N-Acetylcysteine Restores Isoflurane-induced Preconditioning against Myocardial Infarction during Hyperglycemia


Background: Hyperglycemia generates reactive oxygen species and prevents isoflurane-induced preconditioning. The authors tested the hypothesis that scavenging reactive oxygen species with N-acetylcysteine will restore protection against myocardial infarction produced by isoflurane in vivo.

Methods: Barbiturate-anesthetized dogs (n = 45) were instrumented for measurement of systemic hemodynamics. Myocardial infarct size and coronary collateral blood flow were measured with triphenyltetrazolium staining and radioactive microspheres, respectively. All dogs were subjected to a 60-min left anterior descending coronary artery occlusion followed by 3 h of reperfusion. Dogs were randomly assigned to receive an infusion of 0.9% saline or 15% dextrose in water to increase blood glucose concentrations to 600 mg/dl (hyperglycemia) in the absence or presence of isoflurane (1.0 minimum alveolar concentration) with or without pretreatment with N-acetylcysteine (150 mg/kg IV) in six experimental groups. Isoflurane was discontinued, and blood glucose concentrations were allowed to return to baseline values before left anterior descending coronary artery occlusion.

Results: Myocardial infarct size was 27 ± 2% (n = 8) of the left ventricular area at risk in control experiments. Isoflurane significantly (P < 0.05) decreased infarct size (13 ± 2%; n = 7). Hyperglycemia alone did not alter infarct size (29 ± 3%; n = 7) but abolished the protective effect of isoflurane (25 ± 2%; n = 8). N-Acetylcysteine alone did not affect infarct size (28 ± 2%; n = 8) but restored isoflurane-induced cardioprotection during hyperglycemia (10 ± 1%; n = 7).

Conclusions: Acute hyperglycemia abolishes reductions in myocardial infarct size produced by isoflurane, but N-acetylcysteine restores these beneficial effects. The results suggest that excessive quantities of reactive oxygen species generated during hyperglycemia impair isoflurane-induced preconditioning in dogs.

MYOCARDIAL infarction is a major perioperative complication that is associated with significant morbidity and mortality.1 Hyperglycemia related to or independent of diabetes mellitus increases the risk of adverse cardiovascular events,2,5 but the mechanisms responsible for this increase in risk are incompletely understood. Signal transduction pathways required for endogenous myocardial protection against ischemic injury are impaired by hyperglycemia or diabetes. We have previously demonstrated that diabetes and hyperglycemia block ischemic preconditioning and abolish reductions of infarct size produced by activation of adenosine triphosphate-regulated potassium (KATP) channels.4,5 Volatile anesthetic preconditioning (APC) is also markedly attenuated by hyperglycemia and diabetes.6,7 Hyperglycemia generates large quantities of reactive oxygen species (ROS),8 and such reactive intermediates have been strongly implicated in the pathogenesis of ischemia–reperfusion injury.9 Whether hyperglycemia-induced ROS also adversely affects APC is unknown. We tested the hypothesis that the antioxidant N-acetylcysteine restores isoflurane-induced preconditioning during acute hyperglycemia in a canine model of experimental myocardial infarction.

Materials and Methods

All experimental procedures and protocols used in this investigation were reviewed and approved by the Institutional Animal Care and Use Committee of the Medical College of Wisconsin. Furthermore, all conformed to the Guiding Principles in the Care and Use of Animals of the American Physiological 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.10 Briefly, mongrel dogs of either sex were anesthetized with sodium barbital (200 mg/kg) and sodium pentobarbital (15 mg/kg) and ventilated using positive pressure with an air–oxygen mixture after tracheal intubation. Arterial blood pH was maintained within a physiologic range by adjustment of tidal volume and respiratory rate. 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. The canine minimum alveolar concentration of isoflurane used in the present investigation was 1.28%.11 Temperature was maintained with a heating blanket. A 7-French, dual micromanometer–tipped catheter was inserted into the aorta and left ventricle (LV) for measurement.

