Role of Adenosine in Isoflurane-induced Cardioprotection

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Background: This investigation tested the hypothesis that adenosine (A₁) receptor blockade modulates the cardioprotective effects of isoflurane.

Methods: Hemodynamics and percentage segment shortening (%SS) in the left anterior descending coronary artery (LAD) perfusion territory were evaluated in barbiturate anesthetized dogs (n = 31) at selected intervals after pretreatment with the selective A₁ receptor antagonist (8-cyclopentyl-1,3,dipropyl-xanthine; DPCPX 0.8 mg/kg, intravenously) or drug vehicle in the presence or absence of 1 minimum alveolar concentration (MAC) isoflurane. Dogs were subjected to 5 min occlusions and reperusions of the LAD, followed by 180 min of final reperfusion. Isoflurane was administered for 30 min before and during LAD occlusions and reperusions and was discontinued at the onset of final reperfusion. Two other groups of dogs (n = 17) were used to measure interstitial concentrations of purines in the LAD region using a microdialysis technique in the presence and absence of isoflurane.

Results: Dogs receiving drug vehicle or DPCPX exhibited no recovery of %SS after 180 min of reperfusion (5 ± 7 and 5 ± 11% of baseline, respectively; ± SEM). In contrast, dogs receiving isoflurane alone demonstrated complete recovery of %SS at 60 min after reperfusion. DPCPX pretreatment partially attenuated isoflurane-induced enhancement of recovery of %SS (34 ± 11% of baseline 180 min after reperfusion; P < 0.05).

MULTIPLE periods of brief coronary artery occlusion interspersed with reperfusion have been shown to cause reversible, posts ischemic contractile dysfunction known as stunned myocardium.

Conclusions: The results indicate that isoflurane-induced cardioprotection in stunned myocardium is partially mediated by adenosine type 1 receptor activation and is accompanied by decreases in endogenous adenosine release. (Key words: Adenosine, antagonists; DPCPX. Adenosine 5' triphosphate-regulated potassium channels. Anesthetics, volatile; isoflurane. Heart, myocardial stunning.)

Interstitial purine concentrations were increased during multiple occlusions and reperusions of the LAD in dogs not receiving isoflurane, but they were unchanged by coronary artery occlusion and reperfusion in dogs receiving isoflurane.

Volatile anesthetics, including isoflurane, enhance the recovery of stunned myocardium. Recent investigations from our laboratory have demonstrated that the cardioprotective effects of isoflurane in posts ischemic, reperfused myocardium are mediated, at least in part, by KATP channel activation. Although A₁ receptors are known to be coupled to KATP channels, the role of...
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adenosine receptor activation during isoflurane-induced cardioprotection has not been defined. The present investigation tested the hypothesis that isoflurane-induced cardioprotection in stunned myocardium is modulated by $A_2$ receptors. In addition, because $A_2$ receptor activation during ischemic preconditioning may alter interstitial adenosine concentrations, a second group of experiments were performed to evaluate interstitial purine concentrations during brief occlusions and reperusions of the LAD in the presence and absence of isoflurane.

Method and Materials

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

General Preparation

Implantation of instruments has been described in detail previously. Briefly, mongrel dogs were anesthetized with sodium barbital (200 mg/kg) and sodium pentobarbital (15 mg/kg). The lungs of each dog were ventilated via positive pressure with an air and oxygen mixture after tracheal intubation. A double, pressure transducer-tipped catheter was inserted into the aorta and left ventricle for measurement of aortic and left ventricular pressures and the maximum rate of increase of left ventricular pressure (dP/dt max). An ultrasonic transit time flow probe was placed around the ascending thoracic aorta for measurement of aortic blood flow. Heparin-filled catheters were inserted into the left atrial appendage and the right femoral artery for administration of radioactive microspheres and withdrawal of reference blood flow samples, respectively. A precalibrated Doppler ultrasonic flow transducer was placed around the left anterior descending coronary artery (LAD) for measurement of coronary blood flow velocity. A silk ligature was loosely placed around the LAD for subsequent coronary artery occlusion. A pair of ultrasonic segment length transducers was implanted in the LAD subendocardium for measurement of changes in regional contractile function. Segment length and coronary blood flow velocity signals were monitored by ultrasonic amplifiers. Relative diastolic coronary vascular resistance was calculated as the ratio of end-diastolic arterial pressure to peak diastolic coronary blood flow velocity. The pressure-work index, an estimate of global myocardial oxygen consumption, was determined. 20 End-systolic segment length (ESL) was measured at 10 ms before maximum negative left ventricular dp/dt, and end-diastolic segment length (EDL) was measured 10 ms before dp/dt first exceeded 140 mmHg/s (immediately before the onset of left ventricular isovolumic contraction). Percent segment shortening (%SS) was calculated using the formula: %SS = (EDL - ESL) / ESL. All hemodynamic data were continuously monitored on a polygraph and digitized via a computer interfaced with an analog to digital converter.

