Interaction between Anesthetics and the Sodium–Hydrogen Exchange Inhibitor HOE 642 (Cariporide) in Ischemic and Reperfused Rat Hearts

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Background: Sodium (Na⁺)–hydrogen (H⁺) exchange (NHE) inhibitors are effective cardioprotective agents. The potent NHE inhibitor HOE 642 (cariporide) is being evaluated clinically in high-risk patients, including those having coronary artery bypass. Volatile anesthetics are also cardioprotective, most likely via different mechanisms. The potential interaction between anesthetics and HOE 642 was investigated.

Methods: Electrically paced isolated rat hearts were perfused at constant flow. Left ventricular developed pressure and end-diastolic pressure were monitored as determinants of function. Hearts were subjected to 60 min each of total ischemia followed by reperfusion. Isoflurane (0.93 minimum alveolar concentration [MAC]), sevoflurane (1.03 MAC), or sufentanil (1.2 μg/ml) was added 15 min before ischemia and throughout reperfusion, either alone or in combination with HOE 642 (5 μM). The effect of HOE 642 alone was also studied. At the end of reperfusion, hearts were freeze-clamped for subsequent determination of tissue metabolites.

Results: In control hearts, left ventricular developed pressure recovered to 40% of preischemia values, whereas left ventricular end-diastolic pressure increased by 650% after reperfusion. Sevoflurane, isoflurane, or HOE 642 alone significantly enhanced left ventricular developed pressure recovery to more than 90%, although recovery with HOE 642 was more rapid and accompanied by significantly reduced left ventricular end-diastolic pressure. HOE 642 plus volatile anesthetics produced additive effects, with left ventricular developed pressure recovering by more than 100%, although left ventricular end-diastolic pressure was not further reduced. Sufentanil had no effect in terms of developed pressure, but protection with HOE 642 was maintained. HOE 642 with or without volatile anesthetics also preserved adenosine triphosphate levels.

Conclusions: Isoflurane, sevoflurane, and HOE 642 enhance ventricular recovery, but the effect of HOE 642 is also associated with reduced contracture and adenosine triphosphate preservation. A combination of the NHE inhibitor and either volatile agent confers additive and superior protection, which could be relevant for the establishment of ideal cardioprotective strategies during surgery. (Key words: Cardioprotection. Anesthetics, volatile; sevoflurane; isoflurane; sufentanil. Ischaemia. Reperfusion. Sodium–hydrogen exchange.)

SODIUM (Na⁺)–HYDROGEN (H⁺) exchange (NHE) represents an important mechanism for proton extrusion after ischemia-induced acidosis, which acts by extruding protons in exchange for sodium entry in a 1:1 stoichiometric relation. Paradoxically, however, NHE activation also contributes to myocardial ischemic and reperfusion injury, and inhibition of the antiporter has been extensively demonstrated to protect the ischemic and reperfused myocardium against injury (reviewed in references 3–6). The involvement of NHE in cardiac injury likely reflects the fact that the sodium, which enters in exchange for proton efflux, cannot be effectively removed because of reduced Na⁺−K⁺ adenosine triphosphatase (ATPase) activity. The intracellular Na⁺ accumulation then results in elevations in intracellular Ca²⁺ concentrations due to decreased elimination or possibly increased entry of Ca²⁺ via the Na⁺−Ca²⁺ exchange. This ionic imbalance and intracellular calcium overload contributes to cell injury, contracture, and depressed systolic function. Sodium and hydrogen exchange inhibitors have been proposed as superior cardioprotective agents that could be effective as adjuncts to cardioplegic solutions during cardiac surgery. Indeed, the potent and cardiac NHE isoform-specific
NHE INHIBITION AND ANESTHETICS IN MYOCARDIAL ISCHEMIA

