Isoflurane Preconditions Myocardium Against Infarction via Activation of Inhibitory Guanine Nucleotide Binding Proteins

Wolfgang G. Toller, M.D., D.E.A.A.,* Judy R. Kersten, M.D.,† Eric R. Gross, B.S.,‡ Paul S. Pagel, M.D., Ph.D.,§ David C. Warltier, M.D., Ph.D.¶

Background: Isoflurane-induced myocardial protection during ischemia is mediated by adenosine triphosphate–regulated potassium (K\textsubscript{ATP}) channels; however, the intracellular signal transduction cascade responsible for this process has been incompletely evaluated. The authors tested the hypothesis that isoflurane reduces myocardial infarct size through a G\textsubscript{i} protein–mediated process.

Methods: Forty-eight hours after pretreatment with vehicle (0.9% saline) or the G\textsubscript{i} protein inhibitor pertussis toxin (10 \mu g/kg intravenously), barbiturate-anesthetized dogs (n = 43) were instrumented for measurement of aortic and left ventricular pressures and maximum rate of increase of left ventricular pressure. All dogs were subjected to a 60-min left anterior descending coronary artery occlusion followed by 3-h reperfusion. In four separate groups, vehicle- or pertussis toxin–pretreated dogs were studied with or without administration of 1 minimum alveolar concentration isoflurane. In two additional groups, dogs received the direct K\textsubscript{ATP} channel agonist nicorandil (100 \mu g/kg bolus and 10 \mu g \cdot kg\textsuperscript{-1} \cdot min\textsuperscript{-1} intravenous infusion) in the presence or absence of pertussis toxin pretreatment. Myocardial perfusion and infarct size were measured with radioactive microspheres and triphenyltetrazolium staining, respectively.

Results: Isoflurane significantly (P < 0.05) decreased infarct size to 7 ± 2% of the area at risk compared with control experiments (26 ± 2%). Pertussis toxin pretreatment alone had no effects on myocardial infarct size (31 ± 4%) but blocked the beneficial effects of isoflurane (21 ± 3%). Nicorandil decreased infarct size (11 ± 2%), but, in contrast to isoflurane, this effect was independent of pertussis toxin pretreatment (11 ± 1%).

Conclusion: Isoflurane reduces myocardial infarct size by a G\textsubscript{i} protein–mediated mechanism in vivo. (Key words: Myocardial ischemia; pertussis toxin.)
duced by isoflurane but not by the direct $K_{\text{ATP}}$ channel opener nicorandil.\textsuperscript{10}

### Methods

All experimental procedures and protocols used in this investigation were reviewed and approved by the Animal Care and Use Committee of the Medical College of Wisconsin. All procedures were in conformity with the \textit{Guiding Principles in the Care and Use of Animals} of the American Physiologic Society\textsuperscript{11} and were performed in accordance with the \textit{Guide for the Care and Use of Laboratory Animals}.\textsuperscript{12}

#### Surgical Preparation

The experimental methods have been previously described in detail.\textsuperscript{13} Briefly, mongrel dogs (weight $= 23 \pm 1$ kg; mean $\pm$ SEM) were anesthetized with sodium barbital (200 mg/kg) and sodium pentobarbital (15 mg/kg) and ventilated with an air/oxygen mixture (fraction of inspired oxygen $= 0.25$) after tracheal intubation. Tidal volume and respiratory rate were adjusted to maintain arterial blood gas tensions within a physiologic range. A double pressure transducer–tipped catheter was inserted into the aorta and left ventricle (LV) \textit{via} the left carotid artery to measure aortic and LV pressures, respectively. The maximum rate of increase of LV pressure ($+\frac{dP}{dt_{\text{max}}}$) was obtained by electronic differentiation of the LV pressure waveform. The femoral artery and vein were cannulated for the withdrawal of reference blood flow samples and fluid administration, respectively. A thoracotomy was performed at the left fifth intercostal space. A heparin-filled catheter was inserted into the left atrial appendage for administration of radioactive microspheres. A 1.0-cm segment of the left anterior descending (LAD) coronary artery was dissected immediately distal to the first diagonal branch, and a silk ligature was placed around this vessel for production of coronary artery occlusion and reperfusion. Regional myocardial perfusion was measured in the ischemic (LAD) and normal (left circumflex coronary artery) zones using radioactive microspheres. Myocardial infarct size was determined with triphenyltetrazolium chloride staining at the completion of each experiment as previously described.\textsuperscript{4} End-tidal concentrations of isoflurane were measured at the tip of the endotracheal tube by an infrared anesthetic gas analyzer. The canine minimum alveolar concentration value of isoflurane used in the present investigation was 1.28%.\textsuperscript{14} Hemodynamic data were continuously monitored throughout the experiment, recorded on a polygraph, and digitized using a computer interfaced with an analog-to-digital converter.

