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Neutrophils Pretreated with Volatile Anesthetics Lose Ability to Cause Cardiac Dysfunction

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Background: **Volatile anesthetics can precondition the myocardium against functional depression and infarction following ischemia–reperfusion. Neutrophil activation, adherence, and release of superoxide play major roles in reperfusion injury. The authors tested the hypothesis that pretreatment of neutrophils with a volatile anesthetic, *i.e.*, simulated preconditioning, can blunt their ability to cause cardiac dysfunction.**

Methods: **Studies were performed in 60 buffer-perfused and paced isolated rat hearts. Left ventricular developed pressure served as an index of myocardial contractility. Polymorphonuclear neutrophils and/or drugs were added to coronary perfusate for 10 min, followed by 30 min of recovery. Platelet-activating factor was used to stimulate neutrophils. Pretreatment of neutrophils consisted of incubation with 1.0 minimum alveolar concentration (MAC) isoflurane or sevoflurane for 15 min, followed by washout. Additional studies were performed with 0.25 MAC isoflurane. Effects of superoxide dismutase were compared to those of volatile anesthetics. Superoxide production was measured by spectrophotometry. Neutrophil adherence to coronary vascular endothelium was estimated from the difference between neutrophils administered and recovered in coronary venous effluent.**

Results: **Activated neutrophils caused marked, persistent reduction (> 50%) in left ventricular developed pressure. Isoflurane and sevoflurane at 1.0 MAC and superoxide dismutase abolished this effect. Isoflurane and sevoflurane reduced superoxide production of activated neutrophils by 29% and 33%, respectively, and completely prevented the platelet-activating factor–induced increases in neutrophil adherence. Isoflurane at 0.25 MAC blunted, but did not abolish, the neutrophil-induced decreases in left ventricular developed pressure.**

Conclusion: **Neutrophils pretreated with 1.0 MAC isoflurane or sevoflurane lost their ability to cause cardiac dysfunction, while those pretreated with a concentration of isoflurane as low as 0.25 MAC were partially inhibited. This action of the volatile anesthetics was associated with reductions in superoxide production and neutrophil adherence to the coronary vascular endothelium. Our findings suggest that inhibitory actions on neutrophil activation and neutrophil–endothelium interaction may contribute to the preconditioning effects of volatile anesthetics observed *in vivo* during myocardial ischemia–reperfusion.**

RECENT findings have suggested that the volatile anesthetics may possess preconditioning effects in the heart, as evidenced by reduced myocardial infarct size in animal models following ischemia and reperfusion[1–4] and by decreased postoperative release of troponin I and creatine kinase MB isoenzyme in patients undergoing coronary artery bypass graft surgery[5] and enhanced recovery of developed force by human right atrial trabeculae exposed to anoxia *in vitro*.[6]

Experimental studies have sought to clarify the mechanism(s) underlying volatile anesthetic–induced myocardial preconditioning.[1,7–10] A consistent finding has been the ability of adenosine triphosphate–sensitive (K_{ATP}) channel antagonists, *i.e.*, glibenclamide and 5-hydroxydecanoate, to reduce the cardiac protection by volatile anesthetics. This finding, along with the ability of volatile anesthetics to alter K_{ATP} channel activity in isolated myocytes,[11] has led to the suggestion that an action on these channels may be involved in volatile anesthetic–induced preconditioning.[9] While this may be the case, it does not rule out the possibility that effects on other pathways or cell types may also play a role.

Studies over many years using a variety of experimental approaches have implicated the neutrophil–endothelium interaction in the pathophysiology of myocardial reperfusion injury.[12,13] This mechanism involves adherence of neutrophils to the vascular endothelium, followed by their transmigration into the surrounding tissue, where they cause damage through release of oxygen free radicals, including superoxide, and proteolytic enzymes.

Our recent *in vitro* study provided evidence that volatile anesthetics may have an inhibitory effect on the neutrophil–endothelium interaction.[14] It demonstrated that coincubation with isoflurane attenuated superoxide production and coronary vascular adherence of activated neutrophils while reducing neutrophil-induced endothelial dysfunction of the coronary artery segments. These findings were not inhibited by glibenclamide, and thus, they represented a potential K_{ATP} channel–independent pathway for cardiac protection. If the inhibitory effects of the volatile anesthetics on the neutrophil–endothelium interaction persist following washout of

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the anesthetics, they could contribute to myocardial preconditioning.

