

Isoflurane and Sevoflurane Precondition against Neutrophil-induced Contractile Dysfunction in Isolated Rat Hearts

Guochang Hu, M.D.,* M. Ramez Salem, M.D.,† George J. Crystal, Ph.D.‡

Background: The authors tested the hypothesis that pretreatment with isoflurane or sevoflurane can protect the heart against neutrophil-induced contractile dysfunction.

Methods: Studies were conducted in buffer-perfused and paced isolated rat hearts. Left ventricular developed pressure served as an index of contractility. Pretreatment consisted of administration of 1.0 minimum alveolar concentration isoflurane or sevoflurane for 15 min followed by a 10-min washout and was performed in the absence and presence of the adenosine triphosphate-sensitive potassium channel inhibitor glibenclamide (10 μ M). Polymorphonuclear neutrophils and platelet-activating factor were then added to the perfusate for 10 min, followed by 30 min of recovery. Neutrophil retention was assessed from the difference between those administered and collected in coronary effluent and measurements of myeloperoxidase in myocardial samples. Isolated hearts were also used to assess the effect of volatile anesthetic pretreatment on cardiac dysfunction caused by enzymatically generated superoxide. In additional studies, the authors evaluated the effect of volatile anesthetic pretreatment on the adherence of neutrophils to isolated rat aortic segments.

Results: Platelet-activating factor-stimulated neutrophils caused marked and persistent reductions (> 50%) in left ventricular developed pressure. Pretreatment with either isoflurane or sevoflurane abolished these effects, as well as the associated increases in neutrophil retention. Glibenclamide did not alter these actions of the anesthetics. Pretreatment with either volatile anesthetic attenuated the reductions in left ventricular developed pressure caused by exogenous superoxide and abolished the increases in neutrophil adherence in the aortic segments.

Conclusion: Isoflurane and sevoflurane preconditioned the heart against neutrophil-induced contractile dysfunction. This action was associated with an inhibition to neutrophil adherence and likely involved an increased resistance of the myocardium to oxidant-induced injury; the adenosine triphosphate-sensitive potassium channels played no apparent role.

RECENT findings in various animal models¹⁻⁴ and in patients^{5,6} have suggested that volatile anesthetics pos-

sess preconditioning effects in the heart. The adenosine triphosphate-sensitive potassium (K_{ATP}) channels in the cardiomyocytes have been implicated in these effects.^{1,7-10} Whether additional pathways and/or cell types also play a role remains to be determined.

Over the past 15 yr, evidence has accumulated suggesting that neutrophil-endothelial interactions play a significant role in the pathophysiology of myocardial reperfusion injury.¹¹ Adherence of neutrophils to the endothelium *via* the adhesion molecules, *e.g.*, intercellular adhesion molecule 1, is believed to represent a critical step in the process of transmigration and influx of neutrophils into the myocardium, where they cause damage through release of oxygen free radicals, including superoxide, and proteolytic enzymes.¹¹

Several lines of evidence suggest that continuous exposure to a volatile anesthetic can inhibit the neutrophil-endothelium interaction and the inflammatory response.¹²⁻¹⁵ First, we demonstrated that cocubation with isoflurane attenuated superoxide production and coronary vascular adherence of activated neutrophils while reducing neutrophil-induced endothelial dysfunction of coronary artery segments.¹² Furthermore, continuous administration of a volatile anesthetic has been shown to reduce postischemic adhesion of neutrophils in the coronary circulation of isolated rat hearts¹³ and to blunt the decrease in myocardial contractility caused by oxygen free radicals, (generated by electrolysis of the buffer perfusate) in isolated rabbit hearts.¹⁴ Finally, the presence of isoflurane or halothane has been shown to decrease the death of aortic endothelial cells exposed to hydrogen peroxide.¹⁵

Although the preconditioning effects of volatile anesthetics on inflammatory pathways have not been studied extensively, the few results have been promising. We demonstrated that neutrophils pretreated with either isoflurane or sevoflurane lost their ability to cause contractile dysfunction in isolated rat hearts and that this was associated with a reduction in neutrophil adherence.¹⁶ Others have found that pretreatment with isoflurane attenuated cytokine-induced death of cultured human endothelium and rat smooth muscle cells and that it also inhibited endothelium-dependent vasodilation, increase in tumor necrosis factor α , and damage to the vascular endothelium associated with lipopolysaccharide-induced inflammation in rats.^{17,18} It is unknown whether pretreatment of the myocardium with a volatile anesthetic, *i.e.*, anesthetic preconditioning, can provide

* Research Assistant Professor, Department of Anesthesiology, Advocate Illinois Masonic Medical Center, and Department of Anesthesiology, University of Illinois College of Medicine. † Chair, Department of Anesthesiology, Advocate Illinois Masonic Medical Center. Clinical Professor, Department of Anesthesiology, University of Illinois College of Medicine. ‡ Director of Research Laboratory, Department of Anesthesiology, Advocate Illinois Masonic Medical Center. Professor, Departments of Anesthesiology and of Physiology and Biophysics, University of Illinois College of Medicine.