(NHE-1) inhibitor HOE 642 (cariporide) is currently being evaluated in clinical trials as a cardioprotective agent in high-risk patients with acute coronary syndrome, including those having coronary artery bypass surgery. However, interaction between NHE inhibitors and anesthetic agents has not been studied yet. This is particularly important because volatile anesthetics, including sevoflurane and isoflurane, have cardiac effects and indeed have been shown to exert a protective influence on the ischemic myocardium.\textsuperscript{8-20} Although there is evidence for cardioprotective effects of various volatile anesthetics, to our knowledge the influence of sufentanil has not been previously studied, although morphine and activation of opioid receptors have been shown to be involved in mediating the protective effects of myocardial preconditioning.\textsuperscript{21} However, the precise role of opioid receptor activation in myocardial injury has not been elucidated because naloxone, an opioid receptor blocker, has also been shown to protect the ischemic and reperfused myocardium.\textsuperscript{22} Accordingly, the present study was done to determine the effect of HOE 642 on the ischemic and reperfused heart in the presence of either isoflurane or sevoflurane as well as sufentanil and to assess potential interactions between HOE 642 and these agents on the ischemic and reperfused heart.

Materials and Methods

Animals

Male Sprague-Dawley rats (weighing 250–300 g) were purchased from Charles-River Canada (St. Constant, Quebec, Canada) or Harland Sprague-Dawley (Indianapolis, IN). The animals were maintained in the Health Sciences Animal Care Facility of the University of Western Ontario in accordance with the guidelines of the Canadian Council on Animal Care (Ottawa, Ontario, Canada).

Heart Perfusion

Rats were killed by decapitation and the hearts were immediately excised and placed in cold Krebs-Henseleit buffer to stop contractions. The hearts were pickled up by the aorta and prepared for retrograde perfusion using a modified Langendorff method at a constant flow rate of 10 ml/min using a peristaltic pump. The perfusion fluid (pH, 7.4; temperature, 37°C) was Krebs-Henseleit buffer containing 120 mM NaCl, 4.63 mM KCl, 1.17 mM KH\textsubscript{2}PO\textsubscript{4}, 1.25 mM CaCl\textsubscript{2}, 1.2 mM MgCl\textsubscript{2}, 20 mM NaHCO\textsubscript{3}, and 8 mM glucose. The buffer was vigorously gassed with 95% oxygen and 5% carbon dioxide before addition of drugs. Coronary pressure was measured via a side arm of the perfusion cannula connected to a pressure transducer (Spectramed P23XL, Oxnard, CA). A latex water-filled balloon fixed to a pressure transducer was inserted through the mitral valve into the left ventricle to determine left ventricular developed pressure. Positive and negative dP/dt (rates of pressure development or relaxation) were calculated using a differentiator. Left ventricular end-diastolic pressure was adjusted to approximately 5 mmHg before starting the experiment by adjusting the volume in the intraventricular balloon with the aid of a micrometer-equipped syringe. Hearts were electrically paced at three times the voltage threshold with a stimulator set at 5 Hz. Pacing was also maintained during the ischemic period. All determinations of ventricular and coronary pressures were obtained on-line on a Pentium 586 computer using a Biopac data analysis system (Biolyx Scientific Equipment, Montreal, Quebec, Canada).

Experimental Protocol

Hearts were initially equilibrated for 20 min, after which either isoflurane, sevoflurane (delivery and concentrations described subsequently), sufentanil (1.21 nm, calculated to be equivalent to 1.6 \textmu g·kg body weight\textsuperscript{−1·h\textsuperscript{−1}}) was added for an additional 15 min either alone or simultaneously with HOE 642 (5 \textmu M). Other experiments were done in which HOE 642 alone was administered in the absence of anesthetic. Control hearts were perfused with drug-free buffer for an equal period of time.

At the end of the 15-min drug-treatment period, hearts were rendered globally ischemic by completely shutting off the flow for 60 min, after which reperfusion at a normal flow rate was initiated for a further 60 min. The respective drug treatment was maintained throughout the reperfusion period.

