Anesthetic-induced Preconditioning

Previous Administration of Isoflurane Decreases Myocardial Infarct Size in Rabbits

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Background: Experimental evidence suggests that ATP-sensitive potassium channels are involved in myocardial ischemic preconditioning. Because some pharmacologic effects of isoflurane are mediated by K\textsubscript{ATP} channels, the authors tested the hypothesis: Isoflurane administration, before myocardial ischemia, can induce or mimic myocardial preconditioning.

Methods: Myocardial infarct size was measured in three groups of propofol-anesthetized rabbits, each subjected to 30 min of anterolateral coronary occlusion followed by 3 h of reperfusion. Groups differed in their pretreatment: Group 1 (control, N = 13) no pretreatment, Group 2 (ischemic preconditioning, N = 8), 5 min of coronary occlusion and 15 min of reperfusion; Group 3 (isoflurane pretreatment; N = 15), 15 min of isoflurane (1.1% end-tidal) and 15 min of washout. Hemodynamics were monitored serially. Myocardial infarct size and the area at risk were defined using triphenyltetrazolium chloride staining and fluorescent microspheres, respectively, and both were measured using computerized planimetry.

Results: Infarct size expressed as a percentage of area at risk was 23.4 ± 8.5% (mean ± SD) in the isoflurane group compared with 33.1 ± 13.3% in controls, and 8.7 ± 6.2% in the ischemia-preconditioned group. Analysis for coincidental regressions, followed by tests for equality of slope and elevation, showed that the linear relationship between infarct size and area at risk was significantly (P < 0.05) different in all three groups because of differences in line elevation. Minor differences in hemodynamic variables were found between groups, which were unlikely to account for the significant differences in infarct size.

Conclusion: Preadministration of isoflurane, before myocar-

dial ischemia, reduces myocardial infarct size, and mimics myocardial preconditioning. (Key words: Heart: ischemic preconditioning; myocardial infarction; myocardial ischemia. Anesthetics, volatile: isoflurane. Potassium channel: ATP-sensitive.)

MYOCARDIAL “preconditioning” is the name given to the endogenous myocardial protection phenomenon whereby brief periods of myocardial ischemia exert a protective effect against subsequent, more prolonged ischemia. Although preconditioning was originally described after ischemic flow reductions, pre-conditioning-like effects have also been described after a variety of stimuli, including hypoxemia, elevated circulating catecholamines, myocardial stretch, rapid pacing, and pharmacologic interventions. Preconditioning has now been demonstrated in essentially every animal species studied: there is even electrocardiographic evidence for preconditioning in humans.

Because of the potency of the protection provided by myocardial preconditioning, the mechanisms that potentially mediate its effects have been the focus of intense investigation. Several different mechanisms have been implicated, including various types of G-protein-linked receptors (adenosine, α\textsubscript{1}, adrenergic, muscarinic) and protein kinase C. These proposed mechanisms, and others, have been recently reviewed. Accumulating evidence also suggests that myocardial ATP-sensitive potassium channels (K\textsubscript{ATP} channels) play a pivotal role in preconditioning. Recent studies that simultaneously address the role of adenosine and of K\textsubscript{ATP} channels indicate that the opening of myocardial K\textsubscript{ATP} channels, activated by adenosine A1-receptor occupancy, may be a final common effector pathway through which myocardial preconditioning is produced in some models.

Two lines of experimental evidence suggest that volatile anesthetics such as isoflurane may pharmacologically induce preconditioning-like effects. First, adminis-
tration of volatile anesthetics (including isoflurane) during transient myocardial ischemia enhances the recovery of cardiac function and reduces the degree of subsequent myocardial infarction.\textsuperscript{26–29} In addition, more recent studies have shown that isoflurane probably protects the myocardium during ischemia\textsuperscript{29} and vasodilates the coronary circulation\textsuperscript{59} through K\textsubscript{ATP} channel-dependent mechanisms. Based on this evidence, we hypothesized that volatile anesthetics may exert preconditioning-like effects.

