Anesthesiology
82:221–235, 1995

Endothelium-independent Vasoconstricting and Vasodilating Actions of Halothane on Rat Mesenteric Resistance Blood Vessels

W. A. Boyle III, M.D.,* G. M. Maher, B.S.†

Background: **Whether volatile anesthetics produce changes in vascular resistance and blood flow because of direct effects on vascular tissue is unclear. Direct vasoconstricting and vasodilating actions have been demonstrated in isolated conductance arteries *in vitro*, but there is little information regarding direct effects on the small vessels that mediate resistance and flow changes *in vivo*.**

Methods: **We investigated the actions of halothane on 50–200 μM branches of the rat mesenteric artery that were cannulated and studied *in vitro*. The vessels were pressurized to 60 mmHg, and vascular dimensions were continuously monitored using a computer-based real-time image analysis system. The vessel bath was perfused with HCO_3^--buffered saline (37°C) equilibrated with 95% O_2/5% CO_2 (± halothane). The vascular endothelium was mechanically removed before cannulation in some vessels.**

Results: **In unstimulated vessels, halothane had a concentration-dependent vasoconstricting action (EC_{50} = 0.45 mM ≈ 1.5 vol% at 37°C) that was largely transient and was similar to that produced by caffeine. Both halothane and caffeine constrictions were unaffected by bath [Ca^{2+}], nifedipine (1 μM) or Cd^{2+} (100 μM) and were abolished by ryanodine (10 μM). In addition, caffeine responses were attenuated by halothane in a concentration-dependent manner (EC_{50} = 1.6 mM). In vessels preconstricted with KCl (40 mM) or phenylephrine (10^{-6} M), halothane produced transient constriction followed by concentration-dependent vasodilation. Ryanodine, which abolished halothane constrictions, had little effect on the amplitude of KCl- or phenylephrine-induced constrictions or the vasodilating action of halothane. Removal of the endothelium likewise had little effect on the vasoconstricting or the vasodilating actions of halothane in unstimulated, KCl- or phenylephrine-constricted vessels. Halothane completely relaxed KCl and phenylephrine constrictions with EC_{50} values of 0.36 mM (1.2% at 37°C) and 0.75 mM (2.5%), respectively, in intact vessels before ryanodine; 0.25 mM (0.8%) and 0.59 mM (1.9%) in intact vessels after ryanodine; and 0.52 mM (1.7%) and 0.67 mM (2.2%) in endothelium-denuded vessels.**

Conclusions: **Halothane has endothelium-independent vasoconstricting and vasodilating actions in isolated mesenteric resistance blood vessels. The vasoconstricting action appears to involve halothane-induced Ca^{2+} release from caffeine/ryanodine-sensitive intracellular store(s). The vasodilating action in phenylephrine- or KCl-constricted vessels is independent of the Ca^{2+}-releasing action and most likely involves an effect(s) on sarcolemmal-dependent Ca^{2+} signaling (*e.g.*, extracellular Ca^{2+} influx) and/or Ca^{2+} activation of contractile proteins. The magnitude of both the vasoconstricting and the vasodilating actions of halothane in these vessels at clinically relevant concentrations suggests these direct actions contribute to the overall cardiovascular effects of halothane *in vivo*.**
(Key words: Anesthetics: pharmacology. Animal. Caffeine: pharmacology. Endothelium, vascular: drug effects; pharmacology. Halothane: pharmacology. *In vitro*. Mesenteric arteries: drug effects; pharmacology. Muscle, smooth, vascular: drug effects; pharmacology. Phenylephrine: pharmacology. Ryandodine: pharmacology. Vascular resistance. Vasoconstriction. Vasodilation.)

VOLATILE anesthetics produce significant changes in blood pressure and blood flow that may be due to direct and indirect effects on cardiac and vascular tissue.[1–5] Volatile anesthetic-induced depression of baroreflex responses,[6] altered sympathetic neuronal activity,[7,8] and decreases in sympathetic transmitter release[9,10] suggest that central nervous system actions may contribute to the cardiovascular actions of these agents. In addition, changes in parenchymal tissue metabolism and oxygen demand that accompany volatile anesthetic administration indicate that metabolic autoregulation also may play a role in anesthetic-induced alterations of organ blood flow.[11] The cardiovascular actions of volatile anesthetics thus result from the integration of direct effects on cardiac and vascular tissue, effects on

* Assistant Professor of Anesthesiology and of Molecular Biology and Pharmacology.

† Research Technologist, Department of Anesthesiology.

Received from the Departments of Anesthesiology and Molecular Biology and Pharmacology, Washington University School of Medicine, St. Louis, Missouri. Submitted for publication July 6, 1993. Accepted for publication September 14, 1994. Presented at the annual meetings of the American Society of Anesthesiologists, New Orleans, Louisiana, October 17–21, 1992, and Washington, D.C., October 9–13, 1993.

Address correspondence and reprint requests to Dr. Boyle: Department of Anesthesiology, Washington University School of Medicine, Campus Box 8054, 660 South Euclid Avenue, St. Louis, Missouri 63110.

autonomic nervous system activity, and effects on oxygen consumption by parenchymal tissue. The concentration dependence at each of these sites of action and the magnitude of each effect determine their relative importance, which may differ for each anesthetic.

In vitro studies of isolated vessels suggest that direct actions of volatile anesthetics on vascular smooth muscle and vascular endothelium may play an important role in anesthetic-induced changes in arterial tone.[12–27] However, it may not be possible to directly extrapolate actions on the large conductance arterial segments used in prior studies to the smaller resistance sized arteries and arterioles that mediate changes in systemic resistance and blood flow *in vivo*.[28–30] Differences in pharmacology related to vessel size have been described for a variety of vasoactive substances,[31–34] including volatile anesthetics.[24,29,30] Halothane, isoflurane, and enflurane have been shown to produce vasodilation of coronary resistance vessels *in vivo* at clinically relevant concentrations, but these agents have little or no effect on conductance coronary arteries at similar concentrations.[29,30] In addition, significant differences in vasodilatory potency of halothane and isoflurane have been demonstrated *in vitro* among conductance coronary arteries of varying size,[24] further suggesting the potential for important differences in volatile anesthetic action among conductance and resistance arteries. Although direct actions on conductance vessels may be important under certain conditions *in vivo* (*i.e.,* coronary vasospasm), it is clear that resistance vessels mediate the volatile-anesthetic-induced changes in vascular tone *in vivo* and volatile anesthetic pharmacology might differ significantly between conductance and resistance vessels. Because volatile anesthetics actions on resistance vessels *in vivo* also may reflect autonomic nervous system effects or metabolic actions,[34] studies of isolated resistance vessels *in vitro* are important to understand the relative contribution of direct actions on resistance vessels *in vivo*.

We report on the actions of halothane on resistance vessels (50–200 μm outside diameter, OD) studied under physiologic conditions *in vitro*. In resistance arteries isolated from rat mesentery, halothane had a direct vasoconstricting action that appeared to result from intracellular Ca^{2+} release and a direct vasodilating action on vessels preconstricted with either KCl or phenylephrine. Both the vasoconstricting and vasodilating actions of halothane occurred at clinically relevant concentrations, were endothelium-independent, and were of sufficient magnitude to suggest their importance *in vivo*.

Methods

Vessel Isolation

The vessel isolation and cannulation methods were similar to those previously described.[33,35] Mesenteric tissue was removed from halothane-anesthetized Long-Evans rats (200–250 g) and pinned to the sylgard coating in the base of a water-jacketed dissection dish. Proximal arteries were followed distally, and small distal branches (50–200 μm OD) were carefully dissected from surrounding tissue and removed (fig. 1A). The vessels were transferred to a specially constructed vessel bath in which the ends of the vessel were cannulated with fire-polished glass pipettes fabricated from 1.2 mm OD borosilicate glass (tip diameters of 30 μm) and secured to the cannulae with 11-0 suture (fig. 1B).

The perfusate consisted of buffered physiologic saline solution (PSS) of the following composition (mM): NaCl 119; $NaHCO_3$ 24; KCl 4.7; $CaCl_2$ 1.6; KH_2PO_4 1.18; $MgSO_4$ 1.17; [ethylenedinitrilo]tetraacetic acid (EDTA) 0.025; glucose 5.5; and bubbled with 95% O_2/5% CO_2 (*p*H = 7.35).

The perfusion system consisted of a series of glass bath reservoirs with valves to select the reservoir used for perfusion (fig. 1C). The perfusate exited through the bottom of the reservoir and was circulated to the vessel bath by a peristaltic pump. The equilibration gas (95% O_2/5% CO_2) was directed from parallel calibrated flowmeters and control valves to the bubbler for each reservoir. The reservoirs were tightly sealed at the top with a Teflon plug through which passed the equilibration gas inlet line connected to the glass bubbler, a gas outlet line directed to the gas-tight cover over the vessel bath, and a return line for recirculation of buffer from the vessel bath. Temperature was maintained at 37°C throughout the system by water-jacketing around the bath reservoirs and the vessel bath.

