Persistency and pathway of isoflurane-induced inhibition of superoxide production by neutrophils

Persistance et voie de l'inhibition de la production de superoxyde par les neutrophiles induite par isoflurane

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Abstract

Background Our previous work has demonstrated that treatment with isoflurane has a preconditioning-like inhibitory effect on superoxide production (SOP) by polymorphonuclear neutrophils. The current objectives were to determine persistency of this effect and to clarify where in the signalling pathway this inhibition of SOP occurred. The latter was accomplished using two receptor-dependent neutrophil agonists, platelet activating factor (PAF) and formyl-methionyl-leucyl-phenylalanine (fMLP), and two receptor-independent neutrophil stimuli, the protein-kinase C stimulator, phorbol myristate acetate (PMA), and the calcium ionophore, A23187.

Methods Arterial blood samples were obtained from eight dogs under baseline condition (conscious state), during isoflurane (1 MAC) administration, and 24 and 48 hr post-isoflurane (also in conscious state). Neutrophils were isolated and stimulated with 1 μ M concentrations of PAF, fMLP, PMA, and A23187. SOP was measured spectrophotometrically.

Results Isoflurane administration caused (1) an approximate 50% decrease in SOP during PAF or fMLP ($P < 0.01$ vs baseline), which remained evident from 24 to 48 hr following isoflurane; (2) an initial 29% decrease in SOP during PMA ($P < 0.05$ vs baseline), which returned to baseline by 24 hr following isoflurane; and (3) no change in SOP during A23187 ($P > 0.05$ vs baseline).

Conclusions Isoflurane administration caused prolonged (from 24 to 48 hr) decreases in agonist-induced SOP by neutrophils. This effect involved inhibition at site(s) in the signalling pathway upstream from protein kinase C. The current findings suggest that the intraoperative use of isoflurane may result in an extended impairment to the antibacterial host defense mechanism and that neutrophil inhibition may play a role in the delayed tissue protection afforded by treatment with volatile anesthetics.

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Résumé

Contexte Nos travaux précédents ont démontré qu'un traitement à base d'isoflurane a un effet de type pré-conditionnement sur la production de superoxyde (SOP) par les neutrophiles polynucléaires. Les objectifs de cette étude étaient de déterminer la persistance de cet effet et de clarifier l'endroit où cette inhibition avait lieu sur la voie de signalisation. Nous avons effectué cette clarification à l'aide de deux agonistes des neutrophiles dépendants du récepteur, le facteur d'activation plaquettaire (PAF) et le formyl-méthionyl-leucyl-phénylalanine (fMLP), et de deux

stimuli des neutrophiles indépendants du récepteur, le stimulateur de la protéine kinase C, le phorbol myristate acétate (PMA), et l'ionophore de calcium A23187.

Méthode Des échantillons de sang artériel ont été obtenus chez huit chiens avant (état conscient), pendant (1 MAC), et 24 et 48 h après l'administration d'isoflurane (également en état conscient). Les neutrophiles ont été isolés et stimulés avec des concentrations d'1 μ M de PAF, fMLP, PMA et A23187. La SOP a été mesurée par spectrophotométrie.

Résultats L'administration d'isoflurane a causé 1) une réduction d'environ 50% de la SOP pendant la stimulation par PAF ou fMLP ($P < 0,01$ vs valeur de base), ce qui est demeuré évident de 24 à 48 h après l'administration d'isoflurane; 2) une réduction initiale de 29% de la SOP pendant la stimulation par PMA ($P < 0,05$ vs valeur de base), laquelle est revenue à la valeur de base au plus tard 24 h après l'administration d'isoflurane; et 3) aucune modification de la SOP avec la stimulation par A23187 ($P > 0,05$ vs valeur de base).

