Halothane, Isoflurane, and Sevoflurane Reduce Postischemic Adhesion of Neutrophils in the Coronary System

Christian Kowalski, M.D.,* Stefan Zahler, Ph.D.,† Bernhard F. Becker, Ph.D., M.D.,‡ Andreas Flaucher,§ Peter F. Conzen, M.D.,‖ Eckehart Gerlach, M.D.,# Klaus Peter, M.D.**

Background: Polymorphonuclear neutrophils (PMNs) contribute to postischemic reperfusion damage in many organs and tissues, a prerequisite being adhesion of PMNs to vascular endothelial cells. Because adhesion processes involve ordered interactions of membrane proteins, it appeared possible that “membrane effects” of volatile anesthetics could interfere. We investigated the effects of halothane, isoflurane, and sevoflurane on postischemic adhesion of human PMNs in the intact coronary system of isolated perfused guinea pig hearts.

Methods: The hearts (n = 7–10 per group) were perfused in the “Langendorff” mode under conditions of constant flow (5 ml/min) using modified Krebs-Henseleit buffer equilibrated with 94.4% oxygen and 5.6% carbon dioxide. Global myocardial ischemia was induced by interrupting perfusion for 15 min. In the second minute of reperfusion (5 ml/min), a bolus dose of 6 × 10⁶ PMNs was injected into the coronary system. The number of cells reemerging in the coronary effluent was expressed as a percentage of the total number of applied PMNs. Halothane, isoflurane, and sevoflurane, each at 1 and 2 minimal alveolar concentration (MAC), were vaporized in the gas mixture and applied from 1 min before ischemia until the end of the experiment.

Results: Under nonischemic conditions, 24.7 ± 1.3% of the injected neutrophils did not reemerge from the perfused coronary system. Subjecting the hearts to global ischemia augmented retention (36.4 ± 2.8%, P < .05). Application of halothane reduced adhesion of neutrophils to 22.6 ± 2.1% and 24.2 ± 1.8% at 1 and 2 MAC, respectively (P < .05). Exposure to 1 and 2 MAC isoflurane was similarly effective, whereas basal adhesion was not significantly influenced. Sevoflurane-treated hearts (1 and 2 MAC) also showed decreased adhesion of PMNs (23.6 ± 2.3% and 24.8 ± 1.8%, respectively; P < .05) and an identical reduction resulted when sevoflurane (1 MAC) was applied only with the onset of reperfusion.

Conclusions: Although the mechanism of action of volatile anesthetics remains unclear in these preliminary studies, their inhibitory effect on ischemia-induced adhesion of PMNs may be beneficial for the heart during general anesthesia. (Key words: Anesthetics, volatile; halothane; isoflurane; sevoflurane. Blood: neutrophils (polymorphonuclear). Endothelium. Ischemia.)

SHORT periods of myocardial ischemia are often associated with incomplete recovery of contractile function during reperfusion, despite complete preservation of cellular integrity.1 This reversible postischemic myocardial dysfunction is called “myocardial stunning.”2 Such a transient ischemia can occur during general anesthesia and, indeed, prolonged periods of myocardial dysfunction have been shown in these circumstances.3 Another aspect of myocardial reperfusion is the occurrence of dysrhythmias.

Surprisingly, there are relatively few—even partly contradictory—results pertaining to the influence of volatile anesthetics on reperfusion injury and reperfusion dysrhythmias. Halothane has been shown to decrease the incidence of dysrhythmias after ischemia and reperfusion in dogs4 and rats5 and after hypoxia in guinea pigs.3 Halothane also improved recovery of contractile function in isolated guinea pig and rabbit hearts.6 Furthermore, experimental evidence indicates that recovery of contractile function of stunned myocardium is similarly enhanced by isoflurane,8 although this agent seemed to be less effective than halothane.9 In contrast, Tanaka and colleagues7 showed that sevoflurane, administered to isolated rat hearts, did not protect against reperfusion-induced dysrhythmia. Oguchi and associates,10 on the other hand, showed a
reduced incidence of ventricular fibrillation by 1 minimal alveolar concentration (MAC) sevoflurane after 15 min of ischemia in working heart rats. However, the mechanisms underlying the possible cardioprotective effects of volatile anesthetics have not been elucidated yet.