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Received from the Departments of Anesthesiology, Medicine (Division of Cardiovascular Diseases), and Pharmacology and Toxicology, the Medical College of Wisconsin and the Zablocki VA Medical Center, Milwaukee, Wisconsin. Submitted for publication November 5, 2002. Accepted for publication February 11, 2003. This work was supported in part by grants HL-05690 (Dr. Kersten), HL-63705 (Dr. Kersten), HL-54820 (Dr. Warttier), and GM-08377 (Dr. Warttier) from the United States Public Health Service (Bethesda, Maryland).

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ment of aortic and LV pressures and the maximum rate of increase of LV pressure \(\frac{+dp}{dt_{\text{max}}})\). 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 catheter was also inserted into the right femoral vein for fluid or drug administration. A 1-cm segment of the left anterior descending coronary artery (LAD) was isolated immediately distal to the first diagonal branch, and a silk ligature was placed around the vessel for production of coronary artery occlusion and reperfusion. Hemodynamics were monitored continuously on a polygraph and digitized using a computer interfaced with an analog-to-digital converter.

**Experimental Protocol**

Baseline systemic hemodynamics were recorded 90 min after instrumentation was completed and calibrated. All dogs were subjected to a 60-min LAD occlusion followed by 3 h of reperfusion (fig. 1). Dogs were randomly assigned to receive 0.9% saline or 15% dextrose in water to increase blood glucose concentrations to 600 mg/dl in the absence or presence of isoflurane (1.0 minimum alveolar concentration) with or without N-acetylcysteine in six separate experimental groups. N-Acetylcysteine (150 mg/kg IV over a period of 10 min)\(^{12}\) was infused 15 min before administration of dextrose. Isoflurane was administered for 30 min and discontinued 30 min before LAD occlusion.\(^{13}\) Blood glucose concentrations were allowed to return to baseline values 30 min before LAD occlusion. Regional myocardial blood flow was measured at baseline, during LAD occlusion, and after 1 h of reperfusion. Dogs that developed intractable ventricular fibrillation and those with a subendocardial coronary collateral blood flow greater than 0.15 ml-min\(^{-1}\)g\(^{-1}\) were excluded from the analysis.\(^{14}\)

**Measurement of Myocardial Infarct Size**

At the end of each experiment, myocardial infarct size was measured as previously described.\(^{15}\) Briefly, the LV area at risk for infarction (AAR) was separated from the normal area, 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% formaldehyde, infarcted and noninfarcted myocardial areas 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 \(^{141}\)Ce, \(^{103}\)Ru, or \(^{95}\)Nb were used to measure regional myocardial perfusion as described previously.\(^{10}\) 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 thickness. Samples were weighed and placed in scintillation vials, and the activity of each isotope was determined. Similarly, the activity of each isotope in the reference blood flow sample was assessed. Tissue blood flow was calculated as \(Q_r \cdot C_m \cdot C_r^{-1}\), where \(Q_r\) = rate of with-

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**Fig. 1.** Schematic diagram illustrating the experimental protocol. MAC = minimum alveolar concentration; NAC = N-acetylcysteine; OCC = occlusion.
drawal of the reference blood flow sample (ml/min),
C_m = activity (counts per minute per gram) of the
myocardial tissue sample, and C_s = activity (counts per
minute) of the reference blood flow sample. Transmural
blood flow was considered to be the average of subepi-
cardial, midmyocardial, and subendocardial blood flows.

Statistical Analysis
Statistical analysis of data within and between groups
was performed with ANOVA for repeated measures fol-
lowed by Student–Newman–Keuls test. Changes within
and between groups were considered statistically signif-
"icant at a value (two-tailed) of \( P < 0.05 \). All data are
expressed as mean ± SEM.

Results
Forty-six dogs were instrumented to obtain 45 success-
ful experiments. One dog in the isoflurane plus hyper-
glycemia group was excluded because blood glucose
concentrations could not be maintained at 600 mg/dl.
Blood glucose concentrations (table 1) were similar
among groups at baseline. Blood glucose concentrations
during dextrose administration were similar in the pres-
ence or absence of isoflurane or N-acetylcysteine. Blood
glucose concentration returned to baseline values during
reperfusion in each experimental group.