The intravascular system was closed, and transmural pressure was maintained with a pressure servo system (fig. 1D). After initially pressurizing the vessel to 30 mmHg and checking for leaks, the vessel bath was transferred to the stage of a Nikon Diaphot inverted microscope, and intravascular pressure was incrementally raised to 60 mmHg. A micrometer attached to one of the cannula was used to gently elongate the vessel to remove any redundancy in the vessel walls, and a

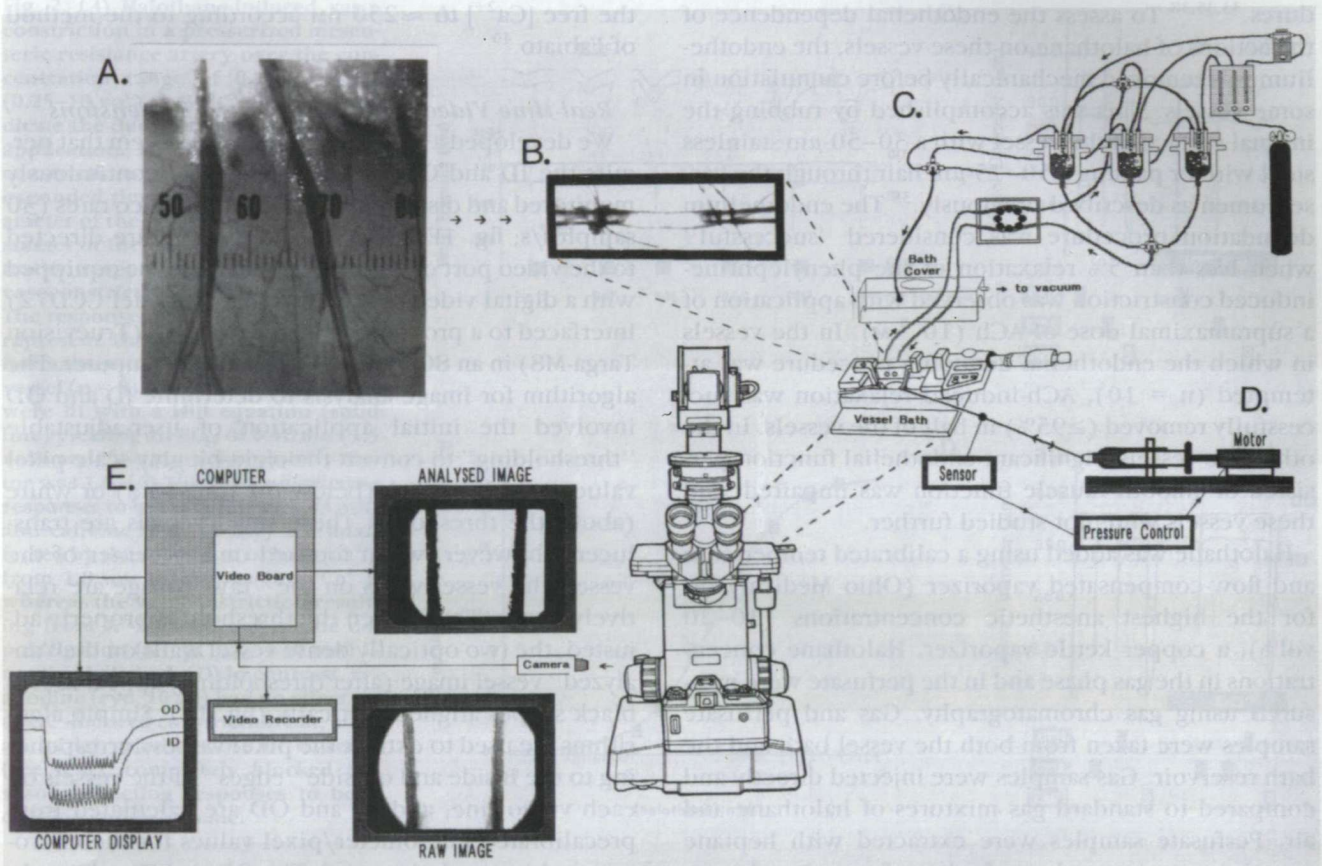

Fig. 1. Methods used for studies of mesenteric resistance blood vessels *in vitro*. (*A*) Vessels measuring 50–200 μm were isolated from terminal branches of the mesenteric vascular tree, shown here as it enters the intestinal wall. The numbered divisions on the micrometer positioned over the vessel represent 1 mm. (*B*) Isolated vessels were cannulated in a specially constructed vessel bath on the stage of an inverted microscope. (*C*) A series of water-jacketed glass bath reservoirs equilibrated with 95% O_2/5% CO_2 (± halothane) were used to perfuse the vessel bath. A series of valves determine which reservoir and equilibration gas were circulated to the vessel bath and bath cover. (*D*) Transmural pressure was maintained at 60 mmHg with a pressure servo system. (*E*) The inside and outside diameter of the vessel were continuously measured and recorded in real-time using a computer-based image analysis system. Thresholding techniques were used to convert raw video images to more basic images for analysis as described in the text. A response to phenylephrine (10^{-6} M), which produced sustained constrictions accompanied by marked oscillations in diameter, is shown in the computer display.

gas-tight cover was placed over the vessel bath to provide an isolated environment through which the equilibration gas (95% O_2/5% CO_2 ± halothane) was circulated.

After a 30-min stabilization, the integrity of the vascular smooth muscle was tested by a brief application of a maximal dose of phenylephrine (10^{-6} M), which produced a sustained (tonic) constriction that was uniform along the length of the vessel. "Healthy" vessels responded to phenylephrine with at least a 50% decrease in inside diameter (ID), and vessels that did not respond in this manner were considered damaged and were not studied further. The integrity of the endothelium was tested by applying a maximal dose of acetylcholine (ACh, 10^{-6} M) to the phenylephrine-containing bath after the phenylephrine constriction had stabilized (≈3–5 min). ACh-induced vasodilation is well known to be endothelium-dependent,[36,37] and complete relaxation of the phenylephrine-induced constriction by ACh was taken as an indication of an intact endothelium. All the vessels that exhibited a healthy response to phenylephrine also met this criterion for an intact endothelium. Endothelial function is characteristically well preserved in this preparation because the endothelial cell surface (*i.e.*, the vascular lumen) is undisturbed during the isolation and cannulation proce-

dures.[33,35,38] To assess the endothelial dependence of the actions of halothane on these vessels, the endothelium was removed mechanically before cannulation in some vessels. This was accomplished by rubbing the intimal surface of the vessel with a 30–50-μm stainless steel wire or passing a 50–75-μm hair through the vessel lumen as described previously.[38] The endothelium denudation procedure was considered "successful" when less than 5% relaxation of the phenylephrine-induced constriction was observed with application of a supramaximal dose of ACh (10^{-5} M). In the vessels in which the endothelial denuding procedure was attempted (n = 10), ACh-induced relaxation was successfully removed (≥95%) in half of the vessels. In the other five vessels, significant endothelial function persisted or smooth muscle function was impaired, and these vessels were not studied further.

Halothane was added using a calibrated temperature and flow-compensated vaporizer (Ohio Medical) or, for the highest anesthetic concentrations (10–20 vol%), a copper kettle vaporizer. Halothane concentrations in the gas phase and in the perfusate were measured using gas chromatography. Gas and perfusate samples were taken from both the vessel bath and the bath reservoir. Gas samples were injected directly and compared to standard gas mixtures of halothane and air. Perfusate samples were extracted with heptane containing an internal standard (isoflurane) and compared to standard mixtures of halothane and isoflurane. In perfusion studies without vessels, halothane equilibrated rapidly (≤2 min) with the perfusate in the bath reservoir; both the gas and perfusate halothane concentrations remained stable throughout the perfusion system. The gas and perfusate halothane concentrations measured during dose-response experiments were all within 10% of the values predicted by the vaporizer setting and the published solubility (*i.e.*, the gas:buffer partition coefficient at 37°C).[39] The halothane concentrations reported here (in mM) represent the mean perfusate concentrations measured at each vaporizer setting (n = 8–14).

Stock solutions for each of the drugs tested were mixed fresh on the day of the experiment and added directly to the volume-calibrated bath reservoirs. The high $[K^+]$ (40 mM) bath (*i.e.*, KCl treatment) consisted of PSS with equimolar substitution of KCl for NaCl. The low $[Ca^{2+}]$ (250 nM) bath consisted of nominally Ca^{2+}-free PSS with the addition of 2 mM ethylene glycol-bis(β-ami-noethylether) N,N,N′,N′-tetraacetic acid and an amount of added Ca^{2+} (1.44 mM) calculated to bring the free $[Ca^{2+}]$ to ≈250 nM according to the method of Fabiato.[40]

Real-time Video Analysis of Vessel Dimensions

We developed a video image analysis system that permits the ID and OD of the vessel to be continuously monitored and displayed in real-time at video rates (30 samples/s; fig. 1E). Images of the vessel are directed to the video port of the inverted microscope equipped with a digital video camera (Dage-MTI model CCD72) interfaced to a programmable video board (Truevision Targa-M8) in an 80386- or 80486-based computer. The algorithm for image analysis to determine ID and OD involved the initial application of user-adjustable "thresholding" to convert the eight-bit gray scale pixel values to either black (below the threshold) or white (above the threshold). These small vessels are translucent; however, when focused on the center of the vessel, the vessel walls on the "raw" image are relatively dense. Thus, when the threshold is properly adjusted, the two optically dense vessel walls on the "analyzed" vessel image (after thresholding) appear as two black stripes aligned vertically (fig. 1E). Simple algorithms are used to extract the pixel values corresponding to the inside and outside "edges" of the vessels on each video line, and ID and OD are calculated from precalibrated micrometer/pixel values for the microscope objective being used. The OD and ID values obtained for each video line are averaged, and these averaged OD and ID values are displayed on the computer video display terminal and recorded on computer hard disk data files in real-time. Direct measurements of vessel diameter from recorded images on video tape indicate that the ID and OD measurements made with this system are accurate.

