Anesthesiology
76:624–634, 1992

A Comparison of the Vasodilating Effects of Halothane and Isoflurane on the Isolated Rabbit Basilar Artery With and Without Intact Endothelium

Niels F. Jensen, M.D., Michael M. Todd, M.D.,† David J. Kramer, B.A.,‡*
Paul A. Leonard, B.S.,§ David S. Warner, M.D.¶

Although volatile anesthetics result in cerebral arterial dilation, the precise mechanisms underlying this effect are not known. *In vitro* tension recordings were used to study the vasodilating potencies of halothane and isoflurane in isolated cerebral vessels and to examine the possible role of the endothelium in modulating any effects observed. Cylindrical segments of the rabbit basilar artery and midline ear artery from the same animal were placed in a flow-through bath of 37° C oxygenated (95% O_2/5% CO_2) physiologic salt solution and stretched to a resting tension of ≈ 2,000 dynes. They were then constricted with 3.0×10^{-2} M K^+, 1.0×10^{-3} M norepinephrine, or 5.0×10^{-6} M serotonin and exposed to either halothane or isoflurane at concentrations of approximately 0.5, 1.0, 1.5, and 2.0 MAC in varied order for 15 min at each concentration. A 30-min period of perfusion with anesthetic-free, vasoconstrictor-containing perfusate separated successive exposures to an anesthetic. Vessels prepared in this fashion retained their responsiveness to both vasoconstrictors and volatile anesthetics for as long as 4 h. They also relaxed appropriately to acetylcholine, indicating that the endothelium was intact. Concentrations of volatile anesthetic in the tissue perfusate were directly measured using gas chromatography, and the relationship between bath concentrations (expressed as MAC fractions) and the degree of relaxation were determined. The data were analyzed by parallel line regression. Halothane was found to be a significantly more potent vasodilator of the isolated basilar artery than was isoflurane. For example, in K^+-constricted vessels, the concentration of halothane needed to produce a 50% reduction in tension was 1.32 MAC, compared with 1.66 MAC for isoflurane. Comparable differences were found in the basilar artery in the presence of other constrictors. However, there was no significant difference between the two agents in their effects upon the ear artery. In a separate series of experiments, the endothelium of basilar artery segments was removed by drying. Removal was confirmed by observing a diminished dilator response to acetylcholine. These vessels were subsequently constricted with K^+, and relaxation dose–response curves were obtained for both halothane and isoflurane. There were no differences in the dose–response curves for deendothelialized *versus* intact vessels, with halothane still the more potent relaxant after endothelial removal. These data demonstrate that halothane and isoflurane cause a dose-dependent relaxation of rabbit cerebral vessels, regardless of the vasoconstrictor used. Halothane was a more potent relaxant of the basilar artery when expressed on a MAC-fraction basis. Removal of the endothelium did not alter the responsiveness of the basilar artery to either volatile agent. These findings support the hypothesis that the vasodilation produced by these agents is neither related to membrane-bound receptor action nor endothelial-dependent. They also suggest that observations made in extracranial vessels may not be readily extrapolated to cerebral arteries. (Key words: Anesthetics, volatile: halothane; isoflurane. Artery, basilar: endothelium. Brain: blood flow. Serotonin. Sympathetic nervous system, catecholamines: norepinephrine.)

VOLATILE ANESTHETICS result in the dilation of many vascular beds. Such vasodilation is manifested either as an increase in flow and/or a reduction in regional vascular resistance. Such changes have been repeatedly demonstrated in the brain, where a number of studies have shown that both halothane and isoflurane produce dose-related increases in cerebral blood flow (CBF).[1–6] Some have also suggested that halothane is a more potent relaxant of the cerebral vasculature than is isoflurane, although there is some evidence that contradicts this belief.[7] However, although measurements of *in vivo* organ flow or vascular resistance are of clear value, they may not provide much information regarding the direct effects of vasoactive compounds on vessels. This is because *in vivo* studies are invariably complicated by innumerable factors that can influence flow and resistance. This is particularly true in the central nervous system, where flow may be under the control of metabolic, hormonal, and/or neurogenic influences.

These studies were undertaken in an effort to better understand the nature of anesthetic-induced vessel relaxation. Such an understanding may provide important insights into the clinical effects of these drugs and may eventually permit a more intelligent manipulation of both peripheral blood flow and CBF.

Materials and Methods

All experiments were approved by the Animal Care and Use Committee of the University of Iowa College of Medicine.

BASIC PREPARATION

New Zealand White rabbits (see table 1 for n numbers) of either sex (weighing 2.8–3.5 kg) were placed in a re-

* Associate, Department of Anesthesiology.
† Professor, Department of Anesthesiology.
‡ Research Assistant, Department of Anesthesiology.
§ Graduate Trainee, Department of Pharmacology.
¶ Associate Professor, Department of Anesthesiology.

Received from the Neuroanesthesia Research Group, Department of Anesthesiology, University of Iowa College of Medicine, and the Veterans Administration Hospital, Iowa City, Iowa. Accepted for publication December 21, 1991. Supported in part by National Institutes of Health grant RO1 NS24517 to Dr. Todd.

Address reprint requests to Dr. Jensen: Department of Anesthesiology, University of Iowa College of Medicine, Iowa City, Iowa 52242.

TABLE 1. Numbers of Rabbits Studied in Each Experimental Group

	Potassium		Norepinephrine		Serotonin	
	Halothane	Isoflurane	Halothane	Isoflurane	Halothane	Isoflurane
Basilar artery: n rabbits	27	27	9	9	11	11
Ear artery: n rabbits	7	6	0	0	15	13
Denuded basilar artery: n rabbits	12	10				

straining box, the right marginal ear vein was cannulated, and a lethal dose of pentobarbital (40 mg/kg) was administered. This drug was chosen for euthanasia because of its widespread use in vessel studies. The brain was rapidly removed from the skull and the basilar artery was removed and placed in cold (4° C) isotonic solution for mammals (ISM).** The vessel was then transferred to an ISM-filled Petri dish and, under a dissecting microscope, was stripped of extraneous, extravascular connective tissue using microforceps and scissors. The distal 8.0 mm (immediately before its bifurcation into the two posterior cerebral arteries) was divided into two 4.0-mm segments ($\approx$ 500 μm OD). Each of the arterial segments was then threaded onto two rigid, triangular clips made from 0.12-mm-diameter stainless steel wire (fig. 1). In a similar fashion, a 6.0-mm segment of the midline ear artery was harvested, cleaned, divided into two 3.0-mm segments and suspended on two clips. Each of the vessel segments were then transferred to one of four ISM-filled Plexiglas perfusion chambers. In each, one wire clip was attached to a high fidelity tension transducer (SensoNor, Horten, Norway) and the other clip was affixed to an adjustable bridge. The perfusion chambers were then sealed.

The four separate vessel chambers were continuously supplied with temperature-controlled (37° C), oxygenated, ISM perfusate. The source of perfusate for each chamber was a sealed, warmed, water-jacketed column that was kept continuously filled with perfusate from a Marriott bottle reservoir. A mixture of 95% O_2/5% CO_2, flowing at a rate of $\approx$ 800 ml/min through calibrated anesthetic vaporizers, was bubbled through these columns to saturate the ISM with O_2, CO_2, and, when desired, a controlled concentration of volatile agent. The gas-equilibrated perfusate was pumped at a rate of 4 ml/min from the columns into the chambers by a peristaltic pump, passing through a warming bath in transit. Simultaneously, the pump effected the evacuation from each chamber of an equal amount of perfusate (which was discarded). In addition to the aqueous delivery of anesthetic, the sealed chambers were connected *via* a glass tube to the top of the bubbling columns and thus to the gaseous phase of the anesthetic agents (fig. 1).