Conclusion L'administration d'isoflurane a provoqué des réductions prolongées (de 24 à 48 h) de la SOP induite par un agoniste par les neutrophiles. Cet effet a impliqué l'inhibition sur un/des site(s) de la voie de signalisation en amont de la protéine kinase C. Ces résultats suggèrent que l'utilisation peropératoire d'isoflurane pourrait avoir pour résultat une détérioration prolongée du mécanisme de défense hôte antibactérien, et que l'inhibition des neutrophiles pourrait jouer un rôle dans la protection tissulaire retardée que procure un traitement à l'aide d'agents anesthésiques volatils.

Introduction

The immune function of neutrophils can be characterized as a double-edged sword. Neutrophils are an integral component of the antibacterial host defense mechanism. The neutrophil response to microbial invasion consists of chemotaxis, phagocytosis, and intracellular killing and digestion.¹ Production of oxygen free radicals, such as superoxide anions and proteolytic enzymes are critical to this bacteriocidal function of the neutrophils. On the other hand, neutrophils play an important role in the pathogenesis of autotissue injury, such as that which occurs following ischemia and reperfusion.^{2,3} In this scenario, signals of inflammation are generated by endothelial and tissue cells, and the neutrophil responses are directed against self. Activation and recruitment of the neutrophils to the ischemic-perfused tissue lead to adherence of the neutrophils to the endothelium *via* the adhesion molecules, e.g., intercellular adhesion molecule-1 (ICAM-1), and

subsequent transmigration and influx of the neutrophils into the reperfused tissue. The activated neutrophils release oxygen free radicals and proteolytic enzymes, which can cause cellular injury, dysfunction, and death.

Previous *in vitro* studies in our laboratory⁴ and others^{5,6} have demonstrated the ability of volatile anesthetics to impair production of superoxide by activated neutrophils. This effect has been invoked to explain the blunted bacteriocidal action⁷ and endothelial-damaging effect⁴ of the anesthetic-treated neutrophils. In a recent study, we demonstrated that the inhibitory effect of isoflurane (or sevoflurane) on the production of superoxide by neutrophils has a “memory.” Neutrophils incubated *in vitro* with either anesthetic exhibited a reduced superoxide production in response to platelet activating factor (PAF). This was associated with reduced vascular adherence and a contractile dysfunction when administered into isolated rat hearts for 30–60 min following washout of the anesthetic. The difficulty in maintaining neutrophil viability for extended durations *in vitro* did not permit an assessment of the time frame that this volatile anesthetic-induced inhibitory effect on neutrophil function persisted. This is an important question, since it has implications relating to both perioperative immunosuppression and tissue protection.

The objective of this study was twofold: (1) To determine the persistency of neutrophil inhibition following treatment with isoflurane. This was accomplished in a dog model by comparing stimulus-induced superoxide production by neutrophils obtained before, during, and 24 and 48 hr following isoflurane administration. (2) To clarify where in the signalling pathway this inhibition of superoxide production by isoflurane was occurring. This was accomplished by use of two receptor-dependent agonists, PAF and formyl-methionyl-leucyl-phenylalanine (fMLP), and two receptor-independent stimuli, the protein-kinase C stimulator, phorbol myristate acetate (PMA), and the calcium ionophore, A23187 (Fig. 1). The rationale was that if isoflurane attenuated the increase in superoxide in response to a particular stimulus, it would indicate that isoflurane was having an inhibitory effect at the site of action of this stimulus or downstream from this site in the superoxide signaling pathway. The study tested the null hypothesis that isoflurane administration does not cause a prolonged decrease in agonist-induced receptor-mediated production of superoxide by neutrophils.

Methods

The study was approved by the Institutional Animal Care and Use Committee of the University of Illinois at Chicago. The eight dogs in this study were also subjects in an investigation of the ability of isoflurane to produce delayed

