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Isoflurane Pretreatment Inhibits Lipopolysaccharide-induced Inflammation in Rats

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Background: **Previous studies have indicated that volatile anesthetic pretreatment protects cells from inflammation *in vitro*; therefore, the authors hypothesized that pretreatment with isoflurane may attenuate the hemodynamic and pathologic changes to the vasculature that are associated with inflammation *in vivo*.**

Methods: **Rats received intravenous lipopolysaccharide or saline placebo with and without pretreatment with isoflurane (1.4% for 30 min immediately before lipopolysaccharide). Mean arterial pressure (MAP) and response to endothelium-dependent (acetylcholine) and -independent (sodium nitroprusside) vasodilators were assessed hourly for 6 h. Tumor necrosis factor-α concentrations, arterial blood gases, and vascular histology were also determined.**

Results: **Lipopolysaccharide decreased MAP and vasodilation to acetylcholine and sodium nitroprusside. Lipopolysaccharide also caused acidosis, endothelial swelling, and endothelial detachment from the smooth muscle. Isoflurane pretreatment prevented the decrease in MAP for 5 h and attenuated the decrease at 6 h. Pretreatment increased the vasodilation to acetylcholine in lipopolysaccharide rats to control concentrations but had no effect on sodium nitroprusside. In control rats, isoflurane pretreatment increased the response to acetylcholine and sodium nitroprusside but had no effect on MAP. Isoflurane pretreatment prevented the acidosis and endothelial damage to mesenteric and aortic vessels, and attenuated the increase in tumor necrosis factor-α associated with lipopolysaccharide-induced inflammation.**

Conclusion: **Pretreatment with 30 min of isoflurane attenuated the decrease in MAP and endothelium-dependent vasodilation, the acidosis, the increase in tumor necrosis factor-α, and the damage to the vascular endothelium associated with lipopolysaccharide-induced inflammation in rats. This study suggests that isoflurane pretreatment may protect the vasculature during inflammation.**

INFLAMMATION associated with endotoxemia decreases systemic pressure and can lead to cardiovascular collapse.[1–4] The hemodynamic effects of inflammation are partly related to the release of cytokines and increases in the inducible nitric oxide synthase (NOS).[1,5] This may lead to loss of vascular smooth muscle tone and endothelial cell dysfunction.[5] The loss of endothelial function and the important physiologic effects of the constitutive endothelial NOS may result in decreased endothelium-dependent vasodilation and altered blood flow to and within various vascular beds.[1,5] Animal models have demonstrated that protection of the endothelium and vascular smooth muscle in lipopolysaccharide-induced inflammation may result in improved hemodynamics and survival.[1,6,7] Various methods for improving endothelial function and hemodynamics during lipopolysaccharide-induced inflammation have included agents that decrease cytokines, inhibit the production of inducible NOS, induce protective heat shock proteins, or are free radical scavengers.[1,2,5,8–10]

Anesthetics may protect the vascular endothelium and smooth muscle during inflammation. We have previously demonstrated that pretreatment with 30 min of 1.4% isoflurane protects cultured endothelial and vascular smooth muscle cells from cytokine and hydrogen peroxide–induced cell death.[11] Other investigators have indicated that volatile anesthetics precondition the myocardium and coronary endothelium from ischemic–reperfusion injury and associated inflammation.[12,13] Furthermore, it has been demonstrated that anesthetics, including halothane, ketamine, and urethane, decrease cytokine concentrations and, therefore, may alter the inflammatory response in rat lipopolysaccharide models.[2,8,14]

Based on the observation that pretreatment with 30 min of isoflurane is protective during cytokine-induced inflammation *in vitro*,[11] we hypothesized that isoflurane pretreatment may have protective effects on the vasculature during lipopolysaccharide-induced inflammation *in vivo*. To test this hypothesis, rats were randomized to receive lipopolysaccharide or placebo with and without 30 min of 1.4% isoflurane pretreatment immediately before lipopolysaccharide. The mean arterial pressure (MAP), vascular response to endothelium-dependent and -independent vasodilators, arterial blood gases, tumor necrosis factor-α (TNF-α) concentrations, and endothelial histology were assessed over a 6-h period.

