8%; preconditioning+glibenclamide, 41.3±3.1%; RP 52891, 39.9±1.7%. Infarct size expressed as a percent of the area at risk was 28.3±5.5% in control animals and was not significantly changed by pretreatment with glibenclamide (31.2±5.9%). In contrast, a 5-minute preconditioning period before the sustained 60-minute occlusion period resulted in a significant (p<0.002) reduction in infarct size (5.9±2.3%) as compared with the control group. Similarly, treatment with the potassium channel opener RP 52891 also resulted in a significant (p<0.03) reduction in infarct size (13.3±2.5%) as compared with the control group. Administration of the KATP channel antagonist glibenclamide 10 minutes before or immediately after the 5-minute preconditioning period totally abolished the protective effect of preconditioning; glibenclamide+preconditioning, 28.4±6.2%; preconditioning+glibenclamide, 30.5±7.8%.
**Table 1. Hemodynamic Data for Six Groups of Dogs During Preconditioning, Occlusion, and Reperfusion**

<table>
<thead>
<tr>
<th>Group</th>
<th>Predrug baseline</th>
<th>Preconditioning or 3 minutes of occlusion</th>
<th>30 Minutes of occlusion</th>
<th>Reperfusion</th>
</tr>
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<tr>
<td></td>
<td></td>
<td>30 Minutes of occlusion</td>
<td>1 Hour</td>
<td>5 Hours</td>
</tr>
<tr>
<td>Control (n=6)</td>
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<tr>
<td>Heart rate (bpm)</td>
<td>153±4</td>
<td>154±4</td>
<td>149±11</td>
<td>153±5</td>
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<tr>
<td>Mean arterial pressure (mm Hg)</td>
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<td>87±8</td>
<td>97±8</td>
<td>99±6</td>
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<td>Rate/pressure product (mm Hg/min/1,000)</td>
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<td>16±1</td>
<td>16±2</td>
<td>17±1</td>
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<tr>
<td>Coronary blood flow (ml/min)</td>
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<td>0</td>
<td>81±12</td>
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<tr>
<td>Glibenclamide (n=7)</td>
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<tr>
<td>Heart rate (bpm)</td>
<td>154±1</td>
<td>155±3</td>
<td>152±1</td>
<td>148±5</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>95±7</td>
<td>91±3</td>
<td>81±5*</td>
<td>90±4</td>
</tr>
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<td>Rate/pressure product (mm Hg/min/1,000)</td>
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<td>16±1</td>
<td>14±1*</td>
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<td>Coronary blood flow (ml/min)</td>
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<td>0</td>
<td>54±11</td>
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<td>Preconditioning (n=7)</td>
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<td></td>
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<tr>
<td>Heart rate (bpm)</td>
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<td>154±2</td>
<td>152±2</td>
<td>154±2</td>
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<tr>
<td>Mean arterial pressure (mm Hg)</td>
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<td>100±5</td>
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<td>Coronary blood flow (ml/min)</td>
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<td>Glibenclamide+preconditioning (n=6)</td>
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<tr>
<td>Heart rate (bpm)</td>
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<tr>
<td>Mean arterial pressure (mm Hg)</td>
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<td>18±2</td>
<td>17±1</td>
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<td>Coronary blood flow (ml/min)</td>
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<td>0</td>
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<td>Preconditioning+glibenclamide (n=7)</td>
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<tr>
<td>Heart rate (bpm)</td>
<td>158±3</td>
<td>156±3</td>
<td>151±2</td>
<td>146±5</td>
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<tr>
<td>Mean arterial pressure (mm Hg)</td>
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<td>102±5*</td>
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<tr>
<td>Heart rate (bpm)</td>
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<td>154±3</td>
<td>156±5</td>
<td>151±4</td>
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<td>Mean arterial pressure (mm Hg)</td>
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<td>97±11*</td>
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<tr>
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<td>49±7</td>
<td>0</td>
<td>0</td>
<td>116±22*†</td>
</tr>
</tbody>
</table>

All values are mean±SEM. bpm, Beats per minute. *p<0.05 vs. baseline value within individual groups. †p<0.05 vs. corresponding time value in the control group.