PRELIMINARY EXPERIMENTS

In preliminary studies, several different types of cerebral and noncerebral vessels in rabbits were isolated. The basilar and median ear arteries were selected for use, primarily because of their accessibility and size similarity (0.5–1.0 mm OD). Because relaxant studies demand the establishment of a stable constricted vessel, a series of experiments were conducted to determine optimal vessel length and vasoconstrictor dose for three commonly used agonists—K^+, serotonin, and norepinephrine. Specifically, length–tension experiments to determine optimal resting tension were performed. We initially were seeking a level of resting tension that would result in the largest response (contraction) to the vasoconstrictor. In our preliminary length–tension and dose–response studies we found that when 3.0-mm ear and 4.0-mm basilar artery segments were passively stretched between 500–3,500 dynes (in 500-dyne increments) by K^+ (range 10–80 mM), the optimal resting tension for both vessels was 2,000 dynes. Knowing this optimal resting tension, we then produced a submaximal constriction with 3.0×10^{-2} M K^+ to perform the studies on acetylcholine and anesthetics. It is important that experimental constrictions be submaximal so that constriction as well as relaxation are possible in the presence of purported vasodilators such as volatile anesthetics. All subsequent work was performed using 30 mM K^+ (ISK-30††). Next, dose–response curves for serotonin and norepinephrine (both from Sigma) were constructed. The resultant median effective dose (ED_{50}) to achieve a maximal constriction to serotonin in both basilar and ear segments was 5.0×10^{-6} M. However, difficulties were encountered with norepinephrine, which produced a much weaker and poorly sustained contraction, particularly in the basilar artery. As a result, we se-

** Composition: 130 mM NaCl, 16 mM NaHCO3, 0.5 mM NaH2PO4, 4.7 mM KCl, 1.8 mM CaCl2, 0.4 mM MgCl2, 13 mM HEPES (Sigma Chemical Co., St. Louis, MO), and 5.5 mM dextrose; *p*H 7.4., osmolality 280–290 mOsm/kg.

†† ISK-30 was prepared by replacing 30 mM Na^+ in the ISM with 30 mM K^+.

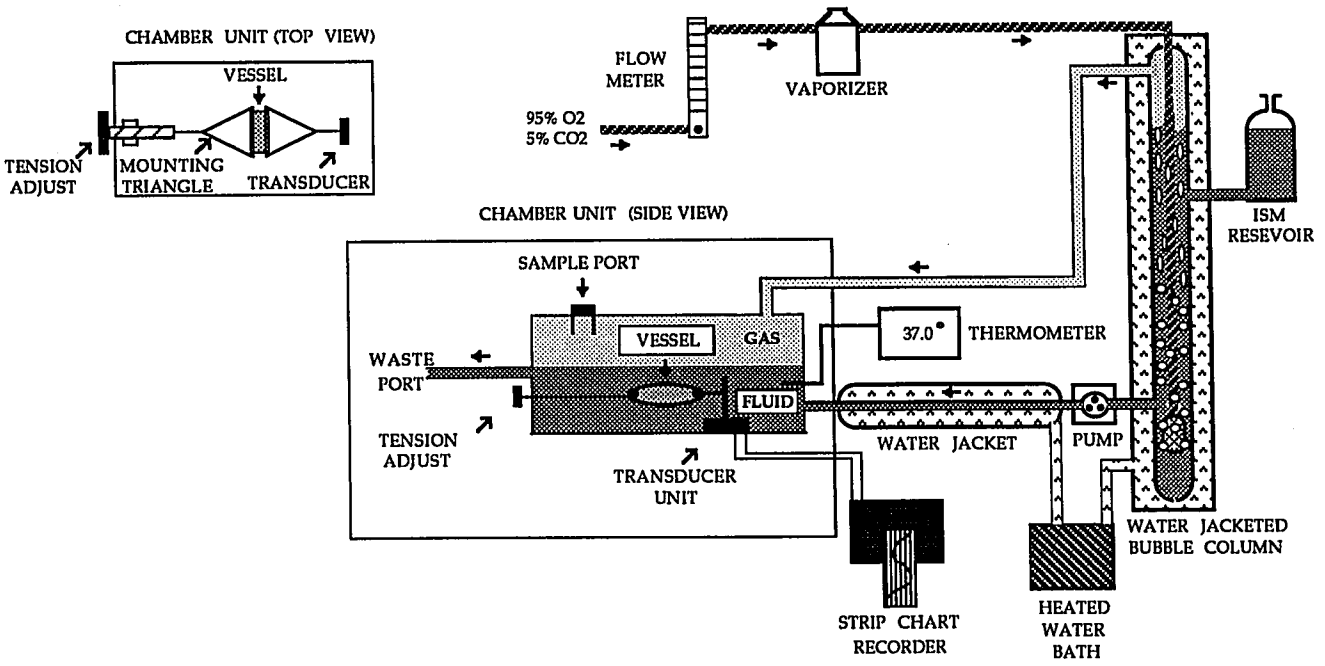

FIG. 1. The experimental system. Gas (95% O_2/5% CO_2) was passed *via* a flow meter, through a calibrated vaporizer, into a water-jacketed bubble column. Anesthetic-saturated fluid was then pumped, *via* a warming bath, into the vessel chamber. Gas drawn from the vapor phase over the liquid in the bubble chamber was also delivered to the vessel chambers, which were sealed after the arterial segments were in place. The vessels themselves were supported on two triangular stainless steel clips, one attached to a force transducer and the other to an adjustable bridge.

lected a dose of norepinephrine (1.0×10^{-3} M) that resulted in a degree of constriction that approximated the constriction achieved with 30 mM K^+ and 5.0×10^{-6} M serotonin, even though this dose represented a near-maximal response to the drug. In addition, lower concentrations of norepinephrine also resulted in unacceptably transient vasoconstriction, making examination of the relaxant effects of volatile agents extremely difficult. It should be noted that even with the chosen norepinephrine dose, a stable constriction could not be sustained for as long as with K^+ and serotonin. This phenomenon has been reported elsewhere and was hence incorporated into the design of our subsequent experiments (see below).

In addition, a series of studies was performed to verify the long-term viability of vessels in this preparation. This viability was examined by monitoring the tension of control vessels (not exposed to volatile agent) constricted with K^+ over a long period of time, and determining that vessels exposed to volatile agent for long periods 1) would still relax further in response to an increase in volatile agent concentration, but 2) would return to normal tensions following volatile agent washout. Time–viability studies were carried out on vessels from eight New Zealand White rabbits. Basilar artery segments slightly longer than usual (12.0 mm) were removed from each rabbit and were divided into three segments. These were handled exactly as described above. After placement in the chambers, the segments were allowed to acclimate in ISM for 30 min with no tension applied and then were stretched to 2,000 dynes. The segments were then constricted by changing the perfusate from ISM to ISK-30 and allowing them to stabilize for 1 h. Calibrated vaporizers were then used to add halothane and isoflurane in approximately 1.0 MAC doses (see below) to the gas mixture flowing into the bubble chambers. One segment was exposed to halothane and one to isoflurane, and one served as a control, unexposed to volatile anesthetic. Tensions were recorded continuously for 4 h, and the 1.0 MAC anesthetic level was not altered during this time. At the end of this period, the vessels were exposed for 30 min to 2.0 MAC doses of volatile agent and then to anesthetic-free ISK-30 for a final 30 min. The tensions achieved in vessels exposed to volatile anesthetic were compared to the initial tensions achieved in these vessels following exposure of anesthetic-free ISK-30.