Fig. 1 Signalling pathway for superoxide (O_2^-) production in the neutrophil indicating the sites at which platelet activating factor (PAF), formyl-methionyl-leucyl-phenylalanine (fMLP), phorbol myristate acetate (PMA), and A23187 act. *PLC* phospholipase C, *IP3* inositol 1,4,5, triphosphate, *DAG* diacylglycerol, *PKC* protein kinase C, *NADPH* nicotinamide adenine dinucleotide phosphate

preconditioning against myocardial stunning; thus, the animals were chronically instrumented for extensive cardiovascular monitoring.⁸ In brief, this included a set of ultrasonic crystals implanted across the left ventricular wall (posterior region) to measure wall thickening (an index of myocardial contractile function); a solid-state miniature pressure gauge inserted through the apex of the ventricle to measure left ventricular pressure, left ventricular dP/dt, and heart rate; and a transit-time ultrasonic flow transducer to measure blood flow through the left circumflex coronary artery. Catheters were implanted in the coronary sinus to obtain coronary venous blood samples and in the aorta to measure arterial pressure and to obtain arterial blood samples. Myocardial oxygen consumption was calculated using the Fick equation. The sampling of blood for neutrophils from these chronically instrumented dogs involved in other experiments was a practical decision based on the cost of the animals and the activities of animal rights groups.

At least 1–2 weeks was allowed for recovery from surgery before the study was initiated. While the animal was conscious and in a relaxed state, an initial 20 mL sample of arterial blood was obtained for neutrophils. Then the dog was anesthetized. In six of the dogs (Group 1), anesthesia induction was accomplished with sodium thiopental ($15 \text{ mg} \cdot \text{kg}^{-1} \text{ iv}$), an endotracheal tube was inserted, and 1 MAC (minimum alveolar concentration) isoflurane 1.4% in oxygen was administered. After 1 hr of isoflurane, a second arterial blood sample was obtained. The isoflurane was discontinued and the animal was allowed to recover before being returned to its cage. Subsequent blood samples were obtained in the conscious state at 24 and 48 hr following discontinuation of isoflurane administration. In two of the dogs (Group 2), isoflurane was administered by mask; thiopental was not used for induction. This was to exclude an inhibitory effect of thiopental on superoxide production by neutrophils. Measurements of hemodynamic variables were obtained at the time of withdrawal of blood samples in the conscious

condition, during isoflurane, and 24 hr after stoppage of isoflurane. A record of these variables was obtained on a physiological recorder.

The 20 mL blood samples were 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). Neutrophils were separated by use of standard methods.⁹ 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 $500 \times g$ at 4°C for 10 min. Contaminating erythrocytes in the pellet were removed by hypotonic lysis for 20 sec with 9 mL of sterile distilled water. A subsequent addition of 3 mL of 0.6 M KCl and 15 mL of buffered HBSS rapidly returned the cells to isotonicity. The leukocyte-rich suspension was centrifuged at $500 \times g$ at 4°C for 10 min, after which time the cells were resuspended in 2 mL of HBSS layered on the top of 3 mL of Ficoll-Paque and centrifuged again at $800 \times g$ at 4°C for 20 min. The resulting pellet was rinsed with HBSS. The neutrophils were resuspended in HBSS, centrifuged, and separated three times in preparation for stimulation and assessment of superoxide production. Approximately 2–3 hr were required for the isolation and multiple washings of the neutrophils. Our procedure for neutrophil isolation yields neutrophil suspensions that are 98% pure and more than 95% viable, as evaluated by trypan blue exclusion.⁴

Superoxide production by the neutrophils in suspension was determined by measuring the superoxide dismutase (SOD) inhibitable reduction of ferricytochrome *c* to ferrocyanochrome *c*.⁴ Neutrophils ($5 \times 10^6 \text{ cells} \cdot \text{mL}^{-1}$) were pre-warmed in a shaking incubator at 37°C with $160 \mu\text{M}$ cytochrome *c* and $5 \mu\text{g} \cdot \text{mL}^{-1}$ cytochalasin B for 5 min and then stimulated with $1 \mu\text{M}$ concentrations of PAF, fMLP, PMA, and A23187. The neutrophils obtained from the dogs of Group 2 were only stimulated with PAF. The doses for the neutrophil stimuli were adapted from

