Halothane-induced Dilatation of Intraparenchymal Arterioles in Rat Brain Slices: A Comparison to Sodium Nitroprusside

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Background: Halothane is a potent dilator of cerebral arteries. The predominant site of cerebrovascular resistance is thought to be intracerebral arterioles, and the effects of halothane on these vessels were not previously examined. This study compared the effects of halothane with those of the vasodilator and nitric oxide donor, sodium nitroprusside, on intraparenchymal microvessel responsiveness in a brain slice preparation.

Methods: Anesthetized Sprague-Dawley rats underwent thoracotomy and intracardiac perfusion and then were decapitated. Hippocampal brain slices were prepared and placed in a perfusion/recording chamber and superfused with artificial cerebrospinal fluid. An arteriole was located within the brain parenchyma and its diameter was monitored with videomicroscopy before, during, and after various concentrations of halothane or sodium nitroprusside were equilibrated in the perfusate. All vessels were preconstricted with prostaglandin $F_{2\alpha}$ before halothane or sodium nitroprusside treatment. An observer blinded to treatment analyzed vessel diameter changes with a computerized videomicrometer.

Results: Baseline microvessel diameter was $18 \pm 2 \mu m$ in the halothane group (n = 14) and $15 \pm 1 \mu m$ in the sodium nitroprusside group (n = 15). Prostaglandin $F_{2\alpha}$ (0.5 μM) preconstricted vessels by approximately 15% from resting diameter in both groups. Halothane significantly and dose dependently dilated intracerebral microvessels by $54\% \pm 6\%$, $74\% \pm 8\%$, $108\% \pm 13\%$, and $132\% \pm 7\%$ (normalized to the preconstricted diameter) at 0.5%, 1.0%, 1.5%, and 2.5% halothane, respectively. This dilatation corresponds to a decrease in a calculated index of cerebrovascular resistance index of up to $117\% \pm 2\%$ at 2.5% halothane. Sodium nitroprusside, in concentrations ranging from 10^{-8} to 10^{-3} M, also dose dependently dilated these intraparenchymal vessels by $129\% \pm 7\%$ at the highest concentration. These alterations in microvessel diameter corresponded to a decrease in the cerebrovascular resistance index of up to $116\% \pm 4\%$ for the largest dose.

Conclusions: Halothane produces dose-dependent vasodilatation of intraparenchymal cerebral microvessels, thus predicting marked decreases in cerebrovascular resistance in this *in vitro* brain slice preparation. The effects of halothane on these cerebral microvessels are similar to those of the potent vasodilator sodium nitroprusside. These findings suggest that direct effects of halothane on cerebral microvessel diameter contribute substantially to alterations in cerebrovascular resistance and flow produced by this agent. (Key words: Anesthetics, volatile: halothane; Brain: brain slices; hippocampus. Vasodilatation. Cerebral arterioles. Sodium nitroprusside.)

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HALOTHANE produces dose-dependent vasodilatation in many experimental preparations, including whole organisms and isolated organs and tissue beds in both arterial and venous blood vessels.[1–7] The effects of halothane on the cerebral vasculature has received significant attention because of the relation between increases in cerebral blood flow and increases in intracranial pressure. Volatile anesthetics are well known cerebral arterial vasodilators.[1–4] Halothane relaxes cerebral arteries *in vivo*[5] and in isolated vessel preparations in which arteries larger than 100 μm in diameter were removed from their neuronal and glial framework.[6–8] Although these arteries are critical in blood and oxygen transport, as in other tissue beds, the predominant site of cerebrovascular resistance, as localized using microspheres and decreases in perfusing vascular pressure,

is attributed to arterioles.[9–11] Whether intracerebral arterioles react to halothane to the same degree and by the same mechanism as observed in cerebral arteries remains unknown. Although it is difficult to measure changes in intracerebral microvasculature directly, previous investigations studying the effects of several different physiologic mediators, including serotonin and norepinephrine, on isolated cerebral microvessels have shown differences in vascular reactivity between cerebral arteries and arterioles.[11–13]

Because cerebral microvessels play an important role in the regulation of cerebrovascular resistance, blood flow, and oxygen delivery, several investigations have studied the effects of halothane on pial microvessels and regional cerebral perfusion using a closed cranial window[14] or laser-Doppler flowmetry.[15–17] Although these studies provide valuable, *in vivo* information, these preparations are limited to either surface blood vessels or regional blood flow rather than showing specific actions at the arteriolar level. In addition, the potentially confounding effects of supplemental baseline anesthetics and hemodynamic alterations are difficult to eliminate, as is the differentiation of indirect *versus* direct vascular effects of the inhalational administration of halothane.

Recently, an *in vitro* brain slice preparation was introduced that allows intracerebral resistance microvessels to be studied in the absence of baseline anesthetics.[18] Microvessels within brain slices remain viable for many hours and may be continuously monitored using computerized videomicroscopy.[18–22] Furthermore, the cerebral arterioles in this preparation remain intraparenchymal and thus presumably responsive to localized neuronal activity and neurohumoral mediators that may be involved in vascular responsiveness to various pharmacologic agents. Although the arteriolar responses to a few physiologic and pharmacologic mediators of vascular reactivity have been evaluated in this preparation,[19–22] the effects of halothane have not been examined.