Our study was performed in isolated rat hearts to evaluate whether pretreatment of neutrophils with the volatile anesthetics isoflurane and sevoflurane, *i.e.*, simulated preconditioning, could blunt the cardiac dysfunction caused by their administration in the presence of platelet-activating factor (PAF). A potential role for modulation of superoxide production was assessed by comparing the findings with the volatile anesthetics to those of the superoxide scavenger, superoxide dismutase (SOD), and by obtaining measurements of superoxide production by the neutrophils. Further mechanistic insights were obtained by evaluating the effects of the volatile anesthetics and SOD on neutrophil adherence in the isolated hearts.

Materials and Methods

Heart Preparation

After approval from the Institutional Animal Care and Use Committee (Chicago, Illinois), studies were conducted in adult Sprague-Dawley rats (Charles River, Wilmington, MA) of either sex (weight, 250–350 g). The rats were anesthetized with pentobarbital sodium (40 mg/kg intraperitoneal). After the chest was opened, 200 U heparin was injected into the vena cava, and the hearts were rapidly excised. The hearts were mounted on a Langendorff apparatus, and retrograde perfusion was initiated immediately with oxygenated (95% O_2/5% CO_2) Krebs buffer at 37°C. The composition of the buffer was as follows: 118 mM NaCl, 4.7 mM KCl, 2.54 mM $CaCl_2$, 1.12 mM $MgSO_4$, 12.5 mM $NaHCO_3$, and 10.0 mM glucose. The hearts were perfused at constant flow by a peristaltic pump, initially titrated to achieve a coronary perfusion pressure (CPP) of 70 mmHg. A balloon-tipped catheter, connected to a microliter syringe and pressure transducer, was inserted into the left ventricle *via* an opening in the left atrium to measure ventricular pressure. Balloon volume was inflated with saline sufficiently to increase left ventricular end-diastolic pressure (LVEDP) to approximately 8–10 mmHg, which was demonstrated in preliminary studies to provide the optimal ventricular preload.

Measurements of left ventricular developed pressure (LVDP; end-systolic minus end-diastolic pressure) and left ventricular dP/dt_{max} served as indices of myocardial contractile function. An in-line, ultrasonic, transit-time flow transducer (Transonic System Inc., Ithaca, NY) was interposed in the perfusion circuit to measure coronary flow. CPP was measured just above the aortic cannula using a Statham transducer. An injection port was situated just proximal to the aortic cannula for infusion of neutrophils and/or drugs. Electrodes were attached to the right ventricle and the heart paced at 300 beats/min (1 volt, 30-ms pulse duration). Coronary flow, CPP, left ventricular pressures, and LV dP/dt were recorded continuously on a physiologic recorder (model 2800; Gould, Cleveland, OH).

Acquisition, Isolation, and Preparation of Neutrophils

Blood (20 ml) was collected from the jugular vein of a conscious dog on the day of the study and anticoagulated with 4.5 ml citric acid, 1.6%, and sodium citrate, 2.5% (pH 5.4), in 10 ml dextran solution, 6%, in buffered Hanks' balanced salt solution (HBSS). Polymorphonuclear neutrophils were separated as described previously.[14] The tubes for blood collection were maintained at room temperature while erythrocytes sedimented (approximately 40 min). The leukocyte-rich plasma layer was carefully aspirated and centrifuged at 500*g* at 4°C for 10 min. Contaminating erythrocytes in the pellet were removed by hypotonic lysis for 20 s with 9 ml sterile distilled water. Subsequent addition of 3 ml KCl, 0.6 M, and 15 ml buffered HBSS rapidly returned the cells to isotonicity. The leukocyte-rich suspension was centrifuged at 500*g* at 4°C for 10 min, after which time the cells were resuspended in 2 ml HBSS, layered on the top of 3 ml Ficoll-Pacque, and centrifuged again at 800*g* at 4°C for 20 min. The resulting pellet was rinsed with HBSS. The neutrophils were resuspended in HBSS in preparation for pretreatment before experimental use. This procedure for neutrophil isolation yields neutrophil suspensions that are 98% pure and more than 95% viable, as evaluated by trypan blue exclusion.[14] Moreover, the neutrophils are not activated by the procedure, as confirmed by the lack of shape changes (epifluorescent microscopy) and by their inability, in the absence of pharmacologic stimulation, to adhere to the coronary endothelium or inhibit endothelium-dependent vasorelaxation of isolated coronary rings.[14]