Regional Myocardial Blood Flow

Carbonized plastic microspheres (15 ± 2 μm [SD] in diameter) labeled with $^{14}Ce$, $^{18}Ru$, $^{51}Cr$, or $^{95}Nb$ were used to measure regional myocardial perfusion as previously described. Briefly, 2 - 3 x 10⁶ microspheres were administered into the left atrium as a bolus during a 10 s period and flushed in with 10 ml of warm (37°C) saline. A few seconds before the microsphere injection, a timed collection of reference arterial flow was started from the femoral arterial catheter and withdrawn at a constant rate of 7 ml/min for 3 min. Transmural tissue samples were selected for mapping of tissue flow in the myocardium at the conclusion of each experiment. The samples were obtained from two regions of the left ventricle: 1) normal zone (myocardium supplied by the left circumflex coronary artery [LCX]); and 2) ischemic zone (distal to the LAD occlusion). Myocardial tissue samples were subdivided into subepicardial, midmyocardial, and subendocardial layers of approximately equal thickness and weight (0.75 g). Samples were weighed, placed in scintillation vials, and the activity of each isotope determined. Similarly, the activity of each isotope in the reference blood sample was assessed. Tissue blood flow (ml·min⁻¹·g⁻¹) was calculated as $Q_t \cdot C_m / C_e$, wherein $Q_t$ is rate of withdrawal of the reference blood flow sample (ml/min); $C_m$ = activity (cpm/g) of the myocardial tissue sample; and $C_e$ = activity (cpm) of the reference blood flow sample. Transmural blood flow was considered as the average of subepicardial, midmyocardial, and subendocardial blood flows.

Experimental Protocol

The experimental design is illustrated in figure 1. Dogs were randomly assigned to receive drug vehicle (50%
polyethylene glycol in 0.1 N sodium hydroxide and normal saline) or the selective $\Lambda_1$ receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX; 0.8 mg/kg, intravenously) in the presence or absence of 1 minimum alveolar concentration (MAC) (1.28% end-tidal concentration) isoflurane. End-tidal concentrations of isoflurane were measured at the tip of the endotracheal tube by an infrared anesthetic analyzer that was calibrated with known standards before and during experimentation.

Thirty minutes after instrumentation was completed, baseline systemic and coronary hemodynamics were recorded. All dogs were subjected to five 5-min periods of LAD occlusion separated by 5-min periods of reperfusion and followed by a final 180 min reperfusion, during which hemodynamics and contractile function were continuously monitored. Regional myocardial blood flow was measured under baseline conditions, during the fifth coronary artery occlusion, and after 60 and 180 min of reperfusion. In one group of experiments, dogs received drug vehicle (5 ml) over 10 min and underwent repetitive occlusions and reperfections 30 min after administration of drug vehicle. In a second group of experiments conducted in an identical fashion, dogs were pretreated with DPCPX (0.8 mg/kg) to examine the effect of $\Lambda_1$ receptor blockade on recovery of function after multiple episodes of ischemia and reperfusion. In two final groups of experiments, the effects of isoflurane on recovery of stunned myocardium were assessed in dogs pretreated with drug vehicle or DPCPX. Immediately after receiving drug vehicle or DPCPX, isoflurane (1 MAC) was administered for 30 min before and during LAD occlusion and reperfusion. Isoflurane was then discontinued at the onset of the final reperfusion.