#### Experimental Protocol

The experimental design is illustrated in figure 1. Forty-eight hours before each dog was subjected to a 60-min LAD occlusion followed by 3 h of reperfusion, they were randomly assigned to receive an intravenous bolus of vehicle (0.9% saline) or PTX (10 $\mu$g/kg; Sigma Chemical, St. Louis, MO). In four separate experimental groups, dogs pretreated with vehicle or PTX were studied in the presence or absence of administration of 1.0 minimum alveolar concentration isoflurane (end-tidal concentration) that was discontinued immediately before the 60-min LAD occlusion. These experiments tested the hypothesis that isoflurane-mediated myocardial protection involves activation of $G_i$ proteins. In two additional groups of experiments, vehicle- or PTX-pretreated dogs received intravenous nicorandil (100 $\mu$g/kg bolus and 10 $\mu$g $\cdot$ kg$^{-1} \cdot$ min$^{-1}$ infusion) initiated 15 min before LAD occlusion and discontinued at the onset of reperfusion. This dose of nicorandil has been previously shown to reduce myocardial infarct size in the absence of systemic hemodynamic effects in dogs.\textsuperscript{15} These experiments tested the hypothesis that direct activation of $K_{\text{ATP}}$ channels protects ischemic myocardium independent of $G_i$ proteins. At the completion of each experiment, dogs received intravenous injections of acetylcholine (4 and 10 $\mu$g/kg), and mean arterial and LV pressure responses were recorded. The latter experiments verified the efficacy of PTX-induced $G_i$-protein inhibition, as previously described.\textsuperscript{9,16}

#### Statistical Analysis

Statistical analysis of data within and between groups was performed using multiple analysis of variance for repeated measures with \textit{post hoc} analysis by the Student $t$ test with Bonferroni’s correction for multiplicity. Changes within and between groups were considered statistically significant at $P < 0.05$. All data are expressed as mean $\pm$ SEM.

### Results

Forty-three dogs were instrumented to obtain 38 successful experiments. Two dogs were excluded because...
of intractable ventricular fibrillation during LAD occlusion or reperfusion (one control; one PTX plus nicorandil). Two dogs were excluded because transmural coronary collateral blood flow exceeded 0.2 ml \( \cdot \) min\(^{-1} \cdot \) g\(^{-1}\) (one control; one PTX plus isoflurane). One dog (control) was excluded because of the presence of heartworms.

**Systemic Hemodynamics**

Pertussis toxin pretreatment significantly \( (P < 0.05) \) reduced mean arterial and LV systolic pressures at baseline (table 1). No other differences in baseline systemic hemodynamics were observed between experimental groups. Isoflurane decreased heart rate, mean arterial and LV systolic pressures, rate-pressure product, and LV +dP/dt\(_{\text{max}}\). Isoflurane produced similar hemodynamic effects in the presence and absence of PTX pretreatment. Nicorandil caused minimal cardiovascular effects. LAD occlusion increased LV end-diastolic pressure in all experimental groups. Hemodynamics were similar between groups during LAD occlusion and reperfusion.

**Regional Myocardial Perfusion**

Transmural myocardial blood flow in the ischemic (LAD) and normal (left circumflex coronary artery) regions is summarized in table 2. There were no intergroup differences in myocardial blood flow before, during, or after LAD occlusion.