Figure 3 demonstrates the relation between infarct size as a percent of the area at risk and collateral blood flow to the inner two thirds of the ischemic region at 30 minutes of occlusion. Because there were no significant differences between the slopes of the lines in groups 1, 2, 4, and 5 and no differences between groups 3 and 6, the data were pooled into two subsets. The regression lines for the two subsets were significantly different by analysis of covariance \( (p=0.005) \) because the line in the preconditioned and RP 52891-treated groups had a flatter slope and was shifted downward. These data indicate that for any given collateral blood flow (below 0.13 ml/min/g) to the inner two thirds of the ischemic region, a smaller infarct would be expected in the two groups in which the \( K_{ATP} \) channels have been activated.

**Myeloperoxidase Measurements**

The data summarizing the amount (units per gram) of MPO activity, an index of neutrophil infiltration in the areas at risk distant from the infarct, the border zone immediately adjacent to the infarct, and the center of the infarct, are shown in Figure 4. There were no differences between groups in MPO activity in the center of the infarct and the area at risk distant from the infarct. In contrast, MPO activity in the border zone immediately adjacent to the infarct was significantly lower in the preconditioned \( (p<0.005) \), glibenclamide+preconditioned \( (p<0.005) \), and RP 52891-treated dogs \( (p<0.05) \).

**Blood Glucose**

The data shown in Table 3 summarize the effects of the various treatments on blood glucose at several times throughout the experiment. Baseline blood glucose values were similar in all groups with the exception of the one in which glibenclamide was administered before preconditioning (significantly lower than the control group). At 30 minutes of occlusion, blood glucose was lower than that of the
control group in the glibenclamide and glibenclamide+preconditioning groups, and at 5 hours of reperfusion, blood glucose was significantly lower than that of the control group in the glibenclamide-treated group in the absence of preconditioning.

**Discussion**

The goal of the present study was to determine the role of the K$_{ATP}$ channel in myocardial preconditioning in dogs. Initially, we observed that a single 5-minute occlusion period before a more sustained 60-minute occlusion produced a marked reduction in myocardial infarct size as compared with a single 60-minute ischemic insult (5.9±2.3% versus 28.3±5.5%). These results are in close agreement with those of Li et al$^3$ in which it was found that a single 5-minute preconditioning period produced a reduction in infarct size from 29.8±4.4% to 3.9±3.1% in an identical canine model as that used in the present study. Subsequently, administration of the specific K$_{ATP}$ channel antagonist, glibenclamide,$^2,12$ 10 minutes before or immediately after preconditioning completely abolished the beneficial effect. Importantly, glibenclamide did not affect infarct size when administered to nonpreconditioned dogs. Thus, the antagonistic effect of glibenclamide was not the result of a direct effect of this compound to increase infarct size independently of preconditioning. In addition, the intravenous administration of a nonhypotensive dose of a new K$_{ATP}$ channel opener, RP 52891,$^{20}$ produced a marked reduction in infarct size (13.3±2.5%) similar to that of preconditioning. The slopes of the lines relating infarct size as a percent of area at risk to collateral blood flow in the inner two thirds of the ischemic region were not different in the preconditioned and RP 52891–treated groups, which suggests a common mechanism of action of these two interventions (Figure 3). Furthermore, the beneficial effects of preconditioning and RP 52891 and the antagonistic effects of glibenclamide on infarct size occurred independently of differences in hemodynamics, coro-
nary collateral blood flow, or ischemic bed size. Taken together, these results clearly suggest an important modulatory role of the K\textsubscript{ATP} channel in the preconditioning phenomenon in the canine heart.

Although the present results clearly implicate the K\textsubscript{ATP} channel in myocardial preconditioning, it is not apparent as to the mechanism by which K\textsubscript{ATP} channel opening protects the myocardium from a subsequent sustained ischemic insult; however, a metabolic basis may offer the most likely explanation. Previous work of Murray and colleagues\textsuperscript{1} indicated that preconditioning in dogs produced by four 5-minute episodes of ischemia followed by a subsequent 40-minute ischemic period markedly reduced infarct size. These investigators subsequently observed that preconditioned hearts developed ultrastructural damage more slowly than nonpreconditioned hearts and that the rate of ATP depletion during the initial phase of the sustained 40-minute ischemic period was reduced as a result of a decrease in ATP utilization.\textsuperscript{8} In addition, these investigators found a decrease in the rate of glycolgen breakdown as well as anaerobic glycolysis and suggested that a preservation of ATP levels or a reduction in the cellular content of toxic metabolites may be responsible for preconditioning. Recently, it has been demonstrated that the K\textsubscript{ATP} channel opens after a small decrease in intracellular ATP in cardiac cells.\textsuperscript{21} Activation of this channel during ischemia has been shown to decrease action potential duration and attenuate membrane depolarization.\textsuperscript{22,23} Theoretically, these effects could lead to a decrease in free cytosolic calcium concentration, a rapid loss of contractile activity, and a reduced level of ATP utilization that would be expected to delay ischemic cell death. In support of this hypothesis, Grover et al.\textsuperscript{13,14} recently showed that two potassium channel openers, cromakalim and pinacidil, reduced myocardial infarct size when given intracoronary to anesthetized dogs, and previous work in our laboratory with a 90-minute LCX occlusion model in anesthetized dogs showed that RP 52891 reduced infarct size when administered systemically at a nonhypotensive dose.\textsuperscript{24} In both of these studies, the reduction in