Anesthetic Delivery and Determination of Concentrations

Volatile anesthetics were injected directly into sealed 4-1 glass bottles each containing 11 preoxygenated perfusate (pH, 7.39 ± 0.02; carbon dioxide pressure, 30 ± 5 mmHg; and oxygen pressure, 818 ± 31 mmHg) as described by Boban et al.\textsuperscript{23} A gas-tight syringe equipped with a gas-tight stainless steel valve (Hamilton 1000 series syringe, Hamilton GTS valve H86560 VWR Canlab, Mississauga, Ontario, Canada) was used to aspirate and
inject the volatile anesthetics. Specific volumes of sevoflurane and isoflurane were injected immediately into the designated sealed glass bottle using a double stopcock system to avoid leakage and volatilization of the anesthetics before and during injection. The sealed perfusate was stirred continuously to facilitate equilibration of the volatile anesthetics between the liquid and gas phases.

To ensure that anesthetic concentrations were maintained throughout the entire perfusion period, samples of perfusate were collected at the aortic outlet just before ischemia and 60 min after reperfusion to determine anesthetic concentrations by gas chromatography using a Varian (Mississauga, Ontario, Canada) 3300 gas chromatograph equipped with a J & W Scientific (VWR Canlab) DB-1 high-resolution gas chromatography column. The anesthetic concentrations before ischemia were 0.308 ± 0.020 mM for isoflurane and 0.307 ± 0.03 mM for sevoflurane, whereas the respective values after 60 min reperfusion were 0.30 ± 0.02 mM and 0.301 ± 0.040 mM. Effective volume percentage concentrations were calculated as 1.42 vol% for isoflurane (0.93 minimum alveolar concentration [MAC]) and 2.17 vol% for sevoflurane (1.03 MAC) before ischemia and 1.39 vol% (0.92 MAC) and 2.13 vol% (1.01 MAC) at the end of reperfusion, respectively, using Krebs-Ringer's solution/gas partition coefficients of 0.55 for isoflurane and 0.36 for sevoflurane at 37°C and 1 atm, as described previously.25

**Metabolite Assays**

At the end of the reperfusion period, hearts were clamped with Wollenberger tongs precooled in liquid nitrogen, removed from the cannula, and stored in liquid nitrogen until enzymatic determination for energy metabolites, as previously described.25

**Data Analysis**

All values are given as mean ± SEM. Multifactorial analysis of variance and Tukey's test for multiple comparisons were used to determine the effects of anesthetics and HOE 642 on ischemic contracture, metabolite content, and hemodynamic function at each time interval during postischemic reperfusion. Differences among the experimental groups were assessed by one-way analysis of variance. Differences were considered significant at \( P < 0.05 \).

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**Results**

**Basal Hemodynamic Function**

As summarized in table 1, there were no significant differences among the groups with respect to baseline hemodynamic function in terms of left ventricular developed pressure after the initial 20-min equilibration perfusion period (BL1). Furthermore, no effects were observed with subsequent 15-min exposure to either sufentanil, sevoflurane, or HOE 642 with respect to any parameter (BL2). Isoflurane produced a modest negative inotropic effect with a maximum contractile depression of 15.7%, but preischemic values for all parameters in the presence of isoflurane were not significantly different from any of the other treatment groups. In addition, left ventricular end-diastolic pressure, which was initially adjusted to 5 mmHg, was completely unaffected by any of the drugs or drug combinations (data not shown).