Initial studies involved pretreatment of the neutrophils with 1.0 MAC isoflurane or sevoflurane, followed by complete removal of the anesthetic and resuspension of the neutrophils. To evaluate potential concentration dependency, additional studies were performed with 0.25 MAC isoflurane. The anesthetics were injected directly into tightly sealed glass tubes (10 ml) containing 6 ml of the neutrophil suspension. The tubes were shaken gently for 15 min at 37°C in a water bath. The neutrophils were washed three times by suspension in fresh HBBS and centrifugation (total elapsed time, 30 min). Complete removal of the volatile anesthetic was confirmed by analysis of an aliquot of the final washing using gas chromatography. Untreated neutrophils were subjected to the same basic protocol, except that incubation was performed in the absence of a volatile anesthetic. Following the pretreatment protocol, neutrophil viability was confirmed (trypan blue technique), and the neutrophils were resuspended in HBSS to achieve a concentration of 1.5×10^7 neutrophils/ml (stock solution).

Experimental Protocols

After stabilization of the heart preparation for approximately 30 min, baseline values for variables of cardiac performance were obtained. Then, neutrophils, either untreated (n = 9) or pretreated with 0.25 or 1.0 MAC isoflurane (n = 6, n = 10, respectively), or 1.0 MAC sevoflurane (n = 10) was evaluated. The neutrophils (1.5×10^7 cells/ml) were initially coincubated with PAF (50 nM) at 37°C for 10 min. Then the PAF–neutrophil suspension was infused at a rate of 2% of coronary flow for 10 min *via* a side arm using a syringe pump, which resulted in a concentration of 3×10^5 neutrophils/ml, in accordance with previous studies,[15] and a final PAF concentration of 1 nM. Measurements of cardiac variables (and neutrophil adherence) were obtained during the neutrophil infusion and for 30 min following the infusion. Prior to infusion of the PAF–neutrophil suspension, an aliquot of the neutrophil suspension was obtained for measurement of superoxide using a spectrophotometric method (see below).

Analogous studies were conducted in which untreated neutrophils, PAF, and SOD (final concentration, 260 U/ml) (n = 9) were first coincubated, as described above, and then infused. "Control" studies were also performed using untreated neutrophils alone (n = 8) and PAF alone (n = 8).

Superoxide Production by Neutrophils

Superoxide production by neutrophils in suspension was determined by measuring the SOD inhibitable reduction of ferricytochrome c to ferrocytochrome c.[14] Neutrophils (1.5×10^7 cells/ml) were prewarmed in a shaking water bath at 37°C with 160 μM cytochrome c and 5 μg/ml cytochalasin B in the absence (control) or presence of the test agents for 5 min and then stimulated with PAF (50 nM). The final reaction volume was 0.5 ml. For each assay, duplicate samples were run. Half of the tubes were provided with an excess of SOD (100 μg/ml) as a control for nonspecific activity or color generation. Five minutes after adding PAF, cytochrome c reduction was measured spectrophotometrically by determining the optical density of the supernatant at 550 nm, using a V_{max} kinetic microtiter plate reader (Molecular Devices, Palo Alto, CA). Superoxide production was calculated using an extinction coefficient of 21 $mM^{-1} \cdot cm^{-1}$ for cytochrome c. Results are expressed as nanomolars of SOD-inhibitable O_2^- produced by a suspension of 1.5×10^7 neutrophils/ml.