**Myocardial Infarct Size**

The area at risk was similar between groups (control, 40 ± 3%; isoflurane alone, 38 ± 2%; PTX alone, 42 ± 2%; PTX and isoflurane, 42 ± 3%; nicorandil, 45 ± 1%; PTX and nicorandil, 40 ± 2% of the LV). Isoflurane significantly \( (P < 0.05) \) reduced myocardial infarct size to 7 ± 2% of the area at risk (fig. 2) compared with control experiments (26 ± 2%). PTX pretreatment abolished the protective effects of isoflurane (21 ± 3%) but had no effect on infarct size when administered alone (31 ± 4%). Nicorandil decreased infarct size independent of PTX (11 ± 1% and 11 ± 2% in the presence and absence of PTX pretreatment, respectively; fig. 2).

---

Anesthesiology, V 92, No 5, May 2000
### ISOFLURANE AND G<sub>i</sub> PROTEINS

#### Table 1. Systemic Hemodynamics

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Preocclusion</th>
<th>30 min CAO</th>
<th>1 h</th>
<th>2 h</th>
<th>3 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (beats/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CON</td>
<td>129 ± 9</td>
<td>129 ± 9</td>
<td>123 ± 7</td>
<td>117 ± 8</td>
<td>117 ± 9</td>
<td>118 ± 10</td>
</tr>
<tr>
<td>ISO</td>
<td>134 ± 9</td>
<td>105 ± 6*</td>
<td>114 ± 8*</td>
<td>114 ± 6*</td>
<td>110 ± 6*</td>
<td>111 ± 7*</td>
</tr>
<tr>
<td>PTX</td>
<td>155 ± 3</td>
<td>155 ± 3†</td>
<td>149 ± 5</td>
<td>140 ± 11</td>
<td>136 ± 10</td>
<td>134 ± 10</td>
</tr>
<tr>
<td>PTX + ISO</td>
<td>147 ± 4</td>
<td>122 ± 5†</td>
<td>129 ± 7</td>
<td>133 ± 10</td>
<td>136 ± 9</td>
<td>133 ± 11</td>
</tr>
<tr>
<td>NIC</td>
<td>126 ± 5</td>
<td>124 ± 5</td>
<td>127 ± 4</td>
<td>122 ± 7</td>
<td>122 ± 7</td>
<td>119 ± 7</td>
</tr>
<tr>
<td>PTX + NIC</td>
<td>142 ± 4</td>
<td>140 ± 6</td>
<td>135 ± 6</td>
<td>121 ± 4*</td>
<td>119 ± 5*</td>
<td>118 ± 5*</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CON</td>
<td>94 ± 4</td>
<td>94 ± 4</td>
<td>82 ± 6</td>
<td>93 ± 7</td>
<td>97 ± 7</td>
<td>97 ± 7</td>
</tr>
<tr>
<td>ISO</td>
<td>112 ± 5</td>
<td>76 ± 3*</td>
<td>94 ± 4*</td>
<td>103 ± 3</td>
<td>107 ± 5</td>
<td>108 ± 4</td>
</tr>
<tr>
<td>PTX</td>
<td>87 ± 4</td>
<td>87 ± 4</td>
<td>88 ± 6</td>
<td>95 ± 7</td>
<td>92 ± 6</td>
<td>96 ± 4</td>
</tr>
<tr>
<td>PTX + ISO</td>
<td>86 ± 3§</td>
<td>68 ± 5†</td>
<td>86 ± 4</td>
<td>91 ± 5</td>
<td>97 ± 2</td>
<td>93 ± 3</td>
</tr>
<tr>
<td>NIC</td>
<td>100 ± 6</td>
<td>91 ± 5</td>
<td>71 ± 4*</td>
<td>88 ± 5</td>
<td>96 ± 4</td>
<td>97 ± 5</td>
</tr>
<tr>
<td>PTX + NIC</td>
<td>92 ± 4</td>
<td>89 ± 4</td>
<td>82 ± 5</td>
<td>90 ± 5</td>
<td>93 ± 8</td>
<td>89 ± 7</td>
</tr>
<tr>
<td>RPP (beats/min · mmHg&lt;sup&gt;-1&lt;/sup&gt; · 10&lt;sup&gt;-3&lt;/sup&gt;)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CON</td>
<td>13.6 ± 1.3</td>
<td>13.6 ± 1.3</td>
<td>11.1 ± 1.2*</td>
<td>11.8 ± 1.4</td>
<td>12.5 ± 1.6</td>
<td>12.6 ± 1.6</td>
</tr>
<tr>
<td>ISO</td>
<td>16.4 ± 1.0</td>
<td>9.2 ± 0.8*</td>
<td>12.0 ± 1.3*</td>
<td>12.9 ± 1.1*</td>
<td>12.9 ± 1.4*</td>
<td>13.2 ± 1.4*</td>
</tr>
<tr>
<td>PTX</td>
<td>15.8 ± 0.7</td>
<td>15.8 ± 0.7</td>
<td>14.7 ± 1.3</td>