Data Analysis

Data files were analyzed using computer playback programs in which maximum, minimum, and average OD and ID values were extracted from user-defined intervals. To determine the vasoconstricting responses to halothane and caffeine, the average diameter of the pretreatment interval was compared to the minimum diameter (*i.e.*, most constricted) in the posttreatment interval. The magnitude of the sustained constrictions produced by KCl and phenylephrine was determined by comparing the averaged diameter before treatment to that obtained during treatment. The marked oscillations in diameter observed during treatment with phenylephrine in endothelial-intact vessels (which oc-

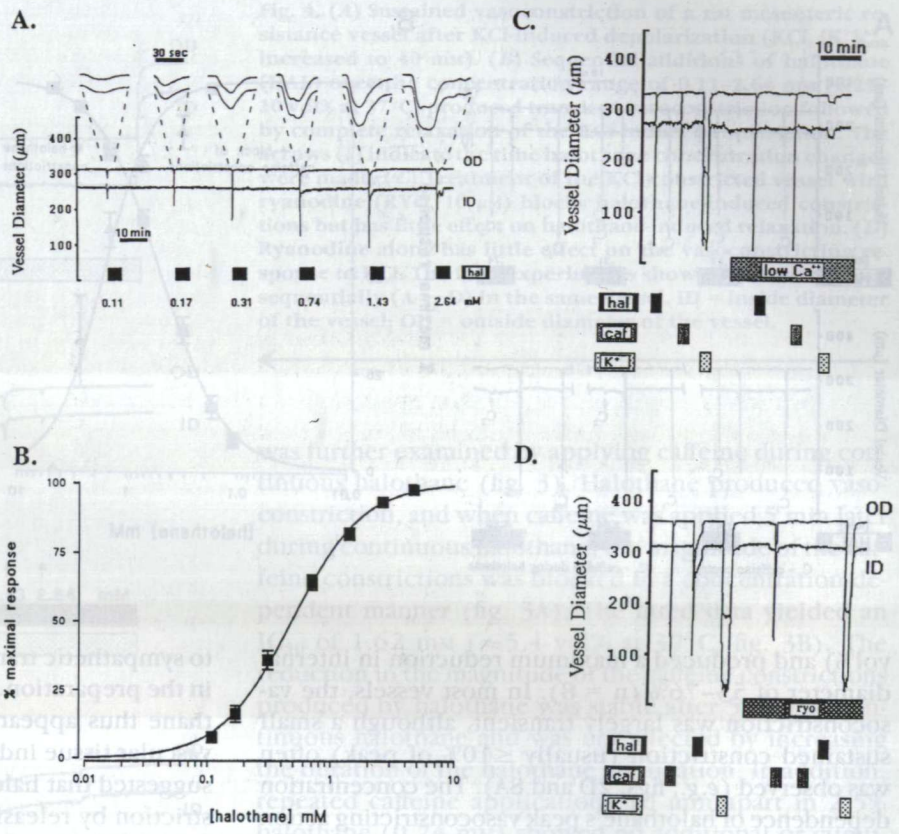

Fig. 2. (*A*) Halothane-induced vasoconstriction in a pressurized mesenteric resistance artery over the concentration range of 0.11–2.64 mM (0.25–10 vol% at 37°C). The bars indicate the duration of the halothane application. The halothane-induced constrictions are shown above on an expanded time scale. ID = inside diameter of the vessel; OD = outside diameter of the vessel. (*B*) The concentration-response relationship for the vasoconstricting effect of halothane. The responses at each concentration represent the peak changes in OD normalized to the maximum in each vessel (n = 8). The mean data (±SEM) were fit with a Hill equation (solid line) yielding an EC_{50} of 0.46 mM (1.5 vol% at 37°C); the cooperativity factor was 1.6. (*C*) The vasoconstricting responses to halothane (hal, 1.43 mM) and caffeine (caf, 25 mM) are unaffected by the reduction in bath $[Ca^{2+}]$ from 1.8 mM to 250 nM (low Ca^{2+}), whereas the vasoconstriction resulting from K^+-induced membrane depolarization (K^+, $[K^+]_{bath}$ = 40 mM) is nearly abolished. (*D*) In contrast, ryanodine (ryo, 10 μM) had no effect on the K^+-induced constriction but, after an initial priming dose of caffeine (see text), completely blocked the vasoconstricting responses to both caffeine and halothane.

curred at a rate of ≈0.2 Hz-*vida infra*) were effectively filtered out by averaging the diameters over several minutes. The vasodilation produced by halothane was determined by comparing the average diameter obtained during the sustained KCl or phenylephrine constrictions to the average diameter after the addition of halothane. OD changes were used for most analyses. ID changes generally gave identical results except that ID was more difficult to estimate during peak constrictions. Halothane vasoconstriction dose-response relationships were determined by normalizing the responses at each dose to the maximal response in each experiment. For the vasodilating dose-response relationships, the maximal response in each vessel was defined as complete (100%) relaxation of the KCl or phenylephrine constriction. The normalized responses from individual experiments at each dose were averaged and fit using Fig P software (Biosoft, Milltown, NJ) with a standard Hill equation where: response (%) = $100/(1 + (1/(([\text{halothane}]/EC_{50})^{-P})))$. The half-maximal halothane concentrations (EC_{50}) and cooperativity values (P) were derived from these fits. STATA software was used for statistical comparisons (Computing Resource Center, Los Angeles, CA). One-way analysis of variance was used for comparing the vasoconstricting responses to halothane, caffeine, and KCl in the normal *versus* low Ca^{2+} bath and before *versus* after ryanodine treatment. Two-way analysis of variance was used to compare the dose-response data for the halothane-induced vasodilation with and without ryanodine and in the presence and absence of an intact endothelium. A P value of ≤0.05 was accepted as indicating a significant treatment effect. The Bonferonni method was used to correct for multiple comparisons in *post hoc* testing where appropriate.

Results

Halothane-induced Vasoconstriction

In pressurized vessels, application of buffer pre-equilibrated with halothane had a marked vasoconstricting action (fig. 2A). The response was concentration-dependent over the range of 0.1–5 mM (0.25–20

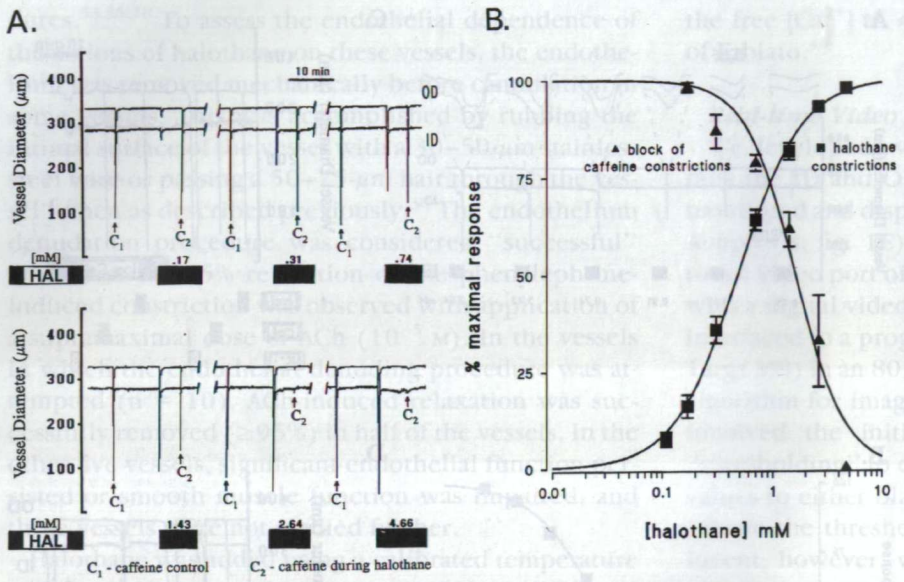

Fig. 3. (*A*) Caffeine (25 mM)-induced vasoconstriction is blocked by halothane (HAL) in a concentration-dependent manner. The vasoconstricting response to caffeine is shown before halothane (C_1) and 5 min after initiating continuous halothane (C_2) at each halothane concentration tested (0.17–4.66 mM). (*B*) The concentration-response relationship for the effect of halothane on caffeine-induced constrictions is shown (▲). The percent maximal response represents the magnitude of the caffeine response during halothane divided by the control response ([C_2 8 C_1] × 100) at each halothane concentration (±SEM, n = 4). The fit to a Hill equation (solid line) yielded an IC_{50} of 1.6 mM; the cooperativity factor was 1.9. For comparison, the concentration-response data for the vasoconstricting effect of halothane are also shown (■).

vol%) and produced a maximum reduction in internal diameter of 53–76% (n = 8). In most vessels, the vasoconstriction was largely transient, although a small sustained constriction (usually ≤10% of peak) often was observed (*e.g.*, figs. 2D and 8A). The concentration dependence of halothane's peak vasoconstricting action is shown in figure 2B. Decreases in OD (±SEM) at each halothane dose were normalized to the maximum response in each vessel, averaged, and fit with a Hill equation (solid line) yielding an EC_{50} of 0.46 mM (1.5 vol% or 1.3 MAC).

Reducing the bath [Ca^{2+}] from 1.8 to 250 nM had no effect on the vasoconstricting action of halothane (fig. 2C) or the transient vasoconstriction produced by caffeine, which releases Ca^{2+} from intracellular stores[41,42] (fig. 2C). In contrast, reducing the bath [Ca^{2+}] significantly attenuated the vasoconstricting response resulting from raising the bath [K^+] ("KCl"), which is due to extracellular Ca^{2+} influx through depolarization-activated sarcolemmal Ca^{2+} channels.[43,44] The peak constricting responses to halothane, caffeine, and KCl were reduced by 3.2 ± 4.5%, 4.0 ± 5.1%, and 84.4 ± 7.2% (mean ± SEM, n = 5), respectively, in the low Ca^{2+} bath ($P < 0.05$). Similar results were obtained when Ca^{2+} influx with blocked with nifedipine (1 μM) or Cd^{2+} (100 μM; data not shown). The vasoconstricting actions of halothane, KCl, and caffeine were not affected by bath additions of the α-adrenergic antagonist, phentolamine (1 μM; n = 3), indicating that the vasoconstricting responses to these agonists were not due to sympathetic transmitter from residual nerve endings in the preparation. The vasoconstricting action of halothane thus appeared to be due to a direct action on vascular tissue independent of Ca^{2+} influx. The results suggested that halothane, like caffeine, produced constriction by releasing Ca^{2+} from intracellular stores.

To further explore the possibility that halothane constrictions resulted from Ca^{2+} release from the same intracellular Ca^{2+} store(s) as caffeine, we examined the effects of ryanodine—a sarcoplasmic reticulum (SR) Ca^{2+} channel agonist that depletes caffeine-sensitive intracellular Ca^{2+} stores[41,42]—on halothane constrictions (fig. 2D). As shown in figure 2D, ryanodine (10 μM) had little effect on the sustained KCl constriction but eliminated the vasoconstricting responses to both caffeine and halothane ($P < 0.05$, n = 4). As ryanodine is an open channel blocker, which depletes the Ca^{2+} store by locking the channel in an open state,[42] caffeine was initially applied to open the channels and thereby deplete the store. Thus, the vasoconstricting response to the initial application of caffeine after ryanodine was largely unaffected (fig. 2D), whereas the responses to subsequent application of either caffeine or halothane were blocked completely. We also tested whether halothane, like caffeine, was able to initiate the ryanodine-induced depletion of the store. As with caffeine, the first response to halothane after ryanodine was not blocked, whereas subsequent responses to halothane or caffeine were blocked completely (not shown).