To investigate whether preconditioning-like effects can be induced by volatile anesthetics, we used a rabbit model of myocardial ischemia and reperfusion, with infarct size as the primary outcome variable. This rabbit model has two advantages: first, it is a well-established animal model of preconditioning in which K\textsubscript{ATP} channel activation is important.\textsuperscript{14,23,24} Second, the innate coronary collateral blood flow of the rabbit myocardium is minimal, essentially nil,\textsuperscript{51,52} which has the advantage of minimizing variability in infarct size caused by differences in the collateral flow.

Methods

Anesthesia and Surgical Preparation

This study was conducted according to the standards of the American Physiological Society and with the approval of our hospital’s animal welfare committee. New Zealand White rabbits (2.6–3.0 kg) were sedated with 70 mg/kg of intramuscular ketamine. An infusion of Diprivan® (Zeneca Pharmaceuticals, Wilmington, DE) (propofol, 10 mg/ml) was started at 0.5–1.0 mg·kg\textsuperscript{-1}·min\textsuperscript{-1} to maintain surgical anesthesia.\textsuperscript{33,54} While administering 100% oxygen by mask, a tracheotomy was performed. Ventilation was controlled using a positive-pressure respirator (Ohio Medical Products, Madison, WI) and an FiO\textsubscript{2} of 1.0, and minute ventilation was adjusted to achieve normocapnia. A carotid artery was isolated and cannulated with a 22-gauge catheter for blood pressure measurement and arterial blood sampling. Ventilation rate was adjusted, and small doses of NaHCO\textsubscript{3} (44 mEq/50 cc) were administered when necessary to maintain a physiologic blood pH (7.35–7.45). Arterial blood gases were measured using a Radiometer® (Copenhagen, Denmark) ABL 2 Acid-Base Laboratory. Endtidal PCO\textsubscript{2} was monitored using a calibrated Puritan-Bennet infrared monitor (Wilmington, MA). Endtidal isoflurane concentration was measured by a Puritan-Bennet Anesthetic Agent Monitor (Wilmington, MA). Core body temperature was maintained between 38–39°C by use of a warming blanket.

Just before median sternotomy, each animal was given a single dose of pancuronium, 0.4 mg, to prevent muscle retraction during electrocautery and thus to minimize bleeding. The heart was then exposed through median sternotomy, and the pericardium was opened. A 2-0 polyester suture was quickly passed around the anterolateral coronary artery\textsuperscript{55} at approximately the midpoint of its epicardial course, and the ends of the suture were passed through a 6-cm long piece of plastic tubing to form a snare. The position of the suture varied considerably between animals as a result of variations in coronary anatomy, and therefore a range of areas at risk (AR) was obtained in each experimental group. The coronary artery was occluded when required by tightening the snare and then by clamping the tube with a hemostat. Three leads were attached to the chest wall for the measurement of heart rate and recording of electrocardiograms. Myocardial ischemia was confirmed by regional cyanosis and S-T segment elevation. Reperfusion was achieved by releasing the snare and was confirmed by visual observation of reactive hyperemia. Surgery was generally completed within 45–60 min.

Experimental Protocols

After placing the snare around the anterolateral coronary artery, rabbits were assigned to one of three pretreatment groups: a control group that received no pretreatment before 30 min of coronary artery occlusion; an ischemia-preconditioning group that received a 5-min coronary artery occlusion followed by 15 min of reperfusion before 30 min of coronary occlusion; and an isoflurane group that received 15 min of 1.1% endtidal isoflurane (0.5–0.6 MAC in rabbits\textsuperscript{56}) followed by a 15-min wash-out period before 30 min of coronary artery occlusion. Based on pilot experiments in our laboratory, we predicted that infarct size would be significantly smaller and less variable in the ischemia-preconditioned group compared with the control group. Experimental group assignments were made using a randomization scheme in which the probability of group assignment was approximately 2:2:1 for the control group, isoflurane group, and ischemia-preconditioned group, respectively. In every case, group assignment was made after completion of the surgical preparation and after placement of the coronary snare. A stabilization period of 20–25 min was allowed after the completion of the surgical preparation, before initia-
tion of the experimental protocol. The duration of the experiments was standardized across all three groups as depicted in figure 1. Each rabbit was anticoagulated with 1500 U beef lung heparin before the initial coronary artery occlusion. Ventricular fibrillation, if it occurred, was reversed via direct mechanical stimulation: an index finger was flicked directly against the right ventricular side of the fibrillating heart one to three times to achieve defibrillation. In all three groups, 3 h of reperfusion followed the 30-min coronary artery occlusion. At the end of 3 h, the heart was stopped by an intravenous bolus of saturated KCl and excised for measurement of infarct size and area at risk.