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Address reprint requests to Dr. Crystal: Department of Anesthesiology, Advocate Illinois Masonic Medical Center, 836 West Wellington Avenue, Chicago, Illinois 60657-5193. Address electronic mail to: gcrystal@uic.edu. Individual article reprints may be purchased through the Journal Web site, www.anesthesiology.org.

protection against local activation of inflammatory pathways.

The current study was conducted in isolated rat hearts to test the hypothesis that isoflurane and sevoflurane can precondition the myocardium against neutrophil-induced contractile dysfunction. Mechanistic insights were obtained from measurements of neutrophil retention and adherence to the vascular endothelium, from the use of the K_{ATP} channel inhibitor glibenclamide and from additional studies in which oxygen free radicals (superoxide anions) were produced by an enzymatic system rather than by neutrophils.

Materials and Methods

The study was composed of three series. Series 1 and 3 were conducted in isolated rat hearts, and series 2 was conducted in isolated rat aortic segments.

Series 1: Neutrophil-induced Cardiac Dysfunction

Heart Preparation. After approval from the Institutional Animal Care and Use Committee (Chicago, Illinois), studies were conducted in 71 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 and mounted on a nonrecirculating Langendorff perfusion apparatus. The heart preparation was supplied with Krebs buffer from either of two heated (37°C) reservoirs. Retrograde coronary perfusion *via* the cannulated aorta was initially *via* a reservoir containing anesthetic-free buffer bubbled with a 95% O_2 –5% CO_2 gas mixture. 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 $NaHCO_3$, 10.0 mM glucose. The hearts were perfused at constant flow, initially titrated to achieve a coronary perfusion pressure (CPP) of 70 mmHg. Coronary flow averaged 19 ± 3 ml/min in the heart preparations. A second reservoir contained buffer that was bubbled with this same gas mixture after it had passed through a calibrated vaporizer (Dräger, Lübeck, Germany) providing 1.0 minimum alveolar concentration (MAC) isoflurane (1.4%) or sevoflurane (2.4%). This procedure was continued for a least 30 min to ensure complete equilibration of the volatile anesthetic as assessed by gas chromatography (mode 5890; Hewlett Packard, Wilmington, DE). With the two-reservoir system, it was possible to switch back and forth between volatile anesthetic-free and volatile anesthetic-equilibrated buffer.

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. The balloon was inflated with

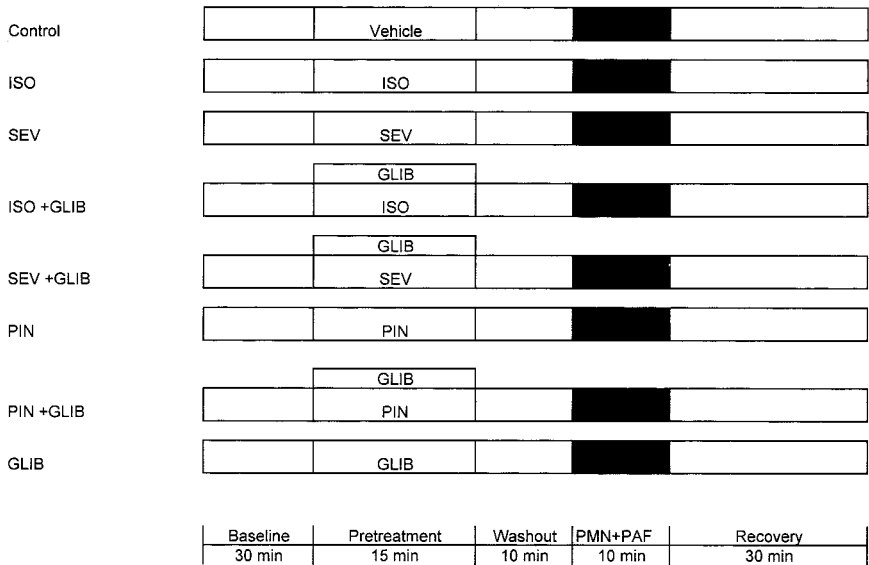
saline sufficiently to increase left ventricular (LV) end-diastolic pressure to approximately 8–10 mmHg, which was shown in preliminary studies to provide the optimal ventricular preload.