Neutrophil Adherence

Neutrophil adherence to coronary vascular endothelium was estimated from the difference between neutrophils administered and recovered in coronary venous effluent.[16] The total number of neutrophils entering the coronary circulation line (neutrophil input) was calculated using the neutrophil concentration, the rate of coronary flow, and the duration of the infusion (10 min). To quantify the number of neutrophils leaving the coronaries (neutrophil output), coronary effluent was collected continuously for 12 min *via* the pulmonary artery from the beginning of the neutrophil administration. Neutrophils were counted using an Automated Hematology Analyzer (SE-900; Toa Medical Electronics Co, Hyogo, Japan). The percentage of neutrophils adherent to the coronary endothelium was calculated as follows:

$$\text{Adhesion (\%)} = [1 - (\text{neutrophil output/neutrophil input})] \times 100$$

Drugs

The following chemicals and reagents were obtained from Sigma Chemical (St. Louis, MO): Ficoll-Pacque, SOD, cytochrome c, and cytochalasin B. PAF and HBSS without Mg^{2+} and Ca^{2+} were obtained from Avanti Polar Lipids (Alabaster, AL) and Meditech, Inc. (Salt Lake City, UT), respectively. All solutions were prepared freshly on the day of the study. In the dog, the 1 MAC values for isoflurane and sevoflurane are 1.4% and 2.36%, respectively.[17,18] Thus, the millimolar equivalents for 1.0 MAC are 0.30 and 0.34 mM in HBSS for isoflurane and sevoflurane, respectively. These concentrations were calculated on the basis of the anesthetic potencies, *i.e.*, the MAC values, and buffer/gas partition coefficients for each anesthetic agent.[19]

Statistical Analysis

The neutrophil-induced changes in LVDP, LV dP/dt_{max}, and CPP were expressed as percent of the baseline (or preneutrophil) value. Within and between treatment changes were analyzed using a two-way analysis of variance for repeated measures followed by *post hoc* analysis with Student-Newman-Keuls test.[20] Effects of volatile anesthetics on superoxide production and adherence by neutrophils were analyzed using the Student *t* test or a one-way analysis of variance combined with the Student-Newman-Keuls test, as required. Data were expressed as mean ± SD. Differences were considered significant when P was less than 0.05.

Results

The baseline values for LVDP, LVEDP, LV dP/dt_{max}, coronary flow, and CPP were not significantly different in the various experimental groups. The grand mean value (± SD) for each of these variables was 74 ± 15 mmHg, 9 ± 1 mmHg, 2,711 ± 815 mmHg/s, 19 ± 4 ml/min, and 70 ± 1 mmHg, respectively.

Untreated, PAF-stimulated neutrophils caused marked reductions (> 50%) in LVDP, which did not recover over time (fig. 1). Pretreatment with either 1.0 MAC isoflurane or sevoflurane, as well as SOD, abolished these effects of the neutrophil–PAF mixture. Neither untreated neutrophils alone nor PAF alone affected LVDP. The

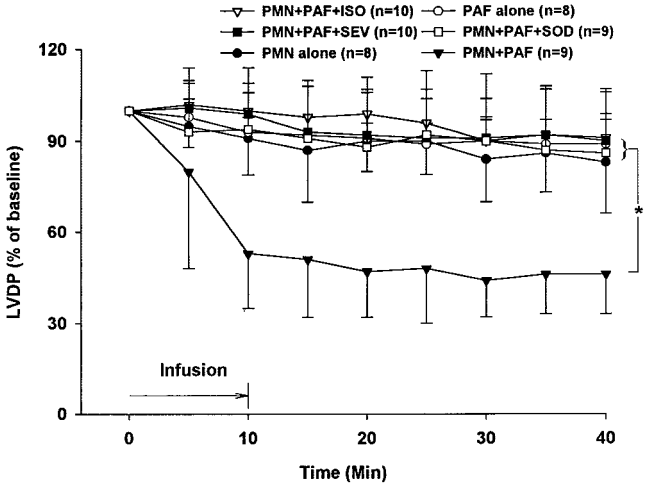

Fig. 1. Left ventricular developed pressure (LVDP) as percent of the preinfusion (baseline) value during infusion of neutrophils and/or drugs for 10 min with 30 min of recovery. Values are mean ± SD. PMN = neutrophils; ISO = isoflurane; SEV = sevoflurane; PAF = platelet-activating factor; SOD = superoxide dismutase. *$P < 0.05$, PMN–PAF *versus* all other groups.

findings for LV dP/dt_{max} were similar to those for LVDP (data not shown).