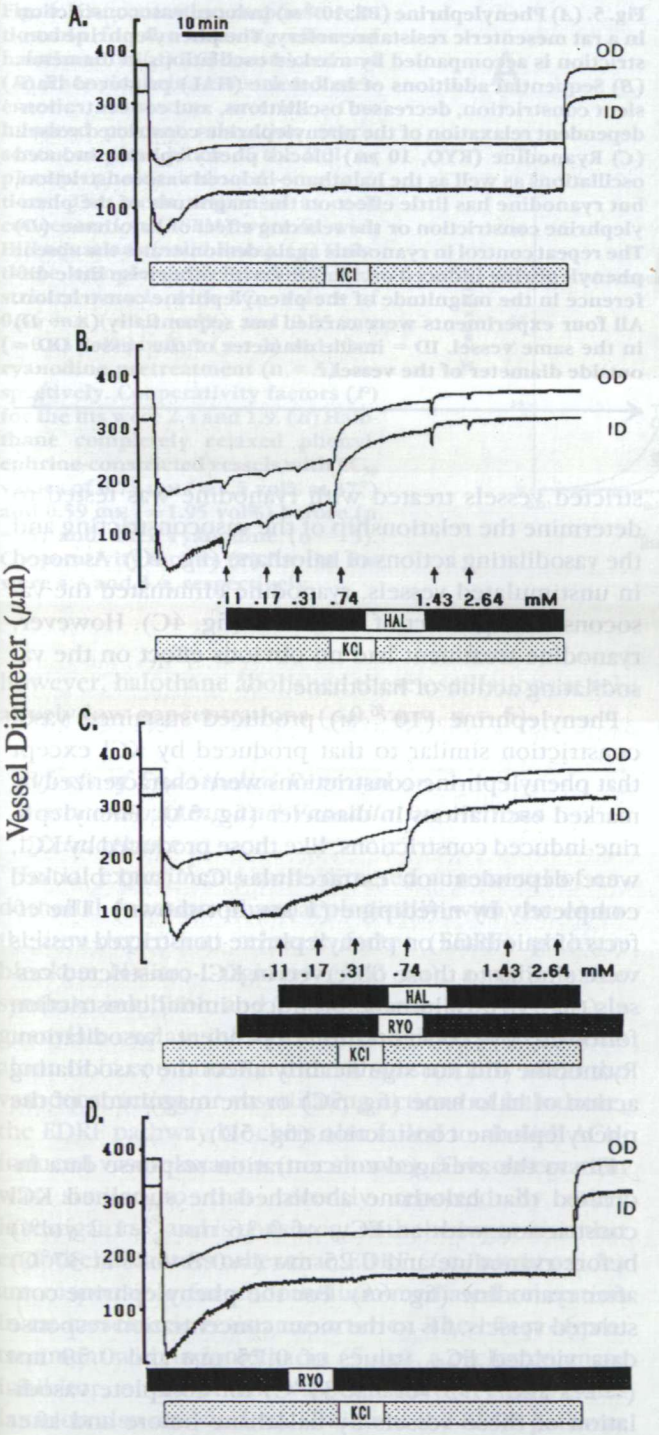

Fig. 4. (*A*) Sustained vasoconstriction of a rat mesenteric resistance vessel after KCl-induced depolarization (KCl, $[K^+]_{bath}$ increased to 40 mM). (*B*) Sequential additions of halothane (HAL) over the concentration range of 0.11–2.64 mM (0.25–10 vol% at 37°C) produced transient vasoconstriction followed by complete relaxation of the KCl-induced constriction. The arrows (↑) indicate the time halothane concentration changes were made. (*C*) Treatment of the KCl-constricted vessel with ryanodine (RYO, 10 μM) blocks halothane-induced constrictions but has little effect on halothane-induced relaxation. (*D*) Ryanodine alone has little effect on the vasoconstricting response to KCl. The four experiments shown were carried out sequentially (A → D) in the same vessel. ID = inside diameter of the vessel; OD = outside diameter of the vessel.

These data indicated that the vasoconstricting effect of halothane was dependent on Ca^{2+} release from the caffeine/ryanodine-sensitive intracellular Ca^{2+} store. The effect of halothane on the integrity of this store was further examined by applying caffeine during continuous halothane (fig. 3). Halothane produced vasoconstriction, and when caffeine was applied 5 min later during continuous halothane, the magnitude of the caffeine constrictions was blocked in a concentration-dependent manner (fig. 3A). The fitted data yielded an IC_{50} of 1.62 mM (≈5.4 vol% at 37°C; fig. 3B). The reduction in the magnitude of the caffeine constrictions produced by halothane was stable after 5 min of continuous halothane and was not affected by increasing the duration of the halothane application. In addition, repeated caffeine applications 10 min apart in 2.5% halothane (0.74 mM) showed no additional or cumulative inhibition (not shown), suggesting that the caffeine-releasable store(s) was able to refill to the same steady-state level during continuous halothane.

Halothane-induced Vasodilation of KCl- and phenylephrine-constricted Vessels

Increasing the bath $[K^+]$ to 40 mM produced sustained vasoconstriction that is well known to be dependent on Ca^{2+} influx through voltage-activated Ca^{2+} channels[43,44]. Consistent with this explanation, KCl constrictions in these vessels were dependent on bath $[Ca^{2+}]$ (fig. 2C) and blocked by the Ca^{2+} channel blockers nifedipine or Cd^{2+} (not shown). KCl-induced constrictions were largely unaffected by ryanodine (fig. 2D), indicating that Ca^{2+} release does not play a major role in the response to KCl. After the sustained KCl constriction had stabilized, halothane (0.1–2.6 mM, ≈0.25–10 vol%) was added sequentially to the high $[K^+]$-containing bath (fig. 4). Halothane produced vasoconstriction in the KCl-constricted vessels, as had been observed in the unstimulated vessels (fig. 2), but this was followed by concentration-dependent vasodilation (fig. 4B). The effect of halothane on KCl-con-

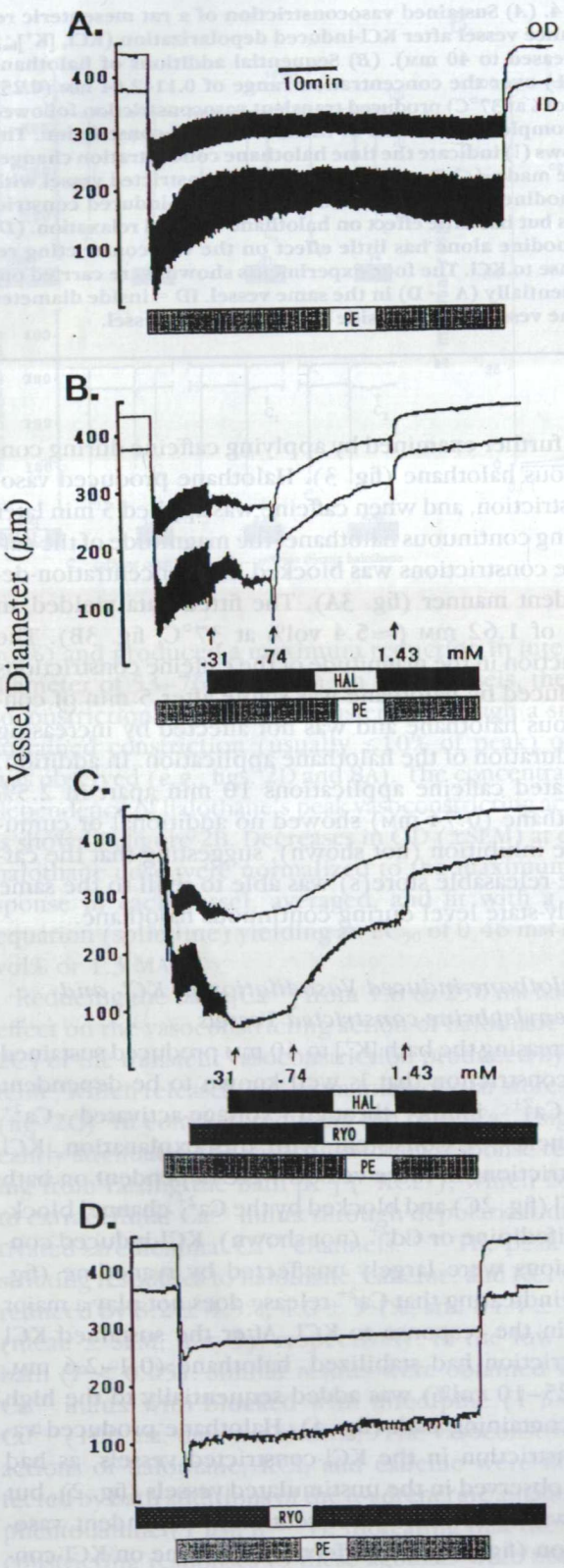

Fig. 5. (*A*) Phenylephrine (PE, 10^{-6} M)-induced vasoconstriction in a rat mesenteric resistance artery. The phenylephrine constriction is accompanied by marked oscillations in diameter. (*B*) Sequential additions of halothane (HAL) produced transient constriction, decreased oscillations, and concentration-dependent relaxation of the phenylephrine-constricted vessel. (*C*) Ryanodine (RYO, 10 μM) blocks phenylephrine-induced oscillations as well as the halothane-induced vasoconstriction, but ryanodine has little effect on the magnitude of the phenylephrine constriction or the relaxing effect of halothane. (*D*) The repeat control in ryanodine again demonstrates the absence phenylephrine-induced oscillations with otherwise little difference in the magnitude of the phenylephrine constriction. All four experiments were carried out sequentially (*A* → *D*) in the same vessel. ID = inside diameter of the vessel; OD = outside diameter of the vessel.

stricted vessels treated with ryanodine was tested to determine the relationship of the vasoconstricting and the vasodilating actions of halothane (fig. 4C). As noted in unstimulated vessels, ryanodine eliminated the vasoconstricting effect of halothane (fig. 4C). However, ryanodine treatment had no obvious effect on the vasodilating action of halothane.

Phenylephrine (10^{-6} M) produced sustained vasoconstriction similar to that produced by KCl except that phenylephrine constrictions were characterized by marked oscillations in diameter (fig. 5A). Phenylephrine-induced constrictions, like those produced by KCl, were dependent on extracellular Ca^{2+} and blocked completely by nifedipine (1 μM; not shown). The effects of halothane on phenylephrine-constricted vessels were similar to those observed in KCl-constricted vessels (fig. 5B). Halothane produced initial constriction followed by concentration-dependent vasodilation. Ryanodine did not significantly affect the vasodilating action of halothane (fig. 5C) or the magnitude of the phenylephrine constriction (fig. 5D).