This investigation evaluated the effects of halothane on intracerebral microvessels using an *in vitro* rat brain slice model. Microvessel responses to halothane were compared with the potent, direct-acting vasodilator, sodium nitroprusside, the responses of which have not been well characterized in this preparation. The evaluation of sodium nitroprusside effects in this model also provides more preparation-specific information regarding the maximum or near-maximum dilatory responses to a direct-acting vasodilator and potential mechanical

or vascular limitations to dilatation at high concentrations of sodium nitroprusside.

Methods

All experimental procedures used in this investigation were reviewed and approved by the Animal Use and Care Committee of the Medical College of Wisconsin, and protocols were completed in accordance with the Guiding Principles in the Care and Use of Laboratory Animals of the American Physiological Society and in accordance with National Institutes of Health guidelines. All animals were housed within the animal facilities of the Medical College of Wisconsin, accredited by the American Association for the Accreditation of Laboratory Care.

General Preparation

Adult male Sprague-Dawley rats weighing 250–350 g and that had no significant weight differences among them were used. Animals were anesthetized in a specially designed animal holding chamber by breathing 2% halothane (Anaquest Inc., Madison, WI) in oxygen. After the animals were adequately anesthetized, a midline thoracotomy was performed and 20 ml buffered saline and 2 ml 0.05% indigo carmine dye in saline were infused intracardially into the left ventricle while a right atrial incision was simultaneously made for blood drainage. The animals were immediately decapitated and the brains rapidly removed and rinsed with nutrient medium (artificial cerebrospinal fluid [aCSF]) composed of 124 mM NaCl, 5 mM KCL, 2.4 mM $CaCl_2$, 1.3 mM $MgCl_2$, 10 mM glucose, 1.24 mM KH_2PO_4, 26 mM $NaHCO_3$, with gaseous equilibration with 95% oxygen and 5% carbon dioxide and *p*H 7.4. Nutrient medium was prepared daily and all measurements of cerebral microvessel diameters were performed on the same day that tissue slices were prepared.

Brains were cut freehand into small blocks containing the hippocampus, followed by immediate sectioning into coronally oriented tissue slices (280–300 μm thick) using a vibratome mechanical tissue slicer (OTS-3000-03, FHC, Brunswick, ME). Throughout the slicing procedure, tissues were continuously bathed in the oxygenated aCSF at or slightly below room temperature (20–23°C). Subsequently, the slices were transferred to a plexiglass holding chamber and maintained at interface with oxygenated aCSF. Individual slices were then transferred for examination to a recording chamber mounted
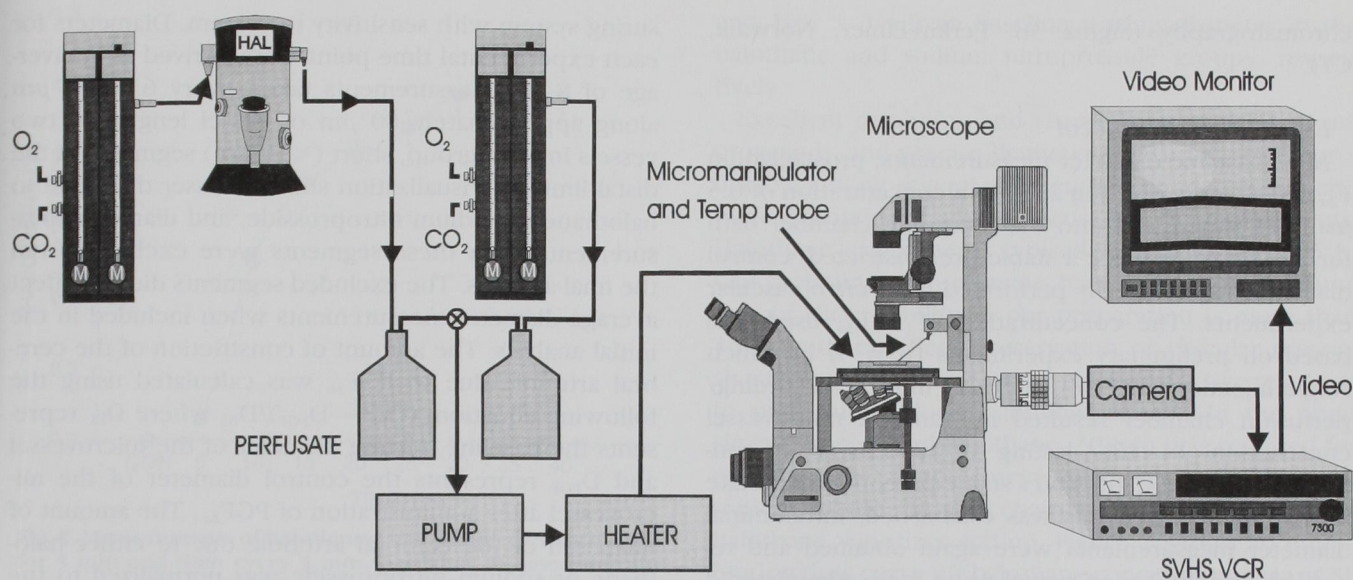
Fig. 1. Brain slice preparation for measuring cerebral microvessel responsiveness. Brain slices were placed in a recording chamber and superfused with oxygenated artificial cerebrospinal fluid. Cerebral microvessels were located and monitored with videomicroscopy and an inverted microscope. Baseline diameter measurements for a selected vessel were recorded using a precalibrated videomicrometer. The experimental conditions were manipulated, and alterations in microvessel diameter were monitored and recorded.

on an inverted halogen transillumination microscope (fig. 1; Nikon Diaphot 200, Yokohama, Japan).