In the isoflurane-pretreated group, propofol infusion was interrupted during administration of isoflurane to maintain a nearly constant level of anesthesia and to avoid hypotension. Hypotension may directly or indirectly affect the ability of various agents to precondition the myocardium. Seven to eight minutes were typically required to achieve a measured end-tidal isoflurane concentration of 1.1%. Once an end-tidal level of 1.1% was reached, end-tidal isoflurane was held constant for 15 min, after which propofol infusion was restarted and isoflurane administration was discontinued. No measurable isoflurane could be detected 4–6 min after the isoflurane had been turned off.

**Hemodynamics**

Measurements of hemodynamics, taken at end-expiration, were made at various times during the experimental protocol (fig. 1). Blood pressure was continuously monitored using a calibrated strain gauge transducer (Abbott Critical Care Systems, North Chicago, IL) and a Grass model 7D polygraph (Grass Instruments, Quincy, MA). Heart rate (HR) was calculated from the electrocardiograph (ECG) and was averaged over several heartbeats during each measurement period. Rate–pressure product was calculated as MAP × HR.

**Myocardial Infarct Size and Area at Risk**

The area at risk was identified by reoccluding the coronary artery of the excised heart and by perfusing the aortic trunk with a solution containing fluorescent microspheres. This solution was prepared by adding 80 mg of 1-20 μm ZnS microspheres (Duke Scientific, Palo Alto, CA) to 20 ml of 10% Dextran 40 containing 0.5 ml of Tween 80 (Sigma, St. Louis, MO). After perfusing the heart with microspheres, each heart was frozen in liquid nitrogen and sliced into 6–10 2-mm-thick sections. Area at risk was defined as the area of myocardial slice not illuminated by microspheres when viewed under ultraviolet light. To discriminate between infarcted and viable myocardium, heart slices were bathed in the vital stain triphenyltetrazolium chloride for 20 min at 37°C, and then submerged in 10% formalin to stop the staining process. Triphenyltetrazolium chloride was prepared by dissolving 2 g of 2,3,5-triphenyltetrazolium chloride in 200 ml of 90 mm phosphate buffer (pH 8.5–8.6 at 37°C). After the slices were flattened in a plastic press at 4°C for approximately 24 h, the area at risk, area of infarct, and total myocardial area on both sides of each slice were traced onto acetate sheets. These traces were enlarged by overhead projection and then digitized using a flat bed scanner (AgFa Division, model Arcus II, Miles, Inc., Wilmington, MA) in conjunction with Adobe Photoshop® software (Adobe Systems Incorporated, Mountain View, CA). Area at risk, area of infarct, and total myocardial area were measured using computerized planimetry (NIH Image 1.60, public domain software), and mean values for each slice were calculated by averaging the values obtained for the two sides. Mean infarct area and area at risk for each slice were divided by total myocardial area, and subsequently
multiplied by slice mass, to obtain approximate values for infarct size and area at risk in grams. The ratio of infarct size:area at risk, the proportion of ischemic myocardium that became infarcted in each heart, was calculated from these data.

