Isoflurane-enhanced Recovery of Canine Stunned Myocardium

Role for Protein Kinase C?

Wolfgang G. Toller, M.D., D.E.A.A.,* Matthew W. Montgomery, B.S., † Paul S. Pagel, M.D., Ph.D.,‡ Douglas A. Hettrick, Ph.D.,§ David C. Warttir, M.D., Ph.D.,¶ Judy R. Kersten, M.D.‖

Background: Isoflurane enhances the functional recovery of postischemic, reperfused myocardium by activating adenosine A1 receptors and adenosine triphosphate–regulated potassium channels. Whether protein kinase C is involved in this process is unknown. The authors tested the hypothesis that inhibition of protein kinase C, using the selective antagonist bisindolylmaleimide, attenuates isoflurane-enhanced recovery of stunned myocardium in dogs.

Methods: Fifty dogs were randomly assigned to receive intracoronary vehicle or bisindolylmaleimide (2 or 8 µg/min) in the presence or absence of isoflurane (1 minimum alveolar concentration). Five brief (5 min) coronary artery occlusions interspersed with 5-min reperfusion periods followed by 180 min of final reperfusion were used to produce myocardial stunning. Hemodynamics, regional segment shortening, and myocardial blood flow (radioactive microspheres) were measured at selected intervals.

Results: There were no differences in baseline hemodynamics, segment shortening, or coronary collateral blood flow between groups. Isoflurane significantly (P < 0.05) decreased heart rate, mean arterial pressure, rate pressure product, and the maximum rate of increase of left ventricular pressure (+dP/dt max) in the presence or absence of bisindolylmaleimide. Sustained contractile dysfunction was observed in dogs that received vehicle (recovery of segment shortening to 12 ± 8% of baseline), in contrast to those that received isoflurane (75 ± 7% recovery). Bisindolylmaleimide at a dose of 2 µg/min alone enhanced recovery of segment shortening (50 ± 7% of baseline) compared with vehicle-pretreated dogs, and isoflurane in the presence of 2 µg/min bisindolylmaleimide further enhanced recovery of contractile function (79 ± 8% of baseline). In contrast, 8 µg/min bisindolylmaleimide alone (32 ± 12%) or combined with isoflurane (37 ± 17%) did not enhance recovery of segment shortening compared with vehicle-pretreated dogs.

Conclusions: The results indicate that protein kinase C inhibition using low doses of bisindolylmaleimide alone produces cardioprotection, and isoflurane further enhances this protection. In contrast, high doses of bisindolylmaleimide are not cardioprotective in the presence or absence of isoflurane. A role for protein kinase C during isoflurane-induced recovery of the stunned myocardium cannot be excluded. (Key words: Bisindolylmaleimide; myocardial ischemia; volatile anesthetics.)

BRIEF, repetitive episodes of myocardial ischemia and reperfusion cause myocardial stunning, which is prolonged reversible contractile dysfunction without tissue necrosis.1,2 Isoflurane enhances the recovery of stunned myocardium when it is administered before or during myocardial ischemia,3-9 but the signal transduction cascade responsible for this myocardial protection has not been completely defined. Previously, we showed that isoflurane-enhanced recovery of postischemic, reperfused myocardium is mediated by activation of adenosine type 1 (A1) receptors9 and adenosine triphosphate–regulated potassium channels.4-8,10 Recent evidence indicates that protein kinase C (PKC) activation may occur as a result of adenosine A1 receptor stimulation and may directly modulate adenosine triphosphate–regulated potassium channel activity in vitro.11-14 These actions may account for the cardioprotective effects of PKC activation during ischemic preconditioning in vivo.15,16 Increased PKC activity has been implicated in anesthetic-induced reductions of myocardial blood flow,17-20 but the role of PKC in isoflurane-enhanced recovery of myocardial stunning is unknown.21-24

* Research Fellow in Anesthesiology.
† Medical Student.
‡ Associate Professor of Anesthesiology.
§ Assistant Professor of Anesthesiology.
‖ Professor of Anesthesiology, Pharmacology and Toxicology, and Medicine (Division of Cardiovascular Diseases), and Vice Chairman for Research of Anesthesiology.

Received from the Departments of Anesthesiology, Pharmacology and Toxicology, and Medicine (Division of Cardiovascular Diseases), the Medical College of Wisconsin and the Zablocki VA Medical Center, Milwaukee, Wisconsin. Submitted for publication November 25, 1998. Accepted for publication April 6, 1999. Supported in part by grants HL 03690 (to Dr. Kersten) and HL 54280 (to Dr. Warltier) and by Anesthesia Research Training grant GM 08377 (to Dr. Warltier) from the United States Public Health Service, Bethesda, Maryland. Dr. Toller received a Max Kade Research Fellowship from the Austrian Science Foundation, Vienna, Austria.

Address reprint requests to Dr. Kersten: Department of Anesthesiology, MEB 462C, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, Wisconsin 53226. Address electronic mail to: jkersten@mcw.edu

Anesthesiology, V 91, No 3, Sep 1999
infarct size, but the role of PKC in enhanced functional recovery of the stunned myocardium produced by isoflurane or other volatile agents is unknown. We tested the hypothesis that inhibition of PKC, using the selective antagonist bisindolylmaleimide, attenuates isoflurane-enhanced recovery of stunned myocardium in barbiturate-anesthetized, acutely instrumented dogs.

**Materials and 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 *Guiding Principles in the Care and Use of Animals* of the American Physiologic Society and were performed in accordance with the *Guide for the Care and Use of Laboratory Animals* (Washington, DC, National Academy Press, 1996).