**Functional Response to Ischemia and Reperfusion**

Figure 1 summarizes the effect of sevoflurane and HOE 642 on recovery of left ventricular developed pressure and elevation in left ventricular end-diastolic pressure during reperfusion. These results show that sevoflurane significantly improved recovery of left ventricular developed pressure from control hearts after 15 min of reperfusion. Although HOE 642 improved the magnitude of recovery to a level not significantly different from sevoflurane, recovery in the presence of the NHE inhibitor occurred more rapidly and to a greater degree during early reperfusion when compared with either control hearts or hearts treated with sevoflurane only (fig. 1). Thus recovery was significantly higher \( (P < 0.05) \) as early as 5 min after initiating reperfusion, which continued throughout reperfusion when compared with control, and for the first 15 min compared with recovery obtained with sevoflurane alone (fig. 1). A combination of sevoflurane plus HOE 642 exerted significantly superior recovery of left ventricular developed pressure compared with all other groups; indeed, functional recovery after 5 min of reperfusion reached 100% of preischemia values (fig. 1). Surprisingly, continued reperfusion in the presence of both sevoflurane and HOE 642 produced a greater than 100% recovery in left ventricular developed pressure (fig. 1).

Figure 1 also demonstrates left ventricular end-diastolic pressure changes during reperfusion and shows that sevoflurane alone failed to depress contracture development. HOE 642 significantly reduced the elevation...
NHE INHIBITION AND ANESTHETICS IN MYOCARDIAL ISCHEMIA

Table 1. Baseline Values of LV Developed Pressure

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Sevo Alone</th>
<th>Iso Alone</th>
<th>Sufn Alone</th>
<th>HOE Alone</th>
<th>Sevo + HOE</th>
<th>Iso + HOE</th>
<th>Sufn + HOE</th>
</tr>
</thead>
<tbody>
<tr>
<td>BL1</td>
<td>49.2 ± 6.8</td>
<td>46.1 ± 6.1</td>
<td>48.0 ± 9.2</td>
<td>48.5 ± 9.2</td>
<td>58.1 ± 9.4</td>
<td>47.3 ± 6.7</td>
<td>45.3 ± 6.5</td>
<td>50.0 ± 6.9</td>
</tr>
<tr>
<td>BL2</td>
<td>51.3 ± 6.7</td>
<td>44.7 ± 6.3</td>
<td>40.5 ± 7.4</td>
<td>53.4 ± 8.8</td>
<td>54.6 ± 10.0</td>
<td>41.5 ± 5.8</td>
<td>44.0 ± 5.5</td>
<td>45.8 ± 5.1</td>
</tr>
<tr>
<td>% change</td>
<td>4.2 ± 6.9</td>
<td>-3.0 ± 7.4</td>
<td>-15.7 ± 8.7</td>
<td>10.1 ± 9.2</td>
<td>-5.9 ± 7.3</td>
<td>-12.1 ± 6.5</td>
<td>-2.9 ± 7.0</td>
<td>-8.4 ± 7.5</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (mmHg). There are no significant differences in BL1, BL2, or percent change between the groups. Left ventricular end-diastolic pressure remained at 5 mmHg at BL1 and BL2 in all groups.

BL1 = baseline LV pressure development after 20 min equilibration period with no drugs present; BL2 = baseline LV pressure development after 15 min exposure to the drugs indicated and just prior to ischemia; Sevo = sevoflurane; Iso = isoflurane; Sufn = sufentanil; HOE = HOE642.

in left ventricular end-diastolic pressure either by itself or in the presence of sevoflurane. However, with respect to the latter drug combination, left ventricular end-diastolic pressure was significantly lower than values obtained with HOE 642 alone during the initial 25 min of reperfusion (fig. 1). Figure 2 shows an example of individual responses in a control heart and in a heart treated with HOE 642 and sevoflurane.

As is shown in figure 3, a virtually identical profile was evident with isoflurane with respect to recovery of developed pressure (fig. 3) and elevation of left ventricular end-diastolic pressure during reperfusion (fig. 3), similar to that observed with sevoflurane. Thus isoflurane significantly improved functional recovery in the absence of any salutary effect on left ventricular end-diastolic pressure. HOE 642 combined with isoflurane produced a greater than 100% recovery in function after reperfusion and significantly attenuated the elevation in left ventricular end-diastolic pressure.