previous studies.^{4,10} The final reaction volume was 0.5 mL. Duplicate samples were run for each assay. Half of the tubes were provided with an excess of SOD ($100 \mu\text{g} \cdot \text{mL}^{-1}$) as a control for non-specific activity or colour generation. Five minutes after adding the test agents, 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, USA). Superoxide production was calculated using an extinction coefficient of $21 \text{ mM}^{-1} \cdot \text{cm}^{-1}$ for cytochrome *c*. Results are expressed as nM of SOD-inhibitable O_2^- produced by a suspension of 5×10^6 neutrophils. Neutrophil stimulation with a given agonist was continued at 24 hr intervals following isoflurane administration until the baseline level of superoxide production was observed. Statistical analysis was performed using the Student's *t* test for paired samples.¹¹ Data are expressed as mean $\pm$ SD. Differences were considered significant when $P < 0.05$.

Results

Table 1 presents systemic hemodynamic and cardiac variables in the conscious state, during isoflurane administration, and 24 hr after stoppage of isoflurane for all eight dogs participating in the study. Isoflurane administration reduced mean arterial pressure (-15%) and two indices of

myocardial contractile function, i.e., left ventricular dP/dt max (-43%) and wall thickening (-31%). Heart rate increased by 15% . Isoflurane administration also reduced myocardial oxygen consumption (-42%), which, in the presence of no change in coronary blood flow, resulted in an increase in coronary venous PO_2 ($+68\%$). Values for hemodynamic variables had returned to baseline by 24 hr following stoppage of isoflurane.

Table 2 presents data for the neutrophils obtained from the dogs anesthetized with isoflurane following induction with thiopental (Group 1). All four stimuli caused marked increases in superoxide production by neutrophils obtained under baseline conditions (prior to anesthesia induction). The increases in superoxide production in response to PAF, fMLP, and PMA were blunted in neutrophils obtained during isoflurane (compared with the baseline responses, superoxide production was reduced by -49% , -44% , and -29% , respectively), although the increases in superoxide production in response to A23187 were not affected. Neutrophils obtained 24 hr after discontinuing isoflurane demonstrated no recovery of the PAF- and fMLP-induced increases in superoxide production, but a return of the PMA-induced increases in superoxide production to the pre-isoflurane baseline values. The increases in superoxide production caused by PAF and fMLP returned to the pre-isoflurane baseline values by 48 hr following stoppage of isoflurane. The values for hemodynamics 48 hr following isoflurane are not presented because variables were already

Table 1 Systemic hemodynamic and myocardial variables at baseline, during 1 MAC isoflurane, and 24 hr after stoppage of isoflurane

	Baseline	Isoflurane	24 hr post-isoflurane
MAP (mmHg)	87 ± 12 (77–97)	$74 \pm 15^*$ (62–87)	94 ± 10 (86–102)
HR (beats $\cdot \text{min}^{-1}$)	91 ± 17 (77–105)	$111 \pm 16^\dagger$ (98–124)	90 ± 20 (73–107)
LVEDP (mmHg)	5.4 ± 2.0 (3.7–7.1)	5.3 ± 2.0 (3.6–7.0)	6.3 ± 2.0 (4.5–8.0)
LV dP/dt max (mmHg $\cdot \text{sec}^{-1}$)	3286 ± 617 (2770–3801)	$1857 \pm 472^\ddagger$ (1462–2252)	3657 ± 394 (3328–3986)
WT (%)	17.8 ± 4.7 (13.9–21.7)	$12.3 \pm 3.7^\ddagger$ (9.2–15.4)	18.2 ± 4.2 (14.7–21.7)
MVO_2 (mL $\cdot \text{min}^{-1}$)	2.6 ± 0.7 (2.0–3.2)	$1.5 \pm 0.7^*$ (0.9–2.1)	2.7 ± 1.5 (1.4–4.0)
CBF (mL $\cdot \text{min}^{-1}$)	20 ± 10 (12–28)	22 ± 7 (16–28)	22 ± 10 (14–30)
PvO_2 (mmHg)	25 ± 7 (19–31)	$42 \pm 7^\dagger$ (36–48)	21 ± 7 (15–27)

Values are mean $\pm$ SD in eight dogs. Numbers in parentheses are 95% confidence intervals