The recording chamber was designed in our laboratory and consisted of a center recording/superfusion compartment of 2.8 ml (30 mm × 22 mm × 8 mm) and an elevated chamber for vacuum suction that prevented any significant fluid waves. Slices were submerged onto a nylon mesh that allowed continuous superfusion of aCSF under and around the brain slices. Flow through the recording chamber was at a rate of 1 ml/min, completely exchanging the volume in the chamber in less than 3 min. The chamber temperature was continuously monitored and maintained at 34°C using a thermoelectric Peltier device coupled to a sensing thermistor. The slices were maintained in this chamber and continuously superfused with the oxygenated aCSF for approximately 1 h before the experimental protocol began.

Microvessel Analysis

A vessel (9–30 μm in diameter) was located within the neuronal tissue and its integrity and diameter were continuously monitored by videomicroscopy. The videomicroscopy equipment consisted of an inverted halogen transillumination microscope and a 40× objective (Olympus WPlanFL 160/0, Tokyo, Japan). The image

was magnified with a ×2.25 video projection lens (Nikon CCTV/Microscope Adapter, Yokohama, Japan), transmitted to video camera (CCD 72; Dage MTI, Michigan City, IN), and projected onto a video monitor (Sony HR Trinitron, Tokyo, Japan). Vessel diameter changes were recorded on videotape using a SVHS video recorder (Sony Trinitron) and analyzed using a microcomputer-based videomicrometer (standardized to an etched/calibrated microscope slide) with sensitivity to 0.1 μm developed in our laboratory.

During the equilibration period, in the recording chamber, an intracerebral microvessel was located. Baseline luminal diameter measurements were obtained after the 60-min equilibration period. The aCSF that superfused the brain slices was aerated with a mixture of oxygen, carbon dioxide, and air sufficient to maintain the pH and partial pressure of carbon dioxide (P_{CO_2}) within normal limits (pH, 7.38–7.42; P_{CO_2}, 34–38 mmHg). The partial pressure of oxygen (P_{O_2}) was maintained at 220 ± 20 mmHg. Gas analysis (Radiometer ABL3, Copenhagen, Denmark) of the superfused fluid was obtained during baseline and every 60 min during the experimental period. The anesthetic concentration in the perfusing fluid was obtained in all experiments by slowly withdrawing 1 ml of fluid from the slice chamber and measuring the millimolar concentration by gas

chromatography (Sigma 3B; Perkin-Elmer, Norwalk, CT).

Experimental Protocol

After baseline diameter measurements, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), dissolved in aCSF to a concentration of 0.5 μM, was superfused into the recording chamber bath for 30 min to produce a stable preconstricted, control diameter, as is typically performed in *in vitro* vascular experiments. The concentration of $PGF_{2\alpha}$ used was based on preliminary experiments (n = 5) in which single injections of $PGF_{2\alpha}$ (0.5 μM) into the recording/perfusion chamber resulted in transient microvessel constriction (14–16%) lasting for 10–12 min. In contrast, during $PGF_{2\alpha}$ infusion a stable preconstricted state was attained. Once this was established, intraluminal diameter measurements were again obtained and recorded, and the experimental drugs were administered during the continuous $PGF_{2\alpha}$ superfusion. Halothane was delivered to one group of slices by volatilizing the anesthetic agent into the aCSF by passing the oxygen, carbon dioxide, and air mixture through a vaporizer (model F100; Ohio Medical Products; Airco Inc., Madison, WI). The time required for halothane to equilibrate in the superfusate in the slice chamber was determined during preliminary experiments. Halothane was administered in graded concentrations. The vaporizer dial settings used were 0.5%, 1%, 2%, 3%, and 5%. Again the reported halothane concentrations in the Results section represent those calculated based on measured values from the slice chamber. After introducing halothane or any change in concentration, at least 30 min was allowed for equilibration. Microvessel diameter was measured at the end of each equilibration period and again 45 min after halothane was discontinued.

Brain slices in the sodium nitroprusside group were superfused with sodium nitroprusside, which was added directly to the aCSF in the recording chamber. During this period, superfusion with $PGF_{2\alpha}$ remained at steady state. Sodium nitroprusside at concentrations of 1×10^{-8} M, 1×10^{-7} M, 1×10^{-6} M, 1×10^{-5} M, 1×10^{-4} M, and 1×10^{-3} M were superfused at 1 ml/min in random order to all slices. After drug administration, changes in cerebral microvessel diameter were monitored, as was the time course when those changes occurred.