Measurements of left ventricular developed pressure (LVDP; end-systolic minus end-diastolic pressure) and LV 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 was paced at 300 beats/min (1 volt, 30-ms pulse duration). Coronary flow, CPP, LV 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 of dextran solution, 6%, in buffered Hanks' balanced salt solution (HBSS). Neutrophils were separated as described previously.¹² 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 500g 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 500g at 4°C for 10 min, after which the cells were resuspended in 2 ml HBSS, layered on the top of 3 ml Ficoll-Paque, and centrifuged again at 800g 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. Our procedure for neutrophil isolation yields neutrophil suspensions that are 98% pure and more than 95% viable as evaluated by trypan blue exclusion.¹²

Experimental Protocols. The experimental protocols for series 1 are illustrated in figure 1. After a stabilization period of 30 min, during which perfusion was *via* the anesthetic-free reservoir, baseline measurements for variables of cardiac performance were obtained. A pretreatment period was initiated by switching perfusion to the anesthetic-equilibrated reservoir. After 15 min of administration of either isoflurane or sevoflurane, a second set of cardiac measurements was obtained, and the heart was returned to the anesthetic-free reservoir for 10 min to allow washout of the anesthetic from the heart. Completeness of washout was confirmed by chromatographic analysis of a sample of effluent obtained at

Fig. 1. Schematic diagram of the experiment protocols. GLIB = glibenclamide; ISO = isoflurane; PAF = platelet-activating factor; PIN = pinacidil; PMN = neutrophils; SEV = sevoflurane.



the end of the washout period. A control group received Krebs solution (vehicle) free of anesthetic during the pretreatment period.

After pretreatment and washout, all hearts were subjected to a 10-min infusion of neutrophils along with platelet-activating factor (PAF) to stimulate the neutrophils.¹² This was followed by a 30-min recovery period. The neutrophils (1.5×10^7 cells/ml) were initially co-incubated with PAF (50 nM) at 37°C for 10 min. Then, the PAF-neutrophil suspension was infused in all groups at a rate of 2% of coronary flow *via* a side arm with use of a syringe pump, which resulted in a concentration of 3×10^5 neutrophils/ml, in accordance with previous studies,^{16,19} and a final PAF concentration of 1 nM. We have demonstrated that this concentration of PAF itself has no effects on cardiac function or CPP in the isolated rat heart preparation.¹⁶ Measurements of cardiac variables were obtained every 5 min during neutrophil infusion and recovery periods.

After observing that both isoflurane and sevoflurane abolished the ability of activated neutrophils to cause cardiac dysfunction, the role of K_{ATP} channels in this effect was evaluated. This was accomplished by administering the K_{ATP} channel inhibitor, glibenclamide (10 μ M),¹² along with 1.0 MAC isoflurane or sevoflurane. Additional validation studies were performed in which (1) the K_{ATP} channel opener, pinacidil (100 μ M), was administered in the absence and presence of glibenclamide; and (2) glibenclamide was administered alone. Pinacidil or/and glibenclamide were infused *via* a side arm connected to the aortic cannula using an infusion pump. The infusions of glibenclamide were initiated 5 min before and maintained during the administrations of pinacidil or the volatile anesthetic-equilibrated perfusate. Additional studies were performed in which neutrophils were administered to untreated hearts in the absence of PAF.

Neutrophil Retention in Isolated Hearts. Neutrophil retention in the myocardium was estimated from the difference between neutrophils administered and recovered in coronary venous effluent.^{13,16} 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 neutrophil administration. Neutrophils were counted using an Automated Hematology Analyzer (SE-900; Toa Medical Electronics Co., Hyogo, Japan). The percentage of neutrophils retained in the coronary endothelium was calculated as: retention (%) = $[1 - (\text{neutrophil output} / \text{neutrophil input})] \times 100$.

Tissue Myeloperoxidase Activity. Myeloperoxidase activity, an index of neutrophil accumulation in the myocardium, was determined using a method described previously.²⁰ At the end of the experiment, the heart was immediately removed, frozen, and stored at -70°C until assay. A sample of myocardium (200 - 400 mg) was obtained from the anterior wall of the left ventricle and homogenized in hexadecyltrimethyl ammonium bromide buffer (100-mg samples/ml). The homogenate was then sonicated three times for 15 s and centrifuged at 10,000 rpm for 30 min at 4°C. A 10- μ l sample of the supernatant was loaded into a cuvette plate. O-dianisidine dihydrochloride with 0.0005% hydrogen peroxide in phosphate buffer (190 μ l) was then added to samples using a multi-petter and analyzed with a spectrophotometer at 460 nm (SPECTRA-max; Molecular Devices, Sunnyvale, CA). Myeloperoxidase activity was expressed as absorbance units (U) \cdot min⁻¹ \cdot g⁻¹ tissue.^{20,21}

Series 2: Neutrophil Adherence to Aortic Vascular Endothelium

Studies were conducted to assess the effect of volatile anesthetic pretreatment on neutrophil adherence *per se*. Isolated neutrophils were labeled with a vital fluorescent dye as described in detail previously.¹² Briefly, 1 ml solution, 4 μM , of PKH26 dye was added to 1 ml dye diluent containing 2×10^7 neutrophils/ml. After the sample was gently mixed, it was incubated at room temperature for 5 min; the labeling reaction was stopped by adding 2 ml plasma and incubating for 1 min. The plasma-stopped sample was diluted with 4 ml HBSS and then centrifuged at 400g for 10 min at 4°C. The resultant cell pellet was transferred to a new tube for additional duplicate washings, and the cells were resuspended. This labeling procedure yields neutrophils possessing normal viability and function.¹²

The studies used 30 aortic segments obtained from seven rats. After being anesthetized as described above, a midsternal thoracotomy was performed, and the thoracic aorta was rapidly excised and placed into cold oxygenated Krebs solution. The vessel was carefully dissected, cleaned of connective and adipose tissue without disturbing the endothelium, and cut into 2- to 3-mm-long rings. The rings were carefully opened without damaging the endothelium to form segments and then incubated in a tube containing 3 ml Krebs solution at 37°C for 15 min, either alone or with 1.0 MAC isoflurane or sevoflurane, followed by three washings.