Finally, because of the crucial role of the endothelium in vascular smooth muscle responsiveness, it was necessary to ensure that our routine preparation did not significantly disrupt the functional integrity of the endothelium. This was determined in two ways. First, six basilar artery segments that had been prepared as described and that had been suspended in the vessel chambers for 5 h during the vessel viability studies were removed from the chambers and fixed in a 2.5% glutaraldehyde buffer solution, and

the endothelial surfaces examined by scanning electron microscopy. Second, the response to acetylcholine (an endothelial dependent relaxant) was examined. Vessels from seven rabbits were cannulated with steel clips and placed in an ISM bath under 2,000 dynes tension. After achieving a stable constriction with ISK-30, the vessels were exposed to 5.0×10^{-6} M acetylcholine. The resulting relaxation of tension was compared to that seen in vessels in which the endothelium had been purposely removed by 14 min of drying (see below).

Volatile Agent Dose–Response Effects

All vessel segments were prepared as noted above. After placement in the chamber, each vessel was allowed to acclimatize in normothermic ISM for 30 min with no tension applied. The vessel segments were then stretched to a final resting tension of 2,000 dynes over a 30-min period. Stretched vessels were then allowed to equilibrate in ISM for 1 h. All segments were then constricted by changing the chamber perfusate from ISM to one containing either 3.0×10^{-2} M K^+ (ISK-30), 1.0×10^{-3} M norepinephrine, or 5.0×10^{-6} M serotonin. Each vessel studied was exposed to only one vasoconstrictor during the course of an experiment. Vessels constricted with serotonin and norepinephrine were allowed to stabilize for 15 min, and a 30-min stabilization interval was allowed for K^+.

After the establishment of stable vessel constriction, calibrated vaporizers were used to add halothane or isoflurane to the 95% O_2/5% CO_2 gas mixture flowing into the bubble chambers. The selected concentration was administered for 15 min, with vessel tension recorded continuously. Vessel tension values were noted immediately before exposure and at the end of the 15-min exposure period. A sample of bath fluid was removed from each chamber at the end of the exposure period for subsequent chromatographic determination of anesthetic concentration. The volatile anesthetic was then discontinued, and each vessel was perfused for either 15 (norepinephrine and serotonin) or 30 min (K^+) with anesthetic-free ISM (still containing vasoconstrictor). Tension was again recorded, and a second concentration of anesthetic introduced, again for 15 min. This sequence was repeated for each of the four target concentrations. An example of a representative tension recording is shown in figure 2.

Target concentrations were equivalent to 0.5, 1.0, 1.5, and 2.0 MAC (assuming rabbit MAC values of 1.39 and 2.05% for halothane and isoflurane, respectively).[8] These target values were equivalent to directly measured bath concentrations of 44.80, 88.96, 133.40, and 177.90 μg/ml (225.30, 450.66, 675.99, and 901.32 μM) of vasoconstrictor solution for halothane, and 43.87, 87.74, 131.60, and 175.50 μg/ml (237.78, 475.55, 713.33, and 951.11 μM) of vasoconstrictor solution for isoflurane. Actual concentrations achieved in each chamber at each dose were determined by gas chromatography as described below. For experiments using K^+ constriction, the order of anesthetic exposure was systematically varied. Every combination beginning with the lowest MAC equivalent (0.5) and every combination beginning with the highest MAC equivalent (2.0) were used on at least two separate occasions. For experiments with norepinephrine and serotonin, only ascending concentration sequences were examined (0.5, 1.0, 1.5, and 2.0 MAC).

Role of the Endothelium

To test the role of the endothelium in modulating volatile anesthetic–induced effects, basilar arteries from 20 rabbits were studied, 10 animals with isoflurane and 10 with halothane. The proximal 4.0 mm served as a control, and the distal 8.0 mm was cannulated with a 30-G needle

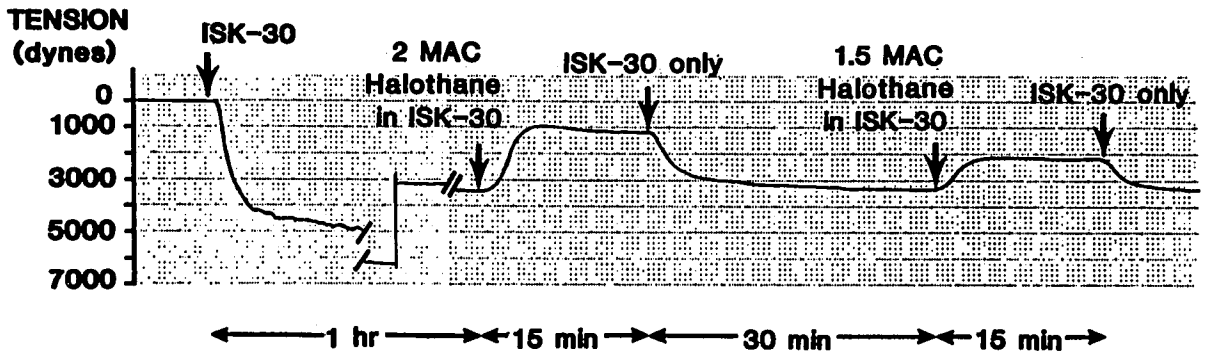

FIG. 2. An example of an actual vessel tension recording. The vessel was stretched to 2,000 dynes and stabilized in isotonic solution for mammals. The strip chart recorder was adjusted to zero tension and the vessel then constricted by 30 mM K^+ (ISK-30). As in this example, sometimes the amplitude of the initial constriction necessitated a halving of the amplification. This gain change occurred at the first break in the tracing. The unit on the Y axis is the tension *after* this change in gain. After 1 h, a randomized dose of the volatile anesthetic was added to the ISK-30. Relaxation was established over 15 min and a perfusate sample was drawn for gas chromatographic analysis. Percent relaxation was calculated by comparing vessel tension immediately before volatile anesthetic exposure to that seen after 15 min of exposure.

that was attached to a 95% O_2/5% CO_2 dry gas source. A gentle stream of this gas was passed through the lumen of the vessel for 14 min, a period of time that was determined to be optimal in terms of denuding the endothelium without damaging the underlying smooth muscle cells. These techniques were adapted from and closely approximate earlier reported work by other investigators on this artery.[9] The middle 4.0 mm was then cut free. Each of these segments was threaded onto a rigid clip and attached to tension transducer in a Plexiglas perfusion chamber, as described above. The vessel chambers were continuously supplied with ISM. A mixture of 95% O_2/5% CO_2 flowing through anesthetic vaporizers was bubbled through the perfusate columns to saturate the perfusate with O_2, CO_2, and controlled concentrations of anesthetic. Each of the segments was perfused for 30 min in ISM, then stretched to 2,000 dynes, and constricted by changing the perfusate to ISK-30. Functional removal of the endothelium was verified by exposing the segments to 5.0×10^{-6} M acetylcholine (Sigma) and comparing relaxation to controls. Both control and experimental vessels were then exposed to 15 min pulses of volatile anesthetic in doses of approximately 0.5, 1.0, 1.5, and 2.0 MAC in a stepwise fashion, with a 30-min period of perfusion with anesthetic-free ISK-30 between each dose.