Methods

In Vivo *Experiments*

These experiments were approved by the Animal Care and Use Committee at the University of Virginia. Male Sprague-Dawley rats weighing 450–550 g were anesthetized with sodium pentobarbital (40 mg/kg, administered intraperitoneally), and anesthesia was maintained by additional injections (15 mg/kg, administered intraperitoneally) hourly. The rats were placed supine on a heating blanket and under a heating lamp to maintain a temperature of 37°C throughout the experiment. A tra-

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cheostomy was performed, and the rats were allowed to breathe spontaneously through a cone placed over the tracheostomy using 100% O_2. A polyethylene catheter was placed in the femoral artery for monitoring MAP. MAP was recorded with a pressure transducer (TRANSPAC; Abbott Laboratories, North Chicago, IL) and a monitor (Datascope 2001A; Datascope Corp., Paramus, NJ). The arterial catheter was infused with saline at a rate of 0.5 ml/h. A polyethylene catheter was also inserted in the femoral vein for injection of pharmacologic agents.

The rats were allowed to stabilize and were then randomized to one of four groups: (1) control, (2) isoflurane–control, (3) lipopolysaccharide, and (4) isoflurane–lipopolysaccharide. The isoflurane groups received isoflurane for 30 min immediately before lipopolysaccharide or saline. Isoflurane 1.4% was delivered through the tracheostomy using a rodent ventilator (model 683; Harvard Apparatus, South Natick, MA) at a rate of 75 breaths/min and a tidal volume of 2.5 ml. The end-tidal isoflurane concentration was measured with a gas analyzer (Capnomac Ultima; Datex, Helsinki, Finland). The control and lipopolysaccharide groups were ventilated without isoflurane for 30 min before saline or lipopolysaccharide. Lipopolysaccharide (10 mg/kg, salmonella; Sigma Chemical, St. Louis, MO) dissolved in 0.5 ml saline or saline alone was injected through the femoral vein catheter. The experiments were conducted such that initiation of isoflurane (or no isoflurane) was time point zero; therefore, lipopolysaccharide was administered at the time point equal to 30 min.

Rats were evaluated either for changes in MAP alone (n = 10 each group) or changes in MAP and endothelium-dependent and -independent vasodilation (n = 9 each group). Endothelium-dependent and -independent vasodilation was evaluated by injections of acetylcholine and sodium nitroprusside every hour for 6 h. Acetylcholine (1 μg/kg) and sodium nitroprusside (7.5 μg/kg) were dissolved in 0.1 ml saline. The drugs were injected in series only after the MAP had returned to baseline and stabilized for 1 min. Drug doses were based on previous experiments by our group.[1]

Exhaled air (100–150 ml) was collected hourly for measurement of nitric oxide (NO) concentration using Sievers 280 Nitric Oxide Analyzer (Sievers Instruments, Boulder, CO).

Tumor Necrosis Factor-α, Blood Gas, and Histology Evaluation

In separate rats (n = 6 each group), arterial blood samples (0.5 ml) were withdrawn at 120 and 270 min for measurement of TNF-α concentration. Blood used for TNF-α assay was centrifuged for 10 min at 3,000 rpm. The plasma was decanted and stored at −70°C until the analysis was performed. TNF-α concentration was measured using enzyme-linked immunosorbent assay kit (Pharmingen, San Diego, CA). Arterial blood gas analysis (Chiron/Diagnostics, Norwood, MA) was performed at the end of the 6 h.

Lung parenchyma, thoracic aorta, mesentery, and renal cortex were harvested for histologic analysis. The harvested tissues were fixed in formalin, embedded in paraffin, cut in 4-μm sections, and subsequently stained with hematoxylin–eosin. A segment of thoracic aorta (diameter = 1.3–2.2 mm) and 5–10 arteries in the lung parenchyma (diameter = 0.2–1.6 mm), mesentery (diameter = 0.3–1.1 mm), and renal cortex (diameter = 0.4–1.1 mm) from each rat were evaluated using light microscopy by a pathologist (L. A. C.) for evidence of endothelial and smooth muscle cell injury. All samples were blinded and scored on a 0–3 scale. The scale was 0 for normal, 0.5 for borderline changes, 1 for decreased number of endothelial cells (nuclei), 2 for additional endothelial cell swelling, and 3 additional detachment of the endothelium from the vascular smooth muscle.