Data Analysis

Intraluminal diameter of cerebral microvessels was measured on-line and off-line using a computerized mea-

suring system with sensitivity to 0.1 μm. Diameters for each experimental time point were derived as an average of 8–13 measurements taken every 6 to 10 μm along approximately 80 μm of vessel length. In two vessels in each group, short (<10 μm) segments at the distal limits of visualization showed lesser dilatation to halothane or sodium nitroprusside, and diameter measurements from these segments were excluded from the final analysis. The excluded segments did not affect average diameter measurements when included in the initial analysis. The amount of constriction of the cerebral arteriole due to $PGF_{2\alpha}$ was calculated using the following equation: $(D_{BL} - D_{PGF})/D_{BL}$ where D_{BL} represents the baseline, resting diameter of the microvessel and D_{PGF} represents the control diameter of the microvessel after administration of $PGF_{2\alpha}$. The amount of dilatation of the cerebral arteriole due to either halothane or sodium nitroprusside was normalized to the amount of constriction produced by $PGF_{2\alpha}$ and was calculated using the following equations: $(D_{HAL} - D_{PGF})/(D_{BL} - D_{PGF})$ and $(D_{SNP} - D_{PGF})/(D_{BL} - D_{PGF})$. D_{HAL} and D_{SNP} were the diameters of the microvessels after administration of halothane or sodium nitroprusside, respectively. An index of cerebral vascular resistance was calculated as the fourth power of the reciprocal of the diameter. The percentage resistance change was calculated in a similar manner, as was the percentage vasodilatation. The resistance change was also expressed as normalized to the resistance change after $PGF_{2\alpha}$.

Artificial CSF fluid samples were obtained from the superfusate for measurements of halothane concentrations, which were subsequently analyzed by gas chromatography. The millimolar concentrations of halothane measured in the bath were converted to equivalent partial pressures in the solution and expressed as percentages of the volatile agent in the gas phase.[23] Thus the halothane concentrations described in the Results section are those calculated based on measured concentrations rather than on vaporizer dial settings.

Statistical Analysis

Microvessel diameter changes were averaged for each experimental manipulation. Both raw and normalized data were analyzed by one-way repeated measures analysis of variance to compare differences within groups at multiple time points. Pairwise comparisons of interventions were performed with contrasts derived from the repeated measures analysis and adjusted for multiplicity by Duncan's modification of the *t* test. Changes in vessel diameter at each concentration of halothane
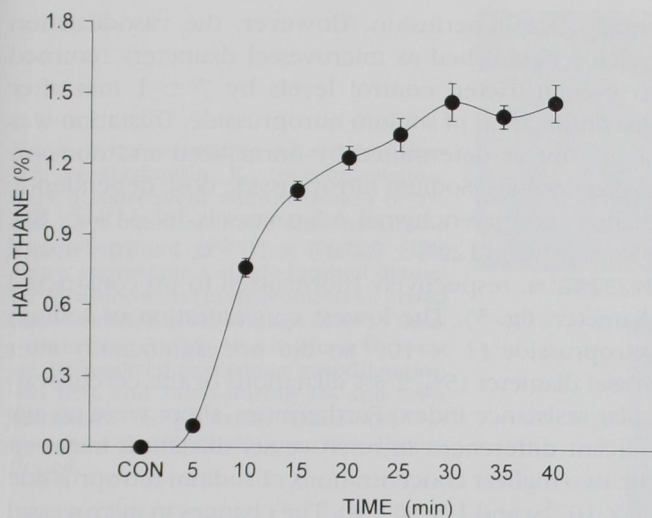
Fig. 2. Measurements of halothane were sampled every minute for 5 min and then every 5 min thereafter to determine the time course of equilibration of the volatile anesthetic in the superfusing artificial cerebrospinal fluid in the recording chamber. Shown is an example of halothane concentrations when setting vaporizer dial concentration from 0% to 2% (n = 5). Stabilization of halothane occurred after 25 to 30 min. Millimolar concentrations were calculated to equivalent partial pressures.

or sodium nitroprusside were compared with the vessel diameter during $PGF_{2\alpha}$ preconstriction and lower concentrations of each agent and were considered significant when the probability value was less than 0.05. All data are expressed as means ± SEM.

Results

A total of 83 hippocampal brain slices were obtained from 55 animals. The protocol with halothane was completed for 14 brain slices, and the protocol with sodium nitroprusside was completed for 15 slices. Six brain slices were used for halothane measurements in aCSF and eight brain slices were used for evaluation of bolus doses of $PGF_{2\alpha}$ to determine the concentration to be used for preconstriction. Brain slices were excluded from analyses if microvessels could not be adequately visualized or if the luminal diameters of the microvessels were not clearly discernible during the experiment. Baseline microvessel diameter ranged from 9 to 27 μm in the halothane group (mean = 18 ± 2 μm) and from 10 to 24 μm in the sodium nitroprusside group (mean = 15 ± 1 μm). Infusion of $PGF_{2\alpha}$ (0.5 μM) resulted in stable cerebral microvessel constriction of 15% ± 2%

and 16% ± 1% from baseline resting diameter in the halothane and sodium nitroprusside groups, respectively.