Gas Chromatography

Volatile anesthetics were extracted from the perfusate samples with n-heptane in 20-ml scintillation vials with Teflon cap liners. A 2-μl sample of the n-heptane phase was then injected into a Hewlett-Packard model 5700A gas chromatograph equipped with an electron capture detector and a Hewlett-Packard model 3392A integrator. The detector response from the unknowns was compared to that response obtained from standard curves developed for isoflurane and halothane. Hamilton gas tight syringes were used in the preparation of standards and in the collection of samples.

Statistics

To examine the relationship between anesthetic dose and relaxation, we chose to express our directly measured bath concentrations as "MAC fractions" (measured bath concentration divided by the pertinent 1 MAC concentration, *i.e.*, 88.96 μg/ml or 450.66 μM for halothane and 87.74 μg/ml or 475.55 μM for isoflurane) rather than as micrograms per milliliter or as partial pressure.

In each case, percent relaxation was determined by the difference in vessel tension just prior to the beginning of volatile agent exposure and tension at the end of a 15-min exposure period divided by tension prior to volatile agent exposure (for an example, see fig. 2). Since a plot of percent relaxation *versus* dose showed an increasing standard deviation with increasing dose, a square root transformation was performed. Plotting the square root of the percent relaxation against the MAC fraction yielded dose–response curves that were homoscedastic and linear up to a halothane concentration of $\approx$ 1.8 MAC.

Halothane points above 1.8 MAC were excluded. The reasons were: 1) as the relaxation induced by the anesthetic approaches the maximum value, the response must become independent of the dose, invalidating the use of points near the maximum for potency comparison; 2) analysis of variance suggested a small but nearly significant nonparallelism between the two curves in some cases if all the data were used, presumably caused by the halothane curve approaching the maximum relaxation; and 3) the response levels (percent relaxation) for halothane were greater than those for isoflurane. Nonparallelism of dose–response curves, which in this case may be due to the high doses of halothane approaching maximal relaxation, would invalidate use of the parallel line technique. The issue of response levels is considered less critical, but potency comparison by parallel line analysis is most appropriate when the responses represented by each curve are comparable to those on the other curves. Disregarding points at the extremes of the dose–response curve is the standard technique for dealing with this situation, because the extremes tell us little about relative potency between two drugs.[10]

Potencies of anesthetics were independently determined by calculating the concentrations needed to obtain 50% relaxation (EC_{50}) from linear regression of these anesthetic dose *versus* relaxation response curves.[11] Potencies were compared by Finney's parallel line bioassay technique.[10] This involves 1) testing the validity of plotting the two dose–response curves as parallel straight lines, and 2) testing for a significant shift to the right or left of one of the lines with respect to the other.

Note that for clarity, data are presented graphically (in figs. 3 and 4) as a constriction index, which is ten minus the square root of the percent relaxation. This was done such that relaxation appeared as a line moving down and to the right. Note that a constriction index of 5 represents a 25% decrease in tension (see legends to figs. 3 and 4).

Differences between intact and deendothelialized vessels were calculated by completely analogous parallel line analysis, testing for a shift of the dose–response curve in deendothelialized vessels relative to intact vessels.

In order to achieve some consistency in the vessel tension produced by the three agonists, vessels that did not attain an initial tension of at least 2,000 dynes above baseline or failed to return to at least 2,000 dynes following a period of volatile anesthetic exposure were excluded from the study.

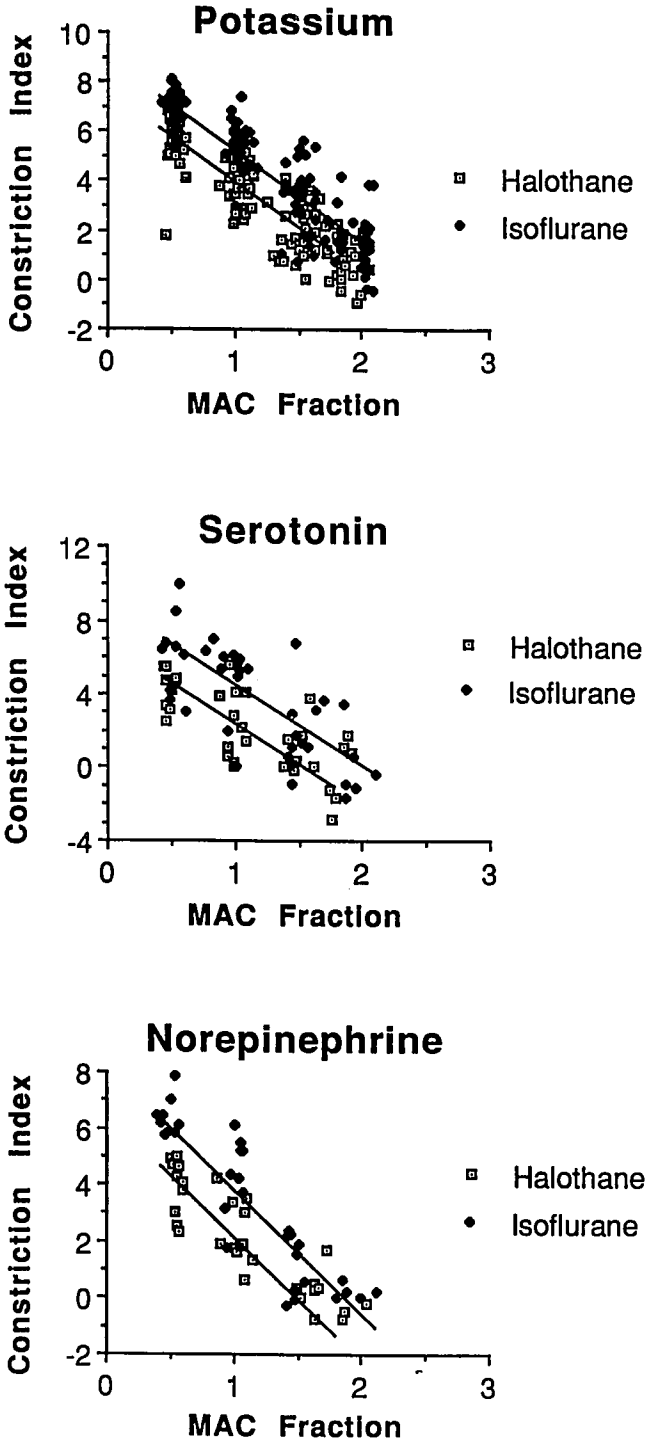

FIG. 3. Inhibition of vasoconstriction by halothane and isoflurane in the basilar artery. The constriction index was defined as 10 minus the square root of percent relaxation. Thus, a constriction index of 5 represents 25% relaxation, and a constriction index of 0 represents 100% relaxation. Note: all points are shown, but those points above 1.8 MAC on the halothane line were not used in the data analysis; thus, the halothane line was not extrapolated beyond that point. (See Results.)

