

Role of Adenosine Receptors in Volatile Anesthetic Preconditioning against Neutrophil-induced Contractile Dysfunction in Isolated Rat Hearts

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Background: **The authors tested the hypothesis that adenosine receptors in polymorphonuclear neutrophils and the heart mediate the preconditioning effects of volatile anesthetics against neutrophil-induced contractile dysfunction.**

Methods: **Studies were conducted in buffer-perfused and paced isolated rat hearts. Left ventricular developed pressure served as index of contractility. Neutrophils and platelet-activating factor were added to perfusate for 10 min followed by 30 min of recovery. The effect of selective pretreatment of the neutrophils and the hearts with 1.0 minimum alveolar concentration isoflurane or sevoflurane on the neutrophil-induced contractile dysfunction was assessed. Studies were performed in the absence and presence of the nonselective adenosine receptor antagonist 8-phenyltheophylline (10 μM). Neutrophil retention was determined from difference between those administered and collected in coronary effluent and from myeloperoxidase concentration in myocardial samples. Superoxide production of neutrophils was measured by spectrophotometry.**

Results: **Under control conditions (no anesthetic pretreatment), activated neutrophils caused marked and persistent reductions in left ventricular developed pressure, associated with increases in neutrophil retention and myeloperoxidase activity. Pretreatment of the neutrophils or the heart with either isoflurane or sevoflurane abolished these effects. Pretreatment of the neutrophils also reduced the platelet-activating factor–induced increase in superoxide production by 29 and 33%, respectively. 8-Phenyltheophylline blunted the effects of anesthetic pretreatment of the neutrophils, whereas it did not alter the effects of anesthetic pretreatment of the heart.**

Conclusion: **An activation of adenosine receptors in neutrophils, but not in the heart, plays a role in the preconditioning effects of volatile anesthetics against neutrophil-induced contractile dysfunction.**

FINDINGS in animal models[1–5] and patients[6,7] have suggested that volatile anesthetics may possess preconditioning effects against myocardial reperfusion injury. A primary pathway for this effect seems to be an action of the anesthetics on the adenosine triphosphate–sensitive potassium (K_{ATP}) channels associated with the myocytes themselves.[1,8–11] However, recent findings have pointed to a second, K_{ATP} channel–independent pathway involving an inhibition of inflammatory cells, *i.e.*, neutrophils, and their interaction with the vascular endothelium.[12–17] As evidence for this pathway, 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.[13] Similar findings were obtained in our subsequent study when the hearts, but not the neutrophils, were pretreated with the volatile anesthetics.[14] Furthermore, 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 the endothelium-dependent vasodilation, increase in tumor necrosis factor α, and damage to the vascular endothelium associated with lipopolysaccharide-induced inflammation in rats.[18,19] The mechanisms underlying the inhibitory effects of anesthetic pretreatment on the inflammatory pathways remain to be clarified.

The heart is endowed with intrinsic protective mechanisms, including the adenosine receptor system, which enhance resistance to the injury of ischemia–reperfusion.[20] Cardiac adenosine concentrations increase rapidly during ischemia. The main cellular source for this adenosine is the myocytes, although inflammatory cells, such as the neutrophils, and the vascular endothelium also contribute. This locally released adenosine acts *via* specific receptors to inhibit activation of neutrophils and their interaction with the vascular endothelium, as well as to have direct protective effects within the myocytes.[20,21] These effects include reduced norepinephrine release, stimulated glycolysis and preserved adenosine triphosphate stores, and reduced Ca^{2+} release from the sarcoplasmic reticulum. Exogenous adenosine has also been shown to have antiinflammatory and anti-ischemic effects in various experimental and clinical investigations.[20,21]

Several experimental studies have implicated the adenosine receptor system in volatile anesthetic preconditioning in the heart.[2,5,9,22] This pathway is thought to involve a G protein coupling to the K_{ATP} channels in the myocytes.[22] Whether the adenosine receptors play a role in the antiinflammatory effects of the volatile anesthetics has not been examined and is unknown. Accordingly, the current study was performed in isolated rat hearts to test the hypothesis that an activation of adenosine receptors mediates the preconditioning effects of the volatile anesthetics, isoflurane

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and sevoflurane, against neutrophil-induced contractile dysfunction. This was accomplished using the adenosine receptor antagonist 8-phenyltheophylline (8-PT). To gain insight into the cellular site of the adenosine receptors, separate studies were conducted in which the neutrophils and the heart were pretreated selectively with the volatile anesthetics with and without 8-PT.