**General Preparation**

The experimental methods used in the current investigation have been described in detail previously. Briefly, mongrel dogs of either sex (weight, 26 ± 1 kg [mean ± SEM]) were fasted overnight, anesthetized with sodium barbital (200 mg/kg) and sodium pentobarbital (15 mg/kg), and ventilated with an air-and-oxygen mixture (fraction of inspired oxygen = 0.25) after endotracheal intubation. Tidal volume and respiratory rate were adjusted to maintain arterial blood gas tensions within a physiological range (pH = 7.36–7.43; carbon dioxide tension = 28–34 mmHg). A double pressure transducer-tipped catheter was inserted into the left ventricle and aorta through the left carotid artery to measure left ventricular (LV) and aortic pressures, respectively. The maximum rate of increase of LV pressure (+dP/dtmax) was obtained by differentiating the LV pressure waveform. A thoracotomy was performed in the left fifth intercostal space, and a transit-time ultrasonic flow probe was placed around the ascending aorta to measure relative (less coronary blood flow) cardiac output. A 1.5- to 2-cm portion of the left anterior descending coronary artery (LAD) immediately distal to the first diagonal branch was isolated, and a precalibrated Doppler ultrasonic flow transducer and a silk ligature were positioned around the vessel to measure coronary blood flow velocity and to produce coronary artery occlusion and reperfusion, respectively. Heparin-filled catheters were inserted into the LAD (via a small catheter [PE-20] that was inserted over a needle [24 gauge; Angiocath; Becton Dickinson, Sandy, UT]), the left atrial appendage, and a femoral vein for intracoronary drug, radioactive microsphere, and fluid administration, respectively. The right femoral artery was cannulated for the withdrawal of reference blood flow samples. A pair of ultrasonic segment-length transducers was implanted in the LAD region to measure regional contractile function using the formula: %regional segment shortening = (EDL − ESL) · 100 · EDL−1, where %segment shortening = the percentage of segment shortening, EDL = end-diastolic segment length, and ESL = end-systolic segment length. Regional myocardial perfusion was measured in the ischemic (LAD) and normal (left circumflex coronary artery) regions using the radioactive microsphere technique. Relative diastolic coronary vascular resistance was calculated as the ratio of end-diastolic arterial pressure to peak diastolic coronary blood flow velocity. Hemodynamic data were monitored continuously during the experiment, recorded with a polygraph, and digitized via a computer interfaced with an analog-to-digital converter.

**Experimental Protocol**

Figure 1 shows the experimental protocol. After the surgical preparation was completed, dogs were assigned randomly to receive intracoronary vehicle (0.5 ml dimethylsulfoxide diluted in 8 ml saline, 0.9%) or bisindolylmaleimide (dissolved in 0.5 ml dimethylsulfoxide and diluted with saline, 0.9%, to a volume of 8 ml) in the presence or absence of isoflurane (1 minimum alveolar end-tidal concentration) in six separate groups of experiments (fig. 1). All dogs were subjected to five brief (5 min) coronary occlusions interspersed with 5-min periods of reperfusion, followed by a final 180-min period of reperfusion to produce stunned myocardium. In all groups, intracoronary drug infusion (0.1 ml/min) was started 30 min before the first LAD occlusion, maintained throughout all five occlusions, and discontinued at the end of the fifth occlusion (total infusion time = 75 min). Two groups of dogs received 2 μg/min bisindolylmaleimide during a period of 75 min (total dose = 150 μg; calculated intracoronary concentration of 100 nm, assuming an LAD blood flow of approximately 40 ml/min). Another two groups of dogs received 8 μg/min bisindolylmaleimide during a period of 75 min (total dose = 600 μg; calculated intracoronary concentration of 400 nm).

**Statistical Analyses**

Statistical analysis of data within and between groups was performed with multivariate analyses of variance for...
repeated measures followed by application of the Student \( t \) test with Bonferroni correction for multiplicity. Changes in segment shortening were analyzed using the Wilcoxon signed rank sum test. Changes within and between groups were considered significant when \( P < 0.05 \). All data are expressed as the mean ± SEM.

Results

Sixty-four dogs were instrumented to provide 50 successful experiments. Ten dogs were excluded from data analysis because transmural coronary collateral blood flow exceeded 0.3 ml min\(^{-1}\) g\(^{-1}\) (two vehicle, three vehicle and isoflurane, two low-dose bisindolylmaleimide, one low-dose bisindolylmaleimide and isoflurane, one high-dose bisindolylmaleimide, and one high-dose bisindolylmaleimide and isoflurane). Four additional dogs were excluded because of myocardial ischemia during the surgical preparation (one vehicle, two vehicle and isoflurane, and one high-dose bisindolylmaleimide).

Hemodynamic Effects of Myocardial Stunning

Systemic and coronary hemodynamics were similar among the groups under baseline conditions. Left anterior descending coronary artery occlusion increased LV end-diastolic pressure and decreased \(+\text{dP/dt}\)max and cardiac output in vehicle-treated dogs (table 1). Decreases in contractile performance were sustained in vehicle-treated dogs and were accompanied by increases in systemic and diastolic coronary vascular resistance after 180 min of reperfusion. In contrast to findings during vehicle alone, isoflurane caused significant (\( P < 0.05 \)) decreases in heart rate, mean arterial and LV systolic pressures, rate pressure product, \(+\text{dP/dt}\)max, and systemic vascular resistance (table 2). Compared with baseline values, LAD occlusion caused similar increases in LV end-diastolic pressure and decreases in \(+\text{dP/dt}\)max and cardiac output in the presence or absence of isoflurane. However, hemodynamics returned to baseline values after 180 min of reperfusion in dogs receiving isoflurane, in contrast to findings during drug vehicle alone.