Hemodynamics and Collateral Blood Flow
There were no significant differences in hemodynam-
ics between experimental groups at baseline (table 2). A
transient increase in \( \text{LV} + \frac{dP}{dt_{\text{max}}} \) was observed during
hyperglycemia. N-Acetylcysteine alone did not alter he-
modynamics. Isoflurane decreased heart rate, mean arte-
rial and LV systolic pressures, and \( \text{LV} + \frac{dP}{dt_{\text{max}}} \) in the
presence or absence of hyperglycemia or N-acetylcyste-
ine. Mean arterial and LV systolic pressures and \( \text{LV} + \frac{dP}{dt_{\text{max}}} \) returned to baseline values 30 min after
isoflurane was discontinued. LAD occlusion and reper-
fusion produced similar increases in LV end-diastolic
pressure and decreases in \( \text{LV} + \frac{dP}{dt_{\text{max}}} \) in all exper-
mental groups. LAD occlusion decreased transmural
myocardial perfusion in the ischemic region (table 3).
There were no significant differences in coronary collat-
eral blood flow between groups.

Myocardial Infarct Size
The LV AAR was similar between groups (control, 39 ± 2%; N-acetylcysteine, 41 ± 1%; isoflurane, 37 ± 1%; hyperglycemia, 37 ± 2%; isoflurane plus hyperglycemia, 37 ± 2%; N-acetylcysteine plus isoflurane plus hypergly-
cemia, 39 ± 3%). Myocardial infarct size expressed as a
percentage of the AAR was 27 ± 2% (\( n = 8 \)) in dogs
receiving 0.9% saline. Isoflurane reduced infarct size to
13 ± 2% (\( n = 7 \)) of the AAR (fig. 2). Hyperglycemia (\( n = 7 \)) alone did not alter myocardial infarct size (29 ± 3%)
but blocked the protective effect of isoflurane (25 ± 2%,
\( n = 8 \)). N-Acetylcysteine did not affect myocardial infarct
size (28 ± 2%, \( n = 8 \)) but restored the protective effect
of isoflurane during hyperglycemia (10 ± 1%, \( n = 7 \)).

Discussion
ROS have traditionally been viewed as toxic mediators
of cellular injury. However, recent evidence indicates
that small quantities of ROS act as important triggers of
intracellular signaling. ROS generated by the mitochon-
drial electron transport chain during brief episodes of
hypoxia or ischemia have been shown to activate signal
transduction pathways that confer protection of isch-
emic myocardium.16,17 Diazoxide, a selective mitochon-
drial K_{ATP} channel agonist, produces preconditioning by
generating ROS. This effect is blocked by antioxidant treat-
ment.20 ROS also seem to be important triggers of APC. Volatile anesthetics cause profound re-
ductions of myocardial infarct size and enhance the
functional recovery of stunned myocardium.13,21–25
These beneficial effects are blocked by antioxidant treat-
ment.23–25 Using confocal fluorescent microscopy with
dihydroethidium staining, we recently demonstrated
that isoflurane directly generates ROS. This effect is
blocked by administration of N-2-mercaptopropionyl gly-

Table 1. Blood Glucose Concentrations

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Intervention</th>
<th>30-min CAO</th>
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<th>3</th>
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<tr>
<td>CON</td>
<td>75 ± 4</td>
<td>62 ± 8</td>
<td>69 ± 5</td>
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<td>66 ± 5</td>
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<tr>
<td>NAC</td>
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<td>76 ± 6</td>
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<td>78 ± 4</td>
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<td>HYP</td>
<td>65 ± 5</td>
<td>577 ± 12*†</td>
<td>69 ± 13</td>
<td>76 ± 10</td>
<td>89 ± 8</td>
</tr>
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<td>ISO</td>
<td>72 ± 4</td>
<td>70 ± 5</td>
<td>69 ± 2</td>
<td>67 ± 4</td>
<td>63 ± 5</td>
</tr>
<tr>
<td>HYP + ISO</td>
<td>75 ± 4</td>
<td>584 ± 4*†</td>
<td>82 ± 13</td>
<td>59 ± 5</td>
<td>67 ± 7</td>
</tr>
<tr>
<td>NAC + HYP + ISO</td>
<td>73 ± 5</td>
<td>577 ± 9*†</td>
<td>149 ± 24*†</td>
<td>72 ± 11</td>
<td>73 ± 8</td>
</tr>
</tbody>
</table>