Materials and Methods

Heart Preparation

After approval from the Institutional Animal Care and Use Committee (Chicago, Illinois), studies were conducted in 135 (70 male and 65 female) adult Sprague-Dawley rats (Charles River, Wilmington, MA) that weighed between 250 and 350 g. Rats of both sexes were well represented in each experimental group. 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 permitting retrograde coronary perfusion *via* the aorta.[13] Electrodes were attached to the right ventricle, and the heart was paced at 300 beats/min (1 V, 30-ms pulse duration). An in-line, ultrasonic, transit-time flow transducer (Transonic System Inc., Ithaca, NY) was interposed in the perfusion circuit to measure coronary flow. Catheters were situated in the inflow line just proximal to the aortic cannula to measure coronary perfusion pressure (CPP) and to infuse neutrophils and drugs.

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 volume was inflated with saline sufficiently to increase left ventricular end-diastolic pressure to approximately 8–10 mmHg, which provides the optimal ventricular preload in this heart preparation.[13] Measurements of left ventricular developed pressure (LVDP; end-systolic minus end-diastolic pressure) and maximum rate of increase of left ventricular pressure (LV dP/dt_{max}) served as indices of myocardial contractile function. The pulmonary artery was cannulated for collection of venous effluent from the heart.

Coronary perfusion was initially *via* a reservoir containing anesthetic-free Krebs buffer bubbled with a 95% O_2–5% CO_2 gas mixture. The hearts were perfused at constant flow, initially titrated to achieve a CPP of 70 mmHg. Coronary flow averaged 19 ± 3 ml/min. 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%). Bubbling 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. 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 of 1.6% citric acid and 2.5% sodium citrate (pH 5.4) in 10 ml of 6% dextran solution in buffered Hanks' balanced salt solution (HBSS). The neutrophils were separated as described previously.[13] The tubes containing the blood 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 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 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.[15]

Experimental Protocols

Series 1: Selective Pretreatment of Hearts with Volatile Anesthetics. The experimental protocols for series 1 are presented in figure 1. After 30 min was allowed for stabilization of the heart preparation, 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 administration of either 1.0 MAC isoflurane or sevoflurane, a second set of cardiac measurements was obtained, and the heart was returned to 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 coronary effluent obtained at 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 neutro-

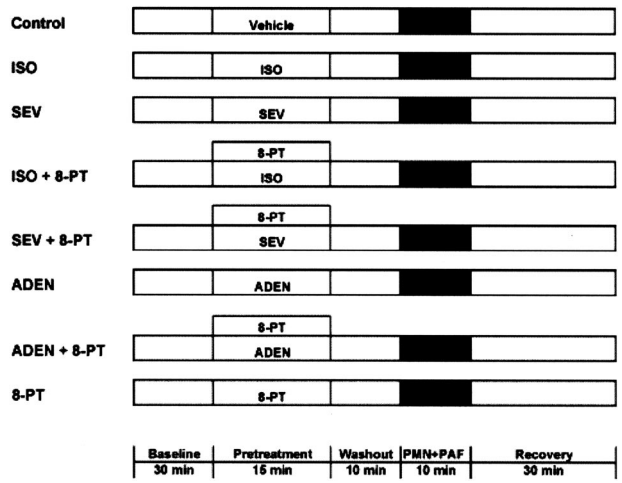

Fig. 1. Schematic diagram of the experiment protocols. 8-PT = 8-phenyltheophylline; ADEN = adenosine; ISO = isoflurane; PAF = platelet-activating factor; PMN = polymorphonuclear neutrophils; SEV = sevoflurane.

phils.[15] This was followed by a 30-min recovery period. 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 into the coronary perfusion line at a rate 2% of coronary flow. This resulted in final concentrations of 1 nM for PAF and 3×10^5 neutrophils/ml, in accordance with previous studies.[13,23] We have demonstrated that these concentrations of PAF or neutrophils themselves have no effects on cardiac function or CPP in the isolated rat heart preparation.[13] Measurements of cardiac variables were obtained every 5 min during the neutrophil infusion and recovery periods. Neutrophil adherence was estimated from calculated values of neutrophil retention and measurements of myeloperoxidase activity in samples of myocardium (see Tissue Myeloperoxidase Activity section).

The role of the adenosine receptors in the ability of the volatile anesthetics to protect the heart from neutrophil-induced contractile dysfunction was evaluated by administering the adenosine receptor antagonist 8-PT (10 μM)[24–26] along with isoflurane or sevoflurane. Additional validation studies were performed in which (1) adenosine (100 μM) was administered in the absence and presence of 8-PT and (2) 8-PT was administered alone. Adenosine, 8-PT, or both were infused *via* a side arm in the coronary inflow line. The infusions of 8-PT were initiated 5 min before and maintained during the administrations of the volatile anesthetics or adenosine.