Platelet-activating factor–stimulated neutrophils increased CPP (fig. 2), which, in the presence of a constant coronary flow, reflected an increase in coronary vascular resistance. Isoflurane or sevoflurane at 1.0 MAC or SOD abolished this effect. Neither untreated neutrophils alone nor PAF alone affected CPP.

Platelet-activating factor stimulation of untreated neutrophils caused a 20-fold increase in superoxide production (fig. 3). Pretreatment of neutrophils with 1.0 MAC isoflurane or sevoflurane reduced the level of superoxide production during PAF by 29% and 33%, respectively.

The presence of PAF doubled the number of adherent

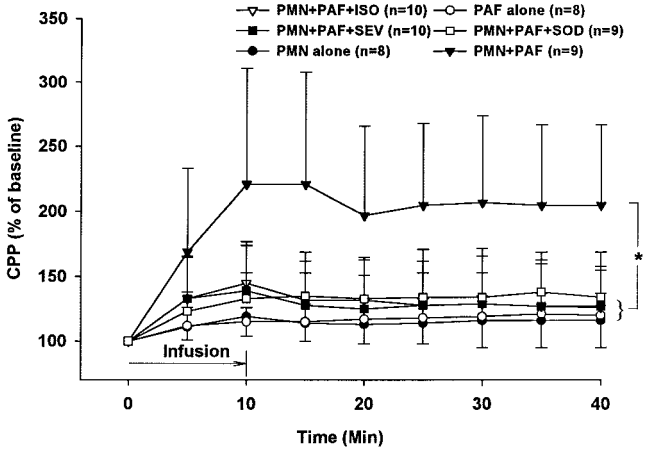

Fig. 2. Coronary perfusion pressure (CPP) as percent of the preinfusion (baseline) value during infusion of neutrophils and/or drugs for 10 min with 30 min of recovery. PMN = neutrophils; ISO = isoflurane; SEV = sevoflurane; PAF = platelet-activating factor; SOD = superoxide dismutase. Values are mean ± SD. *$P < 0.05$, PMN–PAF *versus* all other groups.

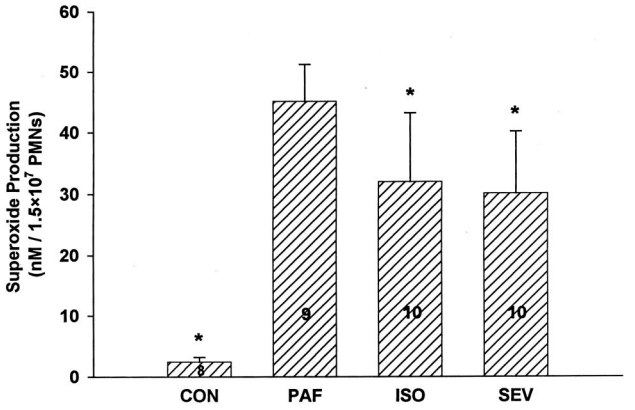

Fig. 3. Effect of pretreatment with isoflurane or sevoflurane on superoxide production by platelet-activating factor (PAF)–stimulated neutrophils. Number in bar represents number of observations. CON = control; PMN = neutrophils; ISO = isoflurane; SEV = sevoflurane. Values are mean ± SD. *$P < 0.05$ *versus* PAF.

neutrophils. Isoflurane or sevoflurane at 1.0 MAC or SOD abolished this effect (fig. 4).

Table 1 presents concentration-dependent effects of isoflurane on neutrophil-induced changes in cardiac function along with the associated changes in superoxide production and adherence. The changes in LVDP, LV dP/dt_{max}, and CPP after 10 min of infusion of activated neutrophils are shown. As is evident in figures 1 and 2, these cardiac variables did not recover during the subsequent 30 min. Pretreatment with 0.25 MAC isoflurane attenuated the decreases in LVDP and LV dP/dt_{max}, as well as the increases in CPP, superoxide production, and adherence, during activation of neutrophils with PAF. With exception of superoxide production and CPP, the effects by 0.25 MAC isoflurane were less than those by 1.0 MAC isoflurane.

