Isoflurane Produces Endothelium-independent Relaxation in Canine Middle Cerebral Arteries


Although it is generally accepted that isoflurane can cause cerebral vasodilation, the sensitivity of the cerebral vessels to this anesthetic agent remains controversial. Furthermore, the mechanism by which isoflurane produces its direct effects on the cerebral vasculature remains unknown. The purpose of this study was to determine if isoflurane-induced relaxation of canine middle cerebral arteries is dose-dependent and/or endothelium-dependent. In an additional series of experiments, isoflurane-induced relaxation was studied in the presence of indomethacin to inhibit prostacyclin release, and endothelium-independent relaxation was examined with sodium nitroprusside. The response to isoflurane was examined in middle cerebral arteries prior to and following pretreatment with 300 μM N\textsuperscript{0}-monomethyl-L-arginine (LnMAA), an inhibitor of endothelium-dependent vasodilation. Vascular rings (2.5 mm in length and 600–800 μm in diameter) were suspended in tissue baths and isometric tension recorded. The rings were constricted with either 0.2 μM 5-hydroxytryptamine or 5 μM prostaglandin F\textsubscript{2α}, and subsequently exposed to increasing concentrations of isoflurane (0.65–4.9%). In separate experiments, the procedure was repeated in vessels with and without endothelium. Isoflurane produced a dose-dependent relaxation in all vessels. This relaxation was not inhibited by LnMAA and was unaffected by the absence of endothelium. The isoflurane response was independent of cyclooxygenase inhibition. These results demonstrate that isoflurane-induced relaxation of canine middle cerebral arteries: 1) is dose-dependent; 2) is not mediated by modulation of endothelium-derived relaxing factor or a release of prostacyclin; and 3) is endothelium-independent. (Key words: Anesthetic, volatile; isoflurane. Brain, circulation; middle cerebral artery. Endothelium-derived relaxing factor; N\textsuperscript{0}-monomethyl-L-arginine. Ions, calcium; calcium ionophore A23187.)

The volatile anesthetic isoflurane is widely used in clinical practice and is often the anesthetic of choice for neurosurgical procedures. Isoflurane has been shown to cause cerebral vasodilation, especially at concentrations required to induce deep planes of anesthesia. However, animal studies have shown that isoflurane causes less cerebral vasodilation than does halothane. Indeed, isoflurane at low concentrations (1 MAC or less) does not appear to affect cerebral blood flow (CBF). Manohar and Parks demonstrated that CBF in swine was not significantly altered at 1 MAC isoflurane. Similar observations were made by Cucchiara and associates in dogs. Several studies have demonstrated that cerebral autoregulation is maintained in the presence of 1 MAC isoflurane, thus possibly explaining the lack of effect of low concentrations of isoflurane on CBF. There is little information available from human studies on the direct effects of isoflurane on CBF. However, Madsen et al. demonstrated in humans that increasing the inspired concentration of isoflurane from 0.75 to 1.5% did not alter CBF.

The role of the endothelium in the vascular response to volatile anesthetics remains controversial. Since it was first demonstrated that the vascular relaxation produced by acetylcholine is endothelium-dependent, many other compounds have been shown to depend on intact vascular endothelium for their actions. Several studies suggest that the volatile anesthetics may induce endothelium-dependent change in tension in isolated vascular rings, either by modulating endothelium-dependent relaxing factor (EDRF)-mediated responses or inhibiting the release of endothelium-dependent constricting factor. Although it has been the focus of numerous investigations, the identity of EDRF remains uncertain. Several studies have demonstrated that EDRF and nitric oxide display similar biologic and pharmacologic properties. Furthermore, nitric oxide is released from cultured endothelial cells in response to stimulation by endothelium-dependent vasodilators. In contrast, recent studies have provided evidence which indicates that EDRF is either nitrosoyisteine or a related nitrosothiol. N\textsuperscript{0}-monomethyl-L-arginine (LnMAA) is a specific inhibitor of nitric oxide formation from L-arginine and has been demonstrated to inhibit the release of nitric oxide (NO) from cultured pig endothelial cells and also to inhibit endothelium dependent relaxation of vascular smooth muscle. Volatile anesthetics may also stimulate endothelial release of the vasodilating prostaglandin, prostacyclin (PGI\textsubscript{2}). Production of PGI\textsubscript{2} by the endothelium is dependent on the cyclooxygenase enzyme, which can be inhibited by indomethacin. The present study was designed to determine if the relaxation of canine middle cerebral arteries produced by isoflurane is: 1) dose-dependent; 2) mediated by EDRF and/or PGI\textsubscript{2} release; or 3) endothelium-dependent.