**Determination of Myocardial Interstitial Purine Concentrations**

The effects of multiple LAD occlusions and reperusions on interstitial purine concentrations were measured in the presence or absence of 1 MAC isoflurane in a separate group of experiments using a cardiac microdialysis technique as previously described. Briefly, each microdialysis probe was constructed with silica tubing placed over each end of a single dialysis fiber, exposing a 2-cm dialysis window. The probe was inserted into the myocardial wall so that the dialysis window was completely imbedded in the midmyocardium of the ischemic region. One end of the probe was glued into a larger piece of silica tubing that was connected to a gas-tight glass syringe filled with a Krebs/Henseleit buffer consisting of 118 mmol/l NaCl, 25 mmol/l NaHCO$_3$, 11 mmol/l glucose, 4.7 mmol/l KCl, 1.25 mmol/l CaCl$_2$, 1.2 mmol/l MgSO$_4$, and 1.2 mmol/l KH$_2$PO$_4$ and equilibrated with 95% N$_2$/5% CO$_2$. The microdialysis fiber was implanted in myocardium perfused by the LAD, and 90 min later, a syringe pump was used to perfuse the probe at 2 μl/min, and the effluent was collected in glass tubes. Effluent samples were obtained at selected intervals before and during multiple occlusions and reperusions in the presence and absence of isoflurane.

High performance liquid chromatography was used to analyze dialysate purines. Briefly, a sample (5 μL)
of the raw dialysate or standard was injected onto a 1090 Series II Liquid Chromatograph (Hewlett Packard Co., Palo Alto, CA) using an autosampler. A microbore column ODS-2 C₁₈ (5 μm) 250 x 1.0 mm (Phenomenex, Palo Alto, CA) with a linear gradient of 10 mmol/l KH₂PO₄ (A) and 10 mmol/l KH₂PO₄ in 50% (v/v) methanol/water (B) (pH = 4.55 adjusted with phosphoric acid) were used for separation. The gradient started at 4–10% B in 10 min, 10–70% B in 5 min, 70–100% B in 12 min, held at 100% B for 2 min, 100–4% B in 1 min, and a 20-min postrun at 4% B. The flow rate was 50 μl/min. A photo diode array detector was used to simultaneously record chromatograms at 254 nm (hypoxanthine, inosine), 260 nm (adenosine), and 270 nm (xanthine) with a 4-nm bandwidth referenced to 450 nm with a 10-nm bandwidth. These are the optimal wavelengths for detection of each compound. Chromatograms were stored and analyzed on a Chem Station (Hewlett Packard Co.). Peak area was calculated and compared with that of known standards to determine the purine concentration of each sample, with a detection limit of 50 fmol.²²

Statistical Analysis

Statistical analysis of data within and between groups under baseline conditions, during drug and anesthetic interventions, and during LAD occlusions and reperfusion was performed with multiple analysis of variance (MANOVA) for repeated measures followed by application of Student’s t test with Bonferroni’s correction for multiplicity. Changes within and between groups were considered statistically significant when the P value was less than 0.05. All data are expressed as mean ± SEM.

Results

Hemodynamic Effects of Ischemia and Reperfusion in Dogs Receiving Drug Vehicle

There were no significant differences in baseline hemodynamics between experimental groups. Arterial blood gases were maintained within the physiologic range in each group throughout the experiment. LAD occlusion significantly (P < 0.05) decreased dP/dt max, cardiac output, and stroke volume in drug vehicle-pre-treated dogs (table 1). No changes in heart rate, mean arterial pressure, or pressure-work index were observed. LAD occlusion caused regional dyskinesia during each 5-min occlusion (fig. 2). Persistent and progressive decreases in regional contractile function were ob-

<table>
<thead>
<tr>
<th>Table 1. Hemodynamic Effects of Myocardial Stunning in Dogs Receiving Drug Vehicle</th>
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<tbody>
<tr>
<td></td>
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<tr>
<td>HR (beats·min⁻¹)</td>
</tr>
<tr>
<td>MBP (mmHg)</td>
</tr>
<tr>
<td>LVSP (mmHg)</td>
</tr>
<tr>
<td>+dP/dt max (mmHg·s⁻¹)</td>
</tr>
<tr>
<td>DCF (mmHg·Hz⁻¹)</td>
</tr>
<tr>
<td>CO (L·min⁻¹)</td>
</tr>
<tr>
<td>SVI (ml·cm⁻²)</td>
</tr>
<tr>
<td>PWI (ml·min⁻¹·100 g⁻¹)</td>
</tr>
</tbody>
</table>