Bisindolylmaleimide produced no hemodynamic effects (tables 3 and 4), and LAD occlusion caused similar hemodynamic effects in dogs that received bisindolylmaleimide or drug vehicle. In addition, bisindolylmaleimide did not alter the systemic or coronary hemodynamic effects of isoflurane (tables 5 and 6), except that sustained reductions in \(+\text{dP/dt}\)max and cardiac output were observed in all bisindolylmaleimide-treated dogs after 180 min of reperfusion.

Contractile Function during Myocardial Stunning

There were no differences in segment shortening among the experimental groups under baseline conditions or during brief LAD occlusions and reperusions. Sustained contractile dysfunction was observed in vehicle-pretreated dogs after 180 min of reperfusion (12 ± 8% of baseline; fig. 2). In contrast, dogs that received isoflurane had enhanced recovery of segment shortening after 60 min (table 2) and achieved recovery to 75 ± 7% of baseline after 180 min of reperfusion. Bisindolylmaleimide alone enhanced recovery of contractile function in the low-dose but not the high-dose groups. However, no differences in recovery were observed between the low- and high-dose bisindolylmaleimide groups alone. Isoflurane in the presence of low-dose bisindolylmaleimide enhanced the recovery of contractile function as early as 60 min after reperfusion (table 5) and to a similar extent compared with isoflurane alone, but it caused signifi-
Table 1. Hemodynamic Effects of Myocardial Stunning in Dogs Receiving Drug Vehicle

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Vehicle</th>
<th>5th Occlusion</th>
<th>Reperfusion (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (bpm)</td>
<td>133 ± 5</td>
<td>132 ± 5</td>
<td>127 ± 6</td>
<td>126 ± 8</td>
</tr>
<tr>
<td>MBP (mmHg)</td>
<td>91 ± 4</td>
<td>92 ± 4</td>
<td>88 ± 6</td>
<td>95 ± 8</td>
</tr>
<tr>
<td>RPP (mmHg · bpm · 10^3)</td>
<td>13.6 ± 0.7</td>
<td>13.7 ± 0.7</td>
<td>12.4 ± 1.0</td>
<td>13.5 ± 1.2</td>
</tr>
<tr>
<td>LVSP (mmHg)</td>
<td>100 ± 4</td>
<td>101 ± 4</td>
<td>97 ± 6</td>
<td>106 ± 7</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>5 ± 1</td>
<td>6 ± 1</td>
<td>13 ± 2</td>
<td>9 ± 1</td>
</tr>
<tr>
<td>RPP (mmHg · bpm · 10^3)</td>
<td>1.640 ± 160</td>
<td>1.620 ± 160</td>
<td>1.310 ± 120*</td>
<td>1.220 ± 100*</td>
</tr>
<tr>
<td>DCBV (Hz · 10^3)</td>
<td>41 ± 4</td>
<td>38 ± 4</td>
<td>—</td>
<td>38 ± 4</td>
</tr>
<tr>
<td>DCRV (mmHg · Hz · 10^3)</td>
<td>2.3 ± 0.3</td>
<td>2.4 ± 0.3</td>
<td>—</td>
<td>2.6 ± 0.4</td>
</tr>
<tr>
<td>CO (l/min)</td>
<td>2.8 ± 0.2</td>
<td>2.6 ± 0.2</td>
<td>2.2 ± 0.2*</td>
<td>2.3 ± 0.2*</td>
</tr>
<tr>
<td>SV (ml)</td>
<td>21 ± 2</td>
<td>20 ± 2</td>
<td>18 ± 2</td>
<td>19 ± 2</td>
</tr>
<tr>
<td>SS (%)</td>
<td>19 ± 2</td>
<td>17 ± 1</td>
<td>—3 ± 1*</td>
<td>4 ± 1*</td>
</tr>
</tbody>
</table>

Data are mean ± SEM; n = 9.
HR = heart rate; MBP = mean aortic blood pressure; RPP = rate pressure product; LVSP and LVEDP = left ventricular systolic and end diastolic pressure, respectively; DCBV = diastolic coronary blood flow velocity; DCRV = diastolic coronary vascular resistance; CO = cardiac output; SVR = systemic vascular resistance; SV = stroke volume; SS = segment shortening.

Table 2. Hemodynamic Effects of Myocardial Stunning in Dogs Receiving Drug Vehicle and Isoflurane

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Vehicle + Isoflurane</th>
<th>5th Occlusion</th>
<th>Reperfusion (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (bpm)</td>
<td>130 ± 7</td>
<td>107 ± 6‡</td>
<td>105 ± 5</td>
<td>104 ± 6*</td>
</tr>
<tr>
<td>MBP (mmHg)</td>
<td>101 ± 3</td>
<td>73 ± 4*</td>
<td>63 ± 3†</td>
<td>93 ± 6</td>
</tr>
<tr>
<td>RPP (mmHg · bpm · 10^3)</td>
<td>14.3 ± 1.1</td>
<td>8.8 ± 0.8†</td>
<td>7.1 ± 0.7†</td>
<td>10.6 ± 1.0*</td>
</tr>
<tr>
<td>LVSP (mmHg)</td>
<td>107 ± 4</td>
<td>77 ± 4‡</td>
<td>64 ± 4‡</td>
<td>99 ± 7</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>4 ± 1</td>
<td>7 ± 1</td>
<td>12 ± 1</td>
<td>9 ± 1*</td>
</tr>
<tr>
<td>RPP (mmHg · bpm · 10^3)</td>
<td>1,700 ± 120</td>
<td>990 ± 50†</td>
<td>840 ± 50†</td>
<td>1,190 ± 60*</td>
</tr>
<tr>
<td>DCBV (Hz · 10^3)</td>
<td>36 ± 6</td>
<td>34 ± 4</td>
<td>—</td>
<td>51 ± 9*</td>
</tr>
<tr>
<td>DCRV (mmHg · Hz · 10^3)</td>
<td>3.5 ± 1</td>
<td>2.5 ± 0.7</td>
<td>—</td>
<td>2.0 ± 0.3</td>
</tr>
<tr>
<td>CO (l/min)</td>
<td>2.2 ± 0.2</td>
<td>1.7 ± 0.2</td>
<td>1.5 ± 0.2*</td>
<td>2.1 ± 0.2</td>
</tr>
<tr>
<td>SV (ml)</td>
<td>17 ± 2</td>
<td>17 ± 3</td>
<td>15 ± 3*</td>
<td>21 ± 3</td>
</tr>
<tr>
<td>SS (%)</td>
<td>14 ± 2</td>
<td>12 ± 2</td>
<td>—4 ± 1*</td>
<td>8 ± 1*</td>
</tr>
</tbody>
</table>