<td>15.0 ± 2.1</td>
<td>13.9 ± 1.9</td>
<td>14.5 ± 1.7</td>
</tr>
<tr>
<td>PTX + ISO</td>
<td>15.2 ± 0.8</td>
<td>10.2 ± 1.0†</td>
<td>13.0 ± 1.3</td>
<td>14.0 ± 1.6</td>
<td>14.9 ± 1.3</td>
<td>14.2 ± 1.5</td>
</tr>
<tr>
<td>NIC</td>
<td>13.8 ± 1.4</td>
<td>12.6 ± 1.3</td>
<td>9.8 ± 0.8*</td>
<td>11.6 ± 0.9</td>
<td>12.5 ± 1.0</td>
<td>12.4 ± 1.1</td>
</tr>
<tr>
<td>PTX + NIC</td>
<td>14.7 ± 0.9</td>
<td>14.0 ± 0.9</td>
<td>12.0 ± 1.1*</td>
<td>11.7 ± 1.0*</td>
<td>11.9 ± 1.5*</td>
<td>11.4 ± 1.4*</td>
</tr>
<tr>
<td>LVSP (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CON</td>
<td>103 ± 4</td>
<td>103 ± 4</td>
<td>87 ± 7</td>
<td>97 ± 8</td>
<td>102 ± 9</td>
<td>102 ± 9</td>
</tr>
<tr>
<td>ISO</td>
<td>120 ± 5</td>
<td>85 ± 3*</td>
<td>101 ± 5*</td>
<td>110 ± 4</td>
<td>114 ± 6</td>
<td>116 ± 5</td>
</tr>
<tr>
<td>PTX</td>
<td>99 ± 3§</td>
<td>99 ± 3</td>
<td>95 ± 5</td>
<td>103 ± 6</td>
<td>100 ± 5</td>
<td>105 ± 4</td>
</tr>
<tr>
<td>PTX + ISO</td>
<td>99 ± 3§</td>
<td>81 ± 4†</td>
<td>98 ± 4</td>
<td>108 ± 8</td>
<td>110 ± 4</td>
<td>108 ± 3</td>
</tr>
<tr>
<td>NIC</td>
<td>105 ± 5</td>
<td>97 ± 5</td>
<td>72 ± 3§</td>
<td>90 ± 3*</td>
<td>102 ± 4</td>
<td>101 ± 6</td>
</tr>
<tr>
<td>PTX + NIC</td>
<td>104 ± 4</td>
<td>99 ± 5</td>
<td>90 ± 7</td>
<td>95 ± 6</td>
<td>98 ± 9</td>
<td>95 ± 9</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CON</td>
<td>6 ± 1</td>
<td>6 ± 1</td>
<td>13 ± 2*</td>
<td>14 ± 2*</td>
<td>14 ± 2*</td>
<td>13 ± 2</td>
</tr>
<tr>
<td>ISO</td>
<td>8 ± 2</td>
<td>9 ± 1</td>
<td>13 ± 1*</td>
<td>11 ± 1</td>
<td>12 ± 1</td>
<td>11 ± 1</td>
</tr>
<tr>
<td>PTX</td>
<td>8 ± 1</td>
<td>8 ± 1</td>
<td>16 ± 2*</td>
<td>15 ± 4</td>
<td>14 ± 2</td>
<td>15 ± 2</td>
</tr>
<tr>
<td>PTX + ISO</td>
<td>6 ± 1</td>
<td>7 ± 1</td>
<td>15 ± 2*</td>
<td>11 ± 2</td>
<td>11 ± 2</td>
<td>12 ± 3</td>
</tr>
<tr>
<td>NIC</td>
<td>9 ± 2</td>
<td>8 ± 2</td>
<td>13 ± 2*</td>
<td>12 ± 2</td>
<td>13 ± 2</td>
<td>13 ± 2</td>
</tr>
<tr>
<td>PTX + NIC</td>
<td>4 ± 1</td>
<td>4 ± 1</td>
<td>13 ± 2*</td>
<td>10 ± 3</td>
<td>6 ± 1</td>
<td>7 ± 1</td>
</tr>
<tr>
<td>+dP/dt&lt;sub&gt;max&lt;/sub&gt; (mmHg/s)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CON</td>
<td>1920 ± 220</td>
<td>1920 ± 220</td>
<td>1420 ± 200*</td>
<td>1410 ± 120*</td>
<td>1400 ± 130*</td>
<td>1360 ± 140*</td>
</tr>
<tr>
<td>ISO</td>
<td>1950 ± 190</td>
<td>1120 ± 80†</td>
<td>1520 ± 110*</td>
<td>1300 ± 120*</td>
<td>1350 ± 160*</td>
<td>1390 ± 150*</td>
</tr>
<tr>
<td>PTX</td>
<td>2340 ± 250</td>
<td>2340 ± 250</td>
<td>1760 ± 200</td>
<td>1540 ± 150*</td>
<td>1530 ± 120*</td>
<td>1470 ± 120*</td>
</tr>
<tr>
<td>PTX + ISO</td>
<td>2010 ± 260</td>
<td>1140 ± 100‡</td>
<td>1580 ± 160</td>
<td>1490 ± 140*</td>
<td>1540 ± 160*</td>
<td>1450 ± 160*</td>
</tr>
<tr>
<td>NIC</td>
<td>1550 ± 150</td>
<td>1580 ± 130</td>
<td>1150 ± 90*</td>
<td>1350 ± 100</td>
<td>1350 ± 90*</td>
<td>1260 ± 80</td>
</tr>
<tr>
<td>PTX + NIC</td>
<td>2370 ± 230</td>
<td>2450 ± 210∥</td>
<td>1810 ± 230*</td>
<td>1430 ± 100*</td>
<td>1410 ± 160*</td>
<td>1430 ± 100*</td>
</tr>
</tbody>
</table>