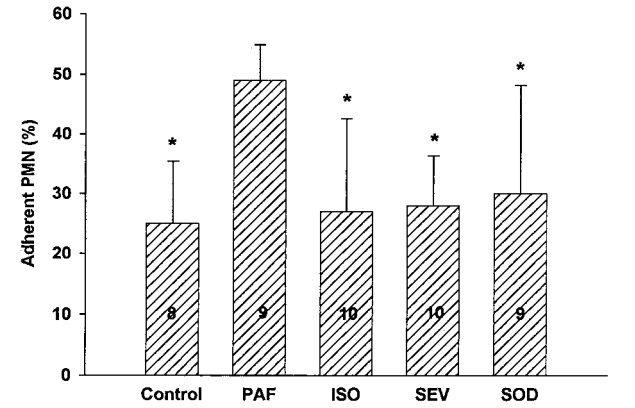

Fig. 4. Effect of pretreatment with isoflurane or sevoflurane, or superoxide dismutase on platelet-activating factor (PAF)–stimulated adherence of neutrophils. Number in bar represents number of observations. PMN = neutrophils; ISO = isoflurane; SEV = sevoflurane; SOD = superoxide dismutase. Values are mean ± SD. *$P < 0.05$ *versus* PAF.

Table 1. Concentration-dependent Effects of Isoflurane on Neutrophil-induced Cardiac Dysfunction along with Associated Changes in Superoxide Production and Adherence

	No Anesthetic	Isoflurane	
		0.25 MAC	1.0 MAC
LVDP (% baseline)	53 ± 18	75 ± 8*	98 ± 10*†
LV dP/dt max (% baseline)	53 ± 17	74 ± 12*	97 ± 12*†
CPP (% baseline)	221 ± 90	120 ± 7*	132 ± 31*
Superoxide production (nM/1.5 × 10^7 PMNs)	45.1 ± 11.1	31.5 ± 7.2*	31.4 ± 11.7*
Adherence (%)	52 ± 4	42 ± 3*	26 ± 8*†

Values are mean ± SD

* $P < 0.05$ *vs.* no anesthetic. † $P < 0.05$ *vs.* 0.25 MAC isoflurane

LVDP = left ventricular developed pressure; CPP = coronary perfusion pressure; PMN = neutrophils; PAF = platelet-activating factor; MAC = minimum alveolar concentration.

Discussion

The main findings in this study are as follows: (1) Pretreatment with either isoflurane or sevoflurane abolished the neutrophil-induced impairment in contractile function in isolated rat hearts; and (2) this inhibitory effect of the volatile anesthetics on neutrophils was associated with an inhibition to neutrophil superoxide production and adherence to coronary vascular endothelium.

Over the past 15 yr, evidence has accumulated suggesting that neutrophil-endothelium interactions and superoxide production play significant roles in the pathophysiology of myocardial reperfusion injury.[12,13] The current findings provide additional support for this hypothesis, by indicating that PAF-activated neutrophils can cause cardiac dysfunction and that this response can be prevented with SOD. Formation of superoxide is the first of several steps in forming other oxygen-derived reactive products, which include hydrogen peroxide and hydroxyl radical.[21] Additional sources of oxygen-derived reactive products during ischemia–reperfusion include xanthine oxidase, the electron transport chain in mitochondria, and the degradation of catecholamines.[21]

Neutrophils activated by PAF can release oxygen free radicals, including superoxide, and proteolytic enzymes, both of which can cause myocardial injury. The ability of the volatile anesthetics to inhibit superoxide production by the neutrophils and of SOD to completely abolish neutrophil-induced cardiac dysfunction point to superoxide as a principal mediator of myocardial injury in this rat model. However, we cannot rule out roles for other factors.