Data are mean ± SEM; n = 8.
HR = heart rate; MBP = mean aortic blood pressure; RPP = rate pressure product; LVSP and LVEDP = left ventricular systolic and end diastolic pressure, respectively; DCBV = diastolic coronary blood flow velocity; DCRV = diastolic coronary vascular resistance; CO = cardiac output; SVR = systemic vascular resistance; SV = stroke volume; SS = segment shortening.

† Significantly different from baseline.
transmural myocardial perfusion or coronary collateral blood flow among the groups.

**Discussion**

Increased activity of an important family of serine-threonine kinase enzymes known collectively as PKC has been implicated in the intracellular signal transduction pathways responsible for myocardial protection during ischemia and anesthetic-induced preconditioning. Bisindolylmaleimide inhibits the activity of all known PKC isoforms, including calcium-dependent and -independent species in conventional, novel, and atypical classes. The results of the present investigation indicate that high but not low doses of bisindolylmaleimide attenuate isoflurane-enhanced functional recovery of stunned myocardium independent of alterations in coronary collateral blood flow or systemic hemodynamics. These results are similar to those of Cope et al., who found that reductions in experimental myocardial perfusion were associated with improved functional recovery.

**Table 3. Hemodynamic Effects of Myocardial Stun-}ing in Dogs Receiving 2 μg/min Bisindolylmaleimide**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>BIS</th>
<th>5th Occlusion</th>
<th>15</th>
<th>30</th>
<th>60</th>
<th>120</th>
<th>180</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (bpm)</td>
<td>131 ± 7</td>
<td>130 ± 7</td>
<td>127 ± 6</td>
<td>125 ± 6</td>
<td>125 ± 5</td>
<td>122 ± 5</td>
<td>122 ± 6</td>
<td>120 ± 7*</td>
</tr>
<tr>
<td>MBP (mmHg)</td>
<td>101 ± 6</td>
<td>98 ± 6</td>
<td>88 ± 6</td>
<td>94 ± 6</td>
<td>96 ± 5</td>
<td>98 ± 5</td>
<td>101 ± 4</td>
<td>99 ± 6</td>
</tr>
<tr>
<td>RPP (mmHg · bpm)</td>
<td>14.5 ± 1.1</td>
<td>14.0 ± 0.9</td>
<td>12.1 ± 1.0</td>
<td>12.9 ± 0.7</td>
<td>13.0 ± 0.6</td>
<td>13.2 ± 0.7</td>
<td>13.3 ± 0.8</td>
<td>13.1 ± 1.1</td>
</tr>
<tr>
<td>LVSP (mmHg)</td>
<td>110 ± 6</td>
<td>105 ± 7</td>
<td>93 ± 6*</td>
<td>104 ± 6</td>
<td>106 ± 5</td>
<td>109 ± 6</td>
<td>108 ± 4</td>
<td>107 ± 6</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>5 ± 1</td>
<td>5 ± 1</td>
<td>7 ± 2</td>
<td>5 ± 1</td>
<td>5 ± 1</td>
<td>5 ± 1</td>
<td>5 ± 1</td>
<td>5 ± 1</td>
</tr>
<tr>
<td>+dP/dt max (mmHg/s)</td>
<td>1,860 ± 140</td>
<td>1,830 ± 80</td>
<td>1,450 ± 90*</td>
<td>1,470 ± 60*</td>
<td>1,510 ± 50*</td>
<td>1,570 ± 70*</td>
<td>1,530 ± 80*</td>
<td>1,460 ± 110*</td>
</tr>
<tr>
<td>DCBFV (Hz · 10(^{-2}))</td>
<td>40 ± 3</td>
<td>41 ± 4</td>
<td>—</td>
<td>47 ± 6</td>
<td>45 ± 5</td>
<td>45 ± 6</td>
<td>46 ± 4</td>
<td>45 ± 4</td>
</tr>
<tr>
<td>DCVR (mmHg · Hz(^{-1} · 10(^{-4}))</td>
<td>2.5 ± 0.3</td>
<td>2.4 ± 0.3</td>
<td>—</td>
<td>2.1 ± 0.3</td>
<td>2.2 ± 0.3</td>
<td>2.4 ± 0.4</td>
<td>2.2 ± 0.2</td>
<td>2.2 ± 0.2</td>
</tr>
<tr>
<td>CO (l/min)</td>
<td>2.5 ± 0.4</td>
<td>2.4 ± 0.4</td>
<td>1.9 ± 0.4*</td>
<td>2.0 ± 0.4</td>
<td>2.0 ± 0.4</td>
<td>2.0 ± 0.3</td>
<td>1.7 ± 0.3*</td>
<td>1.6 ± 0.3*</td>
</tr>
<tr>
<td>SVR (dyne · s · cm(^{-5}))</td>
<td>3,900 ± 610</td>
<td>4,050 ± 710</td>
<td>4,390 ± 540</td>
<td>4,290 ± 500</td>
<td>4,560 ± 620</td>
<td>4,850 ± 930</td>
<td>5,980 ± 1120</td>
<td>5,840 ± 1010</td>
</tr>
<tr>
<td>SV (ml)</td>
<td>19 ± 3</td>
<td>19 ± 3</td>
<td>15 ± 3*</td>
<td>16 ± 3</td>
<td>16 ± 3</td>
<td>17 ± 3</td>
<td>14 ± 2*</td>
<td>14 ± 2*</td>
</tr>
<tr>
<td>SS (%)</td>
<td>17 ± 1</td>
<td>17 ± 1</td>
<td>—</td>
<td>6 ± 2*</td>
<td>8 ± 2*</td>
<td>11 ± 2</td>
<td>11 ± 2*</td>
<td>8 ± 1*\†</td>
</tr>
</tbody>
</table>