Fits to the averaged concentration response data indicated that halothane abolished the sustained KCl constriction with an EC_{50} of 0.36 mM (≈1.2 vol%) before ryanodine and 0.25 mM (≈0.8 vol% at 37°C) after ryanodine (fig. 6A). For the phenylephrine-constricted vessels, fits to the mean concentration response data yielded EC_{50} values of 0.75 mM and 0.59 mM (≈2.5 and 1.95 vol% at 37°C) for complete vasodilation of these vessels by halothane before and after ryanodine treatment, respectively (fig. 6B). Halothane also attenuated the phenylephrine-induced oscillations in diameter, which were abolished by ryanodine as shown in figures 5B and 5C. We did not systemically study the concentration dependence of this effect,

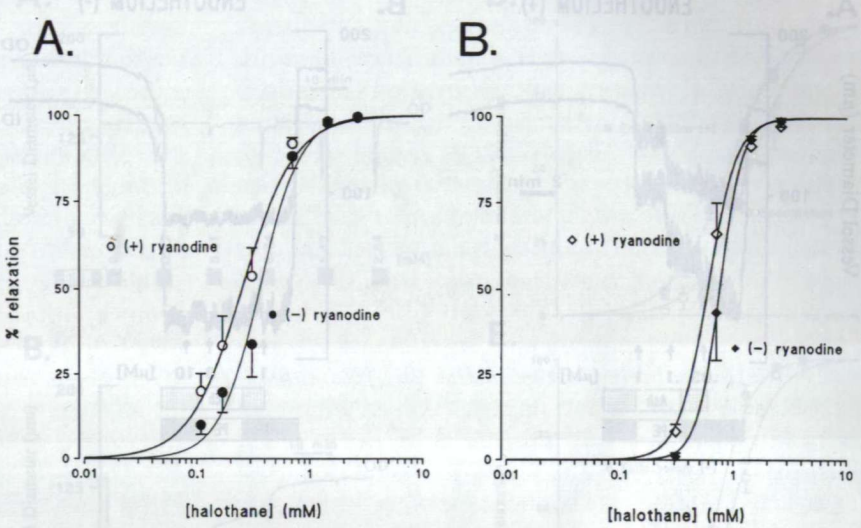

Fig. 6. Concentration-response relationship for the relaxing effect of halothane on (*A*) KCl-constricted and (*B*) Phenylephrine-constricted mesenteric resistance arteries. One hundred percent relaxation represents complete block of the KCl- or phenylephrine-induced constrictions. The mean responses at each concentration (±SEM) were fit with Hill equations (solid lines). (*A*) Halothane completely relaxed KCl-constricted vessels with EC_{50} values of 0.36 mM (≈1.2 vol%) and 0.25 mM (≈0.83 vol%) before (n = 3) and after ryanodine pretreatment (n = 5), respectively. Cooperativity factors (*P*) for the fits were 2.4 and 1.9. (*B*) Halothane completely relaxed phenylephrine-constricted vessels with EC_{50} values of 0.75 mM (≈2.5 vol% at 37°) and 0.59 mM (≈1.95 vol%) before (n = 5) and after ryanodine (n = 5). Cooperativity factors (P) for the fits were 4.3 and 3.4, respectively.

however, halothane abolished these oscillations at relatively low concentrations (≤0.3 mM, n = 5).

Effects of Endothelial Removal on Vasoconstricting and Vasodilating Actions of Halothane

Initial experiments were directed at removal of endothelial function pharmacologically with the endothelium-dependent relaxant factor (EDRF) pathway blockers N-nitro-L-arginine (100 μM), a nitric oxide synthase inhibitor, and/or methylene blue (10 μM), a guanylate cyclase inhibitor. Although these treatments alone or in combination did not substantially affect the vasoconstricting or vasodilating actions of halothane, the EDRF pathway blockers also failed to abolish ACh-induced vasorelaxation (not shown). This observation was similar to that recently reported by other investigators[45] and is consistent with the suggestion that endothelial factors other than EDRF (*i.e.*, nitric oxide) are responsible for endothelium-mediated relaxation in rat mesenteric resistance arteries. The effect of pretreatment with indomethacin (5 μM), a cyclooxygenase inhibitor, on halothane vasoconstriction and vasorelaxation also was studied. Similar to the EDRF pathway inhibitors, indomethacin did not substantially affect the vasoconstricting and vasodilating actions of halothane, nor did it abolish the vasorelaxing effect of ACh (not shown). Because we were therefore unable to pharmacologically remove ACh-induced endothelium-dependent relaxation, which appeared to be multifactorial, we attempted to physically remove the endothelium before cannulation in some vessels to determine whether the vasoconstricting and vasodilating actions of halothane were endothelium-dependent. Physical removal of the endothelium had the added advantage of removing the actions of all endothelial-dependent vasoconstricting and vasodilating factors that might be involved in the actions of halothane.[13,46] Removal of the endothelium was accomplished as described in Methods, and the success of the procedure was based on elimination (≥95%) of ACh-induced relaxation (fig. 7). In addition, the vessels had to display a "healthy" vasoconstricting response to phenylephrine (see Methods). Successful removal of the endothelium, however, did result in attenuation or elimination of the oscillations in diameter typically observed during application of phenylephrine (fig. 7).

As shown in figure 8, removal of the endothelium had little effect on the magnitude of the vasoconstricting and vasodilating actions of halothane. Comparison of the concentration-response data for the vasoconstricting and vasodilating actions of halothane in the endothelial-intact and endothelial-denuded vessels (figs. 8D–8F) suggested that endothelium potentially had some modulating effect on the concentration response relationship for the vasoconstricting action, shifting the EC_{50} to 0.81 mM, compared to 0.48 mM in endothelium-intact vessels (fig. 8D; $P < 0.05$). How-

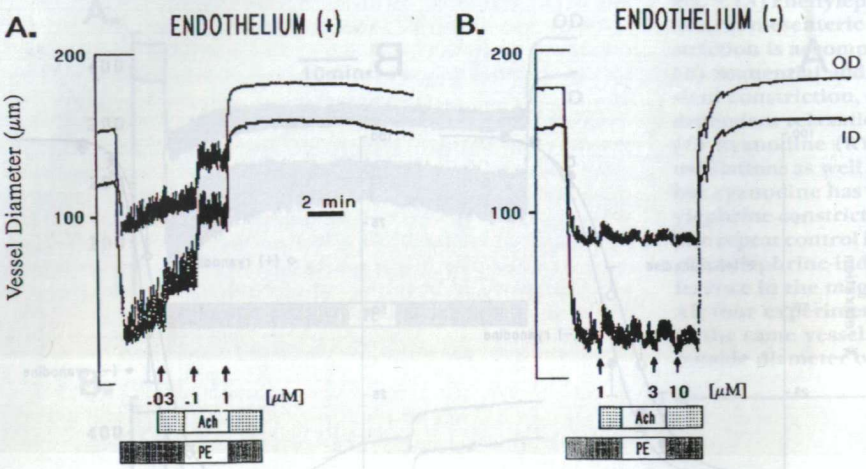

Fig. 7. Phenylephrine (10^{-6} M)-induced sustained vasoconstriction and relaxation by acetylcholine (ACh, 0.03–10 μM) in (*A*) an intact rat mesenteric resistance artery and (*B*) an endothelium-denuded resistance artery. ACh (1 μM) produces complete relaxation of the phenylephrine constriction in the intact vessel, whereas ACh (up to 10 μM) produces little response after removal of the endothelium, which also resulted in attenuation of the phenylephrine-induced oscillations in diameter. ID = inside diameter of the vessel; OD = outside diameter of the vessel.

ever, there were no significant difference between the endothelial-denuded and the intact vessels at any halothane dose. Endothelial removal had no significant effect on the vasodilating action of halothane in KCl- or phenylephrine-constricted vessels at any halothane dose, with EC_{50} values derived from the fits to the mean data of 0.52 and 0.67 mM, respectively, after endothelial removal, compared to 0.36 and 0.75 mM in endothelial-intact vessels (figs. 8E and 8F).

Discussion

In this study, vasoconstricting and vasodilating effects of halothane on intact mesenteric resistance blood vessels are described. The vessels were studied *in vitro,* thereby eliminating the potentially confounding effects of halothane on hemodynamics, organ metabolism, or sympathetic output that may secondarily influence vascular tone *in vivo.*[1–11] In addition, these effects of halothane on isolated vessels were not substantially influenced by physical removal of the endothelium, thereby indicating that they were due to direct actions on vascular smooth muscle. Under conditions of a physiologic temperature and transmural pressure load, the vasoconstricting action of halothane was largely transient and was followed by a vasodilating action in phenylephrine- or KCl-constricted vessels. In contrast to effects previously reported in large conductance arteries, the vasoconstricting and vasodilating actions in resistance vessels occurred at clinically relevant concentrations (*i.e.,* 0.5–2.5 MAC) and were of sufficient magnitude to indicate that they contribute significantly to the cardiovascular effects of halothane *in vivo.*

Because volatile anesthetics, including halothane, generally are viewed as vasodilators,[12–26] the striking vasoconstricting action of halothane—which was clearly evident at clinically relevant (*i.e.,* anesthetic) concentrations ($EC_{50} \approx 1.5$ MAC)—was somewhat unexpected. Muldoon *et al.* previously reported on a constricting effect of halothane in dog femoral artery rings, but that effect was comparatively small and was sustained.[27] Stone and Johns reported on a sustained constricting action of halothane in rat aortic rings, as well as comparable effects of isoflurane and enflurane.[1] However, that effect also was small and, in contrast to the femoral constricting action reported by Muldoon *et al.,* was endothelium-dependent. The constricting action of halothane in that study was only obvious in endothelial intact preparations after treatment with indomethacin. The vasoconstricting action in the mesenteric resistance vessels reported here was largely transient and endothelium-independent and did not depend on indomethacin pretreatment.