Data are mean ± SEM. * Significant (P < 0.05) different from baseline.
served during each 5-min reperfusion period and during 180 min after final reperfusion of the LAD. Impairment of regional contractile function was accompanied by increases in heart rate and diastolic coronary and systemic vascular resistances and decreases in cardiac output, stroke volume, and diastolic coronary blood flow velocity.

**Hemodynamic Effects of Ischemia and Reperfusion in Dogs Receiving DPCPX**

LAD occlusion in DPCPX-pretreated dogs (table 2) caused hemodynamic effects that were similar to those observed in dogs receiving drug vehicle. Regional dyskinesia was observed during each LAD occlusion (fig. 2), and decreases in %SS were demonstrated during each 5-min reperfusion period and throughout 180 min of final reperfusion. Contractile dysfunction during reperfusion was accompanied by increases in mean arterial pressure and systemic vascular resistance and by decreases in dp/dt<sub>max</sub>, cardiac output, and stroke volume at 180 min after reperfusion. No significant differences in systemic and coronary hemodynamics and %SS were observed between drug vehicle- and DPCPX-pretreated dogs (tables 1 and 2).

**Hemodynamic Effects of Ischemia and Reperfusion in Dogs Receiving Isoflurane Alone**

Isoflurane decreased heart rate, mean arterial and left ventricular systolic pressures, dp/dt<sub>max</sub>, diastolic coronary vascular resistance, cardiac output, and pressure-work index (table 3). Diastolic coronary blood flow velocity, systemic vascular resistance, and stroke volume were unchanged. LAD occlusion caused no additional hemodynamic effects in isoflurane-anesthetized dogs. Dogs receiving isoflurane alone demonstrated significantly lower heart rate, mean arterial and left ventricular systolic pressures, dp/dt<sub>max</sub>, systemic vascular resistance, and pressure-work index than dogs receiving drug vehicle or DPCPX alone. Equivalent degrees of systolic dyskinesia occurred during the first LAD occlusion in each group (fig. 2). Dogs receiving isoflurane alone demonstrated complete recovery of %SS to control values during the first reperfusion period and during final reperfusion. Isoflurane-pretreated dogs also demonstrated significantly greater regional contractile function than drug vehicle- or DPCPX-pretreated dogs during the second and fourth 5-min reperusions and throughout 180 min of final reperfusion (fig. 2). Heart rate and pressure-work index were lower in dogs receiving isoflurane alone compared with those receiving drug vehicle or DPCPX during the 180 min of final reperfusion.

**Hemodynamic Effects of Ischemia and Reperfusion in Dogs Receiving Isoflurane and DPCPX**

Isoflurane decreased heart rate, mean arterial and left ventricular systolic pressures, dp/dt<sub>max</sub>, cardiac output
Table 2. Hemodynamic Effects of Myocardial Stunning in Dogs Receiving DPCPX

