Anesthesiology
83:574–582, 1995

Lippincott–Raven Publishers

Inhalation Anesthetics Inhibit the Release of Endothelium-derived Hyperpolarizing Factor in the Rabbit Carotid Artery

Volker Lischke, M.D., Rudi Busse, M.D., Ph.D.,† Markus Hecker, Ph.D.‡*

Background: **Inhalation anesthetics may interfere with the synthesis or action of endothelium-derived vasoactive factors. We investigated the effects of desflurane, enflurane, halothane, isoflurane, and sevoflurane on the release of nitric oxide and endothelium-derived hyperpolarizing factor (EDHF) in the isolated endothelium-intact carotid artery of the rabbit.**

Methods: **Isolated segments of the carotid artery were suspended in Krebs-Henseleit solution (37°C) and preconstricted with phenylephrine (1 μM). Relaxations caused by acetylcholine (ACh) (0.03–10 μM) or sodium nitroprusside (0.01–10 μM) were compared in the presence or absence of the nitric oxide synthase inhibitor N^G-nitro-L-arginine (0.1 mM) in segments exposed to desflurane (8%), enflurane (2–4%), halothane (2–3.5%), isoflurane (2–4%), or sevoflurane (2%) as well as in N^G-nitro-L-arginine–treated segments exposed to enflurane (2%) in combination with the K_{Ca}^+-channel blocker tetrabutylammonium (0.3 mM) or the cytochrome P450 inhibitor clotrimazole (3 μM).**

Results: **Desflurane, enflurane, and sevoflurane selectively inhibited the ACh-induced release of EDHF. Halothane and isoflurane also weakly affected the nitric oxide–mediated relaxant response to ACh. The inhibitory effect of these two anesthetics on EDHF release was concentration-dependent. Relaxations induced by sodium nitroprusside were not inhibited by any of the anesthetics tested. Three structurally unrelated cytochrome P450 inhibitors clotrimazole (0.1 mM), metyrapone (1 mM), and SKF525a (proadifen, 0.1 mM) abolished the EDHF-mediated relaxation elicited by ACh. The pharmacologic profile of the inhibitory effect of enflurane on the release of EDHF closely resembled that of clotrimazole but not that of tetrabutylammonium. Moreover, all anesthetics inhibited the cytochrome P450–catalyzed O-dealkylation of 7-ethoxycoumarin by rabbit liver microsomes in a concentration-dependent manner.**

Conclusions: **Inhalation anesthetics significantly attenuate the EDHF-mediated relaxant response to ACh in the rabbit carotid artery. This effect appears to be attributable to inhibition of the cytochrome P450–dependent synthesis of EDHF by the endothelium. (Key words: Anesthetics, volatile: desflurane; enflurane; halothane; isoflurane; sevoflurane. Arteries: carotid. Endothelium, relaxation: acetylcholine; endothelium-derived hyperpolarizing factor; nitric oxide.)**

THE vascular endothelium appears to play a pivotal role in mediating the effects of anesthetics on vascular tone.[1,2] Most studies thus far have centered on the interaction of inhalation anesthetics with the synthesis of nitric oxide (NO) by the endothelium or its effect on vascular smooth muscle.[3–8] Muldoon *et al.*[3] and Stone and Johns[4] first reported that halothane, enflurane and isoflurane attenuate the endothelium-dependent relaxation evoked by acetylcholine or bradykinin in different vascular beds. These findings were later confirmed by Uggeri *et al.*,[5] Toda *et al.*,[6] and Blaise *et al.*[8] There is however an ongoing debate as to whether these anesthetics in addition to their effect on the agonist-induced release of NO from the endothelium also interfere with the NO-mediated activation of the soluble guanylyl cyclase in the smooth muscle.[7,8]