Statistics

Statistical analysis was performed with Sigma Stat (SPSS Inc., Chicago, IL). Hemodynamic changes over time from baseline within each group were determined by repeated-measures analysis of variance. Differences in MAP, endothelium-dependent and -independent vasodilation, blood gases, and TNF-α between the groups at each time point were evaluated by one-way analysis of variance and a *post hoc* Tukey test. Endothelium-dependent and -independent vasodilation was evaluated by using both the absolute MAP and the percent change in MAP to partially account for the decreased baseline value in the lipopolysaccharide group (results were the same and presented as absolute values). The data are presented as the mean ± SD. Pathology scores were evaluated by analysis of variance for nonparametric values (Kruskal-Wallis) with a *post hoc* Student-Newman-Keuls test and presented as the median with 25th and 75th percentiles. $P < 0.05$ was considered significant.

Results

Hemodynamics, Endothelium-dependent and -independent Vasodilation

Control rats with and without isoflurane pretreatment had no significant change in MAP over the 6-h experiment. In contrast, the rats receiving lipopolysaccharide alone showed a significant decrease in MAP, being less than control from 2 to 6 h. Isoflurane pretreatment prevented this decrease in MAP except for the sixth hour. The isoflurane–lipopolysaccharide rats had a significantly higher MAP than the lipopolysaccharide rats at hours 2–6 (fig. 1). In rats evaluated for endothelium-dependent and -independent vasodilation, the hemodynamic effects of lipopolysaccharide and isoflurane pretreatment were the same as those not receiving

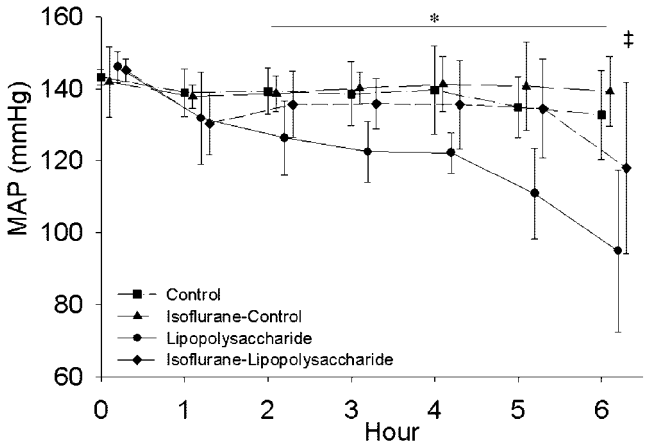

Fig. 1. The effects of lipopolysaccharide with and without isoflurane (1.4% for 30 min) pretreatment on mean arterial pressure (MAP) in rats. Groups are control, isoflurane–control, lipopolysaccharide, and isoflurane–lipopolysaccharide. Lipopolysaccharide is significantly ($P < 0.05$) decreased compared with control. *Isoflurane–lipopolysaccharide is significantly ($P < 0.05$) greater than lipopolysaccharide alone. ‡Isoflurane–lipopolysaccharide is significantly ($P < 0.05$) less than control. All data are mean ± SD.

acetylcholine and sodium nitroprusside; lipopolysaccharide decreased MAP, while isoflurane pretreatment attenuated the decrease in MAP (table 1).

Endothelium-dependent and -independent vasodilation with acetylcholine and sodium nitroprusside did not vary significantly with time in the control group. Isoflurane pretreatment increased endothelium-dependent vasodilation at hours 3–6 in control rats (table 1). Lipopolysaccharide decreased endothelium-dependent vasodilation at hours 3–6. This decrease in endothelium-dependent vasodilation associated with lipopolysaccharide was prevented by isoflurane pretreatment such that endothelium-dependent vasodilation was not different than controls. Isoflurane pretreatment increased endothelium-independent vasodilation in control rats at 2–3 and 5–6 h. Lipopolysaccharide decreased endothelium-independent vasodilation at hours 5 and 6, and this decrease was not attenuated by isoflurane pretreatment.