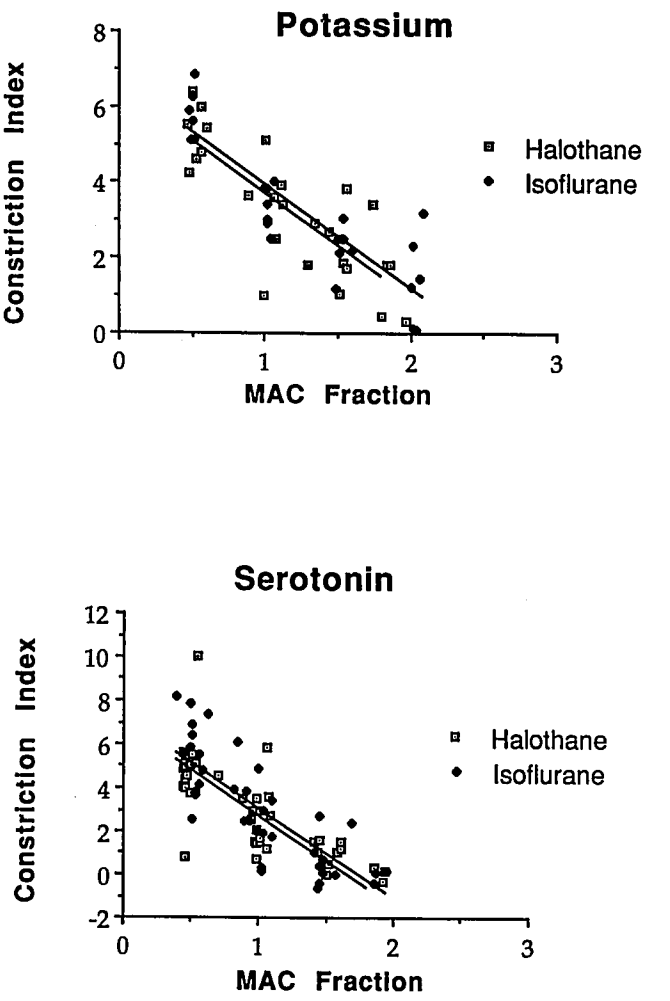

FIG. 4. Inhibition of vasoconstriction by halothane and isoflurane in the ear artery. The constriction index was defined as in figure 3. A before, all points are shown, but those points above 1.8 MAC on the halothane line were not used in the data analysis; thus, the halothane line was not extrapolated beyond that point.

Results

VESSEL VIABILITY–TIME STUDIES

Control segments of basilar artery constricted with K^+ showed only a minor decrease in developed tension over the 4-h observation period. Actual tension present at the end of the 4-h period was 87 ± 4% of that developed upon initial exposure to K^+. When vessels were initially exposed to 1.0 MAC of halothane or isoflurane, tension was reduced to 78 ± 6% and 86 ± 3% of baseline, respectively. At the end of the 4 h of continuous exposure to these volatile agents, tensions were still 77 ± 6% and 78 ± 7%, respectively, of baseline. These vessels still relaxed in response to an increased dose of halothane and isoflurane, evidenced by a further decrease in tension to

39 ± 9% and 43 ± 8%, respectively, of baseline upon exposure to 2.0 MAC volatile agent. Finally, when the volatile agent was discontinued, tension quickly increased to 91 ± 5% (halothane) and 89 ± 7% (isoflurane) of initial values—tensions that did not differ greatly from those seen in control vessels (87 ± 4%) never exposed to volatile agent (but observed for a similar period of time).

Endothelial Integrity Studies

Vessels isolated and prepared as noted above consistently demonstrated significant relaxation when exposed to 5.0×10^{-6} M acetylcholine. The average magnitude of relaxation was 40%. This compared with an average 9% relaxation in vessels in which the endothelium was intentionally removed by drying.

Scanning electron microscopy of six random vessel segments fixed in glutaraldehyde buffer after 5 h of bath suspension (both exposed and unexposed to volatile anesthetics) consistently revealed no significant inadvertent endothelial disruption with steel clip placement or bath exposure, in either the group exposed to volatile anesthetic or the unexposed group.

Volatile Agent Dose–Response Effects

The number of rabbits studied with each vasoconstrictor and volatile agent dose is shown in table 1. Two separate (but identical) sets of experiments using K^+ were performed, separated by 6 months. However, because the dose–response curves in both were indistinguishable, the data were combined for this report, resulting in the large n values.

Analysis of variance suggested that parallel line regression (neglecting halothane points above 1.8 MAC) was appropriate for the dose range examined (see table 2). Analysis of residuals shows no correlation between the degree of anesthetic-mediated relaxation as measured by the constriction index and the preanesthetic constriction, suggesting that 1) the use of percent relaxation in the calculation of the constriction index is appropriate, and 2) vessels that had a lesser initial constriction (down to the cutoff used) were not less viable in their response to drugs than vessels with a greater initial constriction. Figures 3 (basilar artery) and 4 (ear artery) show the parallel line regressions calculated for each vasoconstrictor–volatile anesthetic combination that was examined. The relatively greater vasorelaxant effect of halothane is consistently apparent over the range of MAC fractions studied in the basilar artery, regardless of the vasoconstrictor selected. By contrast, no difference between the anesthetics was found using the ear artery. The comparison of potencies and test for nonparallelism is shown in Table 2. Note that in the basilar artery, halothane was always significantly more potent than isoflurane with either K^+, norepinephrine, or serotonin since the 95% confidence interval for each constrictor included the computed value. By contrast, there was no significant difference in the ear vessels, since the 95% confidence interval for the difference in potency includes 0.0.

The responses we studied predominantly represented a removal of pharmacologic-induced tone, but at higher doses (1.5–2 MAC), anesthetics appear to decrease myogenic tone. The evidence for this is the relaxation below 0 of the constriction index at higher doses, meaning that the vessel had tone before it was contracted (myogenic tone). We did not specifically study myogenic- *versus* agonist-induced tone.

Role of the Endothelium

The dose–response curves for intact and deendothelialized basilar arteries are shown in figure 5. For both isoflurane and halothane, parallel line regression demonstrated that the lines are virtually indistinguishable. Mean preanesthetic constriction by intact vessels was 2,820 dynes, whereas mean preanesthetic constriction by

TABLE 2. Relaxation Data

Vasoconstrictor	Artery	IC_{50}		Difference (with 95% CI)	Test for Nonparallelism
		Halothane	Isoflurane		
Potassium	Basilar	1.32	1.66	0.35 (0.27–0.43)*	$P = 0.699$
	Ear	1.29	1.37	0.07 (−0.15–0.27)	$P = 0.975$
Norepinephrine	Basilar	0.87	1.25	0.38 (0.24–0.54)*	$P = 0.069$
Serotonin	Basilar	0.87	1.35	0.48 (0.27–0.72)*	$P = 0.438$
	Ear	0.96	1.04	0.08 (−0.09–0.25)	$P = 0.254$

IC_{50} indicates the concentration of volatile agent (expressed as MAC fractions) needed to produce a 50% reduction in vessel tension. These were computed from the parallel line regression of the square root of the percent inhibition *versus* the MAC fraction (see figs. 3 and 4).