Series 2: Selective Pretreatment of Neutrophils with Volatile Anesthetics. The procedure for neutrophil pretreatment has been described in detail previously.[13] Briefly, 1 MAC isoflurane or sevoflurane (in the absence and presence of 8-PT [10 μM]) was 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 then 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 no volatile anesthetic or 8-PT was added during incubation. 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). The neutrophils were then coincubated with PAF (50 nM) at 37°C for 10 min to stimulate the neutrophils.[15] The pretreated neutrophils were administered to the hearts as described for series 1. Before infusion of the PAF-neutrophil suspension, an aliquot was obtained for measurement of superoxide concentration using a spectrophotometric method (see Superoxide Production by Neutrophils section).

The following conditions were evaluated in series 2: (1) untreated neutrophils (control, n = 9); (2) isoflurane-pretreated neutrophils with (n = 7) and without 8-PT (n = 10); and (3) sevoflurane-pretreated neutrophils with (n = 7) and without 8-PT (n = 10). Additional validation studies were performed using neutrophils pretreated with adenosine (n = 7) and 8-PT (n = 7) and combined adenosine and 8-PT (n = 7) using the same protocol described in this section for the volatile anesthetics. The concentrations for adenosine and 8-PT were the same as those in series 1.

Neutrophil Retention in the Myocardium

Neutrophil retention in the myocardium was estimated from the difference between neutrophils administered and recovered in coronary venous effluent.[12,13] The total number of neutrophils entering the coronary circulation (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 myocardium (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 retained in the myocardium was calculated as follows: Retention (%) = [1 − (Neutrophil Output/Neutrophil Input)] × 100.

Tissue Myeloperoxidase Activity

Myeloperoxidase activity, an index of neutrophil accumulation in myocardium, was determined using a method described previously.[14] 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 hexadecyltri-

methyl 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-μl sample of the supernatant was loaded into a cuvette plate. O-dianisidine dihydrochloride with 0.0005% hydrogen peroxide in phosphate buffer (190 μl) was then added to samples using a multipipetter and analyzed with a spectrophotometer at 460 nm (SPECTRA-max; Molecular Devices, Sunnyvale, CA). Myeloperoxidase activity was expressed as absorbance units · min^{-1} · g^{-1} tissue.[27,28]

Superoxide Production by Neutrophils

Superoxide production by neutrophils in suspension was determined by measuring the superoxide dismutase–inhibitable reduction of ferricytochrome c to ferrocytochrome c.[15] 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). For each assay, duplicate samples were run. Half of the tubes were provided with an excess of superoxide dismutase (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 Vmax kinetic microtiter plate reader (Molecular Devices, Palo Alto, CA). Superoxide production was calculated using an extinction coefficient of 21 mM^{-1} · cm^{-1} for cytochrome c. Results are expressed as nanomolars of superoxide dismutase–inhibitable O_2^- produced by a suspension of 1.5×10^7 neutrophils/ml.

Drugs

The following chemicals and reagents were obtained from Sigma Chemical (St. Louis, MO): Ficoll-Pacque, superoxide dismutase, cytochrome c, cytochalasin B, adenosine, 8-PT, and dimethyl sulfoxide. 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. The 1.0 MAC values for isoflurane and sevoflurane are 1.4 and 2.4% in the rat[29] and 1.4 and 2.36% in the dog,[30,31] respectively. In series 1, the MAC values for rat were used, and in series 2, those for dog were used. The millimolar equivalents in HBSS for 1.0 MAC isoflurane and sevoflurane are 0.30 and 0.35 mM in the rat and 0.30 and 0.34 mM in the dog, 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.[32]

Statistical Analysis

In series 1, the effect of anesthetic pretreatment of the hearts (both with and without 8-PT) on baseline values for hemodynamic parameters (LVDP, LV dP/dt_{max}, and CPP) was assessed using the Student *t* test for paired samples.[33] Within- and between-group differences for these parameters after neutrophil administration were assessed in both series 1 and 2 using a two-way analysis of variance for repeated measures followed by the Student-Newman-Keuls test.[34] Other statistical analysis was performed using the Student *t* test for unpaired samples or a one-way analysis of variance followed by the Student-Newman-Keuls test, as required. Data were expressed as mean ± SD. Differences were considered significant when $P < 0.05$.