![Graphs illustrating the effects of the different protocols on the area at risk expressed as a percent of the left ventricular weight (AAR/LV) and infarct size expressed as a percent of the area at risk (IS/AAR). There were no differences between groups in AAR/LV; however, preconditioning (PRE) and RP 52891 (RP) produced significant reductions (marked by asterisks) in infarct size as compared with the control (CONT) nonpreconditioned group. Pretreatment with glibenclamide 10 minutes before (G+P) or immediately after (P+G) preconditioning completely abolished the protective effect of preconditioning. Glibenclamide had no effect on infarct size in the absence of preconditioning (GLIB).](http://circres.ahajournals.org/)

![A plot of the relation between infarct size expressed as a percent of the area at risk (AAR) versus collateral blood flow to the inner two thirds of the left ventricular wall in the AAR. In all groups, there is an inverse relation between infarct size as a percent of AAR and regional collateral blood flow. A consolidated regression analysis was performed for the control (CONT) and glibenclamide-treated groups (GLIB, G+P, and P+G) since their individual regression lines were not significantly different (solid line). Similarly, a regression line was calculated for the preconditioned (PRE) and RP 52891–treated (RP) groups (broken line). The regression lines for the two consolidated groups were different by analysis of covariance (p=0.005).](http://circres.ahajournals.org/)
potential beneficial effects of this agent. Furthermore, it has also been shown in isolated rat hearts\textsuperscript{14} as well as in anesthetized dogs\textsuperscript{24} that the $K_{\text{ATP}}$ channel antagonist glibenclamide blocks the beneficial actions of potassium channel openers. Thus, these data as well as the present data with RP 52891 suggest a direct cellular cardioprotective effect of opening the $K_{\text{ATP}}$ channel. In addition, results obtained from our laboratory with the potassium channel opener nitrate compound nicorandil indicate that this compound is cardioprotective in the canine heart\textsuperscript{27,28} as well as the isolated rat heart\textsuperscript{29} and that this compound preserves myocardial ATP levels in both models after ischemia and subsequent reperfusion. Thus, there is strong evidence to support a metabolic basis for the beneficial effect of preconditioning in protecting the ischemic myocardial cell by opening the $K_{\text{ATP}}$ channel; however, further studies are necessary to determine if blockade of this channel prevents the salutary metabolic effects produced by preconditioning or by specific $K_{\text{ATP}}$ channel openers.

Several recent studies suggest an endogenous mechanism by which the $K_{\text{ATP}}$ channel may be opened by specific receptor activation during ischemia or hypoxia. Daut and coworkers\textsuperscript{12} showed that hypoxia and adenosine produced coronary vasodilation via glibenclamide-sensitive potassium channels in isolated guinea pig hearts. Kirsch et al\textsuperscript{11} also found that $K_{\text{ATP}}$ channels in rat ventricular myocytes were activated by adenosine or the specific adenosine-1 ($A_1$) receptor agonist $N^\circ$-cyclohexyladenosine and that activation of the $A_1$ receptor was coupled to the $K_{\text{ATP}}$ channel by a $G$ protein. These results suggest that $A_1$ receptor stimulation may result in activation of $K_{\text{ATP}}$ channels in ischemic cardiac muscle. That adenosine receptors may be importantly involved in myocardial preconditioning has recently been suggested by the studies of Liu et al.\textsuperscript{10} These authors found that blockade of adenosine receptors in the ischemic rabbit heart prevented the effect of preconditioning. Because significant amounts of adenosine are formed from the metabolic breakdown of ATP in ischemic cardiac muscle, it seems reasonable to assume that this diffusible substance could be acting on its own receptor to act as a negative-feedback mechanism to protect the myocardium from a subsequent ischemic insult by opening $K_{\text{ATP}}$ channels.