Sufentanil had no direct cardioprotective effects with respect to developed pressure (fig. 4, top panel) and, when present on its own, produced a sustained elevation in left ventricular end-diastolic pressure during reperfusion (fig. 4, bottom panel). However, the protective influence of HOE 642 was maintained, although with this opioid identical effects of HOE 642 were evident regardless of the presence of sufentanil. Furthermore, HOE 642 prevented the elevation in end-diastolic pressure seen with sufentanil alone (fig. 4, bottom panel).

As summarized in table 2, recovery of ± dP/dt paralleled the responses observed with respect to left ventricular developed pressure in all treatment groups.

There were no significant differences in coronary perfusion pressure among any of the treatment groups (data not shown).

We also studied these treatments to determine if they affect contracture development during the ischemic period in terms of the maximum elevation of left ventricular end-diastolic pressure and the duration required to reach this value. Figure 5 summarizes these results. As shown in this figure, maximum left ventricular end-diastolic pressure reached during ischemia was significantly reduced by HOE 642 but not by any of the anesthetic agents. Furthermore, the time required to reach peak elevation in left ventricular end-diastolic pressure was significantly prolonged only by HOE 642. The beneficial effects of HOE 642 were also seen when the drug was present in combination with any of the anesthetic agents (fig. 5).

Energy Metabolite Contents

Table 3 provides a summary of energy metabolite content in reperfused hearts subjected to various treatments. Significant preservation of adenosine triphosphate (ATP) stores and energy charge was seen in all hearts treated with HOE 642 either by itself or in the presence of volatile anesthetics. Surprisingly, no significant effects on ATP stores or energy charge were evident with HOE 642 in combination with sufentanil. None of the anesthetics by themselves had any affect on these parameters.

Discussion

Our study was done to determine the potential interactions between the NHE inhibitor HOE 642 and general anesthetics, which could have clinical relevance and also further our understanding of the mechanisms underlying the protective effects of these agents. Although NHE represents a major mechanism for recovery of intracellular acidosis in the ischemic myocardium, it also contributes to ischemic and reperfusion injury in the heart through a mechanism that likely involves a secondary elevation in intracellular Ca$^{2+}$ levels.$^{3-6}$

Anesthesiology, V 87, No 6, Dec 1997
sally demonstrated excellent cardioprotection using various experimental models and animal species and NHE inhibitors (reviewed in references 3–6). Therefore we have proposed that NHE inhibition represents an effective and safe approach to cardiac protection and preservation, including as a cardioplegic adjunct in cardiac surgery. 7 HOE 642 is of particular interest because it is highly selective in inhibiting NHE in the myocardium without affecting other membrane ion transport systems, and it exerts marked cardioprotective effects.26 Furthermore, this agent is being evaluated clinically in a multicenter international study in high-risk patients with acute coronary syndrome, including persons hav-

Briefly, this concept reflects the fact that the Na⁺-K⁺ ATPase is inhibited, resulting in inefficient removal of Na⁺ and elevation of intracellular Na⁺ concentrations, followed by elevations in intracellular Ca²⁺ concentrations due either to its reduced removal through the Na⁺-Ca²⁺ exchanger or reverse-mode Na⁺-Ca²⁺ exchange activity resulting in active Ca²⁺ entry. A role for NHE involvement in cardiac ischemic and reperfusion injury is supported by many studies that have univer-

Fig. 1. Effects of sevoflurane (SEVO) or HOE 642 alone or in combination on left ventricular developed pressure and left ventricular end-diastolic pressure during reperfusion after 60 min of ischemia in hearts. Values are mean ± SEM and depict the percentage of preischemic values. *P < 0.05 for SEVO + HOE group versus all other treatments. n = 6 for all groups except control (n = 7). Values for HOE alone were significantly higher compared with control at all times during reperfusion and compared with sevoflurane alone for the first 15 min. The sevoflurane-alone group was significantly higher than controls from 15 min until the end of reperfusion.