Results

Figures 2A–C present the effects of pretreatment of the hearts with isoflurane or sevoflurane, in the absence and presence of 8-PT, on neutrophil-induced changes in LVDP, LV dP/dt_{max}, and CPP (series 1). LVDP and LV dP/dt_{max} decreased modestly during the pretreatment period but returned to baseline levels with washout; CPP was unaffected. In the absence of anesthetic pretreatment (control condition), the activated neutrophils caused pronounced reductions (> 50%) in LVDP and LV dP/dt_{max} and increases in CPP, which did not recover over time. Because flow was held constant, the increases in CPP reflected increases in coronary vascular resistance. Pretreatment of the hearts with either volatile anesthetic abolished the cardiac depression by the activated neutrophils, as well as the associated increases in neutrophil retention (fig. 3A) and myeloperoxidase activity (fig. 3B); these effects were not altered by 8-PT. Anesthetic pretreatment did not affect the increases in CPP caused by the activated neutrophils.

Figures 4–6 present the results for series 2. The main findings are as follows: (1) The "sham" pretreated activated neutrophils (control group) in this series caused similar reductions in LVDP and LV dP/dt_{max} and increases in CPP, neutrophil retention, and myeloperoxidase activity as the untreated activated neutrophils (control group) in series 1. (2) Pretreatment of the neutrophils with either isoflurane or sevoflurane abolished these changes and also reduced the level of stimulated superoxide production by the neutrophils by 29 and 33%, respectively. (3) These effects of the volatile anesthetics were abolished by 8-PT.

Table 1 presents the findings for the validation studies assessing effects of pretreatment of the neutrophils or hearts with adenosine alone and combined with 8-PT. 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. Pretreatment of either the neutrophils or hearts with adenosine abol-

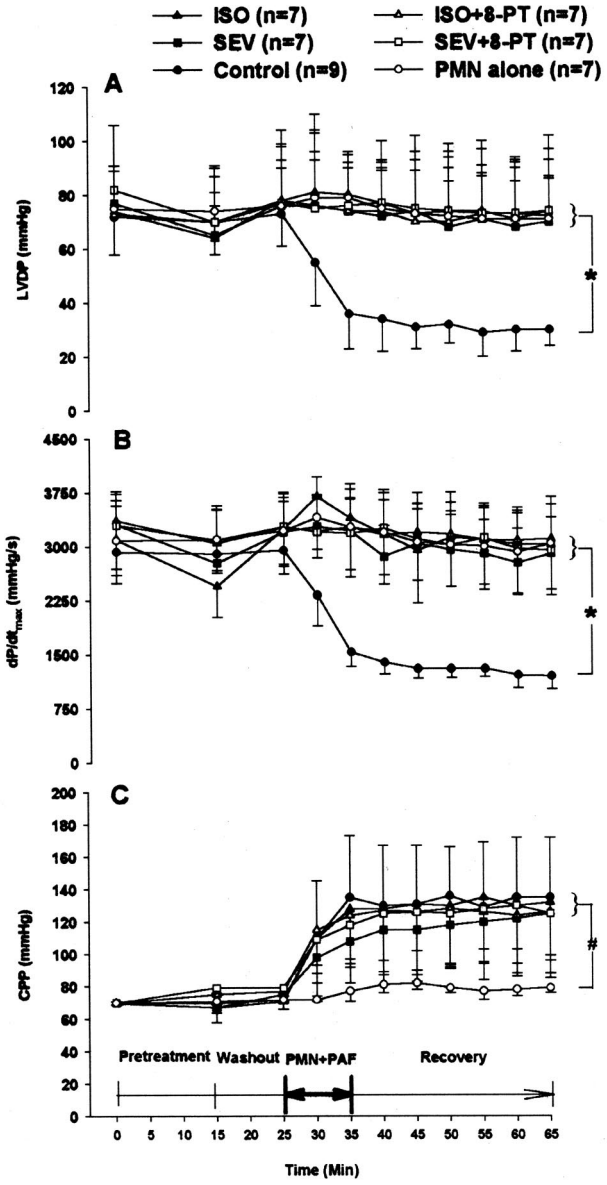

Fig. 2. Effects of pretreatment of hearts with either isoflurane (ISO) or sevoflurane (SEV) in the absence and presence of 8-phenyltheophylline (8-PT) on left ventricular developed pressure (LVDP; *A*), left ventricular dP/dt_{max} (*B*), and coronary perfusion pressure (CPP; *C*) during infusion of polymorphonuclear neutrophils (PMNs) and platelet-activating factor (PAF) for 10 min with 30 min of recovery. Values are presented as mean ± SD. n = number of hearts. * $P < 0.05$, control group *versus* all other groups; # $P < 0.05$, PMN-alone group *versus* all other groups.

ished the decreases in LVDP and dP/dt_{max}, as well as the increases in CPP, caused by the PAF-stimulated neutrophils. These changes were associated with reductions in neutrophil retention and myeloperoxidase activity. Pretreatment with adenosine also decreased superoxide production by activated neutrophils. All of these effects of adenosine were prevented by 8-PT. Neither pretreatment of the neutrophils nor the hearts with 8-PT alone affected the cardiac effects or superoxide production by activated neutrophils (data not shown).