Labeled neutrophils were added to a tube containing 3 ml Krebs solution to achieve a final concentration of 5×10^5 neutrophils/ml. At 10 min after the final washing, the aortic segments were incubated with labeled neutrophils in a shaker bath for 20 min at 37°C. PAF (1.0 μM) was used to stimulate neutrophil adherence. After the incubation period, the vascular segments were removed and flushed gently with HBSS. Adherence was determined by counting the number of neutrophils adhering to the endothelial surface in six separate microscopic fields under epifluorescent microscopy (490-nm excitation, 504-nm emission; Fryer Company, Inc., Huntley, IL), and expressed per square millimeter of endothelium. The following conditions were evaluated in the adherence studies: (1) untreated aortic segment and neutrophils alone; (2) untreated aortic segment, neutrophils, and PAF (control); (3) isoflurane-treated aortic segments, neutrophils, and PAF; and (4) sevoflurane-treated aortic segments, neutrophils, and PAF.

Series 3: Cardiac Dysfunction Caused by Exogenous Superoxide

Studies were conducted to determine the effect of pretreatment with volatile anesthetics on the cardiac dysfunction caused by oxygen free radicals in the absence of neutrophils. Twenty-three isolated rat heart preparations, prepared as describe above, were used.

After stabilization of the preparation, the heart was treated with 1.0 MAC isoflurane or sevoflurane for 15 min, followed by a 10-min washout period. Then, the heart was perfused for 10 min with oxygenated Krebs buffer containing superoxide. Superoxide was generated using an exogenous system comprised of xanthine oxidase and purine,^{22,23} which were infused into the aortic cannula *via* a side arm. The final concentrations of xanthine oxidase and purine were 0.05 U/ml and 3.0 mM, respectively. Preliminary studies showed that administration of these concentrations of xanthine oxidase or purine separately had no effect on LVDP, $\text{dP/dt}_{\text{max}}$ and CPP. After perfusion with the superoxide-containing Krebs buffer, the heart was supplied with normal buffer solution for 15 min. LVDP, LV $\text{dP/dt}_{\text{max}}$, and CPP were obtained every 5 min during the entire experiment. These studies were conducted in three groups: (1) control group: no pretreatment; (2) pretreatment with isoflurane; and (3) pretreatment with sevoflurane.

Drugs

The following chemicals and reagents were obtained from Sigma Chemical (St. Louis, MO): Ficoll-Pacque, PKH26 dye, pinacidil, glibenclamide, dimethyl sulfoxide, purine (7H-Imidazo [4,5-d] pyrimidine), and xanthine oxidase (xanthine: oxygen oxidoreductase; EC 1.1.3.22, grade III from buttermilk). PAF and HBSS without Mg^{2+} and Ca^{2+} were obtained from Avanti Polar Lipids (Alabaster, AL) and Mediatech, Inc. (Salt Lake City, UT), respectively. All solutions were prepared freshly on the day of the study. In the rat, the 1.0 MAC values for isoflurane and sevoflurane are 1.4 and 2.4%, respectively.^{24,25} Therefore, the millimolar equivalents for 1.0 MAC are 0.30 and 0.35 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.²⁶

Statistical Analysis

In series 1, the effect of anesthetic pretreatment (both with and without glibenclamide) on baseline values for hemodynamic parameters (LVDP, LV $\text{dP/dt}_{\text{max}}$, and CPP) was assessed using the Student *t* test for paired samples.²⁷ Within- and between-group differences for these parameters after neutrophil administration were assessed using a two-way analysis of variance for repeated measures followed by the Student-Newman-Keuls test.²⁷ Other statistical analysis was performed using the Student *t* test for unpaired samples or a one-way analysis of variance combined with the Student-Newman-Keuls test as required. Data were expressed as mean \pm SD. Differences were considered significant when $P < 0.05$.