MAP mean arterial pressure, HR heart rate, LVEDP left ventricular end-diastolic pressure, LV dP/dt max maximal rate of rise of left ventricular pressure, WT wall thickening, MVO_2 myocardial oxygen consumption, CBF coronary blood flow, PvO_2 coronary venous oxygen tension

* $P < 0.05$; $^\dagger P < 0.01$; $^\ddagger P < 0.001$ vs baseline

Table 2 Superoxide production (in nM $O_2^-/5 \times 10^6$ PMNs) for neutrophils that were stimulated with 1 μ M concentrations of PAF, fMLP, PMA, and A23187

	Baseline	Hours following isoflurane		
		0	24	48
PAF	13.1 $\pm$ 4.8 (8.1–18.1)	6.7 $\pm$ 3.1* (3.4–10.0)	7.6 $\pm$ 2.8* (4.7–10.5)	14.6 $\pm$ 3.9 (10.5–18.7)
fMLP	7.1 $\pm$ 1.7 (5.3–8.9)	4.0 $\pm$ 2.5 [†] (1.4–6.6)	3.6 $\pm$ 1.4 [†] (2.1–5.1)	6.8 $\pm$ 2.8 (3.9–9.7)
PMA	23.4 $\pm$ 7.1 (15.9–30.9)	16.6 $\pm$ 5.1* (11.2–22.0)	21.8 $\pm$ 5.9 (15.6–28.0)	–
A23187	12.5 $\pm$ 1.7 (10.7–14.3)	12.1 $\pm$ 2.5 (9.5–14.7)	12.5 $\pm$ 1.4 (11.0–14.0)	–

The neutrophils were isolated from blood samples obtained before isoflurane administration (baseline), during isoflurane, and at 24 hr intervals following isoflurane administration. In these studies, thiopental was used for induction of anesthesia (Group 1). Values are mean $\pm$ SD in six dogs. Numbers in parentheses are 95% confidence intervals

PAF platelet activating factor, fMLP formyl-methionyl-leucyl-phenylalanine, PMA phorbol myristate acetate

* $P < 0.05$; [†] $P < 0.01$, vs baseline

at baseline levels at 24 hr following isoflurane. Likewise, neutrophil activation was not performed with PMA or A23187 48 hr after administration of isoflurane because the respective superoxide values had returned to baseline by 24 hr post-isoflurane.

An additional two dogs were anesthetized with isoflurane by mask without induction with thiopental (Group 2), and before and after they were exposed to isoflurane in vivo, their neutrophils were stimulated in vitro with PAF. The findings for these dogs were similar to those presented above for Group 1: (1) The PAF-induced production of superoxide was reduced by approximately 50% in neutrophils obtained during isoflurane and 24 hr after discontinuing isoflurane. The values for superoxide production by neutrophils (in nM $O_2^-/5 \times 10^6$ PMNs) decreased from the baseline values of 12.0 and 14.0 to 7.7 and 7.4, respectively, and they remained at these levels for 24 hr. (2) Superoxide production returned to the pre-isoflurane baseline values (14.0 and 13.0) by 48 hr after stoppage of isoflurane.

Discussion

The main findings from this study were: (1) An exposure to a clinically relevant concentration of isoflurane caused a prolonged (from 24 to 48 hr) reduction in receptor-mediated superoxide production by the neutrophils. (2) This effect involved an inhibitory action of isoflurane at a site in the superoxide signalling pathway upstream from protein kinase C (Fig. 1).