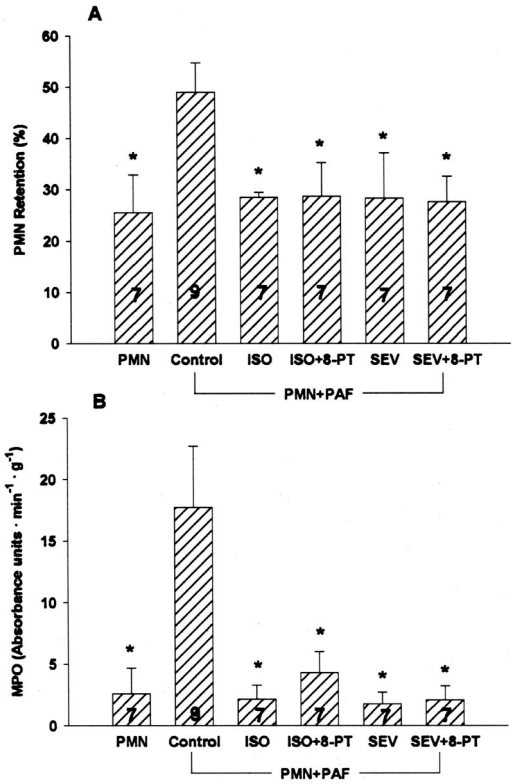

Fig. 3. Effect of pretreatment of hearts with isoflurane (ISO) or sevoflurane (SEV) in the absence and presence of 8-phenyltheophylline (8-PT) on platelet-activating factor (PAF)–stimulated polymorphonuclear neutrophil (PMN) retention in isolated hearts (*A*) and on myeloperoxidase (MPO) activity in end-recovery hearts (*B*). *Number in bar* = number of hearts. Values are presented as mean ± SD. * $P < 0.05$ *versus* control group.

Discussion

The adenosine receptor system is an intrinsic mechanism that has been demonstrated to protect the heart against the injury of ischemia–reperfusion and inflammation.[20,21] Adenosine released from a variety of cells, including the cardiomyocytes, neutrophils, and the vascular endothelium (or administered exogenously), acts *via* specific receptors to cause protective changes within the myocytes as well as to inhibit activation of neutrophils and their interaction with the vascular endothelium.[20,21] The current findings provide additional support for a role for the adenosine receptor system within the neutrophils, by indicating that 8-PT attenuated the ability of the volatile anesthetics to inhibit neutrophil activation (superoxide production) and adherence, and neutrophil-induced cardiac dysfunction.

Currently, four adenosine receptor subtypes encoded by distinct genes have been characterized: A_1, A_{2A}, A_{2B}, and A_3 receptors.[21] The use of the nonselective adenosine receptor antagonist 8-PT in the current study did not permit us to distinguish the contribution of the various adenosine receptor subtypes. However, the previous work from Vinten-Johansen's group[35–37] using selective adenosine receptor agonists and antagonists has sug-