In addition to its beneficial effects on infarct size, ischemic preconditioning has also been reported to markedly decrease the incidence of ventricular arrhythmias during reperfusion. Shiki and Hearse\textsuperscript{5} showed that after a short period of regional ischemia, premature ventricular contractions, tachycardia, and fibrillation were reduced during reperfusion in anesthetized rats that were previously subjected to brief periods of ischemia. A protective effect of preconditioning on reperfusion arrhythmias may appear to argue against the involvement of $K_{\text{ATP}}$ channels in the preconditioning phenomenon since $K_{\text{ATP}}$ channel activation has been proposed by some investigators to be proarrhythmic.\textsuperscript{30,31} However, $K_{\text{ATP}}$ channel activation

![Graphs illustrating the effects of the different treatments on myeloperoxidase activity in the area at risk distant from the infarct, the border zone immediately adjacent to the infarct, and the center of the infarct. There was a significant (*p<0.05) reduction in myeloperoxidase activity in the border zone in animals subjected to preconditioning (PRE), glibenclamide pretreatment before preconditioning (G+P), and RP 52891 (RP) treatment. CONT, control; GLIB, glibenclamide treatment without preconditioning.](http://circres.ahajournals.org/)

infarct size by the potassium channel openers occurred independently of changes in collateral blood flow, peripheral hemodynamics, and area at risk. Conversely, Sakamoto and colleagues\textsuperscript{25} and Imai et al\textsuperscript{26} showed in conscious dogs that systemic administration of the potassium channel opener pinacidil at significant hypotensive doses did not reduce infarct size; however, in these studies a reflex-induced increase in oxygen demand may have masked any
by pinacidil has also been reported to be antiarrhythmic. These conflicting results may be attributable to differences in the type of arrhythmias investigated and the conditions in which they occur, as well as species differences. Nevertheless, K\textsubscript{ATP} channel activation as the mechanism by which preconditioning reduces infarct size is not inconsistent with the finding that preconditioning may also protect against arrhythmias.

Another interesting finding in the present study was the observation that there was a decrease in the amount of MPO activity, an index of neutrophil infiltration, in the border zone immediately adjacent to the infarct in preconditioned animals and in rats subjected to 4-minute preconditioning periods followed by 45 minutes of sustained ischemia. The neutrophil has been implicated in several studies to be involved in tissue damage after reperfusion; thus, the reduction in infarct size by preconditioning or glibenclamide-treated animals suggests that it is unlikely that a decrease in neutrophil-mediated damage. That there was a decrease in neutrophil infiltration in preconditioned dogs pretreated with glibenclamide but not reduction in infarct size as compared with control animals suggests that it is unlikely that a decrease in neutrophil infiltration is the primary mechanism by which preconditioning is produced in the present study. However, preliminary results from our laboratory indicate that both superoxide anion production and luminol-enhanced chemiluminescence by zymosan-activated canine neutrophils was suppressed by the potassium channel opener EMD 52692, and this effect was antagonized by glibenclamide. Thus, an action of preconditioning or specific K\textsubscript{ATP} channel modulators on neutrophil activation or migration cannot be ruled out with certainty.

Finally, another possibility that cannot be ruled out in the present experiments is that glibenclamide may be attenuating preconditioning by a mechanism not directly related to blockade of the K\textsubscript{ATP} channel in the myocardium. Glibenclamide has been shown to decrease coronary blood flow in isolated hearts and to interfere with hypoxic coronary vasodilation. A reduction in blood flow during ischemia or after reperfusion might be expected to result in an increase in infarct size. However, in the present experiments there were no differences in blood flow during ischemia or after reperfusion in the control or glibenclamide-treated animals, so it is unlikely that changes in blood flow were responsible for the effect of glibenclamide to block preconditioning. Another possibility is that glibenclamide might have an adverse metabolic effect on the preconditioned myocardium independent of its effect on the myocardial K\textsubscript{ATP} channel. In the nonischemic heart, glibenclamide has been shown to increase glycolysis by stimulating the uptake of glucose by the heart and to stimulate the oxidation of pyruvate. These actions of glibenclamide might result in an increase in the amount of toxic glycolytic metabolites produced during ischemia and in this manner overcome the protective effects of preconditioning. Alternatively, the accumulation of toxic metabolites such as lactate may not actually occur. Kantor et al recently found that glibenclamide did affect glycolysis in the ischemic heart. In addition, Lampson et al showed that neither glibenclamide nor tolbutamide produced any alterations in myocardial lactate accumulation in ischemic rat hearts. In this study, an increased glycolytic flux without an increase in lactate production was explained by an increase in pyruvate oxidation. An enhanced glycolytic flux during ischemia in sulfonylurea-treated hearts could importantly affect the state of the K\textsubscript{ATP} channel. Accordingly, Weiss and Lamp have shown that glycolytically produced ATP preferentially suppresses K\textsubscript{ATP} channels in isolated ventricular myocytes. Thus, shifts in the compartmentalization of ATP production by agents such as glibenclamide may be factors that might exert important influences on preconditioning in the present study.