"Difference" indicates the difference in MAC fraction needed to achieve comparable degrees of vessel relaxation. *$P < 0.05$ for the differences (*i.e.*, the 95% confidence intervals do not include 0). The differences for all three vasoconstrictors are significant for basilar artery, but not for ear artery.

The test for nonparallelism was ANOVA for statistically significant nonparallelism in the dose–response regression lines. The P value was greater than 0.05 in all cases, so use of parallel lines was not rejected.

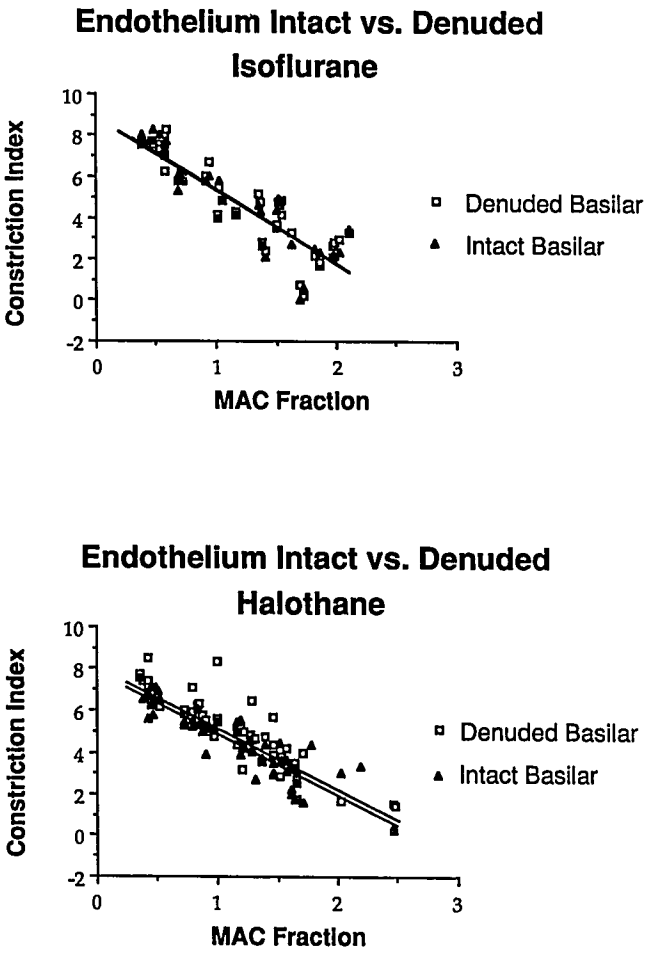

FIG. 5. Lack of effect of deendothelialization on anesthetic-induced relaxation. The constriction index was defined as in figure 3. Potassium was used to preconstrict the vessels. The anesthetics used were halothane (*A*) and isoflurane (*B*).

deendothelialized vessels was 2,580 dynes, again suggesting that little damage to the smooth muscle occurred during the deendothelialization process.

Discussion

The effects of intravenous anesthetics (particularly the barbiturates) upon isolated cerebral vessels have been studied extensively.[12–15] However, although some data exists concerning the influence of volatile anesthetics upon isolated peripheral vessels,[16–30] there is comparatively little information regarding their effects upon isolated cerebral vessels.[31] More importantly, there are no data comparing the effects of different volatile agents on cerebral vessels, and no information concerning their possible interaction with the cerebral vascular endothelium.

The current study was undertaken to compare the effects of halothane and isoflurane on an isolated cerebral vessel and on a similar-sized extracerebral artery. The basilar artery of the rabbit was chosen largely for convenience. Rabbits are readily available and inexpensive, and yet the basilar artery is large enough to be used in an isolated vessel preparation. Vessels from larger animals (*e.g.*, dogs or pigs) are not as easily obtainable or as practical from the standpoint of cost, particularly given the large number of vessels needed. The median ear artery was chosen as the extracranial counterpart to the basilar artery, primarily due to its accessibility and size similarity.

The vessel perfusion/tension apparatus used here has previously been described in connection with studies of pig coronary vessels.[22] This unit was designed specifically to allow the controlled delivery of stable concentrations of volatile agents to the vessel chambers, concentrations that could be easily verified by subsequent gas chromatography. As outlined above, the vessels were continuously perfused with normothermic, fresh physiologic solution. The initial isolation, cleaning, and suspension on metal clips was performed under an operating microscope. As noted, preliminary trials ensured that this preparatory process did not result in vessel damage. This was confirmed by the long-term (4 h) responsiveness of vessel segments, the lack of endothelial damage on microscopic examination, and the presence of endothelial-mediated relaxation to acetylcholine. In short, the apparatus and preparation described, though certainly not unique, does create a stable, near-physiologic environment that can be closely monitored and controlled. Furthermore, vessel viability can be maintained in this environment over a long period, during which volatile anesthetics can be introduced and subsequently measured with a high degree of accuracy.

As noted, we selected three vasoconstrictors to conduct our relaxant studies and chose to express volatile anesthetic concentration in MAC fractions. The use of several vasoconstrictors is typical of *in vitro* studies, allows wider generalization of results, and may provide specific mechanistic information (see below). We suggest that the MAC fraction is a useful parameter for comparison of different volatile anesthetic effects because of the correlation between MAC and lipid solubility. This correlation suggests that the site of action of volatile anesthetics may be hydrophobic lipid membranes, and thus equivalent MAC fractions may represent equivalent numbers of molecules at the membrane level. Because of different solubilities, equimolar concentrations, for example, may not ensure equal numbers of molecules at the active membrane site. An alternative approach would have been to calculate directly the molar concentration of volatile agent dissolved in a hypothetical membrane lipid. However, this approach is unlikely to offer any advantages over use of a benchmark that is familiar to all anesthesiologists.

In this study we demonstrate that halothane and isoflurane both relax the isolated rabbit basilar and ear arteries in a dose-related fashion. However, halothane is consistently more potent than isoflurane in the basilar artery, with the isoflurane dose–response curve being shifted to the right by about 0.4 MAC over the concentration range tested. There were no significant differences between the agents in terms of their effects on the ear artery.

Our data are consistent with those of other investigators who have shown dose-related relaxation by volatile agents in both the rat aorta and in coronary arteries from pigs. Using the model as described here, Bollen *et al.* demonstrated that halothane relaxes K^+-constricted isolated pig coronary artery segments to a greater degree than does isoflurane.[22] In a study of the contractile responses of coronary arteries obtained from human hearts, Villeneuve *et al.* demonstrated that 1.5 MAC halothane attenuated the contractile responses evoked by serotonin, prostaglandin $F_{2\alpha}$, and histamine—three putative mediators of coronary constriction. Isoflurane at the same MAC concentration had no effect on contractions.[28] No dose–response relationships were established for the anesthetics. In the rat aorta, Sprague *et al.* showed that halothane was a more potent inhibitor of phenylephrine-induced constriction than isoflurane.[23] They suggested that the stimulation of cAMP formation may be involved in the mechanism of anesthetic-induced relaxation. Interestingly, these findings may be at variance with *in vivo* observations that suggest that isoflurane decreases peripheral resistance to a greater extent than does halothane.[32–34] However, it should be noted that this information was obtained under conditions in which experimental variables were difficult to control and in which the precise anesthetic concentration at the vessel site was unknown.