Our previous work indicated that the coincubation of a volatile anesthetic (isoflurane) inhibited PAF-induced superoxide production by neutrophils.[14] The current findings extend these observations by indicating that this inhibition persisted following washout of the volatile anesthetic, which is a hallmark of preconditioning. The mechanism(s) underlying this effect are uncertain, but several possibilities can be proposed: (1) a nonspecific stabilization of the cell membrane of the neutrophil; (2) a direct inhibitory action on NADPH oxidase; and (3) an inhibitory effect at a site in the signal transduction pathway regulating NADPH oxidase.[22] These sites include (1) the PAF receptor on the neutrophil membrane; (2) the GTP-binding proteins (G proteins), which are involved in transduction of agonist signals; (3) phospholipase C, which generates 2 second messengers, inositol 1,4,5-triphosphate (IP_3) and diacylglycerol, leading to a rise in cytosolic free Ca^{2+}; and (4) protein kinase C and protein phosphorylation, which have been shown to be involved in the neutrophil respiratory burst. Further investigations are necessary to evaluate these potential mechanisms.

The current study differs notably from previous studies (including one from this laboratory) that demonstrated the ability of volatile anesthetics to reduce adherence of neutrophils to the coronary vascular endothelium.[14,16] First, the current study focused specifically on effects of the volatile anesthetics on the neutrophils; other cell types, including the endothelial cells and myocytes, were not exposed to the anesthetics. Second, the anesthetics were removed before activation and administration of the neutrophils into the coronary circulation, which made the findings relevant to preconditioning.

In PAF-stimulated *in vitro* systems, such as the one used here, adherence of neutrophils is *via* ICAM-1.[23] The ability of the volatile anesthetics to prevent the PAF-induced increase in neutrophil adherence in the current study cannot be due to a direct inhibitory effect on ICAM-1 since the neutrophils but not the endothelial cells were exposed to the anesthetics. However, a direct inhibitory effect on the counterligand CD11/CD18 on the surface of neutrophils cannot be ruled out. Another possibility is that pretreatment with the volatile anesthetics reduced PAF-stimulated superoxide production, which, in turn, prevented up-regulation of ICAM-1.[24,25] This latter mechanism is supported by our findings demonstrating that SOD completely abolished the PAF-induced increases in neutrophil adherence (as well as in cardiac contractile function).

Under normal conditions, neutrophils do not adhere to the coronary vascular endothelium. However, our find-

ings indicated that the basal level of neutrophil adherence, *i.e.*, in the absence of activation by PAF, was approximately 25% (fig. 4), a value comparable to that found in other studies using isolated hearts.[16,26] The mechanism underlying the basal adherence of neutrophils in our isolated hearts is uncertain, but it may be due to the absence of blood components with antioxidant and antiadhesion capabilities or to the effect of the preparation procedures on the neutrophils and endothelial cells. An attenuated release of endothelial nitric oxide, a known inhibitor of neutrophil adherence,[27] must be considered. It is worth noting that the basal adherence of neutrophils did not increase CPP (reflecting vasoconstriction) or impair cardiac contractile function. Apparently, activation of the neutrophils is required before these adverse changes are initiated.

Superoxide production by activated neutrophils can result from adherence-independent and adherence-dependent pathways.[23] Although our findings indicated that pretreatment with the volatile anesthetics reduced adherence-independent superoxide production by only approximately 30%, the reduced adherence of neutrophils suggests that an inhibition to the adherence-dependent pathway for superoxide production also occurred. These combined effects were apparently sufficient to reduce superoxide levels below the level necessary to cause cardiac dysfunction. A predominant role for adherence-dependent superoxide production is suggested by our findings demonstrating that the concentration-dependent inhibitory effects of isoflurane on neutrophil-induced cardiac dysfunction were associated with concentration-dependent inhibitory effects on neutrophil adherence but not on superoxide production of isolated neutrophils (table 1).

Platelet-activating factor–activated neutrophils increased CPP, which reflected an increase in coronary vascular resistance. This could be due to impaired tonic release of a vasodilating substance, *e.g.*, nitric oxide, augmented release of a vasoconstricting substance, *e.g.*, endothelin, or mechanical obstruction of the coronary circulation by neutrophil aggregates. Although total coronary flow was held constant, we can not preclude the possibility that administration of activated neutrophils caused microregions of inadequate flow that contributed to the global contractile dysfunction. The volatile anesthetics, as well as SOD, prevented the increase in coronary vascular resistance caused by the activated neutrophils.