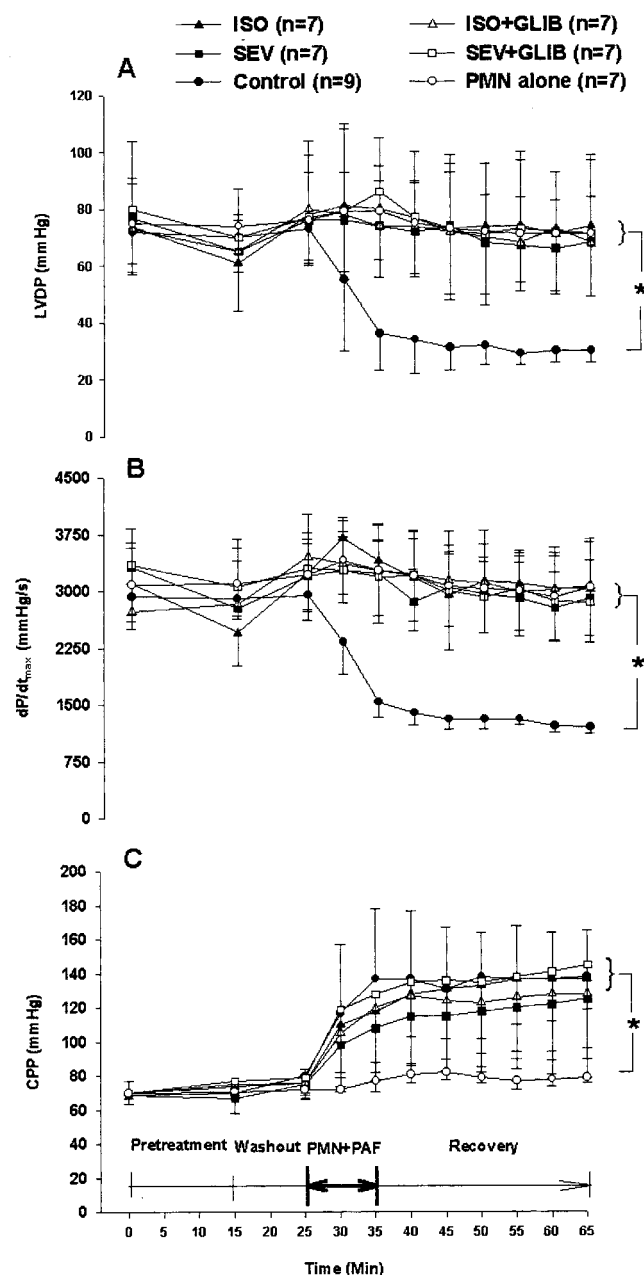


Fig. 2. Effects of pretreatment with 1.0 minimum alveolar concentration isoflurane (ISO) or sevoflurane (SEV) in the absence and presence of glibenclamide (GLIB) on left ventricular developed pressure (LVDP) (A), left ventricular dP/dt_{max} (B), and coronary perfusion pressure (CPP) (C) during pretreatment with volatile anesthetics and infusion of neutrophils (PMNs) and platelet-activating factor (PAF) for 10 min with 30 min of recovery. Values are expressed as mean \pm SD. n represents number of hearts. * $P < 0.05$, control group versus all other groups in A and B and PMN alone group versus all other groups in C.

Results

Initial baseline values for LVDP, dP/dt_{max} , and CPP were similar for the experimental groups (fig. 2). Administration of isoflurane or sevoflurane caused modest reductions in LVDP and LV dP/dt_{max} , which were similar in the absence or presence of glibenclamide; after wash-

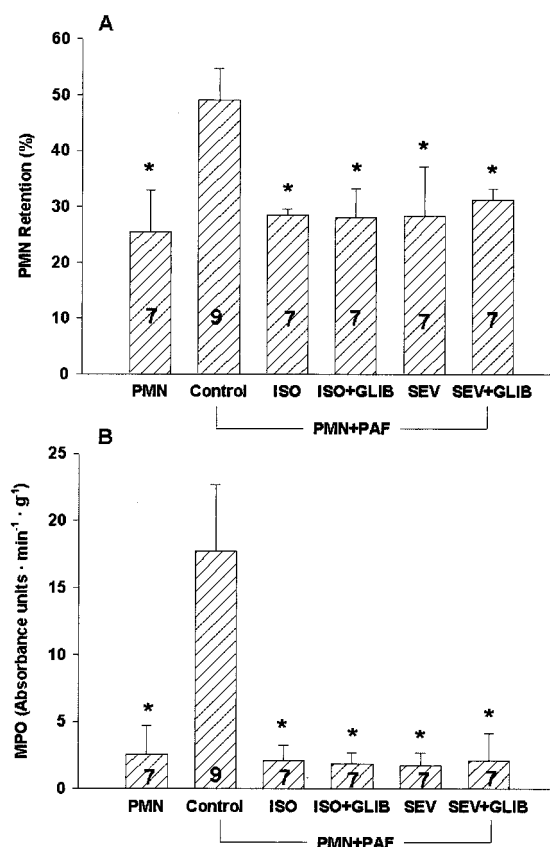


Fig. 3. Effects of pretreatment with isoflurane (ISO) or sevoflurane (SEV) in the absence and presence of glibenclamide (GLIB) on platelet-activating factor (PAF)-stimulated neutrophil (PMN) retention in isolated hearts (A) and on myeloperoxidase (MPO) activity in end-recovery hearts (B). Numbers in bars represent numbers of hearts. Values are expressed as mean \pm SD. * $P < 0.05$ versus control group.

out, these parameters recovered. There was no change in CPP among different groups during pretreatment and washout.