Data are mg/dl, mean ± SEM. Intervention = administration of saline, dextrose or N-acetylcysteine in the presence or absence of isoflurane.
* Significantly \( (P < 0.05) \) different from baseline. † Significantly \( (P < 0.05) \) different from the respective value in control experiments.
CAO = coronary artery occlusion; CON = control; HYP = hyperglycemia; ISO = isoflurane; NAC = N-acetylcysteine.
Table 2. Systemic Hemodynamics

<table>
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<th>Memory</th>
<th>30-min CAO</th>
<th>Reperfusion, h</th>
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<td></td>
<td></td>
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<td><strong>HR, beats/min</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>CON</td>
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<td>137 ± 8</td>
<td>136 ± 7</td>
<td>133 ± 6</td>
<td>121 ± 6*</td>
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<tr>
<td>NAC</td>
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<td>130 ± 7</td>
<td>128 ± 6</td>
<td>122 ± 6†</td>
<td>126 ± 6</td>
</tr>
<tr>
<td>HYP</td>
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<td>128 ± 8</td>
<td>128 ± 8</td>
<td>128 ± 6</td>
<td>113 ± 6*</td>
</tr>
<tr>
<td>ISO</td>
<td>137 ± 4</td>
<td>109 ± 3*†</td>
<td>120 ± 4*</td>
<td>124 ± 4*</td>
<td>117 ± 6*</td>
</tr>
<tr>
<td>HYP + ISO</td>
<td>135 ± 4</td>
<td>111 ± 3*†</td>
<td>127 ± 5</td>
<td>131 ± 4</td>
<td>122 ± 3</td>
</tr>
<tr>
<td>NAC + HYP + ISO</td>
<td>123 ± 7</td>
<td>95 ± 4*†</td>
<td>108 ± 4†</td>
<td>108 ± 5†</td>
<td>104 ± 4*</td>
</tr>
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<td><strong>MAP, mmHg</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>CON</td>
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<td>103 ± 5</td>
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<td>100 ± 5</td>
</tr>
<tr>
<td>NAC</td>
<td>107 ± 3</td>
<td>101 ± 4</td>
<td>101 ± 4</td>
<td>87 ± 4*†</td>
<td>87 ± 7*</td>
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<td>HYP</td>
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<td>100 ± 6</td>
<td>97 ± 4</td>
<td>82 ± 5*</td>
<td>92 ± 4</td>
</tr>
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<td>60 ± 4*†</td>
<td>103 ± 2</td>
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<td>95 ± 2</td>
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<td>91 ± 4</td>
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<td>NAC + HYP + ISO</td>
<td>97 ± 3</td>
<td>61 ± 5*†</td>
<td>97 ± 4</td>
<td>90 ± 6</td>
<td>96 ± 3</td>
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<tr>
<td><strong>LVSP, mmHg</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>CON</td>
<td>114 ± 8</td>
<td>115 ± 10</td>
<td>114 ± 7</td>
<td>102 ± 6</td>
<td>100 ± 6</td>
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<tr>
<td>NAC</td>
<td>117 ± 3</td>
<td>113 ± 5</td>
<td>114 ± 5</td>
<td>92 ± 4*†</td>
<td>90 ± 7*</td>
</tr>
<tr>
<td>HYP</td>
<td>112 ± 3</td>
<td>110 ± 6</td>
<td>107 ± 4</td>
<td>85 ± 6*</td>
<td>96 ± 5*</td>
</tr>
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<td>ISO</td>
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<td>67 ± 3*†</td>
<td>109 ± 3</td>
<td>101 ± 3</td>
<td>98 ± 2</td>
</tr>
<tr>
<td>HYP + ISO</td>
<td>107 ± 3</td>
<td>80 ± 4*†</td>
<td>103 ± 2</td>
<td>88 ± 6</td>
<td>95 ± 4</td>
</tr>
<tr>
<td>NAC + HYP + ISO</td>
<td>104 ± 4</td>
<td>66 ± 6*†</td>
<td>104 ± 4</td>
<td>92 ± 6</td>
<td>89 ± 4*</td>
</tr>
<tr>
<td><strong>LVEDP, mmHg</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CON</td>
<td>6 ± 1</td>
<td>7 ± 1</td>
<td>6 ± 1</td>
<td>16 ± 2*</td>
<td>19 ± 2*</td>
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<tr>
<td>NAC</td>
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<td>5 ± 1</td>
<td>5 ± 1</td>
<td>20 ± 4*†</td>
<td>16 ± 3*</td>
</tr>
<tr>
<td>HYP</td>
<td>7 ± 1</td>
<td>10 ± 2</td>
<td>6 ± 2</td>
<td>11 ± 2*</td>
<td>13 ± 1*</td>
</tr>
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<td>8 ± 1</td>
<td>7 ± 1</td>
<td>12 ± 2*</td>
<td>12 ± 1*</td>
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<tr>
<td>HYP + ISO</td>
<td>5 ± 1</td>
<td>9 ± 3*†</td>
<td>4 ± 1</td>
<td>11 ± 3*</td>
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<tr>
<td>NAC + HYP + ISO</td>
<td>9 ± 2</td>
<td>11 ± 1</td>
<td>7 ± 1</td>
<td>17 ± 4*</td>
<td>16 ± 3</td>
</tr>
<tr>
<td><strong>dP/dt max, mmHg/s</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CON</td>
<td>2,240 ± 160</td>
<td>1,900 ± 110</td>
<td>2,330 ± 160</td>
<td>1,840 ± 110</td>
<td>1,520 ± 70*</td>
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<tr>
<td>NAC</td>
<td>1,910 ± 160</td>
<td>1,820 ± 140</td>
<td>1,800 ± 120†</td>
<td>1,330 ± 90*†</td>
<td>1,420 ± 110*</td>
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<tr>
<td>HYP</td>
<td>1,710 ± 60</td>
<td>2,100 ± 160*</td>
<td>1,950 ± 90*</td>
<td>1,440 ± 120*</td>
<td>1,520 ± 80*</td>
</tr>
<tr>
<td>ISO</td>
<td>1,910 ± 170</td>
<td>970 ± 70*†</td>
<td>1,760 ± 150†</td>
<td>1,660 ± 120</td>
<td>1,510 ± 90*</td>
</tr>
<tr>
<td>HYP + ISO</td>
<td>1,750 ± 100</td>
<td>1,220 ± 40*†</td>
<td>1,680 ± 80†</td>
<td>1,340 ± 150*</td>
<td>1,460 ± 100*</td>
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<tr>
<td>NAC + HYP + ISO</td>
<td>1,870 ± 200</td>
<td>1,110 ± 90*†</td>
<td>1,930 ± 90</td>
<td>1,610 ± 90</td>
<td>1,390 ± 50*</td>
</tr>
</tbody>
</table>