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>DPCPX</th>
<th>5th Occlusion</th>
<th>Final Reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (beats · min⁻¹)</td>
<td>142 ± 7</td>
<td>147 ± 9</td>
<td>147 ± 9</td>
<td>150 ± 8</td>
</tr>
<tr>
<td>MBP (mmHg)</td>
<td>92 ± 6</td>
<td>99 ± 7</td>
<td>97 ± 6</td>
<td>103 ± 6</td>
</tr>
<tr>
<td>LVSP (mmHg)</td>
<td>107 ± 6</td>
<td>112 ± 7</td>
<td>113 ± 6</td>
<td>115 ± 7</td>
</tr>
<tr>
<td>+dP/dt max (mmHg · s⁻¹)</td>
<td>1,920 ± 140</td>
<td>1,850 ± 140</td>
<td>1,560 ± 120*</td>
<td>1,530 ± 100*</td>
</tr>
<tr>
<td>DCBFV (Hz · 10⁻³)</td>
<td>47 ± 4</td>
<td>45 ± 2</td>
<td>0</td>
<td>45 ± 3</td>
</tr>
<tr>
<td>DCVR (mmHg · Hz⁻¹ · 10⁻⁵)</td>
<td>1.9 ± 0.2</td>
<td>2.2 ± 0.2</td>
<td>—</td>
<td>2.4 ± 0.3</td>
</tr>
<tr>
<td>CO (L · min⁻¹)</td>
<td>3.1 ± 0.3</td>
<td>2.6 ± 0.2</td>
<td>2.0 ± 0.1*</td>
<td>2.0 ± 0.2*</td>
</tr>
<tr>
<td>SVR (dyne · s · cm⁻⁵)</td>
<td>2,560 ± 270</td>
<td>3,180 ± 230</td>
<td>4,170 ± 340</td>
<td>4,340 ± 510</td>
</tr>
<tr>
<td>SV (ml)</td>
<td>21 ± 2</td>
<td>17 ± 2</td>
<td>13 ± 1*</td>
<td>13 ± 1*</td>
</tr>
<tr>
<td>PWI (ml · min⁻¹ · 100 g⁻¹)</td>
<td>12.0 ± 1.1</td>
<td>12.6 ± 1.1</td>
<td>11.6 ± 0.7</td>
<td>12.0 ± 0.7</td>
</tr>
</tbody>
</table>

Data are mean ± SEM; n = 9.
HR = heart rate; MBP = mean aortic blood pressure; LVSP = left ventricular systolic pressure; DCBFV = diastolic coronary blood flow velocity; DCVR = diastolic coronary vascular resistance; CO = cardiac output; SV = stroke volume; SVR = systemic vascular resistance; PWI = pressure work index.

* Significantly (P < 0.05) different from baseline.

Table 3. 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>Final Reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (beats · min⁻¹)</td>
<td>130 ± 6</td>
<td>111 ± 7*††</td>
<td>105 ± 7*††</td>
<td>113 ± 6*††</td>
</tr>
<tr>
<td>MBP (mmHg)</td>
<td>94 ± 6</td>
<td>67 ± 8*††</td>
<td>69 ± 3*††</td>
<td>82 ± 4</td>
</tr>
<tr>
<td>LVSP (mmHg)</td>
<td>107 ± 7</td>
<td>81 ± 9*††</td>
<td>78 ± 4*††</td>
<td>93 ± 6</td>
</tr>
<tr>
<td>+dP/dt max (mmHg · s⁻¹)</td>
<td>1,820 ± 140</td>
<td>1,230 ± 140*‡‡</td>
<td>1,030 ± 70*‡‡</td>
<td>1,360 ± 150*</td>
</tr>
<tr>
<td>DCBFV (Hz · 10⁻³)</td>
<td>36 ± 2</td>
<td>40 ± 3</td>
<td>0</td>
<td>35 ± 4</td>
</tr>
<tr>
<td>DCVR (mmHg · Hz⁻¹ · 10⁻⁵)</td>
<td>2.4 ± 0.1</td>
<td>1.5 ± 0.1*</td>
<td>—</td>
<td>2.4 ± 0.3</td>
</tr>
<tr>
<td>CO (L · min⁻¹)</td>
<td>3.1 ± 0.2</td>
<td>2.4 ± 0.2*</td>
<td>2.2 ± 0.1*</td>
<td>2.5 ± 0.2</td>
</tr>
<tr>
<td>SVR (dyne · s · cm⁻⁵)</td>
<td>2,500 ± 230</td>
<td>2,260 ± 190†‡</td>
<td>2,600 ± 220†‡</td>
<td>2,700 ± 190†‡</td>
</tr>
<tr>
<td>SV (ml)</td>
<td>24 ± 2</td>
<td>22 ± 2</td>
<td>22 ± 2</td>
<td>22 ± 2‡</td>
</tr>
<tr>
<td>PWI (ml · min⁻¹ · 100 g⁻¹)</td>
<td>10.9 ± 0.9</td>
<td>7.3 ± 0.9*‡‡</td>
<td>6.9 ± 0.4*‡‡</td>
<td>8.4 ± 0.6*‡‡</td>
</tr>
</tbody>
</table>

Data are mean ± SEM; n = 8.
HR = heart rate; MBP = mean aortic blood pressure; LVSP = left ventricular systolic pressure; DCBFV = diastolic coronary blood flow velocity; DCVR = diastolic coronary vascular resistance; CO = cardiac output; SV = stroke volume; SVR = systemic vascular resistance; PWI = pressure work index.