The mechanism of contractile injury during reperfusion of previously ischemic tissue is still not completely understood and may be distinct from the cause of reperfusion dysrhythmias. The pathogenesis appears to be multifactorial, but the hypothesis that oxygen-derived free radicals contribute to myocardial reperfusion injury has received considerable support.11-13 These reactive products of oxygen can induce structural alterations in cardiac myocytes and vascular endothelial cells,14 leading to a decrease in myocardial contractility and a marked increase in coronary vascular resistance.15,16 A prominent source of such reactive metabolites are activated phagocytic cells, such as monocytes, macrophages, and polymorphonuclear granulocytes (PMNs). The latter are particularly endowed with the enzyme myeloperoxidase, which catalyses the formation of hypochlorous acid.17 Previous studies by Raschke and associates18,19 showed that small numbers of PMN, sufficiently activated, can depress cardiac function after 15–30 min of ischemia. A prerequisite for the depression of cardiac function is the adhesion of PMNs to the coronary microvascular endothelium.20 Interestingly, recent studies have shown enhanced PMN adhesion (50–100% increase) after global ischemia in the intact guinea pig coronary system.21,22

Because adhesion of PMNs to the vascular endothelium seems to be one of the crucial events in reperfusion injury and cardioprotective effects of volatile anesthetics have already been observed, we designed this study to determine if halothane, isoflurane, and sevoflurane can specifically influence the postischemic adhesion of PMNs.

Materials and Methods

Reagents

Halothane (Halothan Hoechst®) was obtained from Hoechst (Frankfurt, Germany), isoflurane (Forene®) from Abbott (Wiesbaden, Germany), and sevoflurane (Sevofo- rane®) from Maruishi Pharmaceuticals (Kobe, Japan). The magnetizable CD15 antibodies were from Miltenyi Biotec GmbH (Bergisch Gladbach, Germany). All other reagents were purchased from Merck (Darmstadt, Germany).

Solutions

The composition of the Krebs-Henseleit buffer was as follows: 126 mM NaCl, 24 mM NaHCO₃, 4.7 mM KCl, 0.6 mM MgSO₄ × 7H₂O, 1.25 mM CaCl₂ × 2H₂O, 1.2 mM KH₂PO₄, 0.3 mM pyruvate; 5.5 mM glucose; and 5 IU/l insulin.

Phosphate-buffered saline (PBS), pH 7.4, contained 120 mM NaCl, 2.7 mM KCl, 4 mM Na₂HPO₄, 5 mM KH₂PO₄, and 0.1% ethylenediaminetetraacetate.

TRIS-buffered Tyrode's solution, pH 7.4, contained 137 mM NaCl, 2.6 mM KCl, 1 mM MgCl₂ × 6H₂O, 3 mM CaCl₂ × 2H₂O, 1 mM TRIS, and 0.1% glucose.

Neutrophil Preparation

Human neutrophils were isolated from venous blood of five healthy donors with the help of magnetizable CD15 antibodies. Blood (about 10 ml) was drawn into propylene syringes containing 0.1% ethylenedi- aminetetraacetate for anticoagulation and centrifuged for 15 min at 750g. The plasma was removed and 700 µl buffy coat was incubated in an Eppendorf cup with 20 µl magnetizable CD15 antibody for 15 min at 8°C. These magnetizable microbeads, with their extremely small size of 50 nm (comparable to the size of a virus), react like macromolecules do, allowing rapid attachment to biological material. They are nontoxic, biodegradable, and rarely affect function or viability of the cells to which they bind.23