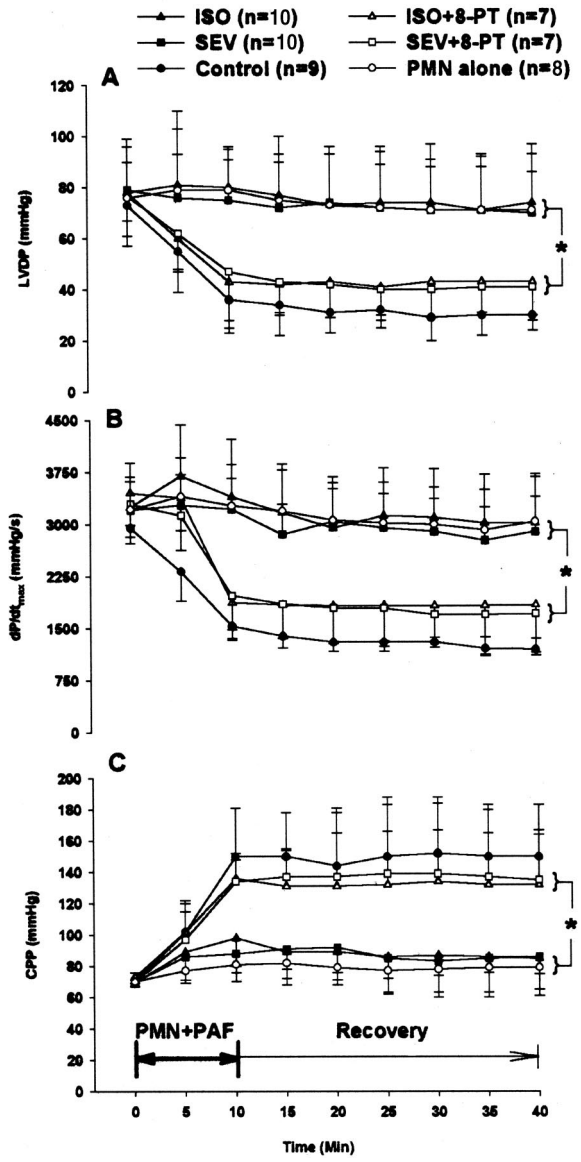

Fig. 4. Effects of pretreatment of neutrophils with either isoflurane (ISO) or sevoflurane (SEV) in the absence and presence of 8-phenyltheophylline (8-PT) on left ventricular developed pressure (LVDP; *A*), left ventricular dP/dt$_{max}$ (*B*), and coronary perfusion pressure (CPP; *C*) during infusion of polymorphonuclear neutrophils (PMNs) and platelet-activating factor (PAF) for 10 min with 30 min of recovery. Values are presented as mean ± SD. n = number of hearts. * $P < 0.05$, volatile anesthetic with 8-PT *versus* corresponding volatile anesthetic alone.

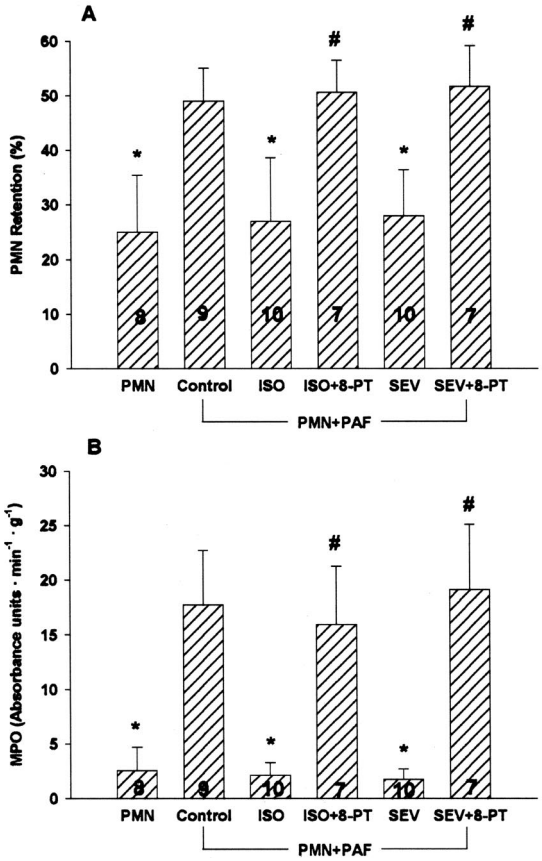

Fig. 5. Effect of pretreatment of neutrophils with isoflurane (ISO) or sevoflurane (SEV) in the absence and presence of 8-phenyltheophylline (8-PT) on platelet-activating factor (PAF)–stimulated polymorphonuclear neutrophil (PMN) retention in isolated hearts (*A*) and on myeloperoxidase (MPO) activity in end-recovery hearts (*B*). *Number in bar* = number of hearts. Values are presented as mean ± SD. * $P < 0.05$ *versus* control group; # $P < 0.05$ *versus* corresponding anesthetics.

gested that adenosine-induced inhibition of neutrophil function and neutrophil-endothelium interactions is mediated by the A_{2A} receptors and A_3 receptors; the A_1 receptors play no apparent role.

The mechanism by which the volatile anesthetics activate adenosine receptors in the neutrophils is uncertain. One possibility is a direct activation of these receptors by the volatile anesthetics. Other potential mechanisms include an increased sensitivity or affinity of adenosine receptors to adenosine contained within the neutrophil or an increased production of adenosine by the neutrophil, all of which could result in an amplification of adenosine receptor–mediated effects within the neutrophil. The latter mechanism seems unlikely because a previous study has shown that isoflurane decreased rather than increased interstitial adenosine concentrations in the heart.[22] However, additional studies are required to determine whether this is also true within the neutrophil.