Arterial Blood Gases, Tumor Necrosis Factor-α, and Exhaled Nitric Oxide

Arterial blood gas analysis demonstrated that lipopolysaccharide caused a significant decrease in pH and a base deficit (table 2). Isoflurane pretreatment had no significant effect in control rats; however, in lipopolysaccharide rats, isoflurane prevented the acidosis. Oxygen tension was also significantly higher in the isoflurane–lipopolysaccharide rats compared with the lipopolysaccharide rats. Carbon dioxide tension and glucose concentrations were lower in the isoflurane–lipopolysaccharide rats than in controls but no different *versus* lipopolysaccharide alone. Calcium and potassium concentrations were not different among the groups.

Tumor necrosis factor-α was low in both controls and isoflurane–controls. Lipopolysaccharide caused a significant increase in TNF-α at both 120 and 270 min (fig. 2). Isoflurane pretreatment significantly attenuated the increase in TNF-α concentrations in lipopolysaccharide rats at both time periods; however, the concentrations remained above control.

Table 1. Endothelium-dependent and -independent Vasodilation

	Hour					
	1	2	3	4	5	6
Control						
Baseline	139 ± 7	139 ± 6	139 ± 9	140 ± 12	134 ± 8	133 ± 12
ΔMAP, acetylcholine	48 ± 12	40 ± 17	39 ± 7	37 ± 7	37 ± 9	42 ± 7
ΔMAP, sodium nitroprusside	37 ± 16	29 ± 18	28 ± 16	37 ± 23	37 ± 10	35 ± 15
Isoflurane–control						
Baseline	139 ± 3	139 ± 5	140 ± 4	141 ± 8	141 ± 12	139 ± 9
ΔMAP, acetylcholine	45 ± 6	54 ± 8	53 ± 6‖	55 ± 9‖	49 ± 11‖	51 ± 9‖
ΔMAP, sodium nitroprusside	45 ± 10	55 ± 16‖	53 ± 9‖	51 ± 13	58 ± 6‖	52 ± 10‖
Lipopolysaccharide						
Baseline	132 ± 13	126 ± 10*	128 ± 8*	122 ± 5*	111 ± 13*	95 ± 22*
ΔMAP, acetylcholine	35 ± 12	27 ± 11	24 ± 6‡	25 ± 4‡	20 ± 7‡	18 ± 4‡
ΔMAP, sodium nitroprusside	44 ± 12	28 ± 8	19 ± 8	19 ± 9	18 ± 9‡	15 ± 6‡
Isoflurane–lipopolysaccharide						
Baseline	130 ± 9	136 ± 9	136 ± 7	136 ± 12	134 ± 14	118 ± 24†
ΔMAP, acetylcholine	39 ± 7	36 ± 10	32 ± 6§	32 ± 10§	32 ± 7§	30 ± 6§
ΔMAP, sodium nitroprusside	48 ± 11	31 ± 10	22 ± 12	21 ± 13	22 ± 14	18 ± 8

All data are mean ± SD. The effects of lipopolysaccharide with and without isoflurane (1.4% for 30 min) pretreatment on endothelium-dependent and -independent vasodilation with acetylcholine and sodium nitroprusside in rats as stated by the change in MAP (ΔMAP).

* Lipopolysaccharide significantly ($P < 0.05$) decreased the baseline in MAP compared with control and isoflurane–lipopolysaccharide. † Isoflurane–lipopolysaccharide baseline is significantly less than controls but greater than lipopolysaccharide alone. ‡ The ΔMAP for lipopolysaccharide is significantly ($P < 0.05$) less than control. § The ΔMAP for isoflurane–lipopolysaccharide is significantly ($P < 0.05$) greater than lipopolysaccharide alone. ‖ The ΔMAP for isoflurane–control is significantly ($P < 0.05$) greater than control.

MAP = mean arterial pressure.

Table 2. Arterial Blood Gases

	Control	Isoflurane–Control	Lipopolysaccharide	Isoflurane–Lipopolysaccharide
pH	7.29 ± 0.02	7.31 ± 0.04	7.17 ± 0.05*	7.28 ± 0.02‡
Pao_2, mmHg	373 ± 35	427 ± 29	326 ± 51	386 ± 18‡
$Paco_2$, mmHg	62 ± 6	62 ± 5	51 ± 16	43 ± 2†
Base excess, mM	2.7 ± 3.4	2.4 ± 2.6	−9.1±4.6*	−5.8 ± 2.2†
Glucose, mg/dl	133 ± 23	138 ± 21	90 ± 46	75 ± 22†
Potassium, mM	4.3 ± 0.9	4.2 ± 0.3	4.9 ± 1.5	4.0 ± 0.8
Calcium, mg/dl	3.4 ± 1.4	4.3 ± 0.4	2.8 ± 1.4	3.1 ± 1.1

The effects of lipopolysaccharide with and without isoflurane (1.4% for 30 min) pretreatment on pH, Pao_2, $Paco_2$, base excess, glucose, potassium, and calcium in rats. All data are mean ± SD.