Neutrophils without PAF had no effect on LVDP, LV dP/dt_{max} , or CPP (fig. 2), although they demonstrated modest retention in the myocardium associated with low-level myeloperoxidase activity (fig. 3).

Platelet-activating factor-activated neutrophils caused pronounced reductions ($> 50\%$) in LVDP and LV dP/dt_{max} and increases in CPP (approximately 100%), which did not recover over time (fig. 2). Because flow was kept constant, the increases in CPP reflected increases in coronary vascular resistance. Pretreatment with the volatile anesthetics abolished the cardiac depression by the PAF-activated neutrophils, although it did not affect the increases in CPP. Pretreatment also abolished the PAF-induced increases in neutrophil retention (fig. 3A) and myeloperoxidase activity (fig. 3B) in the isolated hearts. Glibenclamide did not alter the effects of the volatile anesthetics.

Table 1 presents the effects of pretreatment with pinacidil on the change in cardiac function and associated parameters caused by PAF-activated neutrophils.

Table 1. Effects of Pinacidil Alone and Combined with Glibenclamide on Neutrophil-induced Cardiac Dysfunction Along with Associated Changes in Adherence and Myeloperoxidase

	Control (n = 9)	Pinacidil (n = 7)	Pinacidil + Glybenclamide (n = 7)
LVDP, % baseline	58 ± 18	92 ± 12*	69 ± 19†
LV dP/dt _{max} , % baseline	57 ± 19	95 ± 11*	61 ± 14†
CPP, % baseline	205 ± 88	108 ± 6*	139 ± 12†
Neutrophil retention, %	58.6 ± 6.3	33.2 ± 8.5*	54.1 ± 9.7†
Myeloperoxidase, absorbance U · min ⁻¹ · g ⁻¹ tissue	17.7 ± 11.8	6.5 ± 2.9*	18.7 ± 9.6†

Values are presented as mean ± SD.

* $P < 0.05$ vs. control. † $P < 0.05$ vs. pinacidil.

CPP = coronary perfusion pressure; LV = left ventricular; LVDP = left ventricular developed pressure.

Findings with and without glibenclamide are shown. The changes in LVDP, LV dP/dt_{max}, and CPP after 10 min of exposure to activated neutrophils are presented. As is evident in figure 2, these cardiac variables did not recover during the subsequent 30 min. Table 1 shows that pretreatment with pinacidil abolished the decreases in LVDP and dP/dt_{max}, as well as the increases in CPP, caused by PAF-activated neutrophils. These changes were associated with reductions in neutrophil retention and myeloperoxidase activity. All effects of pinacidil were prevented by glibenclamide.

Platelet-activating factor caused marked increases in neutrophil adherence to the endothelial surface of the aortic segments, which were abolished by pretreatment with the volatile anesthetics (figs. 4 and 5).

Exogenous superoxide caused progressive decreases in LVDP, which maximized at 35% (fig. 6); these effects were reversed when perfusion was returned to normal buffer. Pretreatment with either anesthetic attenuated but did not abolish the decreases in LVDP caused by exogenous superoxide (fig. 6). The results for LV dP/dt_{max} were similar. Exogenous superoxide did not affect CPP in the various experimental groups.

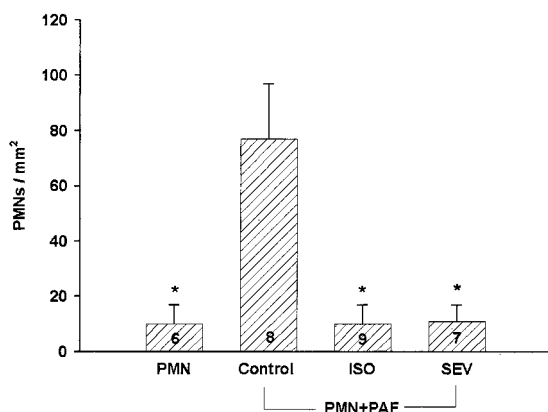


Fig. 4. Effects of pretreatment of aortic segments with isoflurane (ISO) or sevoflurane (SEV) on platelet-activating factor (PAF)-stimulated neutrophil (PMN) adherence to the endothelium. Numbers in bars represent numbers of aortic rings. Values are expressed as mean ± SD. * $P < 0.05$ versus control group (PMN-PAF mixture).

Discussion

The main findings of this study are as follows: (1) Pretreatment of the heart with 1.0 MAC isoflurane or sevoflurane abolished the neutrophil-induced impairment in contractile function. (2) This effect was associated with an inhibition to neutrophil adherence and likely involved an increased resistance of the myocardium to oxidant-induced injury; an opening of K_{ATP} channels played no apparent role.