Excellent resolution and visualization of cerebral microvessels, and precise diameter measurements (reproducible among several independent blinded observers) were obtained for all vessels used in the experiments. Halothane experiments typically lasted less than 5 h. Our preliminary experiments have shown that the duration of slice viability in our preparation is more than 10 h, with excellent preservation of vascular responsiveness as determined by the vasoconstrictive response to potassium chloride administration.[22] In the brain slices that received halothane, 30 min was required for equilibration and to achieve steady-state aCSF concentrations within the slice chamber, after each change in halothane vaporizer setting. Figure 2 shows an equilibration time curve for halothane concentrations in aCSF obtained during preliminary experiments. In all experiments, the actual measured concentration of halothane in the superfused aCSF in the recording chamber, as measured by gas chromatography, was lower than the corresponding vaporizer setting (table 1), but this concentration remained stable for 1 h.

Halothane significantly and dose dependently dilated intraparenchymal microvessels. Changes in vessel diameter were not different between normalized and nonnormalized data. At a concentration as low as 0.5% halothane, significant vasodilatation occurred. Halothane-induced microvessel dilatation was 54% ± 6%, 74% ± 8%, 108% ± 13%, and 132% ± 7%, normalized to the preconstricted diameter (at 0.5%, 1.0%, 1.5%, and 2.5% halothane, respectively; fig. 3). There was no further increase in microvessel diameter when increasing halothane concentration from 2.5% to 4%. The estimated

Table 1. Concentrations of Halothane

Vaporizer Setting (%)	Concentration in Bath (mM)	Calculated Partial Pressure (vol %)
0.5	0.16 ± 0.01	0.5 ± 0.04
1.0	0.30 ± 0.02	1.0 ± 0.05
2.0	0.45 ± 0.04	1.5 ± 0.13
3.0	0.75 ± 0.04	2.5 ± 0.13
5.0	1.26 ± 0.08	4.1 ± 0.08
0% (post-halothane)	0.08 ± 0.02	0.3 ± 0.06

Data are mean ± standard error of the mean; n = 10. Halothane concentrations as set by vaporizer dial in bath (measured by gas chromatography) and calculated partial pressures in the solution (percent of volatile agent in the gas phase).
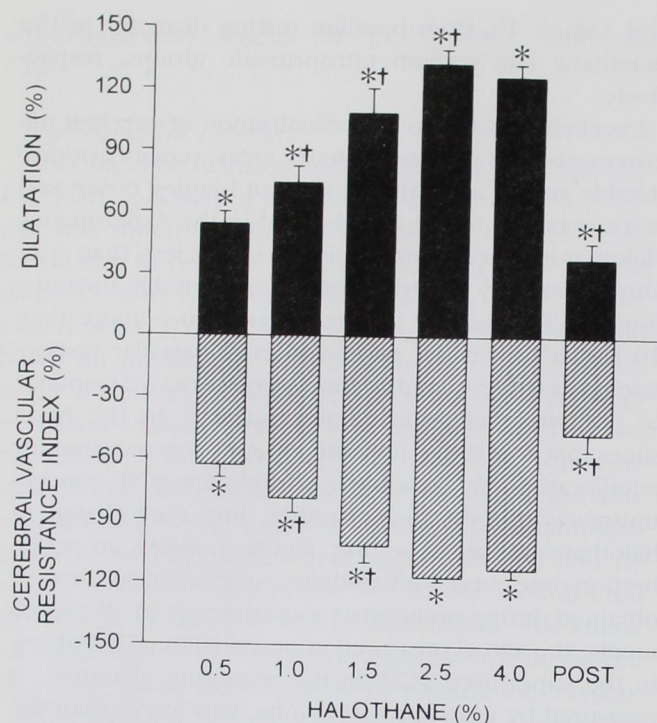
Fig. 3. Percentage of vasodilatation and percentage of change in the calculated cerebral vascular resistance index for measured halothane concentrations of 0.5%, 1.0%, 1.5%, 2.5%, and 4.0%. Percentages of halothane expressed are those calculated from measured artificial cerebrospinal fluid halothane concentrations in the slice chamber. The data are normalized to the amount of constriction produced by prostaglandin $F_{2\alpha}$. *$P < 0.05$ *versus* control, †$P < 0.05$ *versus* immediately lower concentrations.

changes in the calculated cerebrovascular resistance index corresponding to the measured halothane-induced diameter changes were 63% ± 6%, 79% ± 7%, 102% ± 8%, and 117% ± 2% at 0.5%, 1.0%, 1.5%, and 2.5% halothane, respectively (fig. 3). The vasodilatation and reduction in cerebrovascular resistance by halothane was reversible and returned toward control, preconstricted levels after discontinuation of halothane for 30–60 min. Halothane concentrations within the slice chamber remained at 0.08 ± 0.02 mM (0.3% ± 0.06%) for as long as 45 min after halothane was discontinued. Figure 4 depicts a representative intraparenchymal microvessel during control and preconstrictive conditions and during subsequent halothane-induced vasodilatation.