**Data Analysis and Statistics**

Regression analysis showed that a significant linear relationship existed between infarct size and area at risk in the control and isoflurane groups (table 1). We tested for differences in treatment effects using analysis for coincidental regressions, followed by tests for equality of slope and elevation. Because this involved multiple paired tests, the Bonferroni correction was applied to the significance level of each test. Area at risk was compared in the three groups using a one-way analysis of variance (ANOVA).

Hemodynamic variables were analyzed using a repeated measures ANOVA followed by Dunnett’s test for comparison versus control, or by the Student-Newman-Keuls test, as appropriate. Statistical analyses were performed using Systat 5.0 (Systat Inc., Evanston, IL) or Statview 4.0 (Abacus Concepts, Inc., Berkeley CA) statistical software. For all statistical analyses, the fiducial limit of significance was chosen as 5%. Values in tables, figures, and throughout the text are expressed as mean ± SD.

**Results**

**Experimental Animals**

Experiments were performed on 39 rabbits: 14 in the control group, 8 in the ischemic preconditioning group, and 17 in the isoflurane pretreatment group. However, experiments on three rabbits (1 in the control group, 2 in the isoflurane pretreatment group) were excluded because of intractable hypotension that began during coronary occlusion and which precluded 3 h of reprefusion. These excluded experiments were all on animals with large areas of myocardium at risk. Although area at risk in the ischemic preconditioning group was approximately 30% less than that in the control and isoflurane pretreatment groups, this difference was not statistically significant by ANOVA.

**Infarct Size**

Infarct size expressed as a percentage of area at risk was 33.1 ± 13.3% in control rabbits and 23.4 ± 8.5% and 8.8 ± 6.2% in the isoflurane pretreated and ischemic preconditioned rabbits, respectively (fig. 2A). Significant linear relationships existed between infarct size and area at risk in the control and isoflurane pretreated groups (table 1), but not in the ischemic preconditioned group that a plot of infarct size versus area at risk yielded a nearly flat line (fig. 2B, table 1). Pretreatment with isoflurane (1.1%, 0.5–0.6 MAC) significantly altered the linear relationship between infarct size and area at risk compared with the control and ischemic preconditioned groups. The slope of the linear relationship between infarct size and area at risk was not significantly different between groups after Bonferroni correction (0.08 > P < 0.30). However, statistical analysis showed that the elevation of all three lines was different (P > 0.05; table 1). Based on the differences in the

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>Area at Risk (g)</th>
<th>Infarct Size (g)</th>
<th>IS/AR (%)</th>
<th>Regression Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>13</td>
<td>0.98 ± 0.35</td>
<td>0.35 ± 0.23</td>
<td>33.1 ± 13.3</td>
<td>IS = -0.180 ± 0.535(AR) r² = 0.66*</td>
</tr>
<tr>
<td>Isoflurane</td>
<td>15</td>
<td>1.05 ± 0.42</td>
<td>0.26 ± 0.15</td>
<td>23.4 ± 8.5</td>
<td>IS = -0.068 ± 0.315(AR) r² = 0.73*</td>
</tr>
<tr>
<td>Preconditioned</td>
<td>8</td>
<td>0.72 ± 0.25</td>
<td>0.06 ± 0.06</td>
<td>8.7 ± 6.2</td>
<td>IS = -0.018 ± 0.112(AR) r² = 0.26</td>
</tr>
</tbody>
</table>

Values are mean ± SD. The compared lines were significantly different using tests for coincidental regressions and for differences in elevation (Zar, 1984), followed by Bonferroni’s procedure for multiple comparisons. After Bonferroni correction, the slopes of the three regression line were not significantly different. Tests for differences in slope showed:

† P < 0.13, control versus isoflurane.
‡ P = 0.3, isoflurane versus ischemic preconditioning.
§ P = 0.08, control versus ischemic preconditioning.

* Indicates a significant relationship between infarct size and area at risk was present.
regression line elevations and on the mean values for infarct size:area at risk, it is clear that: (1) isoflurane pretreatment can precondition the myocardium; and (2) isoflurane administration (0.5–0.6 MAC, 15 min) was not as effective as a brief episode of ischemia in reducing infarct size caused by a subsequent 30-min coronary occlusion (Fig. 2A).