For high-gradient magnetic-field separation, steel wool-packed columns of 1.5 × 0.6 cm with a binding capacity of about 10⁷ neutrophils were used (Milenyi Biotec GmbH). The flow through the separation columns was restricted using a disposable needle (24 gauge) at the outlet. The steel wool columns were magnetized by placing them in a magnetic field of about 0.6 Tesla, produced by a specially designed permanent magnet system (Mini MACS; Miltenyi Biotec GmbH). After incubation, the labeled cell suspension was placed on top of the separation column in the magnetic field using a pipette. Unbound cells were washed out with 5 × 500 µl buffer (PBS containing 0.5% bovine serum albumin). Next the steel wool column was removed from the magnetic field, placed on a polypropylene tube, and overlayered with 1 ml buffer. This flushed out the "magnetically" labeled cells (PMNs). The neutrophils were washed with 10 ml PBS, centrifuged (at 450g), and finally resuspended in TRIS-buffered Tyr-
ode's solution. The purity (>98%) and viability (>95%) of this cell preparation (preparation time, about 1 h) was routinely controlled by light microscopy (Pappenheim stain), FACScan analysis, and the Trypan blue exclusion test. The cell count (about $7 \times 10^9$ PMNs) was determined (see below) and adjusted to a $6 \times 10^7$ neutrophils/ml buffer. This cell suspension was drawn into a polypropylene syringe (5 ml) immediately before the experiment. To determine neutrophil numbers in Tyrode's solution and in samples of venous effluent, cells were counted in triplicate with a Coulter counter ZM (Coulter Electronics, Luton, UK).

Heart Preparation

Hearts were prepared as previously described. After cervical dislocation, hearts were isolated from male guinea pigs (body weight, 200–300 g) without using any anticoagulants. After median thoracotomy, the ascending aorta was cannulated and the hearts were rapidly excised. The isolated organs were perfused as nonworking “Langendorff” preparations at $37^\circ$C with a modified Krebs-Henseleit bicarbonate buffer, equilibrated with 94.4% oxygen and 5.6% carbon dioxide, providing a $P_{O_2}$ of about 600 mmHg and a pH of $7.4 \pm 0.05$. The veins entering the right atrium and the pulmonary veins were ligated, ensuring that the coronary effluent passed through the pulmonary artery. The pulmonary artery was cannulated to allow us to collect this coronary venous effluent. The perfusion pressure was continuously registered with a pressure transducer. Hearts that did not develop sinus rhythm were rejected from the study.

The care of the animals and all experimental procedures were in full accord with German animal protection laws and officially approved.

Experimental Protocol

Figure 1 shows the perfusion protocol. After isolation and an initial period (10 min) of constant-pressure perfusion at 60 mmHg to finalize preparation, the hearts were perfused at constant flow (5 ml/min) for 24 min. Global myocardial ischemia was induced by interrupting perfusion for 15 min (no flow). The temperature of the hearts was kept constant at $37^\circ$C during ischemia by immersing them in Tyrode’s solution with a pH of 7.4. Myocardial reperfusion was performed at a constant flow of 5 ml/min. After 1 min reperfusion, a 1-ml bolus of PMN in Tyrode’s solution ($6 \times 10^8$ cells) was applied during a period of 1 min into the coronary system via the aortic cannula with an infusion pump (Infors AG, Basel, Switzerland). This resulted in a coronary flow of 6 ml/min during the bolus. Coronary effluent was collected continuously during the bolus and the 140 s that followed to count the PMNs leaving the coronary system (PMN output). Pilot studies (n = 7) in our laboratory previously showed that only a negligible number (<1%) of PMNs ever emerged in the following 5 min of perfusion. In each case, a test bolus of equal volume and duration (1 ml, 1 min) was sampled immediately before the intracoronary application to determine the number of cells actually leaving the syringe (PMN input). The percentage of neutrophils adhering to the endothelium was calculated as follows:

\[
\text{Adhesion (\%)} = \left(1 - \frac{\text{PMN output}}{\text{PMN input}}\right) \times 100
\]

In time-matched control experiments with no ischemia, the neutrophils were injected after 40-min constant flow perfusion. Adhesion in these experiments is called “control adhesion.” These hearts were randomly spaced throughout the study.