Platelet activating factor is a phospholipid mediator that is released in vivo by activated basophils, platelets, endothelial cells, and neutrophils.¹² Formyl-methionyl-leucyl-phenylalanine is a formyl peptide derivative obtained from bacterial metabolites or derived from disrupted mitochondria.¹³ Both PAF and fMLP are chemoattractants that bind to the neutrophil cell surface via members of the membrane-spanning GTP-binding protein (G protein) linked receptor family.^{12–14} Platelet activating factor and fMLP have common and distinct signalling pathways in neutrophils.¹⁴ The main common pathways are as follows.¹⁵ The binding of either PAF or fMLP to its receptor in the neutrophil membrane serves as the first event in a complex signal transduction sequence involving activation of phospholipase C, which generates two second messengers, inositol 1,4,5-triphosphate (IP3) and diacylglycerol.^{15,16} Inositol 1,4,5-triphosphate releases calcium from intracellular calcium stores and increases plasma membrane permeability to calcium. Both calcium and diacylglycerol activate protein kinase C.^{15,16} Activation of protein kinase C results in the production of superoxide by neutrophils via activation and assembly of NADPH oxidase, which is a transmembrane electron transport chain that reduces oxygen to superoxide.^{15–17} It is an inhibitory effect on one or more of the common sites described above that is likely responsible for the more than 24 hr suppression of superoxide production that we observed during stimulation with either PAF or fMLP. These site(s) must be upstream from protein kinase C, since the responses to PMA, although depressed initially, had completely returned to the baseline level by 24 hr after exposure of the neutrophils to isoflurane.

It is well established that the calcium ionophore, A23187, induces an abrupt and sustained increase in neutrophil cytosolic Ca^{2+} , which appears to be due to both release of the cation from intracellular stores and immediate influx from the extracellular environment.¹⁸ Thus, it was a useful experimental tool to clarify the extent to which sites downstream from the membrane receptor contributed to the impaired superoxide responses to fMLP and PAF by the neutrophils exposed to isoflurane. Our findings demonstrated no attenuation of A23187-induced superoxide production by neutrophils from blood samples withdrawn either during or following isoflurane administration. This would seem to rule out a direct inhibitory action on NADPH oxidase in the diminished responses to stimulation with PMA as well as to fMLP and PAF. Although the classical signalling pathway indicates that cytosolic Ca^{2+} exerts its effect on superoxide production via an activation of protein kinase C activation (Fig. 1),¹⁵ recent studies have suggested that high levels of cytosolic Ca^{2+} (those achievable with a Ca^{2+} ionophore) can directly phosphorylate and activate NADPH oxidase.¹⁹

Nakagawara *et al.*⁵ explored mechanisms that may account for their finding that the fMLP-induced production of superoxide by human neutrophils *in vitro* was inhibited in the presence of halothane, enflurane, or isoflurane. These investigators found that the decrease in superoxide production correlated to reductions in Ca^{2+} influx and Ca^{2+} release from intracellular storage sites. Whether an inhibitory effect on Ca^{2+} mobilization is involved in the persistent reduction in neutrophil responsiveness found in the present study remains to be determined experimentally. However, the fact that the site of the inhibition was apparently upstream from protein kinase C is consistent with such a mechanism.

The presence of clinically relevant concentrations of thiopental has been demonstrated to reduce superoxide production of neutrophils pre-incubated with opsonized zymosan.²⁰ Whether this effect persists following removal of thiopental has not, to our knowledge, been determined. However, we could not rule out that thiopental contributed to the persistent decrease in superoxide production by the neutrophils when it was used to induce anesthesia in dogs of Group 1. To evaluate this possibility, we performed additional studies in a limited number of animals in which isoflurane was administered by mask without using thiopental for induction. The findings in these latter studies were similar to those in which thiopental was used, which suggested that the persistent impairment to superoxide production by the neutrophils in the present study was due to an inhibitory action of isoflurane.

In light of the relatively short (6.7 hr) half-life of neutrophils in the dog circulation,²¹ one might wonder how blood samples obtained 24 hr after isoflurane administration could contain neutrophils that were affected by their exposure to the anesthetic. The answer likely lies in the vast reservoir of mature neutrophils that are contained in the bone marrow.²¹ This storage pool is approximately 7.5 times the blood compartment and represents a 3–4 day supply of neutrophils. The marrow delivers about 30% of the storage cells to the peripheral blood daily. The present findings suggest that the neutrophils stored in the marrow were also exposed to isoflurane and are responsible for the prolonged inhibitory effect on agonist-induced superoxide production.