Platelet-activating factor binds to a specific receptor on the neutrophil membrane that acts as the first event in the signal transduction sequence. Ultimately, the production of superoxide by neutrophils results from activation and assembly of nicotinamide adenosine dinucleotide phosphate oxidase, which is a transmembrane electron transport chain that reduces oxygen to superoxide. The current findings indicated that 8-PT completely abolished the reduction in superoxide production by volatile anesthetics, indicating a prominent role for the adenosine receptors in this effect. Previous studies have suggested several mechanisms by which adenosine receptor activation may inhibit superoxide production by neutrophils. One mechanism is an increased

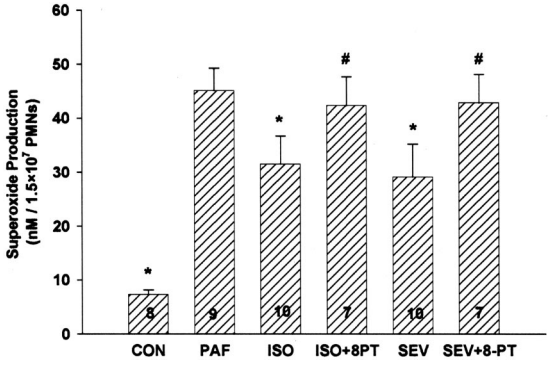

Fig. 6. Effect of pretreatment of neutrophils with isoflurane (ISO) or sevoflurane (SEV) in the absence and presence of 8-phenyltheophylline (8-PT) on superoxide production by platelet-activating factor (PAF)–stimulated polymorphonuclear neutrophils (PMNs). *Number in bar* = number of hearts. Values are presented as mean ± SD. * $P < 0.05$ *versus* control group; # $P < 0.05$ *versus* corresponding anesthetics.

activity of serine/threonine protein phosphatase in the neutrophil plasma memebrane,[38] which would promote dephosphorylation of $p47^{phox}$, a protein involved in the assembly of nicotinamide adenosine dinucleotide phosphate oxidase; dephosphorylation of $p47^{phox}$ has been associated with cessation of superoxide production.[39–41] Other mechanisms are an uncoupling of guanine triphosphate–binding proteins (G proteins) from the agonist, *i.e.*, PAF, receptor,[42] and a facilitation of cyclic adenosine monophosphate–dependent clearance of Ca^{2+} from the cytosol.[43] An increase in cytosolic Ca^{2+} caused by release from intracellular stores and influx through the plasma membrane is essential for the respiratory burst accompanying neutrophil activation.[44]

The current findings confirm previous work indicating that pretreatment of neutrophils with the volatile anesthetics can prevent the PAF-induced increases in vascular adherence.[13] This effect is likely attributable to a down-regulation of expression of CD11/CD18 on the surface of the neutrophils (the counterligand to the endothelial adhesion molecule ICAM-1).[45] The ability of 8-PT to block and of adenosine itself (both here and in the previous studies) and adenosine analogs[35–37,46] to mimic the antiadherence effect of the volatile anesthetics supports a role for adenosine receptors in this effect. The relation between the adenosine receptor–mediated decreases in neutrophil adherence and superoxide production by the volatile anesthetics remains to be defined.

Adenosine triphosphate–sensitive potassium channels have been identified in many cell types, including neutrophils. Treatment with the K_{ATP} channel agonists, such as pinacidil, has been demonstrated by us[15] and others[47] to reduce the level of activation of neutrophils. Previous studies in vascular smooth muscle cells, vascular endothelial cells, and myocytes have suggested involvement of the K_{ATP} channels in adenosine receptor–mediated responses, *e.g.*, vasodilation and cardioprotection.[48,49] However, Zhao *et al.* [47] raised questions about such an adenosine receptor–K_{ATP} channel link in the neutrophil in demonstrating an inability of glibenclamide, a K_{ATP} channel antagonist, to alter adenosine receptor–mediated inhibition of superoxide production. The findings from our current study and previous studies[15] indicating that 8-PT, but not glibenclamide, could blunt volatile anesthetic–induced inhibition of neutrophil superoxide production provide further evidence that the adenosine receptors and K_{ATP} channels may function independently within the neutrophil.

Our previous study suggested that the ability of the volatile anesthetics to precondition the heart against neutrophil-induced contractile dysfunction was related to a reduction in neutrophil adherence (implying an inhibition to the endothelial adhesion molecules P-selectin and ICAM-1), as well as to an increased resistance of the myocardium to oxidants, *e.g.*, superoxide, produced

Table 1. Effects of Adenosine Alone and Combined with 8-Phenyltheophylline on Neutrophil-induced Cardiac Dysfunction along with Associated Changes in Neutrophil Retention, Myeloperoxidase Activity, and Superoxide Production