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Materials and Methods

All experimental procedures strictly adhered to the American Association for Accreditation of Laboratory Animal Care Standards, and all protocols were approved by the Animal Care Committee of the Medical College of Wisconsin.

Adult mongrel dogs of either sex were killed by exsanguination following anesthesia (sodium thiamylal 10 mg/kg intravenously), and their brains were removed. The middle cerebral arteries were identified, carefully dissected, and placed in physiologic saline solution (PSS) of the following composition (in millimolar): NaCl 119, KCl 4.7, MgSO4 1.17, CaCl2 1.6, NaHCO3 27.8, NaH2PO4 1.18, EDTA 0.026, glucose 5.5, and 4-(2-hydroxyethyl)-1-piperazineethane-sulfonic acid (HEPES) 5. The vessels from each dog were cleaned of fat and connective tissue using a dissecting microscope and divided into rings of 2.5 mm in length. Special care was taken to not cause damage to the luminal surface. The vascular rings were mounted on tungsten triangles and suspended in jacketed, temperature-controlled (37°C) tissue baths containing 15 ml of PSS and which were aerated with a mixture of 95.5% O2 and 5% CO2. The pH, PCO2, and PO2 of the salt solution were monitored every 30 min and maintained constant at a pH of 7.38 – 7.42 and PCO2 of 34–36 mmHg. The lower triangle was fixed, and the upper triangle was attached to a force transducer (Grass model FT 103).

Experimental Protocols

The rings were progressively stretched to a final optimal tension of approximately 750 mg. The optimal tension for the cerebral vessels had previously been determined in separate length–tension studies using a standard concentration (40 mM) of KCl. The rings were then allowed to equilibrate for 90 min before any experiments were conducted, and a maximum of a total of 312 vessel rings was isolated from 48 dogs and a maximum of two or three interventions was performed on each vessel ring. Contractile responses were recorded continuously on a polygraph (Grass model 7). The integrity of each ring was examined by the contractile response to 40 mM KCl in the bath medium. The contractile response to KCl usually reached a peak within 2 min. The rings were repeatedly washed, and the tension returned to the initial level within 1 min. After equilibration the rings were constricted with 0.2 μM of 5-hydroxytryptamine (5HT) or prostaglandin F2α (PGF2α) (5 μM) to a stable plateau tension. The integrity of the endothelium in the vascular rings was confirmed by demonstrating a vasodilatory response to the cardiac ionophore A23187 (0.2 μM). The relaxation produced in rabbit aorta by the ionophore A23187 has been shown to be endothelium-dependent, and it is not blocked by cyclooxygenase inhibitors.

Following repeated washouts with fresh PSS until a stable baseline was reestablished, the vessels were again preconstricted with 5HT (0.2 μM) or PGF2α (5 μM). At least 60 min elapsed between successive exposures to 5HT. Using a vaporizer (Dragerwerk, Lubeck, Germany), different concentrations of isoflurane were then bubbled in a randomized fashion into the tissue baths to determine dose–response relationships. The concentration of isoflurane in the baths was determined by gas chromatography, and its vapor content in the carrier gases was measured by mass spectrometry. The millimolar concentration of isoflurane in the baths remained consistently proportional to its measured concentration in the carrier gases throughout the entire study. In the baths the vessel rings were exposed to isoflurane concentrations varying from 0.2 to 1.1 mM, which are equivalent to 0.7–4.9% isoflurane. In dogs, 1.28% isoflurane represented 1 MAC.

In the major component of the study, dogs were divided into two groups. In the first group (68 rings, n = 10 dogs), the effects of isoflurane on 5HT-constricted arteries prior to and 15 min after the addition of LnMMA (300 μM) to the tissue baths was examined. Because the LnMMA effect on vessel rings was resistant to washout, these vessel preparations were not used in any other protocols. In the second group we examined the effects of isoflurane on 5HT-constricted arteries with (68 rings, n = 19 dogs) and without (65 rings, n = 19 dogs) endothelium. We also examined the effects of isoflurane on PGF2α-constricted vessels with (18 rings, n = 5 dogs) and without (18 rings, n = 5 dogs) endothelium. The endothelium was removed by gentle mechanical abrasion of the luminal wall with a finely shaved wooden pick. Vessel rings with and without endothelium were always taken from adjacent segments of the same middle cerebral artery. To determine the effectiveness of LnMMA as an inhibitor of EDRF-mediated responses in cerebral vessels, the response to the endothelium-dependent vasodilator, calcium ionophore A23187, was examined in rings (16 rings, n = 3 dogs) prior to and following treatment with LnMMA (300 μM).