Data are mean ± SEM.

* Significantly (P < 0.05) different from baseline.
† Significantly (P < 0.05) different from the corresponding value in dogs receiving saline (CON).
‡ Significantly (P < 0.05) different from the corresponding value in dogs pretreated with pertussis toxin alone (PTX).
§ Significantly (P < 0.05) different from the corresponding value in dogs receiving isoflurane alone (ISO).
∥ Significantly (P < 0.05) different from the corresponding value in dogs receiving nicorandil alone (NIC).

CAO — coronary artery occlusion; HR — heart rate; MAP — mean aortic blood pressure; RPP — rate-pressure product; LVSP and LVEDP — left ventricular systolic and end-diastolic pressures, respectively; +dP/dt<sub>max</sub> — maximal rate of increase of left ventricular pressure; CON — control; ISO — isoflurane; PTX — pertussis toxin; NIC — nicorandil.

Anesthesiology, V 92, No 5, May 2000
Acetylcholine-induced Hypotension

Mean arterial pressure responses to acetylcholine are depicted in figures 3 and 4. Acetylcholine (4 and 10 μg/kg) decreased mean arterial pressure in control experiments (65 ± 5% and 62 ± 6% of baseline values, respectively). Pretreatment with PTX significantly attenuated these effects.

Table 2. Transmural Myocardial Blood Flow in the Ischemic and Normal Region (ml · min⁻¹ · g⁻¹)