Sodium nitroprusside, infused directly into the brain slice recording chamber, resulted in more rapid vasodilatation that began within 1.5 ± 0.5 min. Sodium nitroprusside-induced vasodilatation remained constant with steady-state superfusion. However, the vasodilatation quickly diminished as microvessel diameters returned to preconstricted control levels by 7 ± 1 min after discontinuation of sodium nitroprusside. Dilatation was significant as determined by normalized and non-normalized values. Sodium nitroprusside dose dependently dilated intraparenchymal microvessels by 34% ± 8%, 95% ± 6%, and 126% ± 7% at 1×10^{-7} M, 1×10^{-5} M, and 1×10^{-4} M, respectively (normalized to preconstricted diameter; fig. 5). The lowest concentration of sodium nitroprusside (1×10^{-8} M) did not significantly alter vessel diameter (5% ± 3% dilatation) or the cerebrovascular resistance index. Furthermore, there were no significant differences in microvessel dilatation between the two highest concentrations of sodium nitroprusside (1×10^{-4} M and 1×10^{-3} M). The changes in microvessel diameter with higher concentrations of sodium nitroprusside resulted in significant calculated decreases in cerebrovascular resistance index (fig. 5) of as much as 116% ± 4% (with 10^{-3} M).

Discussion

Halothane is a potent and dose-dependent vasodilator of cerebral arteries in both humans[1,24] and animals.[2-4] The results of the present investigation show, for the first time, the vasodilatation of intracerebral microvessels by a halogenated volatile anesthetic agent that was both potent and dose dependent. Indeed, the degree of vasodilatation was similar to that produced by high concentrations of the vasodilator sodium nitroprusside. These results suggest that cerebral arterioles significantly contribute to the changes in cerebrovascular resistance and cerebral blood flow produced by halothane. The microvessels in our preparation, while *in vitro,* remained within a neuronal syncytium and neuropil. Thus these vessels may be subject to normal metabolic influences insofar as they are present in this brain slice preparation. Although parenchymal metabolic modulation of cerebral microvessels was not explicitly studied, our preparation is certainly suitable for future studies in this direction. This preparation allows for direct measurement of the microvessel diameter by continuously monitoring the arterioles throughout the experimental protocol. Furthermore, our preparation is not limited to superficial cerebral microvessels, but it is particularly well suited for measuring intraparenchymal cerebral microvessels of a size range that has not been accessible for measuring using other techniques.

Fig. 4. A representative microvessel during prostaglandin $F_{2\alpha}$ preconstriction, during subsequent administration of 1% and 2.5% halothane, and after halothane discontinuation (POST + $PGF_{2\alpha}$). The arrow represents a single luminal diameter measurement (in micrometers); actual data consisted of an average of 8–13 measurements every 6–10 μm along the vessel. Normalized percentage vasodilatation was 69% and 140% during 1% and 2.5% halothane, respectively. Diameters returned to 8% after halothane was discontinued.
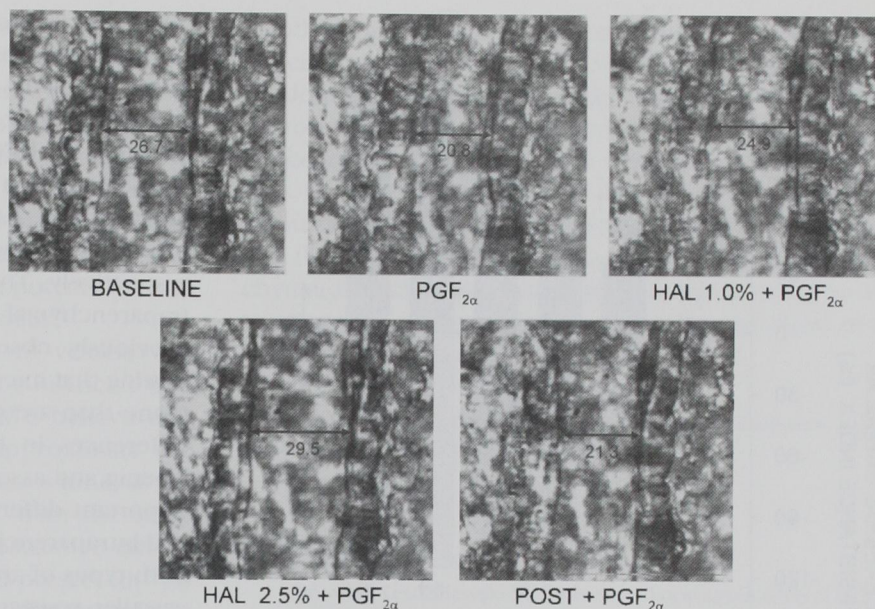
It is well documented that all volatile anesthetics dose dependently increase cerebral blood flow mainly through their direct vasodilator effect on cerebral blood vessels.[1,3,4,25-28] Halothane is believed to be an especially potent cerebral vasodilator with a relatively weak depressant effect on cerebral metabolic oxygen requirements as compared with other volatile anesthetics.[3] Furthermore, the effects of halothane on neocortical *versus* subcortical cerebral blood flow are not homogeneous.[1,27] It was also determined that halothane interfered with autoregulation of cerebral blood flow.[4,28] By the nature of these effects on cerebral blood flow and its implications on intracranial pressure, the use of halothane in neurosurgical procedures has been abandoned. However, halothane has been shown to reduce infarct size and improve postischemic neurological function after transient focal ischemia in the rat.[29] Thus there may yet exist utility in the use of halothane in certain neurosurgical procedures.