Data are mean ± SEM; n = 9. * Significantly (P < 0.05) different from baseline. † Significantly (P < 0.05) different from vehicle (table 1).

**Table 4. Hemodynamic Effects of Myocardial Stun-}ing in Dogs Receiving 8 μg/min Bisindolylmaleimide**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>BIS</th>
<th>5th Occlusion</th>
<th>15</th>
<th>30</th>
<th>60</th>
<th>120</th>
<th>180</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (bpm)</td>
<td>123 ± 4</td>
<td>119 ± 4</td>
<td>112 ± 4</td>
<td>114 ± 5</td>
<td>111 ± 4</td>
<td>108 ± 4</td>
<td>110 ± 6</td>
<td>112 ± 8</td>
</tr>
<tr>
<td>MBP (mmHg)</td>
<td>88 ± 6</td>
<td>89 ± 6</td>
<td>83 ± 9</td>
<td>95 ± 5</td>
<td>90 ± 7</td>
<td>94 ± 5</td>
<td>100 ± 5</td>
<td>99 ± 5</td>
</tr>
<tr>
<td>RPP (mmHg · bpm)</td>
<td>12.0 ± 0.7</td>
<td>11.6 ± 0.6</td>
<td>10.2 ± 1.1</td>
<td>11.7 ± 0.6</td>
<td>11.0 ± 0.9</td>
<td>11.2 ± 0.9</td>
<td>11.8 ± 0.9</td>
<td>12.0 ± 1.1</td>
</tr>
<tr>
<td>LVSP (mmHg)</td>
<td>98 ± 6</td>
<td>96 ± 6</td>
<td>89 ± 8</td>
<td>100 ± 5</td>
<td>98 ± 6</td>
<td>103 ± 4</td>
<td>103 ± 6</td>
<td>103 ± 6</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>7 ± 1</td>
<td>6 ± 1</td>
<td>10 ± 3</td>
<td>7 ± 2</td>
<td>7 ± 2</td>
<td>8 ± 2</td>
<td>8 ± 2</td>
<td>8 ± 2</td>
</tr>
<tr>
<td>+dP/dt max (mmHg/s)</td>
<td>1,760 ± 140</td>
<td>1,610 ± 100</td>
<td>1,280 ± 120*</td>
<td>1,260 ± 60*</td>
<td>1,200 ± 70*</td>
<td>1,210 ± 70*</td>
<td>1,300 ± 100*</td>
<td>1,250 ± 100*</td>
</tr>
<tr>
<td>DCBFV (Hz · 10(^{-2}))</td>
<td>51 ± 7</td>
<td>47 ± 7</td>
<td>—</td>
<td>60 ± 11</td>
<td>54 ± 10</td>
<td>49 ± 8</td>
<td>52 ± 8</td>
<td>54 ± 9</td>
</tr>
<tr>
<td>DCVR (mmHg · Hz(^{-1} · 10(^{-4}))</td>
<td>1.9 ± 0.4</td>
<td>2.2 ± 0.5</td>
<td>—</td>
<td>2.1 ± 0.7</td>
<td>2.2 ± 0.7</td>
<td>2.4 ± 0.7</td>
<td>2.4 ± 0.7</td>
<td>2.2 ± 0.5</td>
</tr>
<tr>
<td>CO (l/min)</td>
<td>2.9 ± 0.3</td>
<td>2.4 ± 0.3</td>
<td>1.9 ± 0.3*</td>
<td>1.9 ± 0.2*</td>
<td>1.9 ± 0.2*</td>
<td>1.7 ± 0.2*</td>
<td>1.6 ± 0.2*</td>
<td>1.6 ± 0.2*</td>
</tr>
<tr>
<td>SVR (dyne · s · cm(^{-5}))</td>
<td>2,490 ± 290</td>
<td>3,130 ± 410</td>
<td>3,640 ± 420</td>
<td>4,010 ± 320*</td>
<td>3,880 ± 480*</td>
<td>4,420 ± 350*</td>
<td>5,160 ± 610*</td>
<td>5,120 ± 490*</td>
</tr>
<tr>
<td>SV (ml)</td>
<td>24 ± 3</td>
<td>21 ± 3</td>
<td>17 ± 3*</td>
<td>17 ± 3</td>
<td>18 ± 2*</td>
<td>16 ± 2*</td>
<td>15 ± 1</td>
<td>15 ± 1</td>
</tr>
<tr>
<td>SS (%)</td>
<td>19 ± 1</td>
<td>18 ± 1</td>
<td>—</td>
<td>5 ± 2*</td>
<td>6 ± 2*</td>
<td>7 ± 3*</td>
<td>8 ± 3*</td>
<td>7 ± 3*</td>
</tr>
</tbody>
</table>

Data are mean ± SEM; n = 7. * Significantly (P < 0.05) different from baseline.