Our findings indicated that the "preconditioning effect" of isoflurane on neutrophils was concentration dependent and that concentrations of isoflurane as low as 0.25 MAC were sufficient to inhibit neutrophil activation and to reduce their ability to cause cardiac dysfunction. These concentration-related findings are consistent with those found in a recent study that evaluated isoflurane preconditioning against myocardial infarction in barbiturate-anesthetized dogs.[4]

The current findings apply specifically to the crystalloid-perfused rat heart preparation and to the concentrations of the volatile anesthetics that were evaluated. Recognized limitations of this model include marked coronary vasodilation and low oxygen transport with high oxygen partial pressure, and an abnormal cardiac workload.

Because the focus of our study was neutrophil-induced cardiac dysfunction *per se*, our experimental design did not include a period of ischemia followed by reperfusion. Neutrophils and their activator, PAF, were infused into the isolated, buffer-perfused rat hearts without an interruption of flow. Other formed elements, *e.g.*, platelets, were excluded from the neutrophil suspensions. This approach greatly simplified interpretation of the findings by providing a controlled stimulus for activation of the neutrophils and by avoiding the complex, multifactorial nature of reperfusion injury.

The use of PAF to stimulate the neutrophils bypassed the normal adherence-dependent, selectin-initiated activation that occurs during ischemia–reperfusion.[12] PAF is an endogenous phospholipid that is a mediator of inflammation. It is released by both neutrophils and endothelial cells *in vivo* in response to chemotactic factors and has been found in the coronary venous effluent following ischemia and reperfusion.[28] PAF binds to a specific receptor on the neutrophil membrane, which is the first event in the signal transduction sequence. Ultimately, the production of superoxide by neutrophils results from activation and assembly of NADPH oxidase, which is a transmembrane electron transport chain that reduces oxygen to superoxide. Superoxide production is accompanied by a rise in cytosolic Ca^{2+} due to release from intracellular stores and influx through the plasma membrane.[22] PAF was chosen for use in the current study because it has been shown to cause more pronounced increases in superoxide production and endothelial adherence by canine neutrophils than the other neutrophil-activating agents, leukotriene B_4 and *N*-formyl-Leu-Phe.[23]

Dose–response studies have demonstrated that PAF itself can cause reductions in developed pressure of isolated rat hearts.[29] Such responses were not evident in the current study, apparently because the buffer concentration of PAF was insufficient, although it was capable of increasing superoxide production and causing cardiac dysfunction when combined with neutrophils.

Canine neutrophils were used in the current study because of the difficulty in acquiring sufficient quantities from the rat. The normal number of neutrophils in the rat circulation is 2.5×10^6 neutrophils/ml.[15] The number used in the current study, 3×10^5 neutrophils/ml, was based on previous work in the isolated rat heart, which demonstrated that, in the presence of a suitable exogenous activator, it was sufficient to cause significant cardiac dysfunction.[15] The lack of cardiac dysfunction during administration of unstimulated canine neutro-

phils in the current study and similar findings in studies employing human neutrophils[15] suggest no adverse reaction when heterologous neutrophils are used in the buffer-perfused rat heart preparation.

In conclusion, the results of this study indicate that neutrophils pretreated with 1.0 MAC isoflurane or sevoflurane lost their ability to cause contractile dysfunction in isolated rat hearts, while those pretreated with a concentration of isoflurane as low as 0.25 MAC were partially inhibited. The mechanism underlying these effects appeared to be an inhibition to superoxide production, leading to a reduced adherence to the endothelium and presumably a reduced migration into the surrounding parenchymal cells, where release of cytotoxic substances would occur. Another factor may have been the ability of the volatile anesthetics to prevent a nonuniform distribution of coronary flow because of microvessel constriction or neutrophil aggregates. Our findings suggest that an inhibition to neutrophil activation and the neutrophil–endothelium interaction may contribute to the preconditioning effect of volatile anesthetics in the heart *in vivo*. The ability of volatile anesthetics to suppress neutrophil activation and the neutrophil–endothelium interactions may have applicability to other clinical conditions characterized by immune cell responses and inflammation, including cardiopulmonary bypass.[30]

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