In separate experiments the effect of sodium nitroprusside (10 μM) on rings preconstricted with 5HT (16 rings, n = 3 dogs) in the presence and absence of LnMMA was determined to ascertain the ability of the vascular rings to relax during inhibition of EDRF. Since it has been suggested that isoflurane also induces the release of a vasodilating cyclooxygenase metabolite from vascular endothelium, a further subset of experiments examined the effects of isoflurane on 5HT-preconstricted, endothelium-intact rings (15 rings, n = 3 dogs), pretreated with indomethacin (10 μM).

Drugs

Solutions of all drugs except isoflurane were prepared on each day of each experiment. 5HT, PGF2α, indomethacin.
methacin, and sodium nitroprusside were obtained from the Sigma Chemical Company (St. Louis, MO). The calcium ionophore A23187 was obtained from Boehringer Mannheim (Indianapolis, IN). Isoflurane was provided by Anaquest (Madison, WI) and the acetate salt of LnMMA was obtained from Calbiochem (La Jolla, CA).

**Statistical Analysis**

The millimolar concentrations of isoflurane measured in the baths were converted to equivalent partial pressures in the PSS and expressed as percentages of the volatile agent in the gas phase. The responses of the middle cerebral arteries to sodium nitroprusside, the calcium ionophore, and isoflurane were expressed as a percentage relaxation of the 5HT- or PGF₂α-induced constriction. The average response of all the vessel rings obtained from each dog was calculated and reported as an n value of 1. The response to each dose of each drug was then compared by analysis of variance with repeated measures. The concentrations of SNP producing 50% maximal dilation in the cerebral vessels (EC₅₀) were compared prior to and following incubation with LnMMA (300 μM) by Student’s t test. Data were expressed as mean ± SEM, and P < 0.05 was considered statistically significant.

**Results**

The mean vessel diameter of the middle cerebral arteries was 800 ± 50 μm. The optimal resting tension was 750 ± 10 mg. KCl (40 μM) produced similar constriction in vessels with and without endothelium (3.8 ± 0.8 g vs. 3.5 ± 0.2 g, respectively). 5HT produced significantly greater constriction in the cerebral vessels stripped of endothelium (1.4 ± 0.1 g), compared to endothelium-intact (1.1 ± 0.1 g) vessels (43 ± 4.2% vs. 27 ± 5.1%, respectively, of potassium-induced contractions). Following incubation with LnMMA (300 μM), 5HT constriction was also increased from 1.2 ± 0.1 to 1.7 ± 0.2 g. However, PGF₂α produced similar constriction in vessels with and without endothelium (1.9 ± 0.2 vs. 2.0 ± 0.2 g, respectively). For the purpose of this study, only vessels that had a prolonged, stable constriction in response to 5HT or PGF₂α were included in the results. Intact vascular rings producing less than 40% relaxation of the 5HT- or PGF₂α-induced preconstriction in response to A23187 (0.2 μM) were discarded. Furthermore, any endothelium-denuded rings that responded to A23187 were also discarded from the study. Of a total of 312 vessel rings, 28 (9%) of the vessel rings were rejected from the study.

Isoflurane produced only relaxation in the canine middle cerebral arteries. The isoflurane-induced relaxation was dose-dependent in all of the groups tested (figs. 1, 2, and 3). Figure 1 is a typical representation of a chart recording demonstrating the potent vasodilatory action of isoflurane on vessels (with and without endothelium) preconstricted with 5HT. The dose-dependent relaxations produced by isoflurane are clearly demonstrated in figures 2 and 3. In all of the experiments the relaxations produced...