That the dose–response curves for the volatile anesthetics are similar regardless of the vasoconstrictor used suggests that perhaps the agents are acting distal to the neurotransmitter receptor. K^+ depolarizes the cell membrane and opens voltage-gated Ca^{2+} channels. Norepinephrine and serotonin, in contrast, activate G-proteins, causing the production of (possibly different) second messengers. Thus, the initial steps in the action of these three vasoconstrictors are different. If volatile anesthetics were acting at these initial sites we would not expect to see the similarity in the anesthetic dose–response curves. Rather, we might see different slopes and/or potencies according to the vasoconstrictor that was used. A later step critical to vasoconstriction may be common to all of the vasoconstrictors, and the volatile anesthetics may in fact act at this common stage.

This study also suggests that the relaxation produced by volatile anesthetics *in vitro* is independent of the endothelium. There was no difference in volatile anesthetic potency between arteries with intact endothelium and arteries in which we documented removal of most of the endothelium by showing minimal acetylcholine-induced vasodilation. Furthermore, there was no correlation between acetylcholine-induced relaxation and anesthetic-induced relaxation. This result is not consistent with the possibility that a small amount of remaining endothelium produced sufficient vasoactive compounds in response to the anesthetic to elicit full vasodilation. This would also imply that a fully intact endothelium might produce maximal vasodilation in the presence of a submaximal dose of anesthetic, an effect that was not seen. The lack of an endothelial effect is consistent with the work of Villeneuve *et al.* on isolated coronaries obtained from humans. These vessels exhibited marked endothelial dysfunction. Their results suggest that halothane attenuates contractions evoked by specific agonists despite the absence of functioning endothelium.[28] The lack of an endothelial effect for halothane is also supported by the recent work of Su *et al.*[29] They found that halothane reduces Ca^{2+} availability in vascular smooth muscle by depleting the sarcoplasmic reticulum of Ca^{2+}. This finding, that halothane induces direct vascular smooth muscle relaxation, had also been suggested previously.[30]

While the role of the endothelium in modulating the constrictor or dilatory effects of many drugs has been demonstrated, its role in determining or modifying the response to volatile agents is largely unknown. In fact, the interaction between the endothelium and volatile anesthetics has only recently been examined in rat aortic vascular rings[21] and canine coronary,[18] femoral, and carotid[19] arterial segments. The results of this work are somewhat confusing. For example, Muldoon *et al.*,[19] working with rabbit aorta and canine femoral and carotid arteries, suggested that halothane has a direct, endothelium-*independent* effect that may produce either an increase or decrease in vessel tone, depending on the specific vessel type. Furthermore, these investigators suggest that halothane interferes with endothelium-derived relaxing factor (EDRF)–mediated relaxation of smooth muscle. Conversely, Stone and Johns,[21] working with rat aorta, found that enflurane and isoflurane have a biphasic effect on vascular tone. At low anesthetic concentrations, an endothelium-dependent vasoconstriction occurred, followed by an endothelium-independent vasodilation at higher concentrations. Although halothane did not cause vasoconstriction at low concentrations, higher doses produced vasodilation similar to that observed with enflurane and isoflurane. Blaise *et al.*,[18] working with canine coronary arteries, demonstrated that isoflurane is capable of attenuating vasoconstrictor-induced contractions in an endothelium-dependent manner. Although Muldoon *et*

al.[19] suggested that halothane does not interfere with guanylate cyclase function (*i.e.*, cyclic GMP production), they (as well as Blaise *et al.*[18] and Stone and Johns[21]) speculate that volatile anesthetics either interfere with the synthesis, release, and transport of EDRF or that volatile anesthetics facilitate the action of EDRF on smooth muscle. Such conclusions are further complicated by the possible existence of substantial regional and species-related heterogeneity in the responses to volatile anesthetics, as they do with other vasoactive agents.[35]

None of the above investigations has examined the endothelial dependency of volatile anesthetics on the cerebral vasculature or compared these effects to a peripheral vessel bed. Although the effects of volatile anesthetics on isolated cerebral vessels have not been studied elsewhere, it should be noted that cerebral vessels have been successfully deendothelialized and the effects of several *intravenous* vasoactive compounds have been examined. For example, Fujiwara *et al.*[36] were among the first investigators to conduct experiments with deendothelialized rabbit basilar artery segments. They reported that endothelium removal potentiated the contractile response to a variety of vasoconstrictors. Furthermore, they suggested that the abolition of the spontaneous release of EDRF was the most probable mechanism of the enhanced vasoconstriction.

The potency differences reported above between volatile anesthetics in intracranial but not extracranial vessels and the lack of endothelial effects in intracranial vessels may represent only another example of the regional heterogeneity of vascular response, both inside[37] and outside[38,39] the brain. Such heterogeneity could result from different numbers and/or populations of receptors, differentiated responsiveness of endothelial cells, or vascular smooth muscle heterogeneity, where identical signals generated by endothelial cells produce different smooth muscle responses.[40] It has been suggested that the heterogeneity of endothelium-dependent responses may in fact serve important hemodynamic purposes.[40] Regional heterogeneity in terms of numbers of receptors may also explain the relatively high concentration of norepinephrine necessary to produce sustainable constrictions of sufficient magnitude in the rabbit basilar artery.

Despite the apparent agreement of our results with *in vivo* studies showing a greater increase in CBF with halothane as compared with isoflurane, we believe that extrapolation of these results to any measurement of CBF is tenuous. The response of CBF to an anesthetic is probably determined by many interacting factors, besides those noted above. Anesthetic effects on cerebral blood volume and perfusion pressure (*i.e.*, blood pressure and intracranial pressure), cardiac output, cerebral metabolism (since CBF and metabolism are closely coupled), cerebral electrical activity, autoregulatory activity, and the activity of the adrenergic and cholinergic nerves and perhaps endogenous hormones and/or peptides seemingly contribute to a final *in vivo* effect. To assume that a change in vessel tension seen *in vitro* can be equated with a change in CBF simply is not warranted at the present time.

Our data demonstrate that halothane and isoflurane cause a dose-dependent relaxation of rabbit cerebral vessels that have been constricted with K^+, norepinephrine, or serotonin. In this *in vitro* study, halothane was a more potent vasodilator of rabbit basilar artery than was isoflurane. In the ear artery, the agents had indistinguishable vasoactive effects. Halothane- and isoflurane-induced relaxation of rabbit basilar artery segments constricted with K^+ was not endothelium-dependent. Endothelium-endogenous production of EDRF might still modulate the response to anesthetics. This study has not clarified the effects of anesthetics upon responses to endothelial dependent agonists, such as acetylcholine or bradykinin.