The volatile anesthetics halothane, isoflurane, and sevoflurane were added to the oxygen/carbon dioxide gas mixture used to equilibrate the Krebs-Henseleit perfusate using calibrated vaporizers (Dräger Vapor 19.1 for halothane and isoflurane; Vapor 19.3 for sevoflurane). Generally, the anesthetics were introduced after the first 10 min of the equilibration period and continued until the end (about 60 min; see fig. 1). However, in one set of experiments, sevoflurane was not administered until the onset of reperfusion. The concentration of the volatile anesthetics (1 and 2 MAC, respectively).
halothane (1 and 2 vol%), isoflurane (1.2 and 2.4 vol%),
and sevoflurane (2 and 4 vol%) in the gas phase was
monitored by piezo electric gas detectors (Dräger
and Datex). All MAC values refer to the guinea pig and
are somewhat greater than the corresponding values for
humans.\textsuperscript{24}

To gain a measure of the severity of myocardial isch-
emia imposed during the protocol, cardiac lactate re-
lease was measured at three time points: twice before
ischemia, first in the absence and then in the presence
of the volatile anesthetics, and again immediately after
ischemia. Lactate in the coronary effluent was deter-
imined enzymatically using lactate dehydrogenase and
nicotine-adenine-dinucleotide, as already described.\textsuperscript{19}

**Statistical Methods**

Each group consisted of seven experiments, except
for the ischemia group without added volatile anesthe-
tics (n = 10). The results are expressed as means ±
SEM. For comparisons among the groups, analysis of
variance was first used to detect a possible overall dif-
ference. Whenever a significant effect was obtained, we
performed multiple comparison tests between the
groups using the Student-Newman-Keul's test. Differ-
ces in the data were considered significant at $P < 0.05$.

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**Results**

**Neutrophil Adhesion**

Under control conditions (no ischemia, constant coro-
nary flow rate of 5 ml/min), about 24% (range, 18–28%)
of the human PMNs injected as a bolus of $6 \times 10^7$ cells
into the coronary arteries of isolated perfused guinea
pig hearts did not emerge from the coronary system.
As shown in figure 2, subjecting the isolated hearts to
global normothermic ischemia lasting 15 min caused a
significant increase ($P < 0.05$) in subsequent adhesion of
the neutrophils to about 36% (range, 30–45%; bolus
injection in the second minute of reperfusion).

Administration of halothane (1 or 2 MAC) before and
during ischemia and reperfusion decreased postisch-
emic adhesion of PMNs to the control level (fig. 2). Use
of isoflurane produced similar results, with enhanced
postischemic PMN adhesion reduced significantly ($P < 0.05$) in the presence of 1 or 2 MAC isoflurane (fig. 3). In
another series of seven experiments, we administered 2
MAC isoflurane without imposing ischemia. Interest-
ingly, isoflurane had no effect (22% adhesion) under
this "control" condition (fig. 3). After exposure to 1 or
2 MAC sevoflurane, the postischemic adhesion of PMNs
was also decreased significantly ($P < 0.05$) to 23% and
25%, respectively (fig. 4). We obtained an equally good
inhibition of PMN adhesion ($P < 0.05$) by administering
sevoflurane (1 MAC) only at the onset of reperfusion (fig. 4).
Effect of 15 min normothermic global ischemia on the adhesion of human neutrophils to guinea pig coronary endothelium as influenced by continuous application of 1 or 2 MAC sevoflurane. Postischemic adhesion was also determined when sevoflurane was given beginning with the onset of reperfusion. Values are means of 7 or 10 (ischemia) experiments. Error bars = SEM. *P < 0.05 versus control, #P < 0.05 versus ischemia.