The ability of glibenclamide to affect insulin and glucose blood levels may also have a negative effect on preconditioning. Omar and coworkers recently showed in an isolated rabbit heart model that preconditioning protection occurs only in the presence of high glucose concentrations in the perfusate during reperfusion. In addition, Bergmann et al demonstrated in isolated rat hearts that insulin or adenosine individually stimulates glucose uptake; however, in combination glucose extraction was no longer enhanced by these two agents. Thus, in the present experiments, a reduction in blood glucose or an increase in insulin levels may be the mechanism by which glibenclamide blocked preconditioning. However, with the relatively low dose of glibenclamide (0.3 mg/kg), little hypoglycemia was observed and, presumably, as inferred from glucose levels, insulin was not greatly increased. Blood glucose levels were only significantly reduced from the control group in the glibenclamide and glibenclamide + preconditioning group during occlusion and only in the glibenclamide group at 5 hours of reperfusion. Therefore, it seems unlikely that a met-

### Table 3. Blood Glucose Data

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline (n=6)</th>
<th>30 Minutes of occlusion (n=6)</th>
<th>5 Hours of reperfusion (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>99±8</td>
<td>96±10</td>
<td>84±4</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>84±5</td>
<td>70±8*</td>
<td>47±5*†</td>
</tr>
<tr>
<td>Preconditioning</td>
<td>83±2</td>
<td>87±6</td>
<td>74±8</td>
</tr>
<tr>
<td>Glibenclamide +</td>
<td>72±6*</td>
<td>66±7*</td>
<td>67±11</td>
</tr>
<tr>
<td>Preconditioning +</td>
<td>89±9</td>
<td>86±8</td>
<td>75±6</td>
</tr>
<tr>
<td>RP 52891 (n=6)</td>
<td>88±2</td>
<td>89±5</td>
<td>93±3</td>
</tr>
</tbody>
</table>

All values are mean±SEM (mg%).

*p<0.05 vs. corresponding time value in the control group.

†p<0.05 vs. baseline value within groups.
abolic effect as a result of increased insulin or decreased blood glucose levels is responsible for the effect of this compound to prevent preconditioning.

The observations of others1-5 and that of the present study in which myocardial preconditioning has been shown to occur in a variety of animal species may have important clinical manifestations. Although certainly speculative, patients that routinely experience intermittent, reversible ischemic episodes before a more intense ischemic insult might be expected to have a more favorable outcome than those suddenly exposed to a sustained ischemic event without prior preconditioning. That preconditioning can occur in humans has been recently demonstrated by Deutsch and coworkers.7 These authors showed that there was less evidence of metabolic, hemodynamic, and electrocardiographic disturbances during the second of two periods of coronary occlusion produced by transluminal angioplasty, which suggests that tolerance to ischemia can rapidly develop in humans. In addition, Muller et al43 recently found that patients with a prior history of angina pectoris who were subjected to thrombolytic therapy for acute myocardial infarction have a more favorable short-term prognosis than those without prior angina. These authors43 suggested that this beneficial effect of antecedent angina may have been the result of ischemic preconditioning. That an endogenous mechanism may exist in the heart that is linked to opening of K,ATP channels suggests that the development of pharmacological agents that selectively act on this channel or a receptor that activates this channel may have great promise as myocardial protective agents that could be administered before or during ischemia in patients undergoing percutaneous transluminal angioplasty, elective coronary artery bypass surgery, or thrombolysis.

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