* Lipopolysaccharide is significantly ($P < 0.05$) different than control.

† Isoflurane– lipopolysaccharide is significantly ($P < 0.05$) different than control.

‡ Isoflurane–lipopolysaccharide is significantly ($P < 0.05$) different than lipopolysaccharide alone.

$Paco_2$ = arterial carbon dioxide tension; Pao_2 = arterial oxygen tension.

Exhaled NO concentrations were less than 2 parts per billion, which is the lower limit of detection, over the entire 6-h period in the control and isoflurane–control rats. Lipopolysaccharide gradually increased exhaled NO concentrations (89 ± 23 parts per billion at 6 h) and was not significantly altered by isoflurane pretreatment (98 ± 23 parts per billion at 6 h).

Histology

Endothelial and smooth muscle cells were evaluated as normal to borderline in the control and isoflurane–control groups. Lipopolysaccharide-exposed rats showed a decrease in the number of endothelial nuclei, increased swelling, and endothelial detachment from the smooth muscle layer in the mesenteric arteries and thoracic aorta (fig. 3). Isoflurane pretreatment prevented the damage to the endothelium of the aorta and mesenteric arteries such that the pathology score was not different than in control vessels (fig. 4). The vascular smooth muscle from all vessels and the endothelium of the lung and renal arteries were not significantly altered by lipopolysaccharide.

Discussion

Based on previous studies that indicated that volatile anesthetics protect cells from ischemic–reperfusion and inflammatory injuries,[11,15] we hypothesized that pretreatment with isoflurane may protect the vasculature from lipopolysaccharide-induced inflammation. This study demonstrated that pretreatment with 1.4% isoflurane administered for 30 min immediately before lipopolysaccharide attenuates the decrease in MAP associated with lipopolysaccharide-induced inflammation in rats for 6 h. Similarly, isoflurane pretreatment increased endothelium-dependent vasodilation, prevented the decrease in pH, attenuated the increase in TNF-α, and attenuated the pathologic damage to the endothelium associated with lipopolysaccharide.

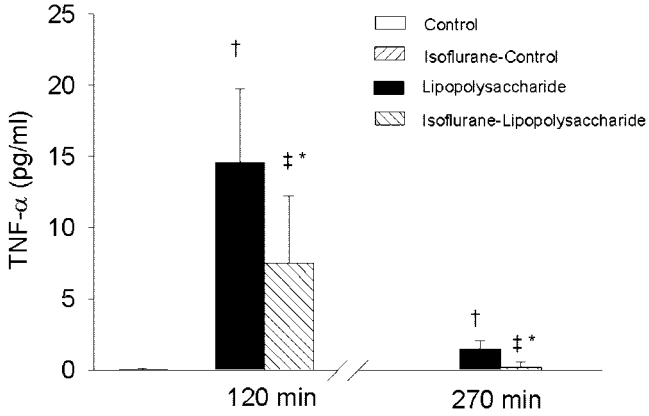

Fig. 2. The effects of lipopolysaccharide with and without isoflurane (1.4% for 30 min) pretreatment on tumor necrosis factor-α (TNF-α) in rats. Groups are control, isoflurane–control, lipopolysaccharide, and isoflurane–lipopolysaccharide. †Lipopolysaccharide significantly ($P < 0.05$) increased TNF-α compared with control. ‡Isoflurane–lipopolysaccharide is significantly ($P < 0.05$) greater compared with control. *Isoflurane–lipopolysaccharide is significantly ($P < 0.05$) less than lipopolysaccharide alone. All data are mean ± SD.