Fig. 2. Examples of developed pressure recordings from a control heart (A) or a heart to which HOE 642 and sevoflurane were added (B). Ischemia (I) and reperfusion (R) were each initiated for 60 min. The horizontal marker bar indicates 20 min, whereas the vertical bar indicates 20 mmHg developed pressure. Note the reduced increase in end-diastolic pressure with the drug combination and the more rapid and pronounced recovery of developed pressure, which reached a maximum value of 127% of preischemia values.

Anesthesiology, V 87, No 6, Dec 1997
NHE INHIBITION AND ANESTHETICS IN MYOCARDIAL ISCHEMIA

exchange at concentrations relevant to clinical anesthesia. Wilde et al. found that at clinically relevant concentrations, volatile anesthetics reduce the Ca\textsuperscript{2+} influx during membrane excitation and reduce the SR release of Ca\textsuperscript{2+}. One report indicated that these agents may decrease the calcium sensitivity in myocardial cells, although this study was limited to hypothermic conditions. Volatile anesthetics also improve postischemic ventricular recovery, which was also obviously evident in our study, which showed increased ventricular functional recovery; in contrast to HOE 642, however, no significant attenuation of contracture development.

Fig. 3. Effects of isoflurane (ISOF) or HOE 642 alone or in combination on left ventricular developed pressure and left ventricular end-diastolic pressure during reperfusion after 60 min of ischemia in hearts. Values are mean ± SEM and depict the percentage of preischemic values. *P < 0.05 for the ISOF + HOE group versus all other treatments. n = 6 for all groups except control (n = 7). Values for HOE alone were significantly higher compared with controls at all times during reperfusion and compared with isoflurane alone for the first 25 min. The isoflurane-alone group was significantly higher than controls from 25 min until the end of reperfusion.

Fig. 4. Effects of sufentanil (SUFN) or HOE 642 alone or in combination on left ventricular developed pressure and left ventricular end-diastolic pressure during reperfusion after 60 min of ischemia in hearts. Values are mean ± SEM and depict the percentage of preischemic values. *P < 0.05 for either the HOE-alone or HOE + SUFN groups compared with the two other treatments. #P < 0.05 from control. n = 6 for all groups except control (n = 7).

Anesthesiology. V 87, No 6, Dec 1997
Table 2. Rates of LV Pressure Development (+dP/dt) and LV Relaxation (−dP/dt) as a Percentage of Baseline during Reperfusion (5, 30, and 60 min)

<table>
<thead>
<tr>
<th></th>
<th>Time after Onset of Reperfusion</th>
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<tbody>
<tr>
<td></td>
<td>5 min</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
</tr>
<tr>
<td>Sevo alone</td>
<td>5.4 ± 3.6</td>
</tr>
<tr>
<td>Iso alone</td>
<td>3.6 ± 3.5</td>
</tr>
<tr>
<td>Sufn alone</td>
<td>14.4 ± 7.5</td>
</tr>
<tr>
<td>HOE alone</td>
<td>54.1 ± 15.2*</td>
</tr>
<tr>
<td>Sevo + HOE</td>
<td>107.4 ± 30.5†</td>
</tr>
<tr>
<td>Iso + HOE</td>
<td>110.3 ± 21.1†</td>
</tr>
<tr>
<td>Sufn + HOE</td>
<td>63.0 ± 13.6*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.
Sevo = sevoflurane; Iso = isoflurane; Sufn = sufentanil; HOE = HOE642.
* P < 0.05 versus controls.
† P < 0.05 versus all other groups.

