Is Isoflurane-induced Preconditioning Dose Related?

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Background: Volatile anesthetics precondition against myocardial infarction, but it is unknown whether this beneficial action is threshold- or dose-dependent. The authors tested the hypothesis that isoflurane decreases myocardial infarct size in a dose-dependent fashion in vivo.

Methods: Barbiturate-anesthetized dogs (n = 40) were instrumented for measurement of systemic hemodynamics including aortic and left ventricular pressures and rate of increase of left ventricular pressure. Dogs were subjected to a 60-min left anterior descending coronary artery occlusion followed by 3 h of reperfusion and were randomly assigned to receive either 0.0, 0.25, 0.5, 1.0, or 1.25 minimum alveolar concentration (MAC) isoflurane in separate groups. Isoflurane was administered for 30 min and discontinued 30 min before left anterior descending coronary artery occlusion.

Results: Infarct size (triphenyltetrazolium staining) was 29 ± 2% of the area at risk in control experiments (0.0 MAC). Isoflurane produced significant (P < 0.05) reductions of infarct size (17 ± 3, 13 ± 1, 14 ± 2, and 11 ± 1% of the area at risk during 0.25, 0.5, 1.0, and 1.25 MAC, respectively). Infarct size was inversely related to coronary collateral blood flow (radioactive microspheres) in control experiments and during low (0.25 or 0.5 MAC) but not higher concentrations of isoflurane. Isoflurane shifted the linear regression relation between infarct size and collateral perfusion downward (indicating cardioprotection) in a dose-dependent fashion.

Conclusions: Concentrations of isoflurane as low as 0.25 MAC are sufficient to precondition myocardium against infarction. High concentrations of isoflurane may have greater efficacy to protect myocardium during conditions of low coronary collateral blood flow.

VOLATILE anesthetics protect myocardium against stunning and infarction. These beneficial actions appear to occur through a signal transduction pathway that is remarkably similar to that observed during ischemic preconditioning (IPC). Activation of adenosine receptors,1–3 protein kinase C,2,4 inhibitory guanine regulatory proteins,5 and mitochondrial and sarcolemmal adenosine triphosphate–regulated potassium (KATP) channels6–9 have been implicated in anesthetic-induced preconditioning. Isoflurane-induced preconditioning and IPC decrease myocardial infarct size by 50–60%.2,7,9–11 Controversy exists as to whether IPC or KATP channel agonists reduce infarct size through threshold- or dose-dependent mechanisms. Whether a threshold concentration of isoflurane less than 1 minimum alveolar concentration (MAC) protects myocardium from infarction is also unknown. We tested the hypothesis that isoflurane decreases myocardial infarct size in a dose-dependent manner using a range of concentrations between 0.25 and 1.25 MAC. We also evaluated the relation between myocardial infarct size and coronary collateral blood flow in the presence and absence of isoflurane to determine if this relation is altered by the anesthetic agent.

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 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.12

General Preparation

Surgical implantation of instruments has been previously described in detail.6 Briefly, dogs were anesthetized with sodium barbital (200 mg/kg) and sodium pentobarbital (15 mg/kg) and ventilated using positive pressure with an air and oxygen mixture after tracheal intubation. End-tidal concentrations of isoflurane were measured at the tip of the endotracheal tube by an infrared anesthetic analyzer. A 7-French, dual micromanometer-tipped catheter was inserted into the aorta and left ventricle (LV) for measurement of aortic and LV pressures and the maximum rate of increase of LV pressure (+dP/dtmax). 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 1-cm segment of the left anterior descending coronary artery (LAD) immediately distal to the first diagonal branch was isolated, and a silk ligature was placed around the vessel for production of coronary artery occlusion and reperfusion. Hemodynamics were continuously monitored on a polygraph and digitized using a computer interfaced with an analog-to-digital converter.