Coronary Perfusion Pressure
Basal coronary perfusion pressure (CPP) after 10 min of constant flow perfusion (5 ml/min) was approximately 55 mmHg and not significantly different among the groups (column 1, table 1). In control hearts (no ischemic intervention) CPP remained practically constant during the experiment. Neither halothane nor sevoflurane at 1 or 2 MAC had significant effects on CPP in preischemic hearts (column 2, table 1). However, after exposure to isoflurane (1 or 2 MAC), the CPP was significantly less than in the hearts without volatile anesthetic (column 2, table 1). All hearts subjected to ischemia showed reactive postischemic coronary dilation compared with the time-matched control group (column 3, table 1). In the fifth minute of reperfusion (i.e., 4th min of the protocol; column 3, table 1), perfusion pressure was decreased in all postischemic groups compared with the preischemic baseline value, although it was somewhat less pronounced in the 2 MAC halothane and 2 MAC sevoflurane groups. Also at this time point, isoflurane (1 or 2 MAC) decreased CPP significantly (P < 0.05). During the next 15 min of reperfusion (i.e., 59th min of protocol), the CPP increased in most groups by about 80–110%. However, significance was not always reached for the change with respect to baseline (compare columns 1 and 4 in table 1), especially not in the case of 2 MAC isoflurane.

Heart Rate
The isolated guinea pig heart preparations did not exhibit reperfusion dysrhythmias, and spontaneous heart rate (212 ± 17 beats/min) was not noticeably influenced by any of the volatile anesthetics.

Release of Lactate
Lactate release during preischemic constant-flow perfusion averaged about 0.82 ± 0.1 μmol/min and did not differ among the groups (baseline column of table 2). After the volatile anesthetics were administered, lactate release was unchanged (table 2). A significant (P < 0.05) increase of lactate release (about 20 times) was observed during the first minute of reperfusion. However, no significant differences were noted among

Table 1. Time Course of Coronary Perfusion Pressure (mmHg)

<table>
<thead>
<tr>
<th></th>
<th>10th min Baseline</th>
<th>24th min ± Anesthetic</th>
<th>44th Reperfusion</th>
<th>59th min Reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>36.0 ± 2.2</td>
<td>41.3 ± 3.2</td>
<td>42.1 ± 3.6</td>
<td>43.6 ± 3.7</td>
</tr>
<tr>
<td>Ischemia</td>
<td>36.6 ± 1.2</td>
<td>43.0 ± 1.6</td>
<td>29.0 ± 2.2†</td>
<td>49.2 ± 3.9†</td>
</tr>
<tr>
<td>Halothane 1 MAC</td>
<td>36.3 ± 3.1</td>
<td>36.1 ± 3.9</td>
<td>23.1 ± 1.6†</td>
<td>40.9 ± 5.2</td>
</tr>
<tr>
<td>Halothane 2 MAC</td>
<td>30.9 ± 2.4</td>
<td>33.6 ± 2.5</td>
<td>23.4 ± 0.9</td>
<td>42.7 ± 3.8†</td>
</tr>
<tr>
<td>Isoflurane 1 MAC</td>
<td>35.1 ± 2.3</td>
<td>28.3 ± 3.2*</td>
<td>20.4 ± 1.1†</td>
<td>45.1 ± 2.9†</td>
</tr>
<tr>
<td>Isoflurane 2 MAC</td>
<td>32.3 ± 1.8</td>
<td>24.6 ± 2.5</td>
<td>18.3 ± 1.1†</td>
<td>34.3 ± 2.3</td>
</tr>
<tr>
<td>Sevoflurane 1 MAC</td>
<td>35.4 ± 3.0</td>
<td>34.6 ± 2.5</td>
<td>25.9 ± 1.5†</td>
<td>45.9 ± 3.1†</td>
</tr>
<tr>
<td>Sevoflurane 2 MAC</td>
<td>36.1 ± 3.7</td>
<td>37.4 ± 3.2</td>
<td>27.7 ± 2.4</td>
<td>42.0 ± 3.7</td>
</tr>
</tbody>
</table>

Measurements were performed at baseline (10th min), after administration of the volatile anesthetics (24th min), and during reperfusion (44th and 59th min of protocol, see fig. 1). Values are mean ± SEM, n = 7 or 10 (ischemia). Control = time-matched protocol without ischemia or anesthetics; Ischemia = group without anesthetics. All hearts received PMN.