The transient vasoconstricting effect of halothane reported by Su and Zhang in KCl-constricted rat aortic rings,[19] although relatively small, was most similar to that which we observed in the mesenteric resistance vessels. In addition, in permeabilized rabbit aortic smooth muscle, they demonstrated that halothane both enhanced caffeine constrictions and potentiated ryanodine-induced block of caffeine responses, suggesting an effect of halothane on Ca^{2+} release.[19] Our data are consistent with those findings and more directly demonstrate that halothane induces transient constrictions due to Ca^{2+} release from caffeine/ryanodine-sensitive Ca^{2+} stores. In the mesenteric vessels, halothane alone

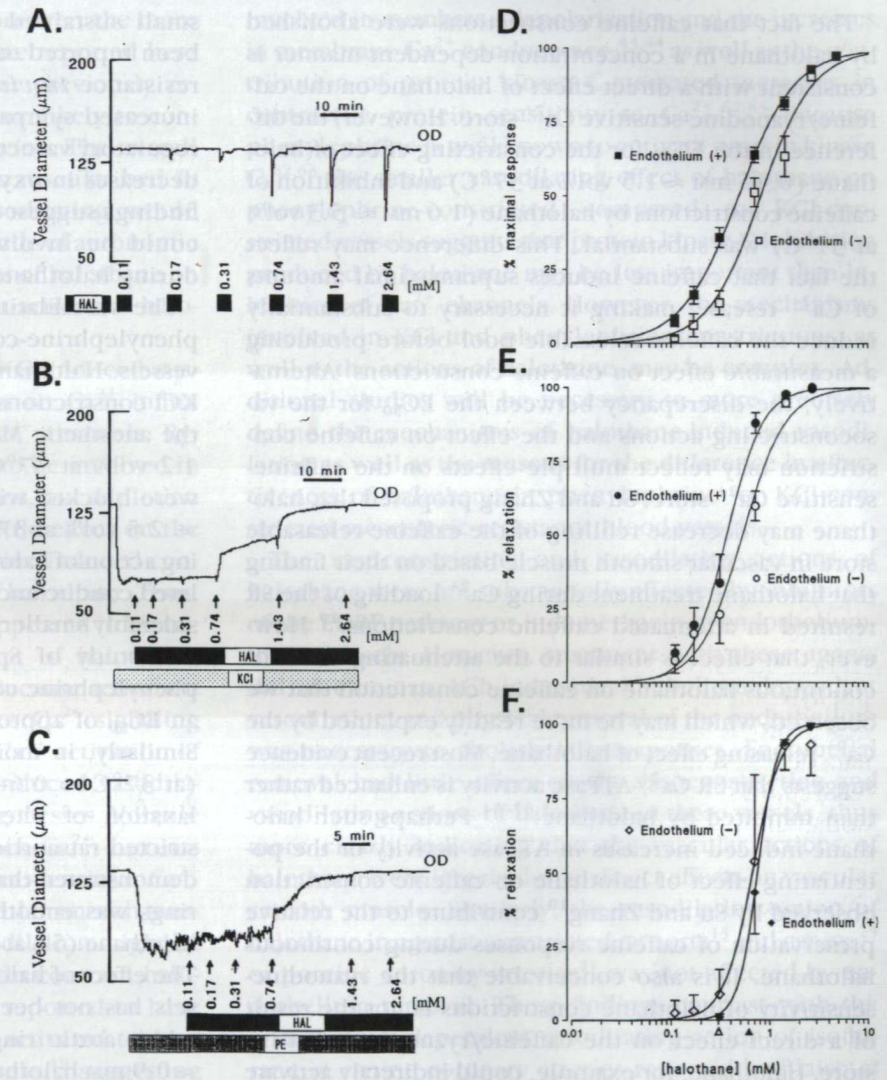

Fig. 8. (*A*) Halothane-induced vasoconstriction and (*B*) relaxation of KCl-constricted and (*C*) phenylephrine-constricted mesenteric resistance arteries after endothelial denudation. The concentration-response relationships for the (*D*) vasoconstricting effect and (*E*) vasorelaxing effect in KCl-constricted vessels and (*F*) phenylephrine-constricted vessels are displayed (±SEM) with fits to Hill equations (solid lines). The concentration-response relationships (*D–F*) observed in the denuded vessels (open symbols) and in the intact vessels (solid symbols) were similar. In denuded and intact vessels, the fits yielded EC_{50} values of (*D*) 0.81 mM (n = 3) and 0.48 mM (n = 8), respectively, for the vasoconstricting effect; (*E*) 0.52 mM (n = 3) and 0.36 mM (n = 3), respectively, for the relaxing effect on KCl-constricted vessels; and (*F*) 0.67 mM (n = 3) and 0.75 mM (n = 5), respectively, for the relaxing effect on phenylephrine-constricted vessels. The open triangles and closed triangles are shown for reference on the x-axis in *F* to denote ≈1 MAC (0.3 mM) and ≈2 MAC (0.6 mM), respectively.

produced constrictions as large as those produced by maximal doses of caffeine, and halothane was as effective as caffeine at accelerating ryanodine-induced depletion of the caffeine-sensitive store. After ryanodine treatment, the constricting responses to both caffeine and halothane were eliminated.

Although we did not directly measure intracellular Ca^{2+} release by halothane, halothane-induced transient increase in $[Ca^{2+}]_i$ has been demonstrated in cardiac muscle,[47] skeletal muscle,[48] and vascular smooth muscle cells.[49,50] In both cardiac and skeletal muscle SR preparations, halothane induced Ca^{2+} efflux that was sensitive to ruthenium red, a specific ryanodine receptor antagonist.[51–53] Thus, the Ca^{2+}-releasing action may be due to a direct effect on ryanodine-sensitive channels. In addition, however, ruthenium red-insensitive Ca^{2+} efflux has been demonstrated in cardiac and skeletal muscle SR,[52–54] and the sensitivity of halothane-induced constrictions or Ca^{2+} release to ruthenium red has not been studied. Although the molecular mechanism(s) involved in the halothane-induced constrictions in mesenteric resistance vessels thus remains unclear, the similarities of the halothane and caffeine responses in these vessels —including the sensitivity of both to ryanodine, the insensitivity of both to extracellular Ca^{2+} influx, and the ability of both to induce ryanodine block—clearly indicate an effect of halothane that involves the caffeine/ryanodine-sensitive Ca^{2+} store that may involve either a direct or an indirect effect(s) on ryanodine-sensitive channels.

The fact that caffeine constrictions were abolished by halothane in a concentration-dependent manner is consistent with a direct effect of halothane on the caffeine/ryanodine-sensitive Ca^{2+} store. However, the difference in the EC_{50} for the constricting effect of halothane (0.46 mM ≈ 1.5 vol% at 37°C) and inhibition of caffeine constrictions by halothane (1.6 mM ≈ 5.3 vol% at 37°C) was substantial. This difference may reflect the fact that caffeine induces supramaximal amounts of Ca^{2+} release, making it necessary to substantially reduce the caffeine-releasable pool before producing a measurable effect on caffeine constrictions. Alternatively, the discrepancy between the EC_{50} for the vasoconstricting actions and the effect on caffeine constriction may reflect multiple effects on the caffeine-sensitive Ca^{2+} store. Su and Zhang proposed that halothane may decrease refilling of the caffeine-releasable store in vascular smooth muscle based on their finding that halothane treatment during Ca^{2+} loading of the SR resulted in attenuated caffeine constrictions.[19] However, that effect is similar to the attenuating effect of continuous halothane on caffeine constriction that we observed, which may be more readily explained by the Ca^{2+}-releasing effect of halothane. Most recent evidence suggests that SR Ca^{2+}-ATPase activity is enhanced rather than inhibited by halothane.[52–54] Perhaps such halothane-induced increases in ATPase activity or the potentiating effect of halothane on caffeine constriction observed by Su and Zhang[19] contribute to the relative preservation of caffeine responses during continuous halothane. It is also conceivable that the ryanodine-sensitivity of halothane constrictions is not the result of a direct effect on the caffeine/ryanodine-sensitive store. Halothane, for example, could indirectly activate the Ca^{2+}-induced Ca^{2+}-release mechanism of the caffeine/ryanodine-sensitive store in vascular smooth muscle.[55] These and other possible mechanism(s) for the vasoconstricting action of halothane should be investigated further in vascular smooth muscle SR preparations.

Although the constricting effect of halothane was largely transient, the magnitude of this effect at clinical concentrations suggests that halothane could produce brief increases in vascular resistance during anesthetic induction or after increases in concentration. After transient constriction, halothane produced sustained vasodilation of KCl- or phenylephrine-constricted vessels, suggesting that the vasodilating action would predominate in stimulated vessels. However, in unstimulated vessels, halothane had a small sustained constricting effect. Halothane has been reported to produce an increase in mesenteric resistance *in vivo*[1–3,5,55] that has been attributed to increased sympathetic tone[56] or to metabolic autoregulatory vasoconstriction due to halothane-induced decreases in oxygen consumption by the gut.[57] Our findings suggest that a direct vasoconstricting action could be involved in mesenteric vasoconstriction during halothane anesthesia.