![Fig. 2. Effect of increasing concentrations of isoflurane on middle cerebral arteries (88 rings, n = 10 dogs) preconstricted with 5-hydroxytryptamine (5HT) before (control) and after treatment with N⁶ monomethyl L-arginine (LnMMA). LnMMA did not alter the cerebral vessel's response to isoflurane. The baseline constricted tensions for control and LnMMA-treated vessels were 1.2 ± 0.1 and 1.7 ± 0.2 g, respectively. Values are mean ± SEM. All responses were significantly different from baseline constriction.](image-url)
by incremental concentrations of isoflurane were significantly different \((P < 0.05)\) from the baseline. In group 1, the addition of LnMMA (300 \(\mu M\)) did not alter the response of the cerebral arteries to increasing concentrations of isoflurane (fig. 2). In group 2, increasing concentrations of isoflurane produced similar responses in the vessel rings with and without endothelium preconstricted with either 5HT or PGF\(_2\alpha\) (figs. 3 and 4). In a subset of vessels preconstricted with 5HT, the addition of indomethacin (10 \(\mu M\)) did not alter the response of the vessel rings to increasing concentrations of isoflurane (fig. 5). In another subset of vessels, the response to the endothelium-dependent vasodilator A23187 was significantly attenuated \((P < 0.05)\) in the presence of 300 \(\mu M\) LnMMA (fig. 6). In another subset of vessels, the addition of LnMMA (300 \(\mu M\)) did not alter the cerebrovascular response to the endothelium-independent vasodilator sodium nitroprusside (fig. 7). The \(EC_{50}\) of sodium nitroprusside in the preconstricted cerebral arteries with and without LnMMA was 51 \(\pm\) 6.3 and 40.9 \(\pm\) 8.5 \(nM\), respectively.

**Discussion**

The results of this study demonstrated that isoflurane, at all concentrations used, induces significant relaxation in canine middle cerebral arteries. This response is dose-dependent and is not due to products of cyclooxygenase metabolism. LnMMA (300 \(\mu M\)), an inhibitor of EDRF-mediated vasodilation, attenuated the response of the cerebral vessels to calcium ionophore but did not alter their response to isoflurane or the endothelium-independent vasodilator sodium nitroprusside. 5HT is an endogenously occurring vasoconstrictor found in large amounts in the platelets and is assumed to be involved in cerebral vasospasm.\(^{26}\) However, 5HT can also evoke endothelium-dependent relaxation.\(^{27}\) These findings were confirmed in the present study, in which a significant increase in vasoconstrictor response to 5HT was observed in vessels preconstricted with 0.2 \(\mu M\) 5-hydroxytryptamine (5HT). Basal tension was not affected by the addition of indomethacin.
stripped of endothelium or treated with LnMMA. Although there was an increase in tension in the LnMMA-treated and stripped vessels, the ratio of isoflurane-induced dilation to preconstricted tension was similar in these vessels to that observed in the control vessels. To normalize the data, we presented the isoflurane-induced relaxations as a percentage of the preconstricted tension.

In addition, we examined the isoflurane responses in vessels with and without endothelium preconstricted with PGE2. In these experiments the basal tone induced by PGE2 was similar in both types of vessels (1.9 ± 0.1 vs. 2.0 ± 0.1 g for intact and stripped vessels, respectively). Again, removal of the endothelium in these vessels did not alter their response to isoflurane, thus substantiating our findings with 5HT.

We chose to examine the canine middle cerebral arteries because, unlike in the heart, large arteries in the brain are important determinants of cerebral vascular resistance. In mammals, several investigators have shown that under normal conditions, large arteries that supply the cerebrum may contribute as much as 60% of total resistance. Previous in vitro studies have shown that no significant change in CBF occurs at 1 MAC isoflurane. In contrast, the results of the present investigation demonstrated significant relaxation of canine cerebral vessels at concentrations as low as 0.5 MAC isoflurane. However, in the intact animal, cerebral autoregulation is maintained at 1 MAC isoflurane, thus possibly preventing the response observed in the present in vitro study. Furthermore, Drummond et al. using rabbits, demonstrated that following depression of the cerebral metabolic rate for oxygen with pentobarbital, isoflurane caused a greater increase in CBF. As an explanation of their findings, the authors suggested that cerebral vasodilation by isoflurane may be the summation of 1) a direct vasodilating effect and 2) a secondary (coupled) vasoconstrictor effect occurring as a result of depression of cerebral metabolic rate by isoflurane.

Other in vitro studies suggest that the volatile anesthetics produced constriction at low concentrations followed by relaxation at higher concentrations in some vascular beds. Our results showed only relaxation occurring in the cerebral rings in response to increasing concentrations of isoflurane. However, it has been suggested that different vascular beds may vary in their response to volatile anesthetics. In a subsequent study using skinned aortic strips, Su and Zhang demonstrated a biphasic effect to halothane in KCl-induced contractions and concluded that halothane-induced tension may be due to increased calcium release from the sarcoplasmic reticulum and the subsequent decrease in KCl contraction may be due to inhibition of calcium flux and/or enhancement of sodium/calcium exchange. However, this mechanism of action may not apply to all vascular beds.

Because it has been suggested that isoflurane stimulates the release of PGI2 (prostacyclin), the effect of isoflurane in the cerebral rings pretreated with indomethacin was also investigated. The addition of indomethacin, which inhibits prostanoid synthesis, did not alter the ce-
reblovascular response to isoflurane. These results support the findings of Blaise et al.,13 who also demonstrated that following inhibition of prostanoid synthesis, there was no attenuation of the vasodilator effects of isoflurane.