Anesthesiology, V 91, No 3, Sep 1999
Table 5. Hemodynamic Effects of Myocardial Stunning in Dogs Receiving 2 μg/min Bisindolylmaleimide and Isoflurane

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>BIS + Isoflurane</th>
<th>5th Occlusion</th>
<th>Reperfusion (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>15</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>123 ± 5</td>
<td>102 ± 4‡</td>
<td>103 ± 4*</td>
<td>106 ± 6*</td>
</tr>
<tr>
<td>MBP (mmHg)</td>
<td>96 ± 4</td>
<td>62 ± 4‡</td>
<td>93 ± 4</td>
<td>106 ± 3*</td>
</tr>
<tr>
<td>RPP (mmHg - bpm · 10⁻⁵)</td>
<td>13.0 ± 0.8</td>
<td>7.5 ± 0.7‡</td>
<td>10.8 ± 0.8‡</td>
<td>12.4 ± 1.1‡</td>
</tr>
<tr>
<td>LVSP (mmHg)</td>
<td>104 ± 4</td>
<td>74 ± 5‡</td>
<td>102 ± 4</td>
<td>115 ± 6</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>6 ± 1</td>
<td>8 ± 1</td>
<td>9 ± 2</td>
<td>8 ± 2</td>
</tr>
<tr>
<td>+dP/dt (mmHg/s)</td>
<td>1,790 ± 100</td>
<td>960 ± 40‡</td>
<td>1,240 ± 60*</td>
<td>1,480 ± 60*</td>
</tr>
<tr>
<td>DCBFV (Hz · 10⁻²)</td>
<td>35 ± 2</td>
<td>38 ± 4</td>
<td>51 ± 7*</td>
<td>38 ± 4</td>
</tr>
<tr>
<td>CO (l/min)</td>
<td>2.7 ± 0.2</td>
<td>2.1 ± 0.1*</td>
<td>2.3 ± 0.2</td>
<td>2.3 ± 0.2</td>
</tr>
<tr>
<td>SS (%)</td>
<td>18 ± 2</td>
<td>15 ± 2</td>
<td>10 ± 1†</td>
<td>15 ± 2†</td>
</tr>
</tbody>
</table>

Data are mean ± SEM; n = 8.

BIS = bisindolylmaleimide; HR = heart rate; MBP = mean aortic blood pressure; RPP = rate pressure product; LVSP and LVEDP = left ventricular systolic and end diastolic pressure, respectively; DCBFV = diastolic coronary blood flow velocity; DCVR = diastolic coronary vascular resistance; CO = cardiac output; SS = systolic shortening.

* Significantly (P < 0.05) different from baseline.
† Significantly (P < 0.05) different from vehicle (table 1).
‡ Significantly (P < 0.05) different from BIS 2 μg/min (table 3).

Cardiac infarct size produced by halothane in rabbits depended on PKC activation. However, we also found that PKC inhibition alone may be cardioprotective, and isoflurane further enhances the protection afforded by low doses of bisindolylmaleimide.

Low doses of bisindolylmaleimide (2 μg/min) specific for PKC inhibition do not block the protective effects of isoflurane and may independently exert cardioprotective effects. The present results are similar to the results of Tosaki et al., found in experiments that evaluated the

Table 6. Hemodynamic Effects of Myocardial Stunning in Dogs Receiving 8 μg/min Bisindolylmaleimide and Isoflurane

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>BIS + Isoflurane</th>
<th>5th Occlusion</th>
<th>Reperfusion (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>15</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>123 ± 3</td>
<td>107 ± 4†</td>
<td>107 ± 5*</td>
<td>108 ± 5*</td>
</tr>
<tr>
<td>MBP (mmHg)</td>
<td>92 ± 4</td>
<td>66 ± 5†</td>
<td>93 ± 4</td>
<td>108 ± 3*</td>
</tr>
<tr>
<td>RPP (mmHg - bpm · 10⁻⁵)</td>
<td>12.5 ± 0.6</td>
<td>8.2 ± 0.8‡</td>
<td>11.3 ± 0.8</td>
<td>12.5 ± 0.9</td>
</tr>
<tr>
<td>LVSP (mmHg)</td>
<td>101 ± 3</td>
<td>75 ± 5†</td>
<td>105 ± 4</td>
<td>112 ± 3</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>5 ± 1</td>
<td>6 ± 1</td>
<td>8 ± 1</td>
<td>8 ± 2</td>
</tr>
<tr>
<td>+dP/dt (mmHg/s)</td>
<td>1,820 ± 130</td>
<td>1,070 ± 80‡</td>
<td>1,300 ± 70*</td>
<td>1,470 ± 110*</td>
</tr>
<tr>
<td>DCBFV (Hz · 10⁻²)</td>
<td>43 ± 7</td>
<td>43 ± 7</td>
<td>48 ± 7</td>
<td>46 ± 7</td>
</tr>
<tr>
<td>CO (l/min)</td>
<td>2.5 ± 0.1</td>
<td>2.0 ± 0.2*</td>
<td>2.1 ± 0.2</td>
<td>1.8 ± 0.2*</td>
</tr>
<tr>
<td>SS (%)</td>
<td>16 ± 1</td>
<td>13 ± 1</td>
<td>7 ± 2*</td>
<td>8 ± 3*</td>
</tr>
</tbody>
</table>

Data are mean ± SEM; n = 9.