* P < 0.05 versus control group at the same time point.
† P < 0.05 versus baseline.
VOLATILE ANESTHETICS AND PMN ADHESION

Table 2. Rates of Release of Lactate (µmol/min) Determined in Coronary Venous Effluent

<table>
<thead>
<tr>
<th></th>
<th>10th min Baseline</th>
<th>20th min ± Anesthetic</th>
<th>39th min Reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ischemia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(no anesthetic)</td>
<td>1.2 ± 0.2</td>
<td>0.9 ± 0.1</td>
<td>16.7 ± 1.7*</td>
</tr>
<tr>
<td>Halothane 1 MAC</td>
<td>0.7 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>13.1 ± 1.4*</td>
</tr>
<tr>
<td>Isoflurane 1 MAC</td>
<td>0.7 ± 0.1</td>
<td>0.7 ± 0.1</td>
<td>16.1 ± 1.9*</td>
</tr>
<tr>
<td>Sevoflurane 1 MAC</td>
<td>0.7 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>15.3 ± 0.9*</td>
</tr>
</tbody>
</table>

Measurements were performed at baseline (10th min), after administration of the volatile anesthetics (20th min), and during the first minute of reperfusion (39th min of protocol, see fig. 1). Values are mean ± SEM, n = 7 or 10 (ischemia).

* P < 0.05 versus baseline and preischemic value.

Discussion

Reperfusion injury of the heart, especially the phenomenon of myocardial dysfunction, is enhanced by PMNs. However, this deleterious action depends crucially on the adhesion of the PMNs to the coronary vascular endothelium (and subsequent interactions with the myocardium). Volatile anesthetics may be able to protect the heart against postsischemic contractile dysfunction, however it has not been determined whether such a protective effect is somehow related to PMN adhesion. Thus we have begun to compare the influence of equianesthetic concentrations of halothane, isoflurane, and sevoflurane on adhesion of PMNs to the intact coronary endothelium of guinea pig hearts after global ischemia. Our principal findings were as follows.

1. Normothermic global ischemia lasting 15 min significantly augmented the adhesion of PMNs to the coronary endothelium.
2. This effect could be completely blocked by halothane, isoflurane, or sevoflurane continuously administered before and during ischemia and reperfusion at 1 and 2 MAC each.
3. Isoflurane given under control conditions without ischemia had no effect on basal PMN adhesion.
4. Administration of sevoflurane just at the onset of reperfusion was as effective as continuous application.
5. Suppression of the postsischemic-enhanced PMN adhesion by the volatile anesthetics was independent of their vasodilating potency.

6. The volatile anesthetics did not influence the severity of ischemic challenge, as judged by myocardial lactate release.

Recently, Raschke and Becker, and Kupatt and coworkers showed an enhanced adhesion of PMN in the coronary system of guinea pig hearts after global ischemia, an effect mediated via endothelial cells. Interestingly, these results were confirmed in the present study, although we used human PMNs instead of guinea pig PMNs and a different PMN preparation technique that minimized preactivation (separation via magnetizable antibodies against CD15 instead of Percoll gradient centrifugation).

These experiments with volatile anesthetics (halothane, isoflurane, and sevoflurane) at clinically relevant concentrations (1 and 2 MAC) suggest an entirely new approach to the problem of reperfusion injury. The consistently observed 50% increase in PMN adhesion in postsischemic hearts was completely blocked by the volatile anesthetics. Because the administration of 2 MAC isoflurane under control conditions (no ischemia) did not influence PMN adhesion, this action of volatile anesthetics seems to be a specific effect existing only during postsischemic reperfusion. The mechanisms by which this occurs are not clear, but several possibilities merit consideration.

First, all three volatile anesthetics may act as systemic and coronary vasodilators, at least under some conditions, with isoflurane being more potent than sevoflurane and halothane. Our results confirm this ranking: Isoflurane reduced the CPP significantly compared with controls, whereas halothane and sevoflurane had practically no influence. Because in our experiments the coronary system was perfused at a constant flow for the entire time, vasodilation could have reduced shear stress at the site of PMN adhesion. However, in recent studies the retained PMNs were localized largely to postcapillary venules and small collecting veins, where altered feed artery and arteriolar shear stress are of no consequence. In addition, the vasodilator iloprost was already found not to influence intracoronary PMN adhesion in our model. Furthermore, suppression of enhanced postsischemic adhesion of PMNs proved to be identical for the three anesthetics tested. Thus vasodilation cannot be a relevant causal factor.