Hemodynamic Variables

There were no significant differences in HR, MAP, or RPP among the three treatment groups at baseline. The hemodynamic effects of the three treatment regimens were similar, although there were some minor differences (Table 2). Administration of isoflurane reduced MAP by 16% and increased HR by 6% compared with baseline values; RPP was not significantly altered. MAP and HR returned to baseline levels in the isoflurane group before coronary occlusion.

Immediately before coronary occlusion, MAP and HR were similar in all three treatment groups. At this time, RPP was slightly lower (−15%) in the ischemia-preconditioned and isoflurane groups as compared with the control group, but these RPP values were not significantly lower than baseline values for the two groups (P = not significant, by ANOVA and Dunnett’s test). During the 30-min ischemic period, hemodynamic values were indistinguishable in the three groups. After 3 h of reperfusion, MAP and RPP were 19% and 21% lower in the control group, respectively, as compared with baseline values. This probably reflects a decline in ventricular performance caused by the mechanical effects of the larger infarct size in the control group.

Incidence of Transient Ventricular Fibrillation

A short period (<90 s) of ventricular fibrillation was recorded in 3 of 13 (23%) control rabbits and 4 of 15 (26%) isoflurane pretreated rabbits during the 30-min occlusion or within the first 15-min of reperfusion. None of the preconditioned rabbits developed ventricular fibrillation.

Discussion

This study demonstrates that pretreatment with a clinically relevant dose of isoflurane (1.1% = 0.5–0.6 MAC in rabbits) induces a preconditioning-like effect in the myocardium, which lasts for at least 15 min. Inhalation of 1.1% end-tidal isoflurane for 15 min, even though this treatment was followed by 15 min of washout, reduced myocardial infarct size by 30% compared with the control group and significantly decreased the elevation of the infarct size:area-at-risk relationship. This was less effective than one 5-min period of ischemic preconditioning, which decreased infarct size by 74% versus control. Pretreatment with isoflurane using this dosage and duration thus partially mimics, but does not duplicate, the potent protective effects of myocardial isch-
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Table 2. Hemodynamics in Rabbits Untreated (Control), Given 5 min of Ischemic Preconditioning, or Exposed to 15 min of Isoflurane (1.1%, 0.5–0.6 MAC) Prior to 30 min of Anterolateral Coronary Occlusion

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Half-way into Isoflurane</th>
<th>Preocclusion</th>
<th>15 min into Occlusion</th>
<th>1 h Reperefusion</th>
<th>3 h Reperefusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 13)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>HR (bpm)</td>
<td>257 ± 36</td>
<td>—</td>
<td>267 ± 29</td>
<td>266 ± 25</td>
<td>273 ± 18</td>
<td>255 ± 25</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>70 ± 11</td>
<td>—</td>
<td>74 ± 11</td>
<td>67 ± 7</td>
<td>66 ± 11</td>
<td>57 ± 11*</td>
</tr>
<tr>
<td>RPP (bpm/mm Hg*10³)</td>
<td>18.3 ± 4.7</td>
<td>—</td>
<td>19.6 ± 3.0</td>
<td>17.9 ± 2.2</td>
<td>18.0 ± 2.9</td>
<td>14.5 ± 3.6*</td>
</tr>
<tr>
<td>Preconditioning (n = 8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>HR (bpm)</td>
<td>255 ± 23</td>
<td>—</td>
<td>254 ± 22</td>
<td>264 ± 22</td>
<td>271 ± 22</td>
<td>256 ± 28</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>68 ± 9</td>
<td>—</td>
<td>65 ± 6</td>
<td>69 ± 11</td>
<td>71 ± 14</td>
<td>59 ± 14</td>
</tr>
<tr>
<td>RPP (bpm/mm Hg*10³)</td>
<td>17.3 ± 2.6</td>
<td>—</td>
<td>16.5 ± 2.6†</td>
<td>18.3 ± 3.1</td>
<td>19.4 ± 5.1</td>
<td>15.1 ± 3.7</td>
</tr>
<tr>
<td>Isoflurane (n = 15)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>249 ± 27</td>
<td>264 ± 31*</td>
<td>248 ± 19</td>
<td>250 ± 23</td>
<td>265 ± 27*</td>
<td>253 ± 23</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>70 ± 12</td>
<td>59 ± 15*</td>
<td>67 ± 12</td>
<td>66 ± 8</td>
<td>68 ± 12</td>
<td>64 ± 12</td>
</tr>
<tr>
<td>RPP (bpm/mm Hg*10³)</td>
<td>17.5 ± 4.3</td>
<td>15.7 ± 5.0</td>
<td>16.7 ± 3.1†</td>
<td>16.7 ± 3.5</td>
<td>18.2 ± 4.3</td>
<td>16.3 ± 3.9</td>
</tr>
</tbody>
</table>