It should be recognized that the procedure we used to isolate neutrophils from a blood sample and prepare them for *in vitro* stimulation would have completely removed any volatile anesthetic present in the sample.²² This meant that all isoflurane-induced reductions in superoxide production, including those found in neutrophils obtained from blood samples withdrawn during isoflurane administration, reflected a preconditioning effect. Our findings indicated that the baseline superoxide responses to PAF were double that to fMLP. This difference in response was also shown

by Zhao *et al.*²³ in their study using canine neutrophils, and it may reflect a difference in receptor density on the surface of the neutrophil membrane for the two agonists.

Blood samples for analysis of neutrophil function were obtained from eight chronically instrumented dogs that were subjects in a broader investigation to assess the ability of isoflurane to produce delayed preconditioning against myocardial stunning; thus, the animals were a convenience sample. Although the sample size in our study was relatively small, our major results were confirmed by rigorous statistical analysis. Use of additional dogs to further evaluate the effect of isoflurane on neutrophil function in the absence of thiopental, i.e., mask induction group, was desirable, but this could not be justified on cost and ethical grounds.

The hemodynamic monitoring in the dogs of this study afforded an opportunity to characterize the conditions under which the blood samples for neutrophils were withdrawn and to assess the physiological integrity of our experimental preparation. The baseline and post-isoflurane values for systemic hemodynamic and myocardial variables are consistent with previous findings in conscious instrumented dogs in a relaxed state.^{24–26} The hemodynamic findings during isoflurane administration in the present study conform to previous work in canine models, which have indicated that 1 MAC isoflurane causes hypotension and has negative inotropic and coronary vasodilating effects.^{25,27,28} Coronary vasodilation was suggested by an uncoupling of coronary blood flow from the prevailing myocardial oxygen demands, with the result that coronary venous PO_2 increased markedly.²⁹

Several limitations of the present study warrant address. First, activation of the neutrophils was performed *in vitro*. Thus, it must be assumed that the *in vitro* responsiveness of isolated neutrophils was a reflection of the *in vivo* physiological capabilities of the cells. Second, the studies were performed in canine neutrophils in order to permit meaningful comparisons with our previous work.^{4,22} It remains to be demonstrated that isoflurane has a similar prolonged inhibitory effect on superoxide production by human neutrophils. Thus, extrapolation of the current findings to the clinical situation must be done with caution. Third, the present study focused exclusively on superoxide production by neutrophils. Whether isoflurane causes prolonged inhibition of other neutrophil functions, e.g., adhesion, chemotaxis, phagocytosis, and degranulation,³⁰ remains to be determined.

A persistent impairment to superoxide production of neutrophils by volatile anesthetics would have both adverse and beneficial effects clinically. On the one hand, it would compromise host resistance to the microbial pathogens encountered in the hospital environment and thus increase the vulnerability of patients to infection. This risk would be

highest in patients requiring a prolonged administration of a volatile anesthetic or patients who are immunocompromised prior to surgery. On the other hand, a prolonged inhibition of neutrophil function by the volatile anesthetics would provide protection against neutrophil-mediated auto tissue injury. Indeed, the current study presents the first evidence that an inhibitory effect on the neutrophils may be involved in the delayed volatile anesthetic-induced preconditioning, i.e., the second window of protection (SWOP), previously demonstrated in a variety of tissues, including the myocardium^{31,32} and brain.³³

In conclusion, the current findings suggest that the intraoperative use of isoflurane (and potentially the other volatile anesthetics) may produce an inhibitory effect on neutrophil production of superoxide that extends more than one day beyond the completion of surgery. This effect would reduce the ability of the neutrophils to participate in antibacterial host defense, while at the same time impairing the ability of these cells to mediate tissue injury. The predominant impact of the inhibitory effect of isoflurane on superoxide production by neutrophils in an individual patient is difficult to predict since it would depend on a number of factors, including the overall immunological status of the patient and the patient's risk of neutrophil-mediated tissue injury.

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Conflicts of interest None declared.

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