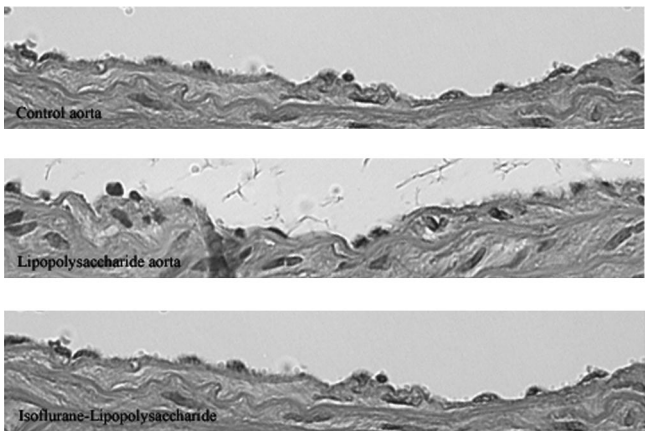

Fig. 3. The effects of lipopolysaccharide with and without isoflurane (1.4% for 30 min) pretreatment on the vascular endothelium from thoracic aorta in rats. Examples are from control, lipopolysaccharide, and isoflurane–lipopolysaccharide. Lipopolysaccharide decreased the number of endothelial cells, increased endothelial swelling, and caused endothelial detachment from the vascular smooth muscle. Isoflurane pretreatment prevented these effects of lipopolysaccharide.

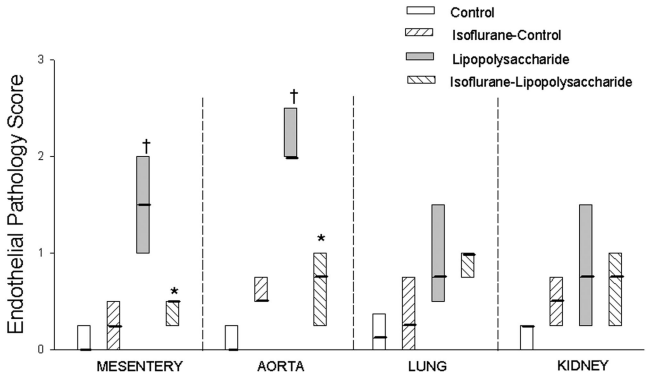

Fig. 4. The effects of lipopolysaccharide with and without isoflurane (1.4% for 30 min) pretreatment on scored pathology of endothelium in rats. The scale was 0 for normal, 0.5 for borderline changes, 1 for decreased number of endothelial cells (nuclei), 2 for additional endothelial cell swelling, and 3 additional detachment of the endothelium from the vascular smooth muscle. Groups are control, isoflurane–control, lipopolysaccharide, and isoflurane–lipopolysaccharide. †Lipopolysaccharide is significantly ($P < 0.05$) increased compared with control. *Isoflurane–lipopolysaccharide is significantly ($P < 0.05$) decreased compared with lipopolysaccharide alone. All data are median (bold line) with 25th and 75th percentiles.

The effects of lipopolysaccharide in this study are consistent with previous studies, including those in our own laboratory, that have evaluated the effects of intraperitoneal or intravenous injection of lipopolysaccharide on hemodynamics.[1,2,10] Previous studies have demonstrated that lipopolysaccharide causes endothelial dysfunction as demonstrated by decreased endothelium-dependent vasodilation.[1,5] Likewise, lipopolysaccharide-induced inflammation is associated with a loss of vascular tone that results in a decrease in MAP, in addition to decreased endothelium-independent vasodilation.[3,9,16] The effects of lipopolysaccharide are consistent with an inflammatory reaction, as indicated by dramatic increases in exhaled NO, elevated TNF-α, and acidosis.[1,2,6,8]

Isoflurane pretreatment attenuated the decrease in MAP associated with lipopolysaccharide but had no effect on MAP in control rats. This indicates that isoflurane inhibits the effects of lipopolysaccharide rather than acting through mechanisms that directly increase MAP. There are no previous studies evaluating the effects of pretreatment with volatile anesthetics on MAP, although other investigators have indicated that continuous administration of some anesthetics may be associated with a higher MAP in lipopolysaccharide rat models.[4,8] Intravenous ketamine administered before and during lipopolysaccharide is associated with a higher MAP in pentobarbital-anesthetized rats, although this may be related to increased catecholamine release.[2] The intravenous anesthetic urethane has also been associated with a higher MAP in lipopolysaccharide rats; however, this may be partly related to its α_2-antagonist effects.[8] While an increase in MAP in the rats exposed to lipopolysaccharide and pretreated with isoflurane suggests that isoflurane pretreatment is protective of the vasculature during lipopolysaccharide-induced inflammation, it is also possible that the increase in MAP reflects an increase in cardiac output secondary to myocardial protection, an effect that is known to occur with anesthetic preconditioning.[17]