The vasodilating action of halothane on KCl- and phenylephrine-constrictions was notable in resistance vessels. Halothane completely and reversibly blocked KCl constrictions with an EC_{50} essentially identical to the anesthetic MAC in this species (*i.e.*, 0.36 mM ≈ 1.2 vol% at 37°C), and phenylephrine constrictions were blocked with an EC_{50} ≈ 2 MAC (*i.e.*, 0.75 mM ≈ 2.5 vol% at 37°C). In contrast, the direct vasodilating action of halothane previously demonstrated in isolated conductance arteries from rat[12,13,15,17] were considerably smaller than those reported here. In the original study of Sprague *et al.*, halothane attenuated phenylephrine constrictions in isolated rat aorta with an EC_{50} of approximately 1.2 mM (≈4% at 37°C).[12] Similarly, in more recent studies, 2–2.3% halothane (at 37°C ≈ 0.6–0.75 mM) produced only ≈20% relaxation of phenylephrine- or norepinephrine-constricted rat aortic rings[15] or strips.[17] Stone and Johns demonstrated that the vasodilating action in rat aortic rings was endothelium-independent, with ≈1.5 mM halothane (5% at 37°C) producing ≈30% relaxation.[13] The effect of halothane on KCl constrictions in rat vessels has not been studied, although KCl-constricted rabbit aortic rings were relaxed only ≈20–30% by ≈0.9 mM halothane (3% at 37°C),[19] and Hatano *et al.* found no effect of ≈0.7 mM halothane (2.3% at 37°C) on KCl-constricted canine coronary strips from "small" coronary arteries (890 ± 10 μm), whereas strips from "large" coronary arteries (2,730 ± 80 μm) were relaxed by ≈10%.[24] Although this latter result suggested the possibility that vasorelaxation may depend on vessel size, Bollen *et al.* found no difference in the vasorelaxation produced by halothane in small (0.5–1.0 mm OD) and medium-sized (1.0–1.5 mm OD) porcine coronary artery rings constricted with KCl,[22] and these vessels were more sensitive to halothane, with ≈50% relaxation by 0.6 mM halothane (2% at 37°C). Although the actions of volatile anesthetics on conductance and resistance vessels have not been compared directly, these results suggest that mesenteric resistance vessels may be more sensitive than conductance vessels to the

vasodilating actions of halothane. Moreover, the more potent vasodilating action of halothane reported here in resistance vessels, rather than that previously reported in conductance vessels, is more likely to contribute to decreases in resistance *in vivo*. The overall effect of halothane on the mesenteric vascular bed *in vivo*, whether vasoconstricting or vasodilating, would be expected to depend on the magnitude of autonomic or metabolic influences and the effects of halothane on these influences, as well as the direct actions of halothane reported here.

Both the phenylephrine- and the KCl-induced sustained constrictions were dependent on Ca^{2+} influx through dihydropyridine-sensitive Ca^{2+} channels. Ryanodine-sensitive Ca^{2+} stores appear to be involved in the phenylephrine-induced oscillations, but depletion of these stores with ryanodine had little effect on the magnitude of the KCl- or phenylephrine-induced constrictions or the vasodilating action of halothane. Thus, the vasodilating effect of halothane appeared to be unrelated to the vasoconstricting effect of halothane, which was eliminated by ryanodine. Because activation of the contractile machinery involves Ca^{2+} signaling (*i.e.*, increased $[Ca^{2+}]_i$) followed by Ca^{2+} activation of contractile proteins,[43,44] halothane-induced vasodilation must involve one of these two processes. Volatile anesthetics have been shown to block Ca^{2+} currents through voltage-gated dihydropyridine-sensitive Ca^{2+} channels in cardiac cells[58,59] and vascular smooth muscle cells,[60,61] suggesting that the vasodilation might be due in part to depressed Ca^{2+} influx. In contrast, halothane (3% at 23°C $\approx$ 1.8 mM) was reported to have only a small effect on maximal Ca^{2+}-activated tension in skinned rabbit aortic smooth muscle,[19] suggesting that halothane has little effect on Ca^{2+} activation of contractile proteins. Our experiments do not provide further illumination regarding the likely mechanism(s) of the vasodilating action, although an effect on Ca^{2+} activation of contractile proteins would have been expected to block the constricting responses to cytoplasmic Ca^{2+} ($[Ca^{2+}]_i$) increases without regard to whether the $[Ca^{2+}]_i$ increases were produced by KCl or caffeine. The fact that caffeine constrictions were relatively less sensitive to block by halothane argues in favor of an effect on Ca^{2+} signaling (influx) rather than a primary effect on Ca^{2+} activation of contractile proteins. The reasons for the relative insensitivity of phenylephrine-induced constrictions compared to KCl constrictions is unclear. Phenylephrine and KCl constrictions may differ with respect to the types of channels involved in membrane depolarization and the increases in membrane Ca^{2+} conductance,[43,44] as well as the contribution of protein kinase C-mediated increases in contractile protein sensitivity to Ca^{2+}.[62,63] Because phenylephrine is well known to activate protein kinase C,[62,63] the smaller vasodilating effect of halothane on phenylephrine-constricted compared to KCl-constricted vessels suggests that protein kinase C inhibition produced by halothane may be less important than inhibition of Ca^{2+} channels. However, the mechanisms involved in KCl and phenylephrine constrictions, as well as the actions of halothane, may be complex. Additional studies will be necessary to more precisely define the mechanisms of halothane-induced vasodilation as well as the reasons for the difference in effectiveness of halothane in phenylephrine- or KCl-constricted mesenteric resistance blood vessels.

The vasoconstricting and vasodilating actions of halothane were not substantially affected by inhibitors of the EDRF pathway or indomethacin in endothelium-intact vessels. However, treatment with these agents did not eliminate ACh-induced endothelium-dependent vasodilation, and physical removal of the endothelium was necessary to abolish ACh responses. Endothelial removal had little effect on the vasoconstricting and vasodilating actions of halothane in these vessels, thus more clearly indicating that the vascular actions of halothane were largely due to direct effects on vascular smooth muscle. Similarly, the vasodilating action of halothane in agonist-constricted rat aorta[13] and canine[25] or porcine coronary arteries[21] was not affected by endothelium removal. These findings contrast with the endothelium-dependent vasodilating action of isoflurane in canine coronary arteries reported by Blaise *et al.*[23] However, other studies indicate that the vasodilating action of isoflurane in rat aorta[14] and canine cerebral arteries[64] is endothelium-independent. Halothane has been well documented to have an inhibitory effect on endothelium-dependent relaxation[15,16,27] that may contribute to halothane-induced vasoconstriction in rat aorta,[13] as discussed above. However, our data suggest that the prevailing effects are due to direct effects on smooth muscle in mesenteric resistance vessels. Halothane attenuated or eliminated the phenylephrine-induced oscillations in these vessels at clinically relevant halothane concentrations even below those associated with vasodilation. These oscillations also were eliminated by endothelial removal, which could reflect an endothelium-dependent effect. Halothane has been reported to have a similar attenuating

effect on norepinephrine-induced oscillations in mesenteric veins.[65,66] Those oscillations, like ours, were ryanodine-sensitive, but they were not blocked by nitric oxide synthase inhibitors.[66] This suggests that the oscillations may be unrelated to endothelial nitric oxide production, although they could be related to other non-EDRF but endothelial-dependent process(es). The importance of the endothelial effect(s) of halothane remains uncertain at this time, although volatile anesthetic effects on the endothelium may have an important modulating role under some conditions.[46]

In summary, endothelium-independent vasoconstricting and vasodilating actions of halothane were demonstrated in rat mesenteric resistance blood vessels at clinically relevant concentrations. The magnitude of these actions, in addition to their concentration dependence, suggest that they can contribute significantly to the cardiovascular actions of halothane *in vivo*. The vasoconstricting action was dependent on Ca^{2+} release from the ryanodine-sensitive intracellular Ca^{2+} store. The vasodilating actions on phenylephrine- or KCl-constricted vessels were independent of the effect of halothane on intracellular Ca^{2+} stores and appeared to be the result of either altered Ca^{2+} signaling or depressed Ca^{2+} activation of contractile proteins in vascular smooth muscle.

The authors thank Dr. Alex Evers, Dr. Joe Henry Steinbach, and Dr. Takashi Akata for discussions regarding this work.

References

1. Eger II EI, Smith MT, Stoelting RK, Cullen DJ, Kadis LB, Whitcher CE: Cardiovascular effects of halothane in man. ANESTHESIOLOGY 32: 396–403, 1970

2. Vatner SF, Smith NT: Effects of halothane on left ventricular function and distribution of regional blood flow in dogs and primates. Circ Res 34:155–167, 1974

3. Hickey RF, Eger II EI: Circulatory effects of inhaled anesthetics: The circulation in anesthesia, Applied Physiology and Pharmacology. Edited by Prys-Roberts C. London, Blackwell Scientific, 1980, pp 441–457

4. Merin RG, Bernard J-M, Doursout M-F, Cohen M, Chelly JE: Comparison of the effects of isoflurane and desflurane on cardiovascular dynamics and regional blood flow in the chronically instrumented dog. ANESTHESIOLOGY 74:568–574, 1991

5. Pagel PS, Kampine JP, Schmeling WT, Warltier DC: Comparison of the systemic and coronary hemodynamic actions of desflurane, isoflurane, halothane, and enflurane in the chronically instrumented dog. ANESTHESIOLOGY 74:539–551, 1991

6. Ebert TJ, Kotrly KJ, Vucins EJ, Pattison CZ, Kampine JP: Halothane anesthesia attenuates cardiopulmonary baroreflex control of peripheral resistance in humans. ANESTHESIOLOGY 63:668–674, 1985

7. Seagard JL, Hopp FA, Bosnjak ZJ, Osborn JL, Kampine JP: Sympathetic efferent nerve activity in conscious and isoflurane-anesthetized dogs. ANESTHESIOLOGY 61:266–270, 1984

8. Christ D: Effects of halothane on ganglionic discharges. J Pharmacol Exp Ther 200:336–342, 1977

9. Roizen MF, Thoa NB, Moss J, Kopin IJ: Inhibition by halothane of release of norepinephrine but not of dopamine-beta-hydroxylase from guinea pig vas deferens. Eur J Pharmacol 31:313–318, 1975

10. Rorie DK, Tyce GM, Mackenzie RA: Evidence that halothane inhibits norepinephrine release from sympathetic nerve endings in dog saphenous vein by stimulation of presynaptic inhibitory muscarinic receptors. Anesth Analg 63:1059–1064, 1984

11. Kenny D, Proctor LT, Schmeling WT, Kampine JP, Warltier DC: Isoflurane causes only minimal increases in coronary blood flow independent of oxygen demand. ANESTHESIOLOGY 75:640–649, 1991

12. 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

13. Stone DJ, Johns RA: Endothelium-dependent effects of halothane, enflurane, and isoflurane on isolated rat aortic vascular rings. ANESTHESIOLOGY 71:126–132, 1989

14. Brendel JK, Johns RA: Isoflurane does not vasodilate rat thoracic aortic rings by endotheliun-derived relaxing factor or other cyclic GMP-mediated mechanisms. ANESTHESIOLOGY 77:126–131, 1992

15. Toda H, Nakamura K, Hataon Y, Nishiwada M, Kakuyama M, Mori K: Halothane and isoflurane inhibit endothelium-dependent relaxation elicited by acetylcholine. Anesth Analg 75:198–203, 1992