Experimental Protocol

Baseline hemodynamics were recorded 90 min after instrumentation was completed. All dogs were subjected
to a 60-min LAD occlusion followed by 3 h of reperfu-
sion. Dogs were randomly assigned to receive 0.0, 0.25,
0.5, 1.0, and 1.25 MAC isoflurane in separate experimen-
tal groups. The canine MAC of isoflurane used in the
current investigation was 1.28%.\textsuperscript{15} Isoflurane was admin-
istered for 30 min and discontinued 30 min before LAD
occlusion. Regional myocardial blood flow was measured
30 min before and during LAD occlusion, and 60 min after
the onset of reperfusion. Dogs that developed intractable
ventricular fibrillation and those with subendocardial col-
lar blood flow greater than 0.15 ml·min\(^{-1}\)·g\(^{-1}\) were
excluded from data analysis.\textsuperscript{14}

**Measurement of Myocardial Infarct Size**
At the end of each experiment, myocardial infarct size
was measured as previously described.\textsuperscript{15} The LV area at
risk (AAR) for infarction was separated from the normal
area (stained with Patent blue dye), and the two regions
were incubated at 37°C for 20–30 min in 1% 2,3,5-
triphenyltetrazolium chloride in 0.1 m phosphate buffer
adjusted to pH 7.4. After overnight storage in 10% form-
aldehyde, infarcted and noninfarcted myocardium
within the AAR were carefully separated and weighed.
Infarct size was expressed as a percentage of the AAR.

**Determination of Regional Myocardial Blood Flow**
Carbonized plastic microspheres (15 ± 2 μm [SD] in
diameter) labeled with \(^{141}\text{Ce}, ^{109}\text{Ru}, \text{or } ^{95}\text{Nb}\) were used to
measure regional myocardial perfusion as previously
described.\textsuperscript{6} Transmural tissue samples were selected
from the ischemic region (distal to the LAD occlusion)
and were subdivided into subepicardial, midmyocardial,
and subendocardial layers of approximately equal thick-
ness. Samples were weighed, placed in scintillation vials,
and the activity of each isotope was determined. Simi-
larly, the activity of each isotope in the reference blood
flow sample was assessed. Tissue blood flow (milliliters
per minute per gram) was calculated as \(Q_r \cdot C_m \cdot C_r\),
where \(Q_r\) indicates the rate of withdrawal of the refer-
ence blood flow sample (milliliters per minute), \(C_m\) in-
dicates the activity (counts per minute per gram) of the
myocardial tissue sample, and \(C_r\) indicates the activity
(counts per minute) of the reference blood flow sample.
Transmural blood flow was considered as the average of
subepicardial, midmyocardial, and subendocardial blood
flows. Coronary collateral blood flow was measured in the
central ischemic zone (LAD perfusion area) after 30 min
of coronary artery occlusion.

**Statistical Analysis**
Statistical analysis of data within and between groups
was performed with analysis of variance for repeated
measures followed by Student-Newman-Keuls test. The
relation between myocardial infarct size and coronary
collateral blood flow was evaluated with linear regres-
sion analysis. Analysis of covariance was used to com-
pare regression relations among groups. Changes within
and between groups were considered statistically signif-
icient when the \(P\) value was < 0.05. All data are ex-
pressed as mean ± standard error of the mean.

**Results**
Forty dogs were instrumented to obtain 36 successful
experiments. Four dogs were excluded from the overall
analysis because subendocardial collateral blood flow
was greater than 0.15 ml·min\(^{-1}\)·g\(^{-1}\) (1, 0.0 MAC; 2,
0.25 MAC; and 1, 0.5 MAC). These four dogs were
included in the specific analysis of the relation between
coronary collateral blood flow and myocardial infarct
size.

**Systemic Hemodynamics**
There were no differences in hemodynamics between
experimental groups during baseline conditions (table
1). Isoflurane caused dose-dependent decreases in heart
rate, mean arterial and LV systolic pressures, and LV
\(+dP/dt_{max}\). Heart rate and mean arterial pressure re-
turned to baseline values within 30 min after discontin-
uation of isoflurane in dogs receiving concentrations less
than 1.0 MAC. In contrast, mean arterial and LV systolic
pressures remained depressed during LAD occlusion and
reperfusion in dogs receiving 1.25 MAC isoflurane as
compared with control experiments. There were no
differences in hemodynamics between groups after 3-h
reperfusion.