HR = heart rate; MAP = mean arterial pressure; RPP = rate-pressure product.

Values are mean ± SD.

* Significantly different from baseline (P < 0.05).
† Significantly different from the same value in control rabbits (P < 0.05).

emeric preconditioning. Further experiments are necessary to establish the optimal dose and duration of isoflurane for preconditioning the myocardium, and the duration of the protective window.

It is unlikely that the isoflurane-induced preconditioning effect discovered in this study was caused by any mechanism other than a direct pharmacologic effect of isoflurane on the myocardium. Although several stimuli other than transient coronary occlusion have been found to cause "inadvertent preconditioning" of the myocardium, all such stimuli were avoided by our experimental design. Such stimuli include hypoxia,2 nonocclusive coronary hypoperfusion,49 myocardial distention and overstretch,5 pacing-induced stress,6 and sympathomimetic stimulation.13 Hypoxia was avoided in all experimental groups by initiating oxygen inhalation immediately on the onset of sedation. Large reductions in MAP, which can cause myocardial hypoperfusion, were avoided by stopping propofol administration during isoflurane exposure.

Additionally, it is unlikely that the reduction in infarct size found in the isoflurane-treated group was caused by any effect other than a preconditioning-like effect. Ischemic-zone collateral blood flow can be an important factor in determining the size of myocardial infarction, particularly in species that have large or highly variable collateral flow.31 Rabbits were chosen for this experiment because their suitability as a preconditioning model is well-established22,24 and because of the known minimal collateral blood flow during ischemia.31,35 Hemodynamic factors such as small differences in blood pressure immediately before ischemia have no significant effect on infarct size in the rabbit model.24,41 In any case, there were no major variations in hemodynamics in the pretreatment phase of the current study.

Although the mechanism(s) of the preconditioning effect of isoflurane cannot be deduced from the current study, there are several possibilities. First, isoflurane may directly or indirectly open KATP channels of ventricular myocytes. Many investigators have found that the opening of KATP channels is a central event in myocardial preconditioning, that inhibitors of KATP channel opening can decrease or eliminate the preconditioning effect22,23,42 and that openers of KATP channels mimic or facilitate preconditioning.7,43 In addition, recent studies addressing the role of KATP channels in preconditioning suggest the hypothesis that the opening of myocardial KATP channels, activated by adenosine A1-receptor occupancy,10-15,44 and mediated by a G-protein mechanism,9 may be a final common effector pathway through which myocardial preconditioning is produced in some models.12,13 With regard to this, evidence from several laboratories, now indicates that some actions of isoflurane and other volatile anesthetics are partially mediated by KATP channels. These include coronary vasodilation,10,45 and myocardial protection when isoflurane is adminis-
tered during ischemia, but not the negative contractility effects caused by isoflurane. Of note, the evidence to date consists mainly of pharmacologic studies using inhibitors of \( K_{ATP} \) such as glibenclamide. It is currently unknown whether the apparent effect of isoflurane on \( K_{ATP} \) channels has a direct effect at the channel or an indirect effect via other modulators of channel activity such as adenosine.