In addition to NO the vascular endothelium is capable of releasing at least two other vasoactive autacoids in response to receptor-dependent stimuli: prostacyclin (PGI_2) and the so-called endothelium-derived hyperpolarizing factor (EDHF). By opening K_{Ca}^+ channels EDHF hyperpolarizes the vascular smooth muscle cells, hence causing relaxation.[9–12] Interestingly, EDHF release seems to account for 40–60% of the endothelium-dependent relaxant response to acetylcholine and bradykinin in different arteries[12–14] and therefore may

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Received from the Center of Physiology and Center of Anesthesiology and Resuscitation, Johann Wolfgang Goethe University Clinic, Frankfurt am Main, Germany. Submitted for publication December 28, 1994. Accepted for publication May 4, 1995. Supported by grants Bu 486/4-3 and He 1587/5-1 from the Deutsche Forschungsgemeinschaft and grant BMH1-CT92-1893 from the Commission of the European Communities. Dr. Hecker is the recipient of a Heisenberg fellowship from the Deutsche Forschungsgemeinschaft.

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Materials and Methods

Carotid Artery Preparation

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Similar to the Ca^{2+}/calmodulin-dependent synthesis of NO by the constitutive NO synthase,[15] the formation of EDHF in endothelial cells is also likely to be a Ca^{2+}-dependent process. Recent experimental evidence suggests that EDHF is a cytochrome P450–derived arachidonic acid metabolite.[13,16,17] As for PGI_2 synthesis,[18] the Ca^{2+}-dependent activation of phospholipase A_2 should thus be rate-limiting for the liberation of arachidonic acid from membrane phospholipids and, as a consequence, also for the cytochrome P450–dependent synthesis of the arachidonic acid epoxide, which is thought to be identical with EDHF.[13,16,17,19,20]

In addition to two preliminary accounts,[21,22] a recent report by Akata *et al.*[23] has addressed the putative action of inhalation anesthetics on the synthesis or release of EDHF in resistance-sized rabbit mesenteric arteries. They found that enflurane, isoflurane, and sevoflurane inhibit the endothelium-dependent relaxant response to acetylcholine mediated by both NO and EDHF as well as the endothelium-independent relaxant response to sodium nitroprusside. We have studied the interaction of inhalation anesthetics with the EDHF-mediated relaxation in a conduit artery, the carotid artery of the rabbit. In addition to examining the effects of desflurane, enflurane, isoflurane, halothane and sevoflurane on the NO-dependent and NO-independent acetylcholine-induced relaxation of preconstricted rabbit carotid artery rings, we have also investigated the putative interaction of these anesthetics with the cytochrome P450–dependent synthesis of EDHF.

Materials and Methods

Carotid Artery Preparation

After institutional approval had been obtained, New Zealand White rabbits of either sex (1.4–3.2 kg body weight) were anesthetized with sodium pentobarbital (60 mg kg^{-1} intravenous). After exsanguination by cutting through the aorta and vena cava, the left and right carotid arteries were removed, cleaned of adventitial adipose and connective tissue, and cut into rings 3–4 mm in width. Four rings were mounted between K30 force transducers (Hugo Sachs Elektronik, March, Germany) and a rigid support for measurement of isometric force. They were incubated in 10-ml organ chambers (made available by Hugo Sachs Elektronik) containing warmed (37°C) oxygenated (95% O_2–5% CO_2) Krebs-Henseleit solution, *p*H 7.4 (millimolar composition: Na^+ 144.0, K^+ 5.9, Cl^- 126.9, Ca^{2+} 1.6, Mg^{2+} 1.2, $H_2PO_4^-$ 1.2, SO_4^{2-} 1.2, HCO_3^- 25.0, and D-glucose 11.1) to which the cyclooxygenase inhibitor diclofenac was added at a concentration of 1 μM. Passive tension was adjusted over a 30-min equilibration period to 2 g, and the Krebs-Henseleit solution exchanged at 10-min intervals. Thereafter the segments were preconstricted with phenylephrine (1 μM) to approximately 2 g tension, and the integrity of the endothelium was tested by applying 1 μM acetylcholine. Segments showing <60% relaxation to acetylcholine were discarded.