Data are mean ± SEM. Intervention = administration of saline, dextrose, or N-acetylcysteine in the presence or absence of isoflurane.

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

CAO = coronary artery occlusion; CON = control; dP/dt max = maximal rate of increase of left ventricular pressure; HR = heart rate; HYP = hyperglycemia; ISO = isoflurane; LVSP and LVEDP = left ventricular systolic and end-diastolic pressures, respectively; MAP = mean aortic blood pressure; NAC = N-acetylcysteine.

cine, N-acetylcysteine, or the mitochondrial K<sub>ATP</sub> channel antagonist 5-hydroxydecanoate. These data provide strong support to the contention that small quantities of ROS are required for APC.

Large quantities of oxygen-derived free radicals are released during ischemia and reperfusion and cause direct lipid peroxidation, mitochondrial damage, and cell death. Excessive amounts of ROS are also generated by the mitochondrial electron transport chain during hyperglycemia, and these reactive intermediates may contribute to myocardial injury as well. We have previously demonstrated that hyperglycemia either independent of or secondary to diabetes mellitus attenuates APC in dogs. An interaction between the dose of volatile anesthetic and severity of hyperglycemia was also observed. Higher blood glucose concentrations were necessary to block the protection of 1.0 compared with 0.5 minimum alveolar concentration isoflurane. This observation led us to hypothesize that volatile anesthetics and glucose may exert opposing actions on K<sub>ATP</sub> channel activity. The current results confirm and extend our previous findings and further suggest that the interaction between isoflurane and hyperglycemia is sensitive to the oxidation–reduction state within the cardiac myocyte.