References

1. Theye RA, Michenfelder JD: The effect of halothane on canine cerebral metabolism. ANESTHESIOLOGY 31:1113–1118, 1968
2. Cucchiara RF, Theye RA, Michenfelder JD: The effects of isoflurane on canine cerebral metabolism and blood flow. ANESTHESIOLOGY 40:561–564, 1974
3. Eintrei C, Leszniewski W, Carlsson C: Local application of 133 Xenon for measurement of regional cerebral blood flow during halothane, enflurane, and isoflurane anesthesia in humans. ANESTHESIOLOGY 63:391–394, 1985
4. Boarini DJ, Kassell N, Coester HC, Butler M, Sokoll M: Comparison of systemic and cerebrovascular effects of isoflurane and halothane. Neurosurgery 15:400–409, 1984
5. Todd MM, Drummond JC: A comparison of cerebrovascular and metabolic effects of halothane and isoflurane in the cat. ANESTHESIOLOGY 60:276–282, 1984
6. Gelman S, Fowler KC, Smith LR: Regional blood flow during isoflurane and halothane anesthesia. Anesth Analg 63:557–565, 1984
7. Hansen TD, Warner DS, Todd MM, Vust LJ, Trawick DC: Distribution of cerebral blood flow during halothane versus isoflurane anesthesia in rats. ANESTHESIOLOGY 69:332–337, 1988
8. Drummond JC: MAC for halothane, enflurane and isoflurane in the New Zealand white rabbit: And a test for the validity of MAC determinations. ANESTHESIOLOGY 66:36–338, 1985
9. Nakagomi T, Kassel NF, Sasaki T, Leman RM, Torner JC, Hongo K, Lee JH: Effect of removal of the endothelium on vascontraction in canine and rabbit basilar arteries. J Neurosurg 68: 757–766, 1988
10. Finney DJ: Statistical Method in Biological Assay. 3rd ed. New York, Macmillan Publishing, 1978
11. Neter J, Wasserman W, and MH Kutner: Applied Linear Regression Models. Homewood, Richard Irwin, Inc., 1983, pp 134–141.
12. Nielsen KC, Owman CH: Contractile response and amine receptor mechanisms in isolated middle cerebral artery of the cat. Brain Res 27:33–42, 1971.
13. Yashon D, Dunker RO, Locke GE, *et al: In vitro* basilar and common

carotid artery reactivity to alpha and beta stimulation. Neurology 21:1085–1087, 1971

14. Edivinsson L, McCullouch J: Effects of pentobarbital on contractile responses of feline cerebral arteries. J Cereb Blood Flow Metab 1:437–440, 1981
15. Wendling WW, Harakal C: Comparative actions of pentobarbital and verapmil on canine cerebral and peripheral arteries *in vitro.* Res Comm Chem Pathol Pharmacol 49:189–202
16. Kassay-Farkas N, Wyse DG: Comparison of responses of helical strips of artery from anesthetized and untreated rats. Can J Physiol Pharmacol 60:986–988, 1982
17. Larach DR, Schuler HG, Derr JA, Larach MG, Hensley FA, Zeils R: Halothane selectively attenuates α_2-adrenoceptor mediated vasoconstriction, *in vivo* and *in vitro.* ANESTHESIOLOGY 66:781–791, 1987
18. Blaise G, Sill JC, Nugent M, Van Dyke RA, Vanhoutte PM: Isoflurane causes endothelium-dependent inhibition of contractile responses of canine coronary arteries. ANESTHESIOLOGY 67: 513–517, 1987.
19. Muldoon SM, Hart JL, Bowen KA, Freas W: Attenuation of endothelium-mediated vasodilation by halothane. ANESTHESIOLOGY 68:31–37, 1988
20. Lunn JJ, Rorie DK: Halothane-induced changes in the release and disposition of norepinephrine at adrenergic nerve endings in dog saphenous vein. ANESTHESIOLOGY 61:377–384, 1984
21. Stone DJ, Johns RA: Endothelium-dependent effects of halothane, enflurane, and isoflurane on isolated rat aortic vascular rings. ANESTHESIOLOGY 71:126–132, 1989
22. Bollen BA, Tinker JH, Hermsmeyer K: Halothane relaxes previously constricted isolated porcine coronary artery segments more than isoflurane. ANESTHESIOLOGY 66:748–752, 1987
23. Sprague DH, Yang JC, Ngai SH: Effects of isoflurane and halothane on contractility and the cyclic 3',5' adenosine monophosphate system in the rat aorta. ANESTHESIOLOGY 40:162–167, 1974
24. Spiss CK, Smith CM, Tsujimoto G, Hoffman BB, Maze M: Prolonged hyporesponsiveness of vascular smooth muscle contraction after halothane anesthesia in rabbits. Anesth Analg 64:1–6, 1985
25. Longnecker DE, Harris PD: Microcirculatory actions of general anesthetics. Fed Proc 39:1580–1583, 1980
26. Longnecker DE, Harris PD: Dilation of small arteries and veins in the bat during halothane anesthesia. ANESTHESIOLOGY 37: 423–429, 1972
27. Witzeling TM, Sill JC, Hughes JM, Blaise GA, Nugent M, Rorie DK: Isoflurane and halothane attenuate coronary artery constriction evoked by serotonin in isolated porcine vessels and in intact pigs. ANESTHESIOLOGY 73:100–108, 1990
28. Villeneuve E, Blaise G, Sill JC, Guerard MJ, Buluran J, Girard D: Halothane 1.5 MAC, isoflurane 1.5 MAC, and the contractile responses of coronary arteries obtained from human hearts. Anesth Analg 72:454–461, 1991
29. Su JY, Zhang CC: Intracellular mechanisms of halothane's effect on isolated aortic strips of the rabbit. ANESTHESIOLOGY 71: 409–417, 1989
30. Laher I, Germann P, Dowd AL, Bevan JA: The selective potentiation of noradrenaline-induced tone by BAY K 8644 in the rabbit basilar artery. J Cereb Blood Flow Metab 9:759–764, 1989
31. Harder DR, Gradall K, Madden JA, Kampine JP: Cellular actions of halothane on cat cerebral arterial muscle. Stroke 16:680–683, 1985
32. Deutsch S, Linde HW, Dripps RD, Price HL: Circulatory and respiratory actions of halothane in normal man. ANESTHESIOLOGY 23:631–638, 1962
33. Eger EI II, Smith NT, Stoelting RK, Cullen DJ, Kadis LB, Whitcher CE: Cardiovascular effects of halothane in man. ANESTHESIOLOGY 32:396–409, 1970
34. Stevens WC, Cromwell TH, Halsey MJ, Eger EI, Shakespeare TF, Bahlman SH: The cardiovascular effects of a new inhalation, anesthetic, Forane, in human volunteers at constant arterial carbon dioxide tension. ANESTHESIOLOGY 35:8–16, 1971
35. Hardebo JE, Hanko J, Owman CH: Species variation in the cerebrovascular response to neurotransmitters and related vasoactive agents. Gen Pharmacol 14:135–136, 1983
36. Fujiwara S, Kassell NF, Tomio S, Nakagomi T, Lehman RM: Selective hemoglobin inhibition of endothelium-dependent vasodilation of rabbit basilar artery. J Neurosurg 64:445–452, 1986
37. Hamel E, Edvinsson L, MacKenzie E: Heterogeneous vasomotor responses of anatomically distinct feline cerebral arteries. Br J Pharmacol 94:423–436, 1988
38. Toda N: Regional differences in response to nicotine in isolated canine arteries. Eur J Pharmacol 35:151–60, 1976
39. Edvinsson L, Owman CH: A pharmacologic comparison of histamine receptors in isolated extracranial and intracranial arteries *in vitro.* Neurology 25:271–76, 1975
40. Vanhoutte PM, Miller VM: Heterogeneity of endothelium-dependent responses in mammalian blood vessels. J Cardiovas Pharmacol 7:S12–S23, 1985