EDRF is an unstable diffusible substance that induces relaxation of vascular smooth muscle by stimulation of soluble guanylyl cyclase, thus elevating smooth muscle cyclic guanosine monophosphate (cGMP) concentrations.34,35 Independent studies36-38 have identified EDRF as nitric oxide. In a recent review, Ignarro14 collected ample evidence to suggest that at least one EDRF released from cultured porcine aortic endothelial cells, intact bovine pulmonary artery and vein, and freshly isolated bovine aortic endothelial cells is either nitric oxide or a chemically related unstable nitroso compound. Although it has been the focus of numerous investigations, the identity of EDRF still remains uncertain. However, recent studies8,19 indicate that EDRF is a nitrosothiol such as S-nitrosocysteine. Whether or not EDRF is a nitrosothiol, it would appear that NO may still have a role to play in endothelium-dependent vasodilatation. According to Myers et al.,18 the incorporation of NO into S-nitrosocysteine may be important in the control of EDRF secretion. The study by Myers et al.18 also suggested that uptake of the nitrosothiol into smooth muscle may be dependent on an active transport mechanism that is reliant on NO, or alternatively, that the nitrosothiol degrades at the cell membrane to deliver efficiently NO into the smooth muscle cytoplasm.

In the present study, LnMMA attenuated the cerebral vessels' response to the endothelium-dependent vasodilator A23187 but did not alter the vessels response to sodium nitroprusside. These results support the findings of Rees et al.,20 who demonstrated that LnMMA inhibits the endothelium-dependent relaxation of the calcium ionophore A23187 in rabbit aorta. However, pretreatment with LnMMA did not attenuate the response of the cerebral vessels to isoflurane, thus suggesting that isoflurane induced relaxation in cerebral vessels under conditions of our experiments is not EDRF-mediated. In contrast, Blaise et al.,13 using coronary vessels, demonstrated that removal of the endothelium altered the response to vasoconstriction in the presence of isoflurane, thus suggesting that this anesthetic effect is endothelium-dependent and is due to an increase in the release of or sensitivity to EDRF. The study by Blaise et al. examined the inhibition of constriction induced by three different agonists (serotonin, phenylephrine, and PGF₂α). However, the phenylephrine-induced constriction was still partially antagonized by isoflurane following the removal of the endothelium, thus suggesting that isoflurane also directly effects coronary vascular smooth muscle.

The results of the present study indicate that neither EDRF nor endothelium is involved in the mechanism of isoflurane-induced relaxation in canine middle cerebral arteries. Our results support the findings of Muldoon et al.,11 who showed no change in response to halothane following the removal of the endothelium in canine femoral and carotid arteries. Our results are also consistent with the conclusions of Marshall and Marshall,39 who demonstrated that the dilating action of the volatile anesthetics halothane, isoflurane, and enfurane on hypoxic pulmonary vasoconstriction in pulmonary arteries is not primarily exerted through EDRF.

Recent studies from our laboratory40 and others41 have shown that volatile anesthetics reduce whole-cell calcium current in canine and rat cardiac cells. This action could also be a mechanism by which isoflurane produces relaxation in cerebral vascular smooth muscle. Another explanation for the direct relaxing action of volatile anesthetics in vascular smooth muscle is an increase in intracellular cyclic 3'-5'-adenosine monophosphate (cAMP), which has been described by Sprague et al.42 in rat aortic smooth muscle following exposure to halothane and isoflurane. This work supported the findings of Yang et al.,43 who demonstrated an increase in cAMP in rat uterine muscle in response to halothane, thus resulting in relaxation. Another possible mechanism of anesthetic-induced relaxation is suggested by the work of Vulliemoz et al.,44 who demonstrated an increase in intracellular cGMP in response to halothane in mouse heart. Increased intracellular cGMP is also associated with relaxation of vascular smooth muscle.44

In summary, results of the present investigation show that isoflurane produces potent dose-dependent relaxation of canine middle cerebral arteries and that this relaxation is endothelium-independent.

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References

5. Manohar M, Parks C: Regional distribution of brain and myocardial perfusion in swine while awake and during 1.0 and 1.5 MAC isoflurane anesthesia produced without or with 50% nitrous oxide. Cardiovasc Res 18:344-355, 1984
7. Madsen JB, Cold GE, Hansen ES, Bardrum B: The effect of iso-