	Control	Adenosine	Adenosine + 8-PT
Pretreatment of neutrophils	n = 9	n = 7	n = 7
LVDP, % baseline	55 ± 17	96 ± 18*	60 ± 15†
LV dP/dt_{max}, % baseline	57 ± 16	98 ± 11*	62 ± 11†
CPP, % baseline	205 ± 78	106 ± 21*	138 ± 12†
Neutrophil retention, %	49.5 ± 6.1	26.2 ± 2.5*	52.3 ± 9.3†
Myeloperoxidase, absorbance $U \cdot min^{-1} \cdot g^{-1}$ tissue	17.7 ± 5.0	4.2 ± 1.9*	20.2 ± 4.8†
Superoxide production, nM/1.5 × 10^7 PMNs	44.8 ± 12.3	27.3 ± 11.5*	44.4 ± 10.5†
Pretreatment of hearts	n = 9	n = 7	n = 7
LVDP, % baseline	59 ± 17	94 ± 11*	66 ± 19†
LV dP/dt_{max}, % baseline	61 ± 23	97 ± 11*	65 ± 22†
CPP, % baseline	206 ± 77	98 ± 9*	141 ± 31†
Neutrophil retention, %	49.0 ± 5.7	26.3 ± 4.0*	49.6 ± 8.1†
Myeloperoxidase, absorbance $U \cdot min^{-1} \cdot g^{-1}$ tissue	17.6 ± 9.8	4.7 ± 2.1*	18.7 ± 5.8†

Values are presented as mean ± SD.

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

8-PT = 8-phenyltheophylline; CPP = coronary perfusion pressure; LV dP/dt_{max} = maximum rate of increase of left ventricular pressure; LVDP = left ventricular developed pressure; PMNs = polymorphonuclear neutrophils.

by the neutrophils *via* both adherence-dependent and adherence-independent pathways.[14] The lack of effect of glibenclamide excluded a role for the K_{ATP} channels in these effects. The current investigation evaluated whether the adenosine receptors were involved. In contrast to the findings in the neutrophil pretreatment studies, the findings in the heart pretreatment studies indicated that 8-PT did not impair the ability of the volatile anesthetics to reduce neutrophil adherence and cardiac dysfunction. This suggests no role for the adenosine receptors in either the endothelial cells or the myocytes. Our findings could mean that the anesthetics did not activate the adenosine receptors within the rat heart model, or that they did so, but because the receptors played a relatively modest role, other antiadhesive and antiinflammatory effects of the volatile anesthetics were able to overshadow them. Whether the observed preconditioning effects of the volatile anesthetics were due to a direct action of the volatile anesthetics on the cardiac cells, *i.e.*, endothelium and myocytes, or to a modulation of another as yet unexplored signaling pathway remains to be determined.

Our findings are consistent with a recent study, also conducted in buffer-perfused rat hearts, suggesting that the A_1 receptors are not involved in isoflurane-induced protection against myocardial stunning.[50] However, they disagree with studies that evaluated the role of adenosine receptors in volatile anesthetic–induced cardioprotection in isolated perfused and *in situ* rabbit hearts,[2,5] open-chest dogs,[22] and human atrial trabecular muscles.[9] An explanation for this apparent conflict is uncertain, but it may be related to differences in species or experimental protocols. With regards to the latter, previous studies caused cardiac injury by ischemia followed by reperfusion or by anoxia, whereas we caused cardiac injury by administering neutrophils (along with their activator PAF) without an interruption of flow. Our approach simplified interpretation of the findings and permitted conclusions specifically related to neutrophil-dependent, inflammatory pathways.

Canine neutrophils were used in the current study because of the difficulty in obtaining sufficient quantities from the rat. The use of neutrophils from larger animals, *e.g.*, dogs and humans, in conjunction with isolated rat hearts has a strong historical precedent and is well accepted.[13,23] Although species-related "chimeric artifacts" cannot be completely ruled out, there is no reason to believe they would change our fundamental conclusions. Our previous study performed with neutrophils and coronary arterial rings both from dogs[15] yielded baseline effects of isoflurane that were consistent with those observed in the current study.

The current findings apply to the crystalloid perfused rat heart preparation and to the concentration of the volatile anesthetics that were evaluated. The advantages and limitations of this heart model to study neutrophil-induced cardiac dysfunction have been discussed in detail in our previous publication.[13] Our concentration for 8-PT was based on previous studies using the same isolated crystalloid-perfused heart preparation.[24–26] We assessed its adequacy in the current study using the well-accepted and classic pharmacologic approach of challenging the adenosine receptor blockade with a high concentration of the receptor agonist adenosine. The ability of 8-PT to abolish the inhibitory effects of exogenous adenosine attested to a highly effective, complete blockade of the adenosine receptors.

In conclusion, the current study demonstrates that the adenosine receptors in the neutrophils (but not in the heart) play an important role in the ability of isoflurane and sevoflurane, when used as a pretreatment, to prevent neutrophil-induced cardiac dysfunction. This pathway would be presumably involved when these volatile anesthetics are used to precondition the myocardium against postischemic reperfusion injury *in vivo*.

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