**Myocardial Infarct Size and Coronary Collateral
Blood Flow**
The LV AAR was similar between groups (control,
37 ± 2; 0.25 MAC isoflurane, 40 ± 2; 0.5 MAC isoflurane,
40 ± 3; 1.0 MAC isoflurane, 38 ± 1; 1.25 MAC isoflurane,
44 ± 2% of LV mass). Myocardial infarct size expressed
as a percentage of the AAR was 29 ± 2% (n = 8) in dogs
that did not receive isoflurane (0.0 MAC). Isoflurane
(0.25, 0.5, 1.0, and 1.25 MAC) reduced infarct size
to 17 ± 3 (n = 8), 13 ± 1 (n = 7), 14 ± 2 (n = 7), and
11 ± 1% (n = 6) of the AAR, respectively (fig. 1). An
inverse relation between myocardial infarct size and
coronary collateral blood flow was observed in dogs
receiving 0.0, 0.25, and 0.5 MAC isoflurane (fig. 2).
Isoflurane also caused a dose-related downward shift in
the regression relation. Infarct size was unrelated to
coronary collateral blood flow during 1.0 and 1.25 MAC
isoflurane (fig. 3). There were no differences in transmu-
ral myocardial perfusion during control conditions and
after 1-h reperfusion among groups (table 2). Coronary
collateral blood flow was also similar among all groups.
Table 1. Systemic Hemodynamics

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Intervention</th>
<th>30 min CAO</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<tr>
<td>HR (beats/min)</td>
<td>CON</td>
<td>129 ± 6</td>
<td>129 ± 7†</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>ISO 0.25 MAC</td>
<td>127 ± 8</td>
<td>124 ± 8†</td>
<td></td>
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<td></td>
<td>ISO 0.5 MAC</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>ISO 1.0 MAC</td>
<td>137 ± 3</td>
<td>111 ± 1*</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td>ISO 1.25 MAC</td>
<td>129 ± 4</td>
<td>95 ± 6*‡</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>CON</td>
<td>106 ± 8</td>
<td>108 ± 8</td>
<td>100 ± 9</td>
<td>107 ± 9</td>
<td>110 ± 8</td>
</tr>
<tr>
<td></td>
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<td>90 ± 4</td>
<td>81 ± 5†‡</td>
<td>87 ± 3</td>
<td>94 ± 2</td>
<td>94 ± 5</td>
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<td>ISO 0.5 MAC</td>
<td>102 ± 4</td>
<td>79 ± 4†‡</td>
<td>92 ± 4</td>
<td>100 ± 3</td>
<td>102 ± 4</td>
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<td></td>
<td>ISO 0.9 MAC</td>
<td>106 ± 3</td>
<td>65 ± 3*‡</td>
<td>92 ± 3</td>
<td>94 ± 4*</td>
<td>99 ± 3</td>
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<td>ISO 1.25 MAC</td>
<td>104 ± 7</td>
<td>56 ± 5*‡</td>
<td>80 ± 5</td>
<td>83 ± 3‡</td>
<td>85 ± 5‡</td>
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<td>LVEDP (mmHg)</td>
<td>CON</td>
<td>116 ± 7</td>
<td>115 ± 7</td>
<td>112 ± 10</td>
<td>117 ± 11</td>
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<tr>
<td></td>
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<td>92 ± 6†‡</td>
<td>95 ± 3</td>
<td>101 ± 3</td>
<td>99 ± 5</td>
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<td></td>
<td>ISO 0.5 MAC</td>
<td>113 ± 5</td>
<td>84 ± 5*†‡</td>
<td>97 ± 4*</td>
<td>104 ± 4</td>
<td>107 ± 4</td>
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<td>115 ± 4</td>
<td>72 ± 3*‡</td>
<td>97 ± 2*</td>
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<td>115 ± 6</td>
<td>60 ± 4*‡</td>
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<td>LVSP (mmHg)</td>
<td>CON</td>
<td>28 ± 1</td>
<td>4 ± 1</td>
<td>15 ± 3*</td>
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<td></td>
<td>ISO 0.25 MAC</td>
<td>37 ± 1</td>
<td>5 ± 1</td>
<td>9 ± 2‡</td>
<td>11 ± 2*</td>
<td>9 ± 2</td>
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<td></td>
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<td>38 ± 2</td>
<td>9 ± 1†‡</td>
<td>14 ± 2</td>
<td>19 ± 3*</td>
<td>15 ± 2*</td>
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<td></td>
<td>ISO 1.0 MAC</td>
<td>37 ± 1</td>
<td>9 ± 1†‡</td>
<td>15 ± 2*</td>
<td>15 ± 2*</td>
<td>15 ± 3*</td>
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<td>ISO 1.25 MAC</td>
<td>36 ± 2</td>
<td>8 ± 2‡</td>
<td>19 ± 3*‡</td>
<td>19 ± 2*</td>
<td>18 ± 3*</td>
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<tr>
<td>+dP/dtmax (mmHg/s)</td>
<td>CON</td>
<td>1,920 ± 160</td>
<td>1,870 ± 150</td>
<td>1,610 ± 180</td>
<td>1,630 ± 210</td>
<td>1,580 ± 150</td>
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<tr>
<td></td>
<td>ISO 0.25 MAC</td>
<td>1,870 ± 90</td>
<td>1,720 ± 190†</td>
<td>1,630 ± 110</td>
<td>1,510 ± 120</td>
<td>1,420 ± 130</td>
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<tr>
<td></td>
<td>ISO 0.5 MAC</td>
<td>1,750 ± 120</td>
<td>1,180 ± 60‡</td>
<td>1,540 ± 130</td>
<td>1,400 ± 50*</td>
<td>1,430 ± 40*</td>
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<td></td>
<td>ISO 1.0 MAC</td>
<td>2,020 ± 140</td>
<td>1,030 ± 50‡</td>
<td>1,590 ± 100*</td>
<td>1,470 ± 100*</td>
<td>1,480 ± 80*</td>
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<tr>
<td></td>
<td>ISO 1.25 MAC</td>
<td>1,760 ± 110</td>
<td>830 ± 50‡</td>
<td>1,300 ± 120*</td>
<td>1,380 ± 70</td>
<td>1,290 ± 140</td>
</tr>
</tbody>
</table>