††Significantly (P < 0.01) different from baseline.
†Significantly (P < 0.05) different from drug vehicle-pretreated dogs (table 1).
‡Significantly (P < 0.05) different from DPCPX-pretreated dogs (table 2).
and pressure-work index in dogs pretreated with DPCPX (table 4). In contrast to dogs receiving isoflurane alone, diastolic coronary vascular resistance was unchanged in DPCPX-pretreated dogs during administration of isoflurane. No differences in hemodynamics were observed in dogs receiving DPCPX and isoflurane compared with those receiving isoflurane alone during coronary artery occlusions or after final reperfusion. Dogs pretreated with DPCPX and isoflurane demonstrated equivalent degrees of systolic dyskinesia during each LAD occlusion compared with dogs receiving isoflurane alone. However, %SS failed to recover to control values during final reperfusion of the LAD, and %SS was decreased in isoflurane-anesthetized dogs receiving DPCPX compared with dogs receiving isoflurane alone 180 min after final reperfusion (fig. 2).

**Effects of Myocardial Stunning on Regional Myocardial Perfusion**

No differences in myocardial perfusion to the ischemic or normal regions (table 5) at baseline or during coronary artery occlusion and reperfusion were observed between groups. Multiple LAD occlusions and reperusions decreased transmural blood flow in the ischemic zone to equivalent degrees in all four experimental groups.

**Effects of Myocardial Stunning on Interstitial Purine Concentrations**

Multiple episodes of ischemia and reperfusion caused increases in interstitial adenosine, inosine, hypoxanthine, and xanthine in control dogs (fig. 3). Administration of isoflurane caused initial decreases in adenosine (fig. 3A) and xanthine (fig. 3B) concentrations that returned to baseline values after 30 min equilibration at 1 MAC. Isoflurane abolished increases in interstitial purine concentrations during multiple occlusions and reperfusion of the LAD in contrast to the increases observed in the absence of this volatile anesthetic.

**Discussion**

The cardioprotective role of adenosine during myocardial ischemia and reperfusion injury has received considerable recent attention. Adenosine reduced myocardial damage after a 15-min coronary artery occlusion and reperfusion\(^2\), or after multiple brief coronary artery occlusions and reperfusion\(^3\). Functional recovery of stunned myocardium was enhanced in dogs receiving...
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Table 5. Effects of Myocardial Stunning on Transmural Myocardial Perfusion (ml·min⁻¹·g⁻¹)

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>5th Occlusion</th>
<th>Final Reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>60 min</td>
<td>180 min</td>
<td></td>
</tr>
<tr>
<td>Ischemic (LAD) region</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drug vehicle</td>
<td>0.94 ± 0.04</td>
<td>0.08 ± 0.02*</td>
<td>0.95 ± 0.06</td>
</tr>
<tr>
<td>DPCPX</td>
<td>0.86 ± 0.11</td>
<td>0.07 ± 0.02*</td>
<td>0.92 ± 0.09</td>
</tr>
<tr>
<td>Vehicle + isoflurane</td>
<td>0.83 ± 0.09</td>
<td>0.10 ± 0.02*</td>
<td>0.88 ± 0.10</td>
</tr>
<tr>
<td>DPCPX + isoflurane</td>
<td>0.64 ± 0.08</td>
<td>0.10 ± 0.02*</td>
<td>1.04 ± 0.16</td>
</tr>
<tr>
<td>Normal (LCCA) region</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drug vehicle</td>
<td>0.95 ± 0.04</td>
<td>1.05 ± 0.06</td>
<td>1.02 ± 0.07</td>
</tr>
<tr>
<td>DPCPX</td>
<td>0.93 ± 0.11</td>
<td>1.03 ± 0.08</td>
<td>1.04 ± 0.08</td>
</tr>
<tr>
<td>Vehicle + isoflurane</td>
<td>0.88 ± 0.07</td>
<td>1.06 ± 0.09</td>
<td>0.91 ± 0.07</td>
</tr>
<tr>
<td>DPCPX + isoflurane</td>
<td>0.67 ± 0.04</td>
<td>0.89 ± 0.07</td>
<td>0.98 ± 0.11*</td>
</tr>
</tbody>
</table>