<table>
<thead>
<tr>
<th></th>
<th>Preocclusion</th>
<th>Coronary Artery Occlusion</th>
<th>Reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ischemic region</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CON</td>
<td>0.95 ± 0.11</td>
<td>0.06 ± 0.01*</td>
<td>1.70 ± 0.25*</td>
</tr>
<tr>
<td>ISO</td>
<td>0.72 ± 0.08</td>
<td>0.07 ± 0.02*</td>
<td>1.10 ± 0.16</td>
</tr>
<tr>
<td>PTX</td>
<td>1.20 ± 0.14</td>
<td>0.09 ± 0.02*</td>
<td>1.64 ± 0.29</td>
</tr>
<tr>
<td>PTX + ISO</td>
<td>0.82 ± 0.12</td>
<td>0.11 ± 0.02*</td>
<td>1.51 ± 0.15</td>
</tr>
<tr>
<td>NIC</td>
<td>0.87 ± 0.10</td>
<td>0.08 ± 0.01*</td>
<td>1.42 ± 0.22*</td>
</tr>
<tr>
<td>PTX + NIC</td>
<td>1.30 ± 0.27</td>
<td>0.10 ± 0.01*</td>
<td>1.23 ± 0.13</td>
</tr>
<tr>
<td><strong>Normal region</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CON</td>
<td>1.35 ± 0.30</td>
<td>1.21 ± 0.12</td>
<td>1.37 ± 0.19</td>
</tr>
<tr>
<td>ISO</td>
<td>0.96 ± 0.11</td>
<td>0.96 ± 0.11</td>
<td>1.09 ± 0.13</td>
</tr>
<tr>
<td>PTX</td>
<td>1.58 ± 0.19</td>
<td>1.41 ± 0.23</td>
<td>1.15 ± 0.24</td>
</tr>
<tr>
<td>PTX + ISO</td>
<td>1.02 ± 0.14</td>
<td>1.11 ± 0.08</td>
<td>1.19 ± 0.15</td>
</tr>
<tr>
<td>NIC</td>
<td>1.14 ± 0.16</td>
<td>0.94 ± 0.05</td>
<td>0.90 ± 0.06</td>
</tr>
<tr>
<td>PTX + NIC</td>
<td>1.63 ± 0.25</td>
<td>1.34 ± 0.22</td>
<td>0.93 ± 0.09*</td>
</tr>
</tbody>
</table>

Data are mean ± SEM.
* Significantly (P < 0.05) different from preocclusion.
CON = control; ISO = isoflurane; PTX = pertussis toxin; NIC = nicorandil.

Discussion

Experimental evidence indicates that volatile anesthetics exert protective actions during ischemia and reperfusion by activating KATP channels.4–7 Opening of KATP channels has also been shown to play a pivotal role in mediating the protective effects of ischemic preconditioning (IPC).17 These findings suggest that the intracellular signal transduction pathways responsible for both APC and IPC may be similar. The mechanism by which APC or IPC activates the KATP channel is incompletely understood. It has been proposed that IPC causes activation of adenosine A₁ receptors, which are coupled to G₁ proteins. Activation of PKC18,19 may subsequently...

Fig. 2. Histograms depicting myocardial infarct size as a percentage of area at risk in dogs pretreated with vehicle (control, left) and pertussis toxin (right).

Fig. 3. Alterations in left ventricular (LV) pressure tracings recorded during administration of intravenous acetylcholine in representative dogs pretreated with vehicle (control, left) and pertussis toxin (right).

Anesthesiology, V 92, No 5, May 2000
phosphorylate and enhance K\textsubscript{ATP} channel opening by decreasing its sensitivity to inhibition by ATP.\textsuperscript{20} The beneficial effects of IPC are abolished by pharmacologic blockade of A\textsubscript{1} receptors,\textsuperscript{17} G\textsubscript{i} proteins,\textsuperscript{9,21,22} and PKC.\textsuperscript{23} Blockade of both A\textsubscript{1} receptors and PKC also attenuates the protective effects of isoflurane to enhance recovery of stunned myocardium\textsuperscript{1,2} and blocks the protective effects of isoflurane\textsuperscript{3,5} and halothane\textsuperscript{3} during experimental myocardial infarction. These findings suggest that G\textsubscript{i} proteins may also be involved in signal transduction during APC.

The present results indicate that G\textsubscript{i} proteins are an essential element of isoflurane-induced K\textsubscript{ATP} channel activation. G\textsubscript{i}-protein blockade with PTX alone did not alter myocardial infarct size but completely abolished the protective effects of isoflurane independent of the alterations of systemic hemodynamics produced by this volatile agent. In contrast, PTX pretreatment did not block the protective effects of the direct K\textsubscript{ATP}-channel agonist nicorandil. These findings indicate that K\textsubscript{ATP} channels remain functionally intact during inhibition of G\textsubscript{i} proteins, and direct stimulation of these channels by nicorandil, at a site presumably independent of the ATP inhibition site, is capable of producing a cardioprotective effect. Thus, the present findings support the contention that volatile anesthetics may activate K\textsubscript{ATP} channels through second messengers.