Endothelium-dependent vasodilation was increased by isoflurane pretreatment after 3 h in control experiments and in lipopolysaccharide rats. Increased endothelium-dependent vasodilation in lipopolysaccharide rats suggests that isoflurane pretreatment provided functional protection of the vascular endothelium. Preserved endothelium-dependent vasodilation is consistent with our study results, that isoflurane pretreatment protects endothelial cells from cytokine-induced cell death *in vitro*.[11] The observation that endothelium-dependent vasodilation is also increased in control rats indicates that isoflurane up-regulates endogenous endothelial vasodilators. Whether up-regulation of endogenous vasodilators is protective or is a marker of protection in lipopolysaccharide rats is uncertain. Endothelium-dependent vasodilation is primarily increased by elevated constitutive endothelial NOS, a factor that is up-regulated by continuous isoflurane[18] and protective of the endothelium.[19] Endothelium-derived hyperpolarizing factor and products of the cyclooxygenase pathway may also play a role in endothelium-dependent vasodilation, although their regulation by isoflurane or their protective effects are not well known.[9,20]

Endothelium-independent vasodilation was increased in control rats by isoflurane pretreatment. Since sodium nitroprusside provides NO at the level of the vascular smooth muscle, this indicates that distal portions of the NO–3'5'cyclic guanosine monophosphate pathway may be up-regulated. The etiology of this is unclear since isoflurane is thought to have either no effect or inhibit guanylate cyclase activity.[21,22] Endothelium-independent vasodilation was decreased by lipopolysaccharide but not altered by isoflurane pretreatment. It would be expected that if isoflurane pretreatment increases endothelium-dependent vasodilation in lipopolysaccharide rats, that endothelium-independent vasodilation would also be increased since endothelium-dependent vasodilation evaluates the entire NO–3'5'cyclic guanosine monophosphate pathway. However, it is possible that alterations of endothelium-derived hyperpolarizing factor or the cyclooxygenase pathway may be responsible for the increase in endothelium-dependent vasodilation, which would leave endothelium-independent vasodilation unaltered. It is also possible that the response to exogenous NO (*i.e.*, sodium nitroprusside) is different from the response to endogenous NO (*i.e.*, acetylcholine)[20,23] or that lipopolysaccharide masks the effects of sodium nitroprusside on endothelium-independent vasodilation. While the effects of acetylcholine and sodium nitroprusside indicate that endothelium-dependent and -indepen-

dent vasodilation are affected by isoflurane pretreatment, we cannot rule out that part of their hemodynamic effects may be due to alterations in cardiac output or pulmonary vascular resistance, or that lower baseline pressure and hypoglycemia significantly affected the response in the lipopolysaccharide (but not control) rats.

In none of the experiments was endothelium-dependent or -independent vasodilation altered in the first hour. Rather, these observations occur after 2–3 h. If this occurred only in lipopolysaccharide rats, this may be explained by the time required to produce endothelial or smooth muscle damage (2–3 h); however, this also occurred in control rats. This indicates there are changes in the endothelium or vascular smooth muscle that occur over this period as a result of the isoflurane pretreatment in both control and lipopolysaccharide rats. The most likely explanation is that isoflurane increases protein expression, since protein activity should have been observed immediately. Endothelial NOS may be up-regulated and result in increased endothelium-dependent vasodilation; however, other factors that may be up-regulated and protect endothelial cells include protein kinase C[20] or adenosine triphosphate–sensitive potassium channels.[9,20] Both protein kinase C and adenosine triphosphate–sensitive potassium channels are thought to be involved in preconditioning of endothelium and cardiac muscle *in vivo*[12,20] and protect against cytokine-induced injury of endothelial and smooth muscle cells in culture.[11,12,20] Heat shock proteins have also been demonstrated to be protective[24]; however, they normally are protective at periods beyond the time evaluated in this study. Further studies will be required to determine which proteins or channels may be up-regulated by isoflurane pretreatment and how these affect MAP, endothelium-dependent and -independent vasodilation, and different components of the NO–3'5'cyclic guanosine monophosphate pathway.