BIS = bisindolylmaleimide; HR = heart rate; MBP = mean aortic blood pressure; RPP = rate pressure product; LVSP and LVEDP = left ventricular systolic and end diastolic pressure, respectively; DCBFV = diastolic coronary blood flow velocity; DCVR = diastolic coronary vascular resistance; CO = cardiac output; SS = systolic shortening.

* Significantly (P < 0.05) different from baseline.
† Significantly (P < 0.05) different from vehicle (table 1).
‡ Significantly (P < 0.05) different from BIS 8 μg/min (table 4).

Anesthesiology, V 91, No 3, Sep 1999
role of PKC during ischemic preconditioning. The PKC antagonist, calphostin C, did not alter the protection afforded by ischemic preconditioning when administered at low concentrations (100 nM), enhanced protection at moderate concentrations (200 nM), and blocked the cardioprotective effects of ischemic preconditioning on arrhythmogenesis at high concentrations. Thus, the response to PKC inhibition using calphostin C was shown to be biphasic. Blockade of some PKC isoforms at low concentrations may have caused cardioprotective effects, whereas high concentrations may have differentially blocked the “cardioprotective” isoforms of PKC.

In the current investigation, the estimated coronary concentrations of bisindolylmaleimide used were 100 and 400 nM (low and high doses, respectively). Because the half-maximal inhibitory concentration for PKC inhibition of calphostin C and bisindolylmaleimide are similar (40 nM and 10 nM, respectively), the results of our study also may indicate that the response to PKC inhibition is biphasic. This contention is further supported by findings that PKC inhibition with bisindolylmaleimide or chelerythrine enhanced recovery of LV developed pressure and reduced infarct size in hearts subjected to global or regional ischemia and reperfusion, whereas high doses of chelerythrine abolished the cardioprotective effects of ischemic preconditioning. These findings were interpreted to indicate that cardioprotection during PKC activation is isoform specific and may be variable according to the end point of cardioprotection used.

The effects of isoflurane or other volatile anesthetic agents on the activity of specific PKC isoforms are unknown. Increases in calcium-activated force in skinned arterial smooth muscle strips produced by isoflurane were blocked by bisindolylmaleimide but not by a PKC inhibitor specific for calcium-dependent isoforms. These data suggested that isoflurane may increase the activity of e-PKC, a prominent calcium-independent PKC isoform prevalent in canine myocardium and one

---

**Table 7. Transmural Myocardial Blood Flow (ml · min⁻¹ · g⁻¹) in the Ischemic (LAD) and Normal (LCCA) Regions**

<table>
<thead>
<tr>
<th>LAD region</th>
<th>Baseline</th>
<th>5th Occlusion</th>
<th>60</th>
<th>180</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>1.06 ± 0.08</td>
<td>0.13 ± 0.02*</td>
<td>0.97 ± 0.11</td>
<td>0.91 ± 0.12</td>
</tr>
<tr>
<td>Vehicle + isoflurane</td>
<td>1.33 ± 0.23</td>
<td>0.13 ± 0.03*</td>
<td>1.30 ± 0.18</td>
<td>1.02 ± 0.08</td>
</tr>
<tr>
<td>BIS (2 µg/min)</td>
<td>1.16 ± 0.10</td>
<td>0.15 ± 0.03*</td>
<td>1.15 ± 0.18</td>
<td>1.23 ± 0.22</td>
</tr>
<tr>
<td>BIS (8 µg/min)</td>
<td>1.03 ± 0.07</td>
<td>0.13 ± 0.03*</td>
<td>1.04 ± 0.09</td>
<td>1.11 ± 0.07</td>
</tr>
<tr>
<td>BIS (2 µg/min) + isoflurane</td>
<td>1.21 ± 0.18</td>
<td>0.08 ± 0.01*</td>
<td>1.17 ± 0.24</td>
<td>1.07 ± 0.11</td>
</tr>
<tr>
<td>BIS (8 µg/min) + isoflurane</td>
<td>1.31 ± 0.15</td>
<td>0.16 ± 0.03*</td>
<td>1.32 ± 0.14</td>
<td>1.30 ± 0.17</td>
</tr>
<tr>
<td>LCCA region</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>1.06 ± 0.07</td>
<td>1.11 ± 0.07</td>
<td>1.01 ± 0.09</td>
<td>0.94 ± 0.07</td>
</tr>
<tr>
<td>Vehicle + isoflurane</td>
<td>1.26 ± 0.19</td>
<td>1.18 ± 0.16</td>
<td>1.30 ± 0.18</td>
<td>1.31 ± 0.29</td>
</tr>
<tr>
<td>BIS (2 µg/min)</td>
<td>1.27 ± 0.11</td>
<td>1.14 ± 0.08</td>
<td>1.31 ± 0.19</td>
<td>1.40 ± 0.23</td>
</tr>
<tr>
<td>BIS (8 µg/min)</td>
<td>1.01 ± 0.07</td>
<td>1.26 ± 0.18</td>
<td>1.04 ± 0.06</td>
<td>1.21 ± 0.06</td>
</tr>
<tr>
<td>BIS (2 µg/min) + isoflurane</td>
<td>1.24 ± 0.18</td>
<td>1.00 ± 0.18</td>
<td>1.36 ± 0.18</td>
<td>1.24 ± 0.18</td>
</tr>
<tr>
<td>BIS (8 µg/min) + isoflurane</td>
<td>1.27 ± 0.15</td>
<td>1.18 ± 0.14</td>
<td>1.29 ± 0.14</td>
<td>1.24 ± 0.15</td>
</tr>
</tbody>
</table>

Data are mean ± SEM.
BIS = bisindolylmaleimide; LAD = left anterior descending coronary artery; LCCA = left circumflex coronary artery.
* Significantly (P < 0.05) different from baseline.
that has been implicated as a critical mediator of ischemic preconditioning.\textsuperscript{27,28} Although such a hypothesis may be highly plausible, direct evidence linking the ε-PKC or any other specific PKC isoform to myocardial protection afforded by isoflurane has yet to be identified and represents an important goal of future research.