The ability of halothane to dilate cerebral vessels was first demonstrated in large- and medium-sized cerebral arteries with diameters greater than 100 μm.[7,8,30,31] However, these blood vessels were isolated and thus devoid of their natural surrounding neural and glial tissue. Furthermore, although large arteries clearly contribute to cerebrovascular resistance,[9,10] intracerebral arterioles appear to play the primary role in local regulation of vascular resistance and blood flow. Compared with the present study in which 1.5% halothane re-

sulted in 108% microvessel dilatation, Flynn *et al.*[30] showed that 2 minimum alveolar concentration of halothane (2.2%) caused larger cerebral arteries to dilate approximately 85% (of preconstriction), suggesting that cerebral microvessels are more responsive than large cerebral arteries to the effects of halothane.

There are several differences in vascular reactivity between cerebral arteries and arterioles, such as in their response to sympathetic and adrenergic stimulation, acetylcholine, and serotonin.[11-13] Isolated cerebral arterioles are generally more responsive to vasodilators and less responsive to certain vasoconstrictors then are isolated cerebral arteries. The potent dilatory effect of halothane on intraparenchymal microvessels is consistent with these segmental differences in cerebrovascular reactivity.

Previously, Lee and colleagues[15,16] and Smith *et al.*[17] assessed the effects of halothane on cerebrocortical blood flow using laser-Doppler flowmetry. In their studies, halothane produced dose-dependent hyperemic responses of approximately 10%, 22%, and 42%, during 1.1%, 1.6%, and 2.2% end-tidal halothane administration, respectively. Assuming that the relative increases in laser-Doppler flow are analogous to relative decreases in total cerebral vascular resistance, these halothane-mediated decreases in cerebrovascular resistance were somewhat smaller than those observed in the present study with similar concentrations of halothane. Using similar methods of calculation, the predicted reductions
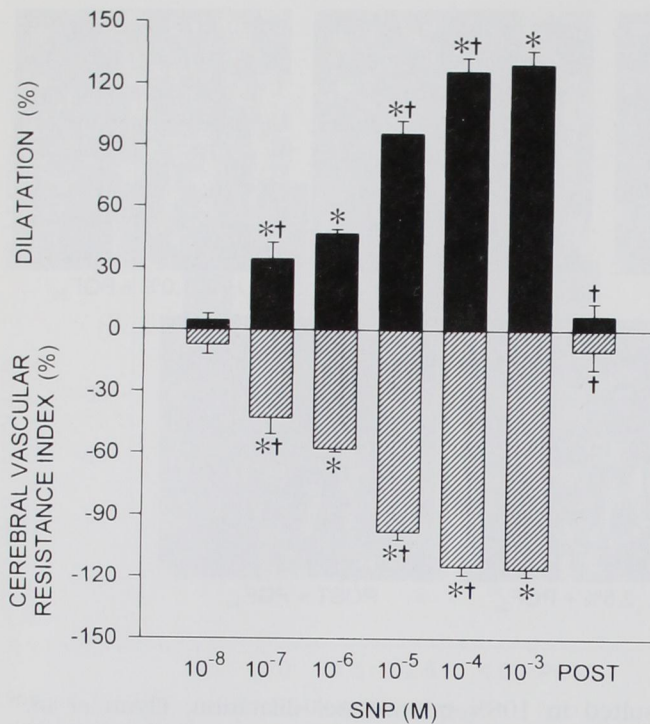
Fig. 5. Percentage vasodilatation and percentage change in the calculated cerebral vascular resistance index for sodium nitroprusside (SNP) concentrations of 1×10^{-8} M, 1×10^{-7} M, 1×10^{-6} M, 1×10^{-5} M, 1×10^{-4} M, and 1×10^{-3} M. The data are normalized to the amount of constriction produced by prostaglandin $F_{2\alpha}$. *$P < 0.05$ *versus* control, †$P < 0.05$ *versus* immediately lower concentrations.

in cerebrovascular resistance in the present experiments are 38%, 47%, and 55% during administration of 1.0%, 1.5%, and 2.5% halothane. Obviously the change in resistance of a single vascular segment may not reflect the change in total cerebral vascular resistance, as the latter is the result of the net effect of alterations in various cerebral vessels, including large conduit arteries, arterioles, veins, and venules. Further reasons for the differences in these findings may be associated with the use of a baseline anesthetic *in vivo* and the possible influence of intravascular pressure and flow, which were absent in our preparation. Nevertheless, this comparison of resistance changes is important because it suggests that the predominant vascular effects of halothane occur at the intraparenchymal microvascular level.