Isoflurane pretreatment prevented the acidosis at 6 h associated with lipopolysaccharide. This was secondary to a trend toward both a decrease in the base deficit and arterial carbon dioxide partial pressure, both of which did not reach statistical significance. Isoflurane is known to alter ventilation,[25] although the precise mechanism that may explain a decrease in arterial carbon dioxide partial pressure with isoflurane pretreatment in lipopolysaccharide rats is unclear. Prevention of acidosis most likely involves an improvement in perfusion, although further studies will be needed to determine if isoflurane pretreatment alters cardiac output in larger animal lipopolysaccharide models. It is possible that the acidosis associated with lipopolysaccharide partly contributed to the decrease in MAP and endothelium-dependent vasodilation in lipopolysaccharide rats; however, this did not contribute to the increase in endothelium-dependent vasodilation with isoflurane pretreatment in the control rats. Isoflurane pretreatment also increased arterial oxygen partial pressure in the lipopolysaccharide rats, suggesting improved lung function.

There was a trend toward lower glucose concentrations in the lipopolysaccharide rats, a finding that is consistent with other studies[1]; however, this was only statistically significant in the rats also pretreated with isoflurane. The observation that isoflurane pretreatment did not prevent the decrease in glucose is in contrast with the use of inducible NOS inhibitors, which has been demonstrated to prevent the decrease in glucose concentrations associated with lipopolysaccharide.[1] Similarly, isoflurane did not prevent the large increase in exhaled NO, a factor that is also prevented by inducible NOS inhibitors.[1] This indicates that the mechanism of endothelial and vascular protection secondary to isoflurane pretreatment may be different from those caused by inducible NOS inhibitors.

Isoflurane pretreatment significantly attenuated the increase in total (unbound and bound) TNF-α. Previous studies have suggested that continuous halothane administration attenuates the increase in cytokines associated with intratracheal administration of lipopolysaccharide.[14] *In vitro*, halothane and isoflurane decrease cytokine production by macrophages.[14] Likewise, ketamine and urethane attenuate the increase in total TNF-α associated with lipopolysaccharide in rats at the same time periods we evaluated.[2,8] The anesthetic urethane has been shown to decrease mRNA expression of TNF-α.[8] Although we only measured TNF-α, other studies have indicated that interleukin-6 is similarly attenuated by continuous administration of intravenous and volatile anesthetics.[2,14]

Endothelial protection by isoflurane pretreatment was demonstrable by light microscopy. Endothelial cells of the thoracic aorta and mesenteric arteries of lipopolysaccharide rats were decreased in number, swollen, and detached from the underlying vascular smooth muscle. In both the thoracic aorta and mesenteric arteries, isoflurane pretreatment prevented the endothelial damage. This histologic finding is also consistent with the preserved systemic endothelium-dependent vasodilation in lipopolysaccharide rats pretreated with isoflurane. On the other hand, endothelium in arteries from the renal cortex and lung parenchyma were not significantly altered by lipopolysaccharide, as was all vascular smooth muscle, indicating the diverse effects of lipopolysaccharide in different organs. Since we only evaluated the effects of one dose of lipopolysaccharide, one dose of isoflurane, and one time period, we cannot state whether the protection of the vascular endothelium holds true in all vascular conditions involving inflammation or if there are more subtle differences that may be observed by electron microscopy. It is also uncertain if this short-term protection alters longer-term pathology or hemodynamics, or if isoflurane pretreatment alters mortality from inflammation.

In conclusion, 30 min of isoflurane pretreatment attenuated the decrease in MAP, the decrease in endothelium-dependent vasodilation, the acidosis, and the increase in TNF-α concentrations associated with lipopolysaccharide-induced inflammation. Isoflurane pretreatment also prevented the pathologic changes in the endothelium of aortic and mesenteric arteries. While the precise mechanism by which isoflurane pretreatment is protective of the endothelium is unknown, this *in vivo* study is consistent with growing evidence that isoflurane pretreatment protects against cellular injury resulting from inflammation.

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