Data are mean ± SEM.
LAD = left anterior descending coronary artery; LCCA = left circumflex coronary artery.
* Significantly (P < 0.05) different from baseline.

intracoronary adenosine before and during ischemia but not during reperfusion alone. Increases in endogenous adenosine attained through blockade of adenosine deaminase25-28 or inhibition of nucleoside transport29 also enhanced contractile function of posts ischemic, reperfused myocardium.

The mechanisms by which adenosine exerts its cardioprotective effects are not fully understood. Stimulation of A1 receptors by adenosine has been firmly linked to activation of KATP channels in ventricular myocardium.23-26 KATP channel activation has been shown to enhance functional recovery of stunned myocardium, decrease myocardial infarct size, and mimic the effects of ischemic preconditioning in vivo, actions which are blocked by the selective KATP channel antagonist, glyburide (glibenclamide).27,29,30 It has been hypothesized that KATP channel activation represents the end-effector in an endogenous cardioprotective signal transduction system that becomes activated during myocardial ischemia.25,26 Stimulation of A1 receptors by adenosine may play an important role in this process because these receptors are coupled to KATP channels via inhibitory G proteins in ventricular myocytes.25

A recent investigation18 from our laboratory demonstrated that isoflurane-induced cardioprotection in stunned myocardium is abolished by glyburide pretreatment. This finding suggests that isoflurane activates KATP channels in myocardium. The role of adenosine in the modulation of cardioprotective signal transduction during isoflurane anesthesia has not been previously investigated. The present results show that the cardioprotective effects of isoflurane are attenuated by pretreatment with DPCPX. DPCPX, a xanthine derivative, previously has been shown to be 700 times more selective for A1 versus A2 receptors using radioligand binding studies and in vitro functional assays.31 Although isoflurane enhanced recovery of function of stunned myocardium to baseline values, DPCPX pretreatment in dogs receiving isoflurane attenuated recovery of segment shortening after 180 min of reperfusion. These results indicate that isoflurane-induced cardioprotection is partially mediated by A1 receptor activation. Interestingly, Yao et al. demonstrated that cardioprotection induced by cyclopentyladenosine, a selective A1 receptor agonist, was blocked by glyburide, indicating that an important interaction exists between A1 receptors and myocardial KATP channels. The results of the present and previous investigations demonstrate that the myocardial protective effect of isoflurane involves activation of A1 receptors and KATP channels, although it is unclear at present whether the effect is sequential or mediated by independent effects on the receptor and the channel. The findings also indicate that DPCPX partially attenuates, whereas glyburide completely abolishes, the beneficial effects of isoflurane on functional recovery of stunned myocardium, results which support the hypothesis that KATP channel activation represents the end-effector in isoflurane-induced cardioprotection. The mechanism of KATP channel-induced cardioprotection is incompletely understood, but it may result from decreases in action potential duration, decreased intracellular calcium accumulation, and preservation of celluar energy stores. Whether isoflurane modulates other signaling pathways or other elements in the aden-
osine/K_{ATP} pathway of cardioprotective signal transduction has not been examined during this investigation and is unknown.

The actions of DPCPX to attenuate rather than completely abolish the enhanced recovery of stunned myocardium by isoflurane were probably not related to the use of an insufficient dose of DPCPX. During preliminary studies, a higher dose of DPCPX (1 mg/kg, intravenously) exacerbated myocardial stunning and caused significantly greater reductions in %SS during repertusion when compared with dogs receiving drug vehicle alone. The dosage of DPCPX (0.8 mg/kg) used during the present investigation did not adversely affect the recovery of stunned myocardium compared with control dogs. Similarly, the dosage of glyburide used in our previous investigation had no effect on recovery of stunned myocardium. Nevertheless, glyburide abolished the beneficial effects of isoflurane on functional recovery of stunned myocardium, demonstrating that isoflurane-induced cardioprotection critically depends on K_{ATP} channel activation.