Fig. 5. Changes in maximum left ventricular end-diastolic pressure (LVEDP) and the time to peak LVEDP during 60 min of ischemia. Values are mean ± SEM. C = control; S = sevoflurane (Sevo); I = isoflurane (Isof); SF = sufentanil (Sufn); H = HOE 642. * P < 0.05 compared with unlabeled groups.
NHE INHIBITION AND ANESTHETICS IN MYOCARDIAL ISCHEMIA

Table 3. Energy Metabolite Concentrations

<table>
<thead>
<tr>
<th></th>
<th>ATP</th>
<th>CP</th>
<th>LACT</th>
<th>ADP</th>
<th>AMP</th>
<th>GLY</th>
<th>HEP</th>
<th>TAN</th>
<th>ENCH</th>
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<tbody>
<tr>
<td>Control</td>
<td>9.89</td>
<td>1.23</td>
<td>19.98</td>
<td>5.59</td>
<td>14.83</td>
<td>4.91</td>
<td>11.44</td>
<td>2.12</td>
<td></td>
</tr>
<tr>
<td>Sevo alone</td>
<td>10.19</td>
<td>1.25</td>
<td>17.00</td>
<td>1.90</td>
<td>7.70</td>
<td>2.31</td>
<td>7.45</td>
<td>1.14</td>
<td></td>
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<tr>
<td>Iso alone</td>
<td>9.58</td>
<td>0.54</td>
<td>17.82</td>
<td>1.42</td>
<td>8.49</td>
<td>3.71</td>
<td>6.96</td>
<td>0.66</td>
<td></td>
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<tr>
<td>Sufn alone</td>
<td>9.13</td>
<td>1.17</td>
<td>14.82</td>
<td>1.29</td>
<td>7.01</td>
<td>2.29</td>
<td>6.81</td>
<td>0.52</td>
<td></td>
</tr>
<tr>
<td>HOE alone</td>
<td>13.00</td>
<td>2.51*</td>
<td>18.85</td>
<td>2.91</td>
<td>13.48</td>
<td>3.81</td>
<td>7.48</td>
<td>0.56</td>
<td></td>
</tr>
<tr>
<td>Sevo + HOE</td>
<td>13.35</td>
<td>1.00*</td>
<td>21.98</td>
<td>1.98</td>
<td>7.70</td>
<td>1.45</td>
<td>8.51</td>
<td>0.97</td>
<td>1.79</td>
</tr>
<tr>
<td>Iso + HOE</td>
<td>14.34</td>
<td>1.68*</td>
<td>22.39</td>
<td>2.80</td>
<td>8.13</td>
<td>0.71</td>
<td>7.43</td>
<td>0.94</td>
<td>1.64</td>
</tr>
<tr>
<td>Sufn + HOE</td>
<td>9.67</td>
<td>0.68</td>
<td>14.36</td>
<td>1.54</td>
<td>11.39</td>
<td>1.84</td>
<td>7.78</td>
<td>0.83</td>
<td>1.68</td>
</tr>
</tbody>
</table>

ATP = adenosine triphosphate; CP = creatine phosphate; LACT = lactic acid; ADP = adenosine diphosphate; AMP = adenosine monophosphate; GLY = glycogen; HEP = total high energy phosphates; TAN = total adenine nucleotides; ENCH = energy charge.

Values are mean ± SEM (μmol/g dry weight). See table 1 for abbreviations.

* P < 0.05 versus control, Sevo alone, Iso alone, Sufn alone, and Sufn + HOE.