Data are mean ± standard error of the mean.

* Significantly (P < 0.05) different from baseline. † Significantly (P < 0.05) different from the respective value during 1.25 minimum alveolar concentration (MAC) isoflurane. ‡ Significantly (P < 0.05) different from the respective value during control (0.0 MAC) experiments.

CAO = coronary artery occlusion; HR = heart rate; MAP = mean aortic pressure; LVSP = left ventricular systolic pressure; LVEDP = left ventricular end-diastolic pressure; +dP/dtmax = maximal rate of increase of left ventricular pressure; CON = control; ISO = isoflurane.

Discussion

A minimum duration of ischemia is required to activate endogenous protective signal transduction during IPC. For example, an ischemic duration between 2 and 3 min alone is insufficient to produce IPC. This threshold may be pharmacologically altered. Administration of K<sub>ATP</sub> channel agonists or an allosteric enhancer of the A<sub>1</sub> adenosine receptor in doses that are insufficient to produce myocardial protection is unknown. The current study was designed to examine if isoflurane-induced preconditioning is dose-related.

Our results indicate that concentrations of isoflurane as low as 0.25 MAC are sufficient to precondition myocardium against infarction. These data contrast with our previous findings demonstrating that 1 MAC sevoflurane does not protect against infarction when this anesthetic is washed out 30 min before coronary artery occlusion. Sevoflurane appears to retain a significantly shorter “memory” than that characteristic of isoflurane and IPC, which allows myocardium to remain resistant to infarction after the initial preconditioning stimulus is removed. Nevertheless, previous exposure to sevoflu-
dent of coronary collateral blood flow. A single 5-min preconditioning episode shifted the regression relation downward when compared with control experiments. The importance of coronary collateral blood flow as a determinant of infarct size during low but not high concentrations of isoflurane is similar to findings observed during IPC. The extent of infarction has been demonstrated to be inversely related to collateral blood flow in pigs subjected to a 3-min episode of preconditioning ischemia, and the regression relation is shifted downward when compared with control experiments. A 10-min preconditioning episode shifted the regression relation further downward to such a degree that infarct size was no longer dependent on collateral blood flow. Thus, the current and previous results suggest that evaluation of the relation between infarct size and collateral blood flow may be a sensitive method of determining whether anesthetic-induced preconditioning is dose-related.