Over many years, data have accumulated that implicate neutrophil-endothelium interactions and superoxide production in the pathophysiology of myocardial reperfusion injury.^{11,28} The current findings provide additional support for this hypothesis by indicating that PAF-activated neutrophils can cause cardiac dysfunction. Our previous study showed that this dysfunction could be prevented with superoxide dismutase, thus suggesting an integral role for superoxide.¹⁶ Formation of superoxide is the first of several steps in forming other oxygen-derived reactive products, which include hydrogen peroxide and hydroxyl radical.²⁹ Additional sources of oxygen-derived reactive products during ischemia-reperfusion include xanthine oxidase, the electron trans-

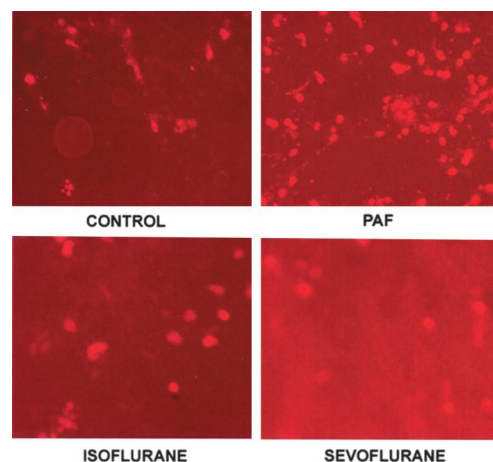


Fig. 5. Photomicrographs showing neutrophil adherence to the endothelial surface of isolated aortic segments under control, during administration of platelet-activating factor (PAF), and during administration of PAF after pretreatment with either isoflurane or sevoflurane (magnification ×40).

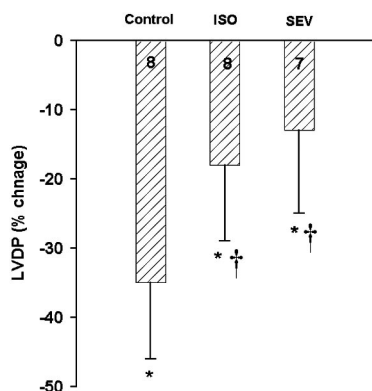


Fig. 6. Effect of pretreatment with 1.0 minimum alveolar concentration isoflurane (ISO) or sevoflurane (SEV) on reductions in left ventricular developed pressure (LVDP) caused by exogenous superoxide produced by xanthine oxidase and purine. Presented are maximal changes, which occurred after 10 min exposure. Values are expressed as mean \pm SD. * $P < 0.05$ versus baseline value. † $P < 0.05$ versus control group (no pretreatment).

port chain in mitochondria, the endothelium, and the degradation of catecholamines.²⁹

In our previous study using the same rat heart model, we demonstrated that selective pretreatment of neutrophils with either isoflurane or sevoflurane could prevent their ability to cause contractile dysfunction when they were administered with PAF.¹⁶ The current study differs notably from that study in that it focused specifically on effects of the volatile anesthetic pretreatment on cells other than neutrophils, including the coronary vascular endothelium and myocytes. The volatile anesthetics were administered into the coronary circulation and then washed out of the heart well before untreated neutrophils along with PAF were administered.

Pretreatment of the heart with either volatile anesthetic abolished the PAF-induced increases in myeloperoxidase activity and retention of neutrophils. This could have resulted from inhibitory effects on neutrophils adhering to the vascular endothelium, present as microemboli in the microvascular, or migrated into the parenchyma in proximity to the myocytes. However, the latter mechanism seems unlikely because neutrophil migration takes several hours to accomplish.¹¹ An ability of pretreatment with the volatile anesthetics to reduce neutrophil adherence to the vascular endothelium *per se* is demonstrated by the findings obtained in the isolated aortic segments. These various lines of evidence suggest that pretreatment of the heart with the volatile anesthetics reduced neutrophil adherence and that this action was a major contributor to the cardioprotective effects of these drugs.

The main endothelial adhesion molecules are P-selectin, which mediates the initial "rolling" and slowing of neutrophils along the endothelial surface, and intercellular adhesion molecule 1, which mediates the later firm adherence of neutrophils to the endothelial sur-

face.^{11,30,31} The volatile anesthetics could have reduced neutrophil adherence to either or both of these adhesion molecules by inhibiting transcription or translation of DNA or messenger RNA encoding them, thus reducing the adhesion molecule number, or by producing alterations in the affinity of the molecules.³² Another possibility was that pretreatment with the volatile anesthetics blunted the production of oxygen free radicals from sites within the heart, *e.g.*, the nicotinamide adenosine dinucleotide phosphate oxidase complex in the vascular endothelium and the myocytes, which occurs secondary to the interaction of neutrophils with the vessel wall.³³ Previous studies have shown that oxygen free radicals are potent stimuli for up-regulation of endothelial adhesion molecules.^{34,35}

Möbert *et al.*³⁶ found that pretreatment of human umbilical vein endothelial cells with 1.0 MAC isoflurane or sevoflurane did not inhibit neutrophil adherence. An explanation for the apparent discrepancy between these findings and those from the current study is uncertain, but it may be related to methodologic differences, including those related to species, tissues, protocols, and neutrophil activator (PAF *vs.* *N*-formyl-methionyl-leucyl-phenylalanine).