The myocardial signal transduction pathways responsible for endogenous protection from ischemic injury exhibit substantial biologic redundancy. Preconditioning-induced reductions of myocardial infarct size elicited through multiple brief periods of ischemia and reperfusion appear to be relatively resistant to attenuation by PKC inhibitors. In contrast, cardiac muscle preconditioned by a single brief period of ischemia may be more susceptible to pharmacologic inhibition of PKC activity.\textsuperscript{29,30} Multiple signal transduction cascades may be recruited during repetitive episodes of ischemia and reperfusion, and pharmacologic strategies using specific receptor antagonists or enzyme inhibitors of known constituents of these pathways may reveal the relative contribution of unique or redundant signaling to myocardial protection.\textsuperscript{30} Consistent with this hypothesis, we have shown that isoflurane-induced protection against reversible\textsuperscript{4,8} and irreversible\textsuperscript{10} ischemic injury is mediated by adenosine triphosphate–regulated potassium channel activation. This energy-dependent ion channel has been shown to be the putative end-effector of both ischemic and anesthetic-induced preconditioning \textit{in vitro} and is activated by various signaling processes, including adenosine A\textsubscript{1} and A\textsubscript{2a} adrenergic receptors, G\textsubscript{i/o} proteins, and PKC, to reduce ischemic damage.\textsuperscript{11,12} Evidence suggests that PKC may provide an important mechanistic link between stimulation of adenosine A\textsubscript{1} receptors and increases in adenosine triphosphate–regulated potassium channel activity.\textsuperscript{11,12} The current results with the PKC inhibitor, bisindolylmaleimide, lend further support to the hypothesis that myocardial protection produced by isoflurane occurs as a result of a complex, multifactorial process involving A\textsubscript{1} receptors and adenosine triphosphate–regulated potassium channels in which PKC and its intracellular translocation\textsuperscript{32} may play a role.

Signal transduction pathways other than PKC may also have been involved in cardioprotection mediated by isoflurane. A role for tyrosine kinase in ischemic preconditioning\textsuperscript{33} was implicated by recent findings indicating that beneficial reductions in infarct size after a brief ischemic stimulus were abolished by the combination of PKC and tyrosine kinase inhibitors but not by either drug alone.\textsuperscript{34} These findings raise the interesting possibility that isoflurane and PKC may participate in parallel cardioprotective pathways, as previously suggested to occur during ischemic preconditioning.\textsuperscript{35} Low-dose bisindolylmaleimide may have inhibited the activity of specific (detrimental) isoforms of PKC, whereas isoflurane may have directly stimulated a tyrosine kinase pathway. Accordingly, high-dose bisindolylmaleimide may have inhibited the activity of all PKC isoforms and tyrosine kinase in the current investigation. The results suggest that complex intracellular signal transduction pathways mediate cardioprotection during isoflurane, and a complete elucidation of the involved pathways will require further \textit{in vitro} investigation into the role of specific protein kinase isoforms.

The current results must be interpreted within the constraints of several potential limitations. The relative lack of selectivity of other PKC inhibitors, including staurosporine and polymyxin B, often has been a limiting factor in previous studies that evaluated the role of PKC in intracellular signaling during myocardial ischemia, because these drugs may also inhibit cyclic adenosine monophosphate–dependent protein kinase, myosin light chain kinase, and tyrosine kinase.\textsuperscript{19} Bisindolylmaleimide has been shown to be highly PKC selective,\textsuperscript{18} but higher doses of this drug may also inhibit the activity of other protein kinases.\textsuperscript{18} Bisindolylmaleimide was administered through an intracoronary route to attain plasma concentrations that previously were shown effectively to inhibit PKC \textit{in vitro},\textsuperscript{18} but plasma bisindolylmaleimide concentrations in the coronary circulation were not specifically measured in the current investigation. Interpretation of the data may also have been influenced by the load-dependent nature of regional segment shortening. However, this potential source of bias was probably minimal, because no differences in loading conditions during reperfusion were observed among the groups. Differential alterations in myocardial oxygen consumption are unlikely to account solely for the cardioprotective effects of isoflurane or bisindolylmaleimide in the current investigation. Isoflurane, but not bisindolylmaleimide, decreased the rate pressure product, yet low- but not high-dose bisindolylmaleimide was cardioprotective, regardless of previous exposure to isoflurane. However, coronary sinus oxygen tension was not determined, and myocardial oxygen consumption was not measured directly.

In conclusion, the current results show that isoflurane enhances the functional recovery of canine stunned myocardium. Inhibition of PKC with bisindolylmaleimide causes biphasic, dose-dependent effects on recovery of stunned myocardium. Whereas cardioprotective ef-
fects of low-dose bisindolylmaleimide are enhanced by isoflurane, high-dose bisindolylmaleimide, alone or combined with isoflurane, produces no cardioprotective effects. The findings provide additional evidence that isoflurane-induced myocardial protection occurs by a signal transduction pathway similar to that observed in ischemic preconditioning, and a role for PKC during isoflurane-induced cardioprotection cannot be excluded.

The authors thank David Schwabe for technical assistance; Angela Barnes for assistance in manuscript preparation; and Drs. Werner F. List and Helfried Metzler, Department of Anesthesiology and Intensive Care Medicine, University of Graz, Austria, for their gracious support.

References

20. Steinberg SF, Goldberg M, Rybin VO: Protein kinase C isozyme diversity in the heart. J Mol Cell Cardiol 1995; 27:141–53