Isoflurane produced dose-related decreases in heart rate, arterial pressure, and LV dP/dt\textsubscript{max}. Isoflurane was discontinued 30 min before the LAD occlusion, but the hemodynamic effects of the 1.25 MAC concentration persisted into the reperfusion period. Alterations in myocardial metabolism during and after the administration of isoflurane may be partially responsible for the protection against infarction observed in dogs receiving higher concentrations of this agent. We and other investigators have previously demonstrated that the protective effects of volatile anesthetics were abolished by \( K_{ATP} \) channel antagonists. This action was observed despite the presence of similar hemodynamic conditions with or without \( K_{ATP} \) channel blockade. Thus, it appears unlikely that hemodynamic effects of higher concentra-
tions of isoflurane are solely responsible for reductions in infarct size observed in the current or previous investigations. However, the results of experiments conducted in barbiturate-anesthetized dogs may or may not be similar to those observed in conscious dogs or humans.

The area of the LV at risk for development of infarction and degree of coronary collateral blood flow are important determinants of the extent of myocardial infarction. However, no differences in these variables accounting for the current findings were observed among experimental groups. The relation between collateral blood flow and infarct size was also directly evaluated and compared between groups. The contribution of specific signal transduction elements to the protection afforded by different concentrations of isoflurane was not evaluated in the current investigation. Evidence suggests that multiple episodes of IPC may activate both protein kinase C- and tyrosine kinase-mediated pathways, in contrast to a single preconditioning stimulus. Whether higher concentrations of or prolonged exposure to isoflurane recruits additional pathways that may also be responsible for myocardial protection will require additional investigation. It is also possible that low concentrations of isoflurane activate only one $K_{ATP}$ channel subtype, whereas high concentrations activate both sarclemal and mitochondrial $K_{ATP}$ channels. Both channels have been shown to be important during ischemic and anesthetic-induced preconditioning in dogs. This hypothesis will require further evaluation in vitro.

In conclusion, the results demonstrate that low concentrations of isoflurane are sufficient to precondition against infarction, but the efficacy of 0.25 or 0.5 MAC isoflurane to decrease infarct size may be diminished in the presence of low coronary collateral blood flow. High concentrations of isoflurane (1.0 or 1.25 MAC) produce profound and equivalent protective effects independent of the extent of coronary collateral perfusion.

The authors thank David Schwabe, B.S., for technical assistance, and Mary Lorence-Hanke, A.A. (Department of Anesthesiology, Medical College of Wisconsin, Milwaukee, WI) for assistance in preparation of the manuscript.

### References


### Table 2. Transmural Perfusion in the Ischemic (LAD) Region (ml · min$^{-1}$ · g$^{-1}$)

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>30 min CAO</th>
<th>1 h Reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON</td>
<td>0.75 ± 0.10</td>
<td>0.06 ± 0.01*</td>
<td>2.01 ± 0.18*</td>
</tr>
<tr>
<td>ISO$_{0.25}$ MAC</td>
<td>1.03 ± 0.13</td>
<td>0.07 ± 0.01*</td>
<td>2.06 ± 0.31*</td>
</tr>
<tr>
<td>ISO$_{0.5}$ MAC</td>
<td>0.70 ± 0.07</td>
<td>0.06 ± 0.01*</td>
<td>1.68 ± 0.15*</td>
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<tr>
<td>ISO$_{1.0}$ MAC</td>
<td>0.63 ± 0.08</td>
<td>0.06 ± 0.01*</td>
<td>1.75 ± 0.35*</td>
</tr>
<tr>
<td>ISO$_{1.25}$ MAC</td>
<td>0.81 ± 0.13</td>
<td>0.05 ± 0.01*</td>
<td>1.29 ± 0.14*</td>
</tr>
</tbody>
</table>

Data are mean ± standard error of the mean.

* Significantly (P < 0.05) different from baseline.

LAD = left anterior descending coronary artery; CAO = coronary artery occlusion; CON = control; ISO = isoflurane.
potassium (KATP) channel opener, nicorandil, lowers the threshold for ischemic preconditioning in barbital-anesthetized dogs. Heart Vessels Suppl 1997; 12: 175–7