Myocardial ischemia results in ATP depletion and the
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degradation of adenine nucleotides to adenosine and inosine. During reperfusion, adenosine and inosine are released into the interstitial space, taken up by endothelial cells, and further metabolized to hypoxanthine and xanthine. It has been suggested that during a brief period of ischemia, adenosine release, acting through activation of A1 receptors and possibly via K_{ATP} channels, results in reductions in high-energy phosphate use during a subsequent prolonged period of ischemia. Whereas A1 receptor activation may trigger ischemic preconditioning, interstitial and venous adenosine concentrations are paradoxically reduced in preconditioned myocardium and in response to K_{ATP} agonists, respectively. The results of the present investigation demonstrate that isoflurane abolishes increases in interstitial adenosine during multiple occlusions and reperusions of the LAD, extending the findings of previous investigations during which isoflurane was shown to preserve high-energy phosphate content of postischemic, reperfused myocardium. These anesthetic actions to attenuate ATP breakdown during ischemia are similar to those observed during ischemic preconditioning and in response to other K_{ATP} channel agonists. These collective findings indicate that isoflurane either activates A1 receptors directly or indirectly enhances the sensitivity of A1 receptors to decreased amounts of endogenously released adenosine. Interstitial purine concentrations measured during this investigation provide an index of ATP depletion during ischemia, with a microdialysis efficiency of 59% ± 1%. A phase lag may exist between changes in tissue purine concentration and their subsequent collection and measurement. Such a phase delay would be expected to be similar among groups and thus not alter the results. In addition, tissue trauma caused by microdialysis fiber implantation was not likely to have altered the results because interstitial purine concentrations were stable 90 min after fiber implantation.

The cardioprotective effects of isoflurane demonstrated in the present investigation were probably not related to differential alterations in myocardial oxygen consumption. The pressure-work index, a global estimate of myocardial oxygen consumption, was similar in dogs receiving isoflurane in the presence or absence of DPCPX. There also were no differences in systemic hemodynamics between dogs receiving isoflurane with or without DPCPX pretreatment. Only dogs receiving isoflurane alone without DPCPX demonstrated complete recovery of segment shortening after 180 min of reperfusion. These results indicate that isoflurane-induced alterations in the primary determinants of myocardial oxygen supply and demand relations can, at best, account for only a fraction of the beneficial actions of this volatile anesthetic during ischemia. Coronary sinus oxygen tension was not determined, and direct measurements of myocardial oxygen consumption were not made in the present investigation.

Isoflurane previously has been shown to produce coronary vasodilation, and recently this action has been attributed to K_{ATP} channel activation. In the present investigation, isoflurane decreased diastolic coronary vascular resistance in drug vehicle but not DPCPX-pretreated dogs. Although direct activation of A1 receptors causes coronary vasodilation, these results suggest that stimulation of A1 receptors by isoflurane indirectly contributes to anesthetic-induced coronary vasodilation. The results support the contention that isoflurane may act via a similar pathway involving stimulation of A1 receptors coupled to K_{ATP} channels in coronary vascular smooth muscle cells and ventricular myocytes to cause coronary vasodilation and antiischemic effects, respectively. Although isoflurane alone reduced diastolic coronary vascular resistance, alterations in myocardial perfusion do not account for the cardioprotective effects of isoflurane observed in the present investigation. Myocardial perfusion was similar in dogs receiving isoflurane with or without DPCPX. In addition, transmural collateral blood flow was very low (<0.10 ml min^-1 g^-1), and no differences were observed between experimental groups.

In summary, the present results demonstrate that the cardioprotective effects of isoflurane in stunned myocardium are mediated, in part, by A1 receptor activation. These effects are accompanied by decreases in endogenous adenosine release from the ischemic zone, findings similar to those found during ischemic preconditioning. The results provide evidence to support the hypothesis that isoflurane-induced cardioprotection occurs via direct activation or enhanced sensitivity of A1 receptors coupled to K_{ATP} channels in canine myocardium.

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