Koenig *et al.*[14] used a closed cranial window with an intravital microscopy technique to assess the effects of halothane on pial microvessels (approximately 40 to 62 µm in diameter). Halothane volatilized in suffused aCSF

produced dose-dependent dilatation of both pial arterioles and venules. These investigators determined that halothane at 1%, 2%, and 3% concentrations produced dose-dependent dilatation of the pial arterioles of approximately 10%, 15–17%, and 18–25% above baseline diameter. When we recalculated our data using similar methods, 1%, 1.5%, 2.5%, and 4% halothane resulted in microvessel vasodilation of 14%, 20%, 25%, and 30%, respectively. Thus halothane-induced alterations of intraparenchymal vessels were slightly greater than the previously observed alterations in pial vessels, suggesting that microvessels may be more affected by halothane than are pial vessels. Clearly there were several differences in the experimental conditions between Koenig and associates' and our study. Nevertheless, an important difference in the regulatory function of pial and intraparenchymal microvessels may exist. Although both types of arterioles contribute to the total cerebrovascular resistance, the pial arterioles have been proposed to act as pressure modulators, conducting and distributing blood from large cerebral arteries to the penetrating arterioles.[32,33] In contrast, the intraparenchymal arterioles appear to play a principal role in regulating local cerebral blood flow to functional units of the cerebral cortex.[33]

In this study, the vasodilatation produced by halothane was dose-dependent up to a measured concentration of 2.5%. Above this concentration of halothane, no further vasodilation appeared to occur. Previous *in vivo* data also showed dose-dependent increases in cerebrocortical flow by halothane.[15–17] However, these experiments could not assess the effects of halothane concentrations of 4% due to the inherent systemic and hemodynamic effects. The lack of further vasodilation in intracerebral arterioles at the highest halothane concentration may occur because cerebral metabolic requirements for oxygen are decreased, thus limiting the metabolic requirement for vasodilatation. But this mechanism is unlikely, because there was a similar limitation to the sodium nitroprusside-induced vasodilatation.

Fergus *et al.*[20] previously showed that the nitric oxide donor, sodium nitroprusside, vasodilates intraparenchymal microvessels. A complete dose-response curve to sodium nitroprusside and the vasodilatory limits of responsiveness in this preparation was not assessed in that study. In the present study, sodium nitroprusside at similar concentrations used by Fergus and associates[20] dilated intraparenchymal vessels to a similar extent. At higher concentrations, sodium nitroprusside continued to significantly dilate microvessels until a maximum dila-

tation of 129%. Temporal arterial infusion of sodium nitroprusside increases cerebral blood flow without peripheral cardiovascular changes.[34] In contrast, when infused intravenously, sodium nitroprusside resulted in marked hypotension without changes in cerebral blood flow. When the systemic hypotension to sodium nitroprusside is prevented by the coadministration of angiotensin, a marked increases in cerebral blood flow is observed, which is consistent with the potent cerebral vasodilator effect of sodium nitroprusside.[34]

In this preparation, preconstriction of vessels was achieved by $PGF_{2\alpha}$, an endogenous vasoconstrictor that provides a baseline, myogenic tone. We believe that the amount of preconstriction may approximate the amount of normal physiologic spontaneous tone, as we have found that after preconstriction with the same concentration of $PGF_{2\alpha}$, the normal vasoactive effects of hypercarbia and hypocarbia are well preserved.[21] In the presence of an alternative potent pharmacologic vasoconstrictor, potassium chloride, halothane also significantly dilates intraparenchymal microvessels.[35] Furthermore, although halothane-induced dilatation can also be elicited in the absence of preconstriction, these changes were small in magnitude.[35]

Smith and colleagues[17] used laser-Doppler flowmetry to delineate the mechanism(s) involved in halothane-induced cerebral hyperemic response and suggested that nitric oxide may play a permissive role in halothane-induced cerebral vasodilatation. Koenig *et al.*[14] also suggested an important role of nitric oxide in mediating vasodilatation of pial arterioles. Although we did not directly assess the role of nitric oxide in these experiments, the amount of intraparenchymal microvessel vasodilatation produced by halothane was similar to the vasodilatation to relatively large doses of the nitric oxide donor sodium nitroprusside. Nitric oxide appears to modulate the resting tone of intraparenchymal microvessels[20] and the microvascular response to norepinephrine.[36] Whether nitric oxide is involved in the response of intraparenchymal microvessels to halothane is unknown. Future studies will examine the role of nitric oxide in halothane-induced vasodilatation of intraparenchymal microvessels.

In conclusion, we present a brain slice preparation in which intracerebral microvessels can be continuously monitored by videomicroscopy to assess their responses to vasoactive agents. The results show that halothane and sodium nitroprusside are both potent and dose-dependent dilators of intraparenchymal microvessels. We have shown that the vasodilatory responses to

halothane are comparable in magnitude to those of high concentrations of the potent vasodilator and nitric oxide donor, sodium nitroprusside. Furthermore, the vasodilatory response to halothane reaches a plateau at a concentration of 2.5%. Of all cerebral vessels studied thus far, the dilator effect of halothane appears to be greatest in intracerebral arterioles. These findings suggest that halothane-induced vasodilatation of intraparenchymal microvessels may contribute significantly to alterations in cerebrovascular resistance and flow produced by halothane.

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