Second, ischemia in hearts treated with volatile anesthetics could be alleviated because of their potential to
exert a negative inotropic effect. After more moderate ischemia, production of radicals in early reperfusion should be diminished. This could be relevant because oxygen radicals (superoxide and hydroxyl radicals) and oxidants (hydrogen peroxide) induce leukocyte adhesion. However, the unaltered coronary venous release of lactate that we observed during early reperfusion in the presence of the anesthetics refutes such a mechanism. An unchanged lactate release from the isolated guinea pig heart after 50 min of global ischemia when treated with halothane has also been reported by Buljubasic and colleagues. The belief that negative inotropy is not responsible for diminished PMN adhesion is enhanced by the fact that halothane, the strongest cardio-depressant, did not decrease postischemic PMN adhesion more than the other two volatile anesthetics did. Most importantly, sevoflurane given immediately after ischemia was just as effective as when given before ischemia.

Third, volatile anesthetics could act on the production or the effects of reactive oxygen metabolites in reperfused heart tissue by mechanisms independent of the previous ischemic insult. Oxygen radicals and oxidants contribute to the development of reperfusion damage. In addition, free radicals induce peroxidation of cell membrane lipids and alter the structural integrity of the membrane. As already mentioned, free radicals and oxidants are also assumed to enhance adhesion of PMNs to the endothelium. Indeed, Kuppert and associates recently showed that enhanced postischemic PMN adhesion decreases to basal values in the presence of the radical scavengers uric acid and nitric oxide. Therefore, oxygen radicals may be involved in the enhanced postischemic adhesion of PMNs in guinea pig hearts. In this respect, the findings of Tanguy and colleagues merit attention. These authors showed in isolated beating rabbit hearts that oxygen-derived free radicals (produced by electrolysis) decreased coronary flow and left ventricular pressure. The effects were attenuated by halothane, enflurane, and isoflurane, as was PMN adhesion in our studies. Because of their general chemical stability, it is not likely that all these actions of volatile anesthetics can result from their direct interaction with free radical substances and oxidants. Nevertheless, indirect effects on radical production in the heart are feasible and need to be investigated.

Fourth, the adhesion of PMNs to the microvascular endothelium is mediated by interacting sets of cell adhesion molecules, with chemotactants and, perhaps, radicals and oxidants augmenting binding. Volatile anesthetics may reduce PMN adhesion by preventing or reducing the expression of cell adhesion molecules or by changing the binding affinity of these molecules. Such actions could easily arise from "membrane" effects of the lipophilic anesthetics. However, further studies are required to substantiate this hypothesis.

In conclusion, this study is the first to compare the effects of halothane, isoflurane, and sevoflurane on adhesion of neutrophils in an intact coronary system (guinea pigs) after global ischemia. At clinically used concentrations, all three volatile anesthetics reduced the increased postischemic adhesion of PMNs to control levels. Of necessity, some aspects of the work presented are preliminary and the model used is far from perfect. For instance, only a limited number of fresh PMNs were available for each bolus, and this number was not varied systematically. In addition, only one condition of ischemia and reperfusion was investigated. Furthermore, influences of additional blood constituents need to be evaluated with respect to reperfusion damage by PMNs and the action of volatile anesthetic compounds. Unfortunately we did not pursue the issue of a dose-response relation of the volatile anesthetics (1 and 2 MAC being equieffective in all cases), and thus differences in the threshold concentration of individual volatile anesthetics required to evoke an effect cannot be excluded. Until now we have not tested the effect of anesthetics on the PMN-induced postischemic diminished ventilator function and increased vascular permeability. This will be the focus of a subsequent study. Despite these shortcomings and although the mechanism of action remains unclear, given such an inhibitory effect on PMN adhesion also in vivo, volatile anesthetics may protect hearts directly against reperfusion injury.

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