The direct effects of volatile anesthetics on the K\textsubscript{ATP} channel \textit{in vitro} remain unclear. Using patch-clamp techniques in rabbit ventricular myocytes, Han \textit{et al.}\textsuperscript{24} demonstrated that isoflurane directly inhibits K\textsubscript{ATP}-channel activity but paradoxically increases the probability of K\textsubscript{ATP}-channel opening. The latter action probably occurred through an effect of isoflurane to decrease channel sensitivity to inhibition by ATP. Adenosine has also been shown to enhance K\textsubscript{ATP}-channel opening by altering channel sensitivity to ATP, and this effect is mediated by activation of A\textsubscript{1} receptors and G\textsubscript{i} proteins.\textsuperscript{25,26} Recent evidence indicates that isoflurane does not potentiate K\textsubscript{ATP}-channel activity in the presence of adenosine in a cell-free environment\textsuperscript{27} but increases K\textsubscript{ATP}-channel current in whole ventricular myocytes. These data suggest that cellular mechanisms underlying anesthetic-induced activation of K\textsubscript{ATP} channels require the presence of an intracellular second messenger system. The present results support the latter hypothesis because blockade of G\textsubscript{i} proteins abolished the protective effects of isoflurane but not the actions of the direct K\textsubscript{ATP}-channel agonist nicorandil, whose actions are thought to occur at a site distinct from the ATP regulatory site.\textsuperscript{10,26}

Pertussis toxin was used in the present investigation to block G\textsubscript{i} proteins, and the mechanism and duration of action of this toxin have been previously characterized. Endoh \textit{et al.}\textsuperscript{28} demonstrated in isolated rat atria that intravenous administration of PTX blocked the G\textsubscript{i} protein-mediated negative chronotropic and inotropic effects of the muscarinic cholinergic agonist carbachol. These effects were time-dependent and most pronounced 48 h after administration. Accordingly, several \textit{in vitro}\textsuperscript{22,29,30} and \textit{in vivo}\textsuperscript{9,16,21,31} studies have used PTX to block G\textsubscript{i} proteins, and the dose used in the present investigation has been shown to be effective in the dog.\textsuperscript{16} The efficacy of PTX to block G\textsubscript{i} proteins was confirmed in the present investigation using an acetylcholine challenge, as previously described.\textsuperscript{9,16} Intravenous acetylcholine causes pronounced decreases in arterial pressure mediated through activation of G\textsubscript{i} proteins, and pretreatment with PTX modulates this response. We observed marked attenuation of acetylcholine-induced decreases in arterial pressure in PTX-pretreated dogs, indicating that G\textsubscript{i} proteins were effectively blocked in the present investigation. PTX pretreatment is well tolerated in a variety of animal species, but decreases in baseline arterial pressure are often observed.\textsuperscript{9,31,32} In the present investigation, PTX pretreatment also caused slight decreases in baseline mean arterial and LV systolic pressures. However, it is unlikely that these small hemodynamic changes were responsible for the failure of isoflurane to reduce myocardial infarct size in PTX-pretreated dogs.
The present findings must be interpreted within the constraints of several other potential limitations. Isoflu-
urane-induced decreases in heart rate, mean arterial pres-
sure, and myocardial contractility may have caused fa-
vorable alterations in myocardial oxygen supply–
demand relations and contributed to a reduction in in-
farct size. However, blockade of G_i proteins with PTX
completely abolished the protective effect of isoflurane
without affecting the hemodynamic actions of this anes-
thetic agent. Nevertheless, coronary venous oxygen ten-
sion was not measured and myocardial oxygen con-
sumption was not directly quantified in the present
investigation. Interpretation of the present findings
should also be qualified because only a single end-tidal
concentration of isoflurane was used. Higher inspired
concentrations of isoflurane may have produced reduc-
tions of myocardial infarct size via effects on K_{ATP} chan-
nels despite pretreatment with PTX. Experiments with
nicorandil were completed as positive controls to dem-
strate that PTX does not prevent direct K_{ATP} channel
activation and reductions of myocardial infarct size. Nic-
orandil possesses nitrate-like characteristics that could
contribute to cardioprotection independent of K_{ATP}
channels. However, Mizumura et al. demonstrated that
the infarct size–reducing effect of nicorandil is specific-
ally mediated by activation of K_{ATP} channels in vivo
and is not blocked by nitric oxide inhibition with meth-
ylene blue. Nicorandil has also been shown to activate
mitochondrial K_{ATP} channels, and these channels have
been suggested to be critical mediators of ischemic pre-
conditioning. The subcellular location (sarcogolmem
al ves mitochondrial) of K_{ATP} channels modulated by
isoflurane is unknown, but preliminary results with desflurane suggest that mitochondrial K_{ATP} channels
are also involved in anesthetic-mediated myocardial
protection. Whether PTX-induced G_i-protein inhibi-
tion differentially alters sarcosomal versus mitochondrial K_{ATP} channel–linked cardioprotective mecha-
nisms is unknown.