Second, protein kinase C (PKC), a mediator of intracellular protein phosphorylation, has been implicated as a participating mechanism in preconditioning. Volatile anesthetics such as isoflurane and halothane are known to affect the actions of PKC, although the reported actions have been contradictory. For example, PKC-induced contractions in rat coronary arteries are inhibited by halothane but enhanced by isoflurane. Ozhan et al. found that neither isoflurane or halothane inhibited PKC-induced contractions in swine coronary arteries. In another model, PKC-mediated alterations in hepatic vascular tone were inhibited by isoflurane and by halothane. The differences among these studies may be a result of species or tissue-specific differences in anesthetic-PKC interaction or to other as yet-unexplained factors. However, these studies suggest that, if PKC is confirmed to be an important factor in preconditioning, volatile anesthetics could affect the preconditioning process at this level.

Ventricular Fibrillation
Isoflurane, although it induced a preconditioning-like effect, did not appear to affect the incidence of ventricular fibrillation in this experiment. The incidence of ventricular fibrillation was 26% in the isoflurane-treated rabbits versus 23% in the control group. This suggests that isoflurane-mediated protection against infarction may be qualitatively different from protection against fibrillation. This is in agreement with the work of Haessler et al., who studied the influence of different anesthetic regimens on the effectiveness of preconditioning in a similar rabbit model. In that study, the incidence of ventricular fibrillation was found to be affected by the type of anesthetic used during the ischemic period, but not by the presence or absence of preconditioning.

Other Considerations: Effects of Basal Anesthesia in Preconditioning Studies
The choice of basal anesthetic used may determine the susceptibility of the preparation to preconditioning or may affect the threshold level of stimulus that is required to induce preconditioning. For example, Thornton et al. found that glibenclamide, a blocker of \( K_{ATP} \) channels, was pros ischemic and did not eliminate preconditioning in rabbits anesthetized with pentobarbital. In contrast, Toombs et al. used a nearly identical rabbit model with the only difference that ketamine-xylazine anesthesia was used. They found that \( K_{ATP} \) channel blockade with glibenclamide reduced preconditioning. This was subsequently verified by Walsh and Thornton. Thus, basal anesthesia, by unknown mechanisms, can determine the absolute susceptibility to preconditioning.

In the current study, propofol was used as the basal anesthetic. Propofol has not, to our knowledge, previously been used as a basal anesthetic in preconditioning studies. We chose this basal anesthetic because preliminary studies indicated that addition of a significant MAC dose of isoflurane to a full pentobarbital anesthetic or a full ketamine-xylazine anesthetic in rabbits leads to hemodynamic instability. Propofol has the distinct advantage that intravenous infusion can be discontinued during administration of a second, test anesthetic such as isoflurane. In this manner, the profound hemodynamic effects of a double anesthetic can be minimized.

Limitations of the Current Study
One potential disadvantage of our experimental design is that the basal anesthetic, propofol, was not administered in the same way to each group. It was turned off during isoflurane administration and turned back on when isoflurane was discontinued. The unacceptable alternatives would be to allow isoflurane administration to cause severe hypotension or to use other drugs to support blood pressure. Because \( \alpha \)-adrenergic stimulation has been reported to cause preconditioning, the latter option was unacceptable. In addition, because hypotension during isoflurane administration could directly or indirectly influence the effect of isoflurane on post ischemic infarct size. We chose to decrease the basal anesthetic while isoflurane was administered.

Summary of Conclusions
Preadministration of isoflurane partially protects the heart from subsequent ischemia by decreasing myocardial infarct size. This effect is analogous to the protective effect observed with preconditioning ischemia. The optimal dose, the maximal protective effect, the duration of the "protective window," and most importantly, the cellular mechanisms of anesthetic-induced preconditioning remain to be determined. In the larger con-
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text, our findings may present a method to pharmacologically induce myocardial preconditioning and thereby to protect the heart in clinical situations where transitory intraoperative myocardial ischemia is expected.

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