The mitochondrial K<sub>ATP</sub> channel plays a central role in both APC and ischemic preconditioning. However, a direct cause-and-effect relationship between mitochondrial K<sub>ATP</sub> channel opening and the production of ROS has not been demonstrated conclusively during APC or ischemic preconditioning. ROS enhance mitochondrial K<sub>ATP</sub> channel activity but are also released when the channel is opened. Superoxide anion generated by xanthine oxidase activates mitochondrial K<sub>ATP</sub> channels from bovine ventricular myocardium reconstituted in lipid bilayers. The mitochondrial K<sub>ATP</sub> channel agonist diazoxide also generates ROS measured with a...
mitochondria-specific fluorescent probe. However, it is possible that this action of diazoxide may not be a result of direct activation of the channels but instead may result from nonspecific uncoupling of electron transport. Interestingly, diazoxide markedly reduces ROS production in cardiac mitochondria subjected to hypoxia and reoxygenation. Thus, the protective effects of mitochondrial KATP channel agonists may occur as a consequence of triggering by ROS that subsequently prevents the release of larger quantities of these reactive intermediates during reperfusion injury. Experiments conducted in isolated mitochondria demonstrate that a triggering quantity of ROS may exceed a critical threshold that results in a transition in mitochondrial inner membrane permeability and the subsequent release of a burst of ROS in a process that has been called "ROS-induced ROS release." This transition in mitochondrial permeability precedes necrotic or apoptotic cell death, and glutathione is a primary defense against this event. The current and previous results suggest that hyperglycemia may accelerate, but volatile anesthetics or other mitochondrial KATP channel agonists may prevent this mitochondrial permeability transition in an oxidant-sensitive manner. This intriguing hypothesis remains to be specifically tested. There is also evidence to suggest that different ROS exert opposing actions on mitochondrial KATP channel activity, especially during conditions of high oxidative stress, such as hyperglycemia. Dismutation of superoxide anion leads to production of secondary ROS, including hydrogen peroxide, hydroxyl radical, and peroxyxynitrite, and these radicals may differentially alter potassium channel activity. For example, superoxide anion and hydrogen peroxide enhance but peroxyxynitrite decreases calcium-activated potassium channel activity in rat cerebral arteries. An important goal of future research will be to determine the actions of different species of free radicals on KATP channel activity and their impact on APC.

Nitric oxide is also a critical mediator of endogenous cardioprotective signal transduction. Nitric oxide directly activates mitochondrial KATP channels and potentiates agonist-induced increases in channel activity. Diazoxide has been demonstrated to produce early and delayed preconditioning against infarction by activating mitochondrial KATP channels through a nitric oxide-dependent mechanism. In contrast, hyperglycemia decreases the availability of nitric oxide by enhancing ROS production. Superoxide anion reacts with and inactivates nitric oxide and leads to the formation of peroxyxynitrite. Many of the adverse consequences of hyperglycemia and diabetes are thought to result from decreased nitric oxide activity and increased generation of reactive intermediates. However, far less is known about the deleterious interactions among ROS, nitric oxide, and KATP channels during diabetes or how such interactions might affect APC.

The current results should be interpreted within the constraints of several potential limitations. First, ROS were not directly measured in the present investigation. However, we have shown previously that isoflurane directly produces ROS and that this effect is abolished by mitochondrial KATP channel antagonists and ROS scavengers, including N-acetylcysteine. The ability of hyperglycemia to generate large quantities of ROS has also been well documented, and using dihydroethidium staining, we have recently demonstrated that hyperglycemia dose-dependently increases ROS in canine myocardium. N-Acetylcysteine was used to scavenge ROS. This drug is a sulfhydryl-containing glutathione precursor that exerts antioxidant effects by contributing to glutathione synthesis, serving as a glutathione peroxidase substrate, and directly scavenging several ROS through the actions of reduced glutathione. The results indicate that hyperglycemia impairs APC. This effect is reversed by N-acetylcysteine, suggesting a role for ROS in this process. However, the identity of a specific ROS.