In summary, the present results indicate that G_i pro-
teins play a critical role in isoflurane-mediated reduc-
tions of experimental myocardial infarct size in dogs and
support the contention that ischemic preconditioning
and volatile anesthetics activate similar signal transduc-
tion pathways.

The authors thank Drs. Werner List and Helfried Metzler (Depart-
ment of Anesthesiology and Intensive Care Medicine, University of
Graz, Austria) for their gracious support, and David Schwabe for
technical assistance.

Anesthesiology, V 92, No 5, May 2000

References
2. Toller WG, Montgomery MW, Pagel PS, Hettrick DA, Warltier DC, Kersten JR: Isoflurane-enhanced recovery of canine stunned myocar-
3. Cope DK, Impastato WK, Cohen MV, Downey JM: Volatile anes-
thesics protect the ischemic rabbit myocardium from infarction. Anes-
thesiology 1997; 86:699–709
4. Kersten JR, Schmeling TJ, Pagel PS, Gross GJ, Warltier DC: Isoflu-
rane mimics ischemic preconditioning via activation of K_{ATP} channels: Reduction of myocardial infarct size with an acute memory phase. Anesthesiology 1997; 87:361–70
5. Ismaeil MS, Tkachenko I, Gamperl AK, Hickey RF, Cason BA: Mechanisms of isoflurane-induced myocardial preconditioning in rab-
bits. Anesthesiology 1999; 90:812–21
6. Toller WG, Kersten JR, Pagel PS, Hettrick DA, Warltier DC: Sevoflurane reduces myocardial infarct size and decreases the time
threshold for ischemic preconditioning in dogs. Anesthesiology 1999;
91:1457–64
7. Toller WG, Gross ER, Gross GJ, Kersten JR, Pagel PS, Warltier DC: Mitochondrial adenosine triphosphate-sensitive potassium (K_{ATP})
channels mediate the cardioprotective effects of desflurane (abstract). Anesthesiology 1999; 91:625
9. Schultz JE, Hsu AK, Barbieri JT, Li PL, Gross GJ: Pertussis toxin abolishes the cardioprotective effect of ischemic preconditioning in int-
12. Committee to Revise the Guide for the Care and Use of Laboratory Animals: Clark JD, Baldwin RL, Bayne KA, Brown MJ, Gebhart GF,
Gonder JC, Gwathmey JK, Keeling ME, Kohn DF, Robb JW, Smith OA,
Steggerda JD, Vandenbergh JG, White WJ, Williams-Bängero S, Vande-
Berg JL: Guide for the Care and Use of Laboratory Animals. Edited by
17. Auchampach JA, Gross GJ: Adenosine A_1 receptors, K_{ATP} chan-
nels, and ischemic preconditioning in dogs. Am J Physiol 1993; 264:
H1327–36
18. Hu K, Li GR, Nattel S: Adenosine-induced activation of ATP-
sensitive K⁺ channels in excised membrane patches is mediated by PKC. Am J Physiol 1999; 276:H488–95
27. Fujimoto K, Bosnjak ZJ, Kwok WM: Effect of isoflurane on K_{ATP} channels activated by adenosine (abstract). Anesthesiology 1999; 91: A328
34. Sato T, Sasaki N, O’Rourke B, Marban E: Nicorandil activates mitochondrial ATP-dependent potassium channels in rabbit ventricular cells (abstract). Circulation 1998; 98(Suppl):A1799