16. Uggeri MJ, Proctor GJ, Johns RA: Halothane, enflurane, and isoflurane attenuate both receptor and non-receptor-mediated EDRF production in rat thoracic aorta. ANESTHESIOLOGY 76:1012–1017, 1992

17. Hart JL, Jing M, Bina S, Freas W, Van Dyke RA, Muldoon SM: Effects of halothane on EDRF/cGMP-mediated vascular smooth muscle relaxations. ANESTHESIOLOGY 79:323–331, 1993

18. Clark SC, MacCannell KL: Vascular responses to anesthetic agents. Can Anesth Soc J 22:20–33, 1975

19. Su J, Zhang CC: Intracellular mechanisms of halothane's effect on isolated aortic strips of the rabbit. ANESTHESIOLOGY 71:409–417, 1989

20. Bollen BA, Tinker JH, Hermsmeyer K: Halothane relaxes previously constricted isolated porcine coronary artery segments more than isoflurane. ANESTHESIOLOGY 66:748–752, 1987

21. Witzeling TM, Sill JC, Hughes JM, Blaise GA, Nugent M: Isoflurane and halothane attenuate coronary artery constriction evoked by serotonin in isolated porcine vessels and in intact pigs. ANESTHESIOLOGY 73:100–108, 1990

22. Bollen BA, McKlveen RE, Stevenson JA: Halothane relaxes preconstricted small and medium isolated porcine coronary artery segments more than isoflurane. Anesth Analg 75:9–17, 1992

23. 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

24. Hatano Y, Nakamura K, Yakushiji T, Nishiwada M, Mori K: Comparison of the direct effects of halothane and isoflurane on large and small coronary arteries isolated from dogs. ANESTHESIOLOGY 73: 513–517, 1990

25. Blaise GM, Hughes JM, Sill JC, Buluran JN: Attenuation of contraction of isolated canine coronary arteries by enflurane and halothane. Can J Anaesth 38:111–115, 1991

26. Villeneuve E, Blaise G, Sill JC, Guerard MJ, Buluran J, Girard D: Halothane 1.5 MAC, isoflurane 1.5 MAC, and the contractile re-

sponses of coronary arteries obtained from human hearts. Anesth Analg 72:454–461, 1991

27. Muldoon SM, Hart JL, Bowen KA, Freas W: Attenuation of endothelium-mediated vasodilation by halothane. ANESTHESIOLOGY 68: 31–37, 1988

28. Longnecker D, Harris P: Microcirculatory actions of general anesthetics. Fed Proc 39:1580–1583, 1980

29. Sill JC, Bove AA, Nugent M, Blaise GA, Dewey JD, Grabau C: Effects of isoflurane on coronary arteries and coronary arterioles in the intact dog. ANESTHESIOLOGY 66:273–279, 1987

30. Conzen PF, Habazetti H, Vollmar B, Christ M, Baier H, Peter K: Coronary microcirculation during halothane, enflurane, isoflurane, and adenosine in dogs. ANESTHESIOLOGY 76:261–270, 1992

31. Schnaar RL, Sparks HV: Response of large and small coronary arteries to nitroglycerin $NaNO_2$ and adenosine. Am J Physiol 223:223–228, 1972

32. Cohen MV, Kirk ES: Differential response of large and small coronary arteries to nitroglycerin and angiotensin. Circ Res 33:445–453, 1973

33. Nakayama K, Osol G, Halpern W: Differential contractile responses of pressurized porcine coronary resistance-sized and conductance coronary arteries to acetylcholine, histamine and prostaglandin F_2 alpha. Blood Vessels 26:235–245, 1989

34. Mulvany MJ, Aalkjaer C: Structure and function of small arteries. Physiol Rev 70:921–961, 1990

35. Mulvany MJ, Halpern W: Contractile properties of small arterial resistance vessels in spontaneously hypertensive and normotensive rats. Circ Res 41:19–26, 1977

36. Ignarro LJ: Biological actions and properties of endothelium-derived nitric oxide formed and released from artery and vein. Circ Res 65:1–21, 1989

37. Moncada S: From endothelium-dependent relaxation to the l-arginine:NO pathway. Blood Vessels 27:208–217, 1990

38. Osol G, Cipolla M, Knutson S: A new method for mechanically denuding the endothelium of small (50–150 μm) arteries with a human hair. Blood Vessels 26:320–324, 1989

39. Renzi F, Waud BE: Partition coefficients of volatile anesthetics in Krebs' solution. ANESTHESIOLOGY 47:62–63, 1977

40. Fabiato A: Computer programs for calculating total from specified free or free from specified total ionic concentrations in aqueous solutions containing multiple metals and ligands. Methods Enzymol 157:378–417, 1988

41. Wantanabe C, Yamamoto H, Hirano K, Kobayashi S and Kanaide H: Mechanisms of caffeine-induced contraction and relaxation of rat aortic smooth muscle. J Physiol (Lond) 456:193–213, 1992

42. Su JY: Effects of ryanodine on SR Ca^{2+} release channels demonstrated with caffeine-induced tension transients in skinned striated and vascular smooth muscle. Adv Exp Med Biol 311:439–443, 1992

43. Hathaway DR, March K, Lash JA, Adam L, Wilensky RL: Vascular smooth muscle: A review of the molecular basis of contractility. Circulation 83:382–390, 1989

44. Frelin C: Mechanisms of vasoconstriction. Am Heart J 121: 958–960, 1991

45. Garland CJ, McPherson GA: Evidence that nitric oxide does not mediate the hyperpolarization and relaxation to acetylcholine in the rat small mesenteric artery. Br J Pharmacol 105:429–435, 1992

46. Johns RA: Endothelium, anesthetics, and vascular control. ANESTHESIOLOGY 79:1381–1391, 1993

47. Wheeler DM, Rice T, Hansford RG, Lakatta EG: The effect of halothane on the free intracellular calcium concentration of isolated rat heart cells. ANESTHESIOLOGY 69:578–583, 1988

48. Klip A, Hill M, Ramlal T: Halothane increases cytosolic Ca^{2+} and inhibits Na^+/H^+ exchange in L6 muscle cells. J Pharmacol Exp Ther 254:552–559, 1990

49. Sill JC, Uhl C, Eskuri S, Van Dyke R, Tarara J: Halothane inhibits agonist-induced inositol phosphate and Ca^{2+} signaling in A7r5 cultured vascular smooth muscle cells. Mol Pharmacol 40:1006–1013, 1991

50. Iaizzo PA: The effects of halothane and isoflurane on intracellular Ca^{2+} regulation in cultured cells with characteristics of vascular smooth muscle. Cell Calcium 13:513–520, 1992

51. Herland JS, Julian FJ, Stephenson DG: Halothane increases Ca^{2+} efflux via Ca^{2+} channels of sarcoplasmic reticulum in chemically skinned rat myocardium. J Physiol (Lond) 426:1–18, 1990

52. Frazer MJ, Lynch C: Halothane and isoflurane effects on Ca^{2+} fluxes of isolated myocardial sarcoplasmic reticulum. ANESTHESIOLOGY 77:316–323, 1992

53. Louis CF, Zualkernan K, Roghair T, Michelson JR: The effects of volatile anesthetics on calcium regulation by malignant hyperthermia-susceptible sarcoplasmic reticulum. ANESTHESIOLOGY 77:114–125, 1992

54. Blanck TJJ, Peterson CV, Baroody B, Tegazzen V, Lou J: Halothane, enflurane and isoflurane stimulate calcium leakage from rabbit sarcoplasmic reticulum. ANESTHESIOLOGY 76:813–821, 1992

55. Herrmann-Frank A, Darling A, Meissner G: Functional characteristics of the Ca^{2+}-gated Ca^{2+} release channel of vascular smooth muscle sarcoplasmic reticulum. Pflugers Arch 418:353–359, 1991

56. Tverskoy M, Gelman S, Fowler KC, Bradley EL: Intestinal circulation during inhalation anesthesia. ANESTHESIOLOGY 62:462–469, 1985

57. Loick HM, Tokyay R, Abdi S, Traber DL, Nichols RJ, Herndon DL: Halothane markedly reduces mesenteric blood flow but does not impair gut mucosal oxygenation in pigs. Eur J Pharmacol 201:91–96, 1991

58. Terrar DA, Victory JGG: Effects of halothane on membrane currents associated with contraction in single myocytes isolated from guinea pig ventricle. Br J Pharmacol 94:500–508, 1988

59. Bosnjak Z, Supan F, Rusch N: The effects of halothane, enflurane, and isoflurane on calcium current in isolated canine ventricular cells. ANESTHESIOLOGY 74:340–345, 1991

60. Buljubasic N, Flynn NM, Marijic J, Rusch NJ, Kampine JP, Bosnjak ZJ: Effects of isoflurane on K^+ and Ca^{2+} conductance in isolated smooth muscle cells of canine cerebral arteries. Anesth Analg 75: 590–596, 1992

61. Buljubasic N, Rusch NJ, Marijic J, Kampine JP, Bosnjak ZJ: Effects of halothane and isoflurane on calcium and potassium channel currents in canine coronary arterial cells. ANESTHESIOLOGY 76:990–998, 1992

62. Morgan J, Perreault C, Morgan K: The cellular basis of contraction and relaxation in cardiac and vascular smooth muscle. Am Heart J 121:961–968, 1991

63. Wantanabe J, Keitoku M, Hangai K, Karibe A, Takashima T: Alpha-adrenergic augmentation of myogenic responses in rat arterioles: Role of protein kinase C. Am J Physiol 264:H547–H552, 1993

64. Flynn N, Buljubasic N, Bosnjak Z, Kampine J: Isoflurane produces endothelium-independent relaxations in canine middle cerebral arteries. ANESTHESIOLOGY 76:461–467, 1992

65. Marijic J, Madden JA, Kampine JP, Bosnjak ZJ: The effect of halothane and isoflurane on norepinephrine responsiveness in rabbit small mesenteric veins. ANESTHESIOLOGY 73:479–484, 1990

66. Stadnicka A, Flynn NM, Bosnjak ZJ, Kampine JP: Enflurane, halothane, and isoflurane attenuate contractile responses to exogenous and endogenous norepinephrine in isolated small mesenteric veins of the rabbit. ANESTHESIOLOGY 78:326–334, 1993