### Table 3. Transmural Myocardial Perfusion in the Ischemic (LAD) Region

<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>1.24 ± 0.14</td>
<td>0.07 ± 0.02*</td>
<td>1.68 ± 0.17*</td>
</tr>
<tr>
<td>NAC</td>
<td>0.76 ± 0.06</td>
<td>0.05 ± 0.01*</td>
<td>1.47 ± 0.14*</td>
</tr>
<tr>
<td>HYP</td>
<td>1.27 ± 0.16</td>
<td>0.05 ± 0.01*</td>
<td>2.00 ± 0.32*</td>
</tr>
<tr>
<td>ISO</td>
<td>0.78 ± 0.11</td>
<td>0.08 ± 0.01*</td>
<td>1.65 ± 0.33*</td>
</tr>
<tr>
<td>HYP + ISO</td>
<td>1.11 ± 0.10</td>
<td>0.05 ± 0.01*</td>
<td>1.80 ± 0.24*</td>
</tr>
<tr>
<td>NAC + HYP + ISO</td>
<td>0.77 ± 0.16</td>
<td>0.07 ± 0.02*</td>
<td>1.21 ± 0.16*</td>
</tr>
</tbody>
</table>

Data are ml · min⁻¹ · g⁻¹ mean ± SEM.

* Significantly (P < 0.05) different from baseline.

CAO = coronary artery occlusion; CON = control; HYP = hyperglycemia; ISO = isoflurane; LAD = left anterior descending coronary artery; NAC = N-acetylcysteine.

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Fig. 2. Histogram illustrating myocardial infarct size as a percentage of the left ventricular area at risk (AAR) in dogs receiving 0.9% saline (CON) or 15% dextrose in water to increase blood glucose concentrations to 600 mg/dl (HYP) in the absence or presence of isoflurane (ISO; 1.0 minimum alveolar concentration [MAC]) and N-acetylcysteine (NAC; 150 mg/kg intravenously). Significantly (P < 0.05) different from CON. †Significantly (P < 0.05) different from HYP + ISO.
involvement in the interaction between hyperglycemia and 
APC was not determined in the present investigation,
and it is possible that the beneficial effect of N-acetylcyste-
teine occurred through an alternative pathway, such as
inhibition of advanced glycation end products. It is
likely that the hemodynamic effects of isoflurane and
N-acetylcysteine alone had few hemodynamic effects, yet their presence signi-
ficantly influenced the protective effect of isoflurane.
Although it is unlikely that the hemodynamic effects of
isoflurane, N-acetylcysteine, or hyperglycemia were re-
ponsible for the observed differences in infarct size,
coronary venous oxygen tension was not measured and
myocardial oxygen consumption was not directly quan-
tified in the present investigation. Thus, changes in myo-
cardial metabolism during the administration of isoflur-
ane in the presence or absence of hyperglycemia or
N-acetylcysteine cannot be completely excluded from
the analysis. Experiments were conducted in an estab-
lished model of myocardial infarction using acutely in-
strumented, barbiturate-anesthetized dogs, but these re-
results may not be directly comparable to those in
anesthetized humans.

In summary, the current results confirm our previous findings indicating that hyperglycemia abolishes the
myocardial protection produced by isoflurane and dem-
strate that pretreatment with the antioxidant N-acetyl-
cysteine before the onset of hyperglycemia restores the
beneficial effects of isoflurane in dogs. The current re-
results further suggest that cardioprotective signal trans-
duction during administration of isoflurane is sensitive to the
oxidation–reduction state within the cardiac myo-
cyte. Defining the precise relationships between ROS,
mitochondrial K(ATP) channels, and nitric oxide and their role in APC represent important goals of future research.

The authors thank David Schwabe, B.S. (Research Scientist), and Mary Lorence-
Hauke, A.A. (both from the Department of Anesthesiology, Medical College of
Wisconsin, for assistance in preparation of this manuscript.)

References