It is known that the oxygen free radicals can cause cardiac depression through reduction in Ca^{2+} sensitivity of cardiac myofilaments and impairment to excitation-contraction coupling.²² Oxygen free radicals can also have deleterious effects on membrane function and compromise membrane integrity by reacting with unsaturated lipids and sulfhydryl groups in proteins, resulting in cytoplasmic Ca^{2+} overload.^{14,37} Therefore, another potential mechanism by which pretreatment with volatile anesthetics blunted neutrophil-induced cardiac dysfunction was by enhancing the resistance of the myocardium to oxidant-induced injury. To explore this mechanism, we exposed hearts to enzymatically generated superoxide in the absence and presence of volatile anesthetic pretreatment. The results suggested that pretreatment conferred resistance against oxidant-induced cardiac injury, which pointed to this pathway as contributing to the ability of the volatile anesthetics to protect the myocardium against neutrophil-induced dysfunction. The mechanism by which the volatile anesthetics protect the heart against oxidant-induced injury is uncertain, but it may involve a limitation of intracellular Ca^{2+} accumulation and a stabilization of the myocardial cell membrane.^{38,39}

The K_{ATP} channels have been identified in myocytes, vascular smooth muscle cells, and endothelial cells.⁴⁰⁻⁴² These channels are located on the sarcolemmal membrane and inner mitochondrial membrane. Studies using K_{ATP} channel inhibitors, such as glibenclamide, have implicated the K_{ATP} channels in both ischemic and volatile anesthetic precondition in the heart.¹ This involvement is supported by work indicating the ability of K_{ATP}

channel openers, such as pinacidil, to mimic myocardial preconditioning.⁴³ Pinacidil has also been shown to reduce neutrophil adherence and neutrophil-induced endothelial dysfunction in isolated canine coronary arterial segments.¹² The current pinacidil findings and their reversal with glibenclamide (table 1) provide additional support for a K_{ATP} channel-mediated pathway for cardiac and endothelial protection. However, because glibenclamide did not alter the ability of the volatile anesthetics to attenuate neutrophil adherence or neutrophil-induced contractile dysfunction, these effects were apparently independent of the K_{ATP} channels.

Platelet-activating factor-activated neutrophils increased CPP, which reflected an increase in coronary vascular resistance. This may be attributable to impaired release of a vasodilator, *e.g.*, nitric oxide, augmented release of a vasoconstrictor, *e.g.*, endothelin, or mechanical obstruction of the coronary circulation by neutrophil aggregates. Although total coronary blood flow was kept constant, it cannot be ruled out that activated neutrophils caused microregions of inadequate flow that contributed to the global cardiac dysfunction. Volatile anesthetic pretreatment was effective in abolishing the neutrophil-induced cardiac dysfunction, but the increased CPP persisted. This suggests that these responses may be through different pathways.

The advantages and limitations of the isolated rat heart model to study neutrophil-induced cardiac dysfunction have been discussed in detail in our previous article.¹⁶ One limitation is marked coronary vasodilation because of the low oxygen content of the erythrocyte-free perfusate. A limited vasodilator reserve in the basal state could explain the inability of both isoflurane and sevoflurane, demonstrated coronary vasodilators *in vivo*,^{40,44} to reduce CPP, *i.e.*, coronary vascular resistance, during the pretreatment period. Another limitation of the model is the basal level of neutrophil retention, *i.e.*, in the absence of activation by PAF. The cause for this phenomenon remains to be determined, but it may be related to the lack of blood components with antioxidant and antiadhesion capabilities or to the effect of the preparation procedures on the neutrophils and endothelial cells.

Our concentration for glibenclamide was based on previous studies indicating that it was sufficient to completely block the K_{ATP} channel.^{45,46} Its adequacy is supported by an ability to abolish responses to the K_{ATP} channel opener pinacidil in our preparation.

The absence of anesthetic in samples of effluent provided evidence of washout before administration of the neutrophil-PAF mixture. However, because we did not perform measurements of anesthetic concentration in the myocardium, we cannot rule out the possibility that trace amounts of anesthetic remained in the tissue.

In conclusion, isoflurane and sevoflurane had profound preconditioning effects on the heart; 1.0 MAC was sufficient to completely abolish neutrophil-induced car-

diac dysfunction. The associated reductions in neutrophil adherence implicate an inhibitory effect on endothelial adhesion molecules in volatile anesthetic preconditioning. The ability of the anesthetics to attenuate the cardiac dysfunction caused by exogenous superoxide (in the absence of neutrophils) suggests that a direct protective effect on the myocardium may also be involved. An opening of K_{ATP} channels played no apparent role in volatile anesthetic preconditioning against neutrophil-induced cardiac dysfunction. The current findings compliment those from our previous study in the same heart model indicating that selective pretreatment of neutrophils with isoflurane or sevoflurane abolished their ability to cause contractile dysfunction.¹⁶ The work to date suggests that effects on neutrophils, endothelial cells, and myocytes may all contribute to the preconditioning effects of the volatile anesthetics *in vivo*.

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