(increase in left ventricular end-diastolic pressure) during ischemia or reperfusion was evident. Furthermore, with respect to comparisons between the two drug groups, it is interesting that although the magnitude of ventricular recovery was identical, HOE 642-treated hearts recovered significantly faster after restoration of flow. This difference is consistent with distinct and separate mechanisms of action for cardioprotection by volatile anesthetics. In this regard, it has been proposed that activation of ATP-sensitive potassium channels (K\text{ATP}) likely represents the mechanism for the salutary actions of isoflurane because glyburide, a K\text{ATP} blocker, prevents the protection afforded by this agent.19,20 However, using isolated rabbit cardiomyocytes, investigators have reported that isoflurane inhibits K\text{ATP}.32 These authors also showed, on the other hand, that isoflurane decreases channel sensitivity to ATP which, in effect, would support the involvement of K\text{ATP} in the cardioprotective effect of this anesthetic. Although the mechanism for K\text{ATP} involvement is not known with certainty, one proposed explanation suggests that this would result in decreased action potential duration and decreased Ca\text{2+} entry, although this concept is not universally accepted (reviewed by Grover17). Because volatile anesthetics and NHE inhibitors may act by decreasing intracellular Ca\text{2+} levels, the dissimilar protective profile shown in our study argues against decreased Ca\text{2+} entry and energy use associated with action potential shortening as the sole mechanism. Alternatively, it is possible that the inability of volatile anesthetics to inhibit contracture may reflect additional effects of these drugs, which counter the protective influence such as inhibition of Na\text{+} - Ca\text{2+} exchange,24 which is a route of Ca\text{2+} removal from the cardiac cell during diastole. Therefore, inhibition of Na\text{+} - Ca\text{2+} exchange by volatile anesthetics may actually increase intracellular Ca\text{2+} levels during ischemia and reperfusion, resulting in decreased cardioprotection.

An interesting and surprising finding of our study was the excellent recovery of function observed in hearts treated with the combination of volatile anesthetic and HOE 642, which often exceeded preischemic function. Although this likely represented additive effects due to distinct mechanisms of protection by these agents, the basis for more than 100% recovery observed in some hearts, as illustrated in figure 2, can only be a matter of speculation. The phenomenon may possibly reflect myofibrillar sensitization to Ca\text{2+} in the reperfused heart due to the drug combination, although this concept needs to be addressed with further studies. It should also be emphasized that the superior functional recovery when hearts were treated with a combination of HOE 642 and the volatile anesthetic was particularly evident during the first 30 min of reperfusion, with contractility declining modestly with continued perfusion. Because we did not reperfuse these hearts for more than 60 min, the possibility that the additive effects of volatile anesthetics and HOE 642 represent a phenomenon related to early reperfusion only cannot be excluded.

The importance of preservation of energy metabolic status, particularly with respect to high-energy phosphate content, requires comment. In our study, we did not find improved high-energy phosphate preservation except with HOE 642 alone or combined with either isoflurane or sevoflurane when ATP levels were higher and also associated with the most favorable recovery and diminished contracture. These results suggest that

high-energy phosphate preservation at the end of reperfusion does not contribute extensively to improved recovery with volatile anesthetics alone; however, preserved ATP stores with the HOE 642 and volatile anesthetic combination may contribute, at least in part, to attenuation of contracture. Other studies have, however, reported that volatile anesthetics preserve ATP stores in stunned canine myocardium\textsuperscript{16} and in isolated rat hearts subjected to brief (15 min) periods of ischemia.\textsuperscript{17} The reasons for the apparent differences between those reports and our findings are uncertain but may reflect different experimental designs or durations of ischemia. We must also add that a recent study found that both isoflurane and halothane increased ATP content in preconditioned reperfused ischemic rat hearts in the absence of improved ventricular recovery, thereby suggesting a dissociation between energy metabolic status and contractile recovery.\textsuperscript{35} Thus the exact role of high-energy phosphate preservation in mediating the salutary effects of volatile anesthetics requires further studies.

Despite the absence of a precise mechanistic basis for the effect, our studies suggest that the use of HOE 642 in combination with the volatile anesthetics isoflurane or sevoflurane exerts not only protective effects but also an additive protective influence resulting in superior recovery of function. Sufentanil, on the other hand, had no protective effects and produced a sustained elevation in left ventricular end-diastolic pressure during reperfusion. Although the mechanism for the latter effect is unknown, it was completely prevented by HOE 642. Furthermore, the salutary actions of HOE 642 in terms of improved functional recovery after reperfusion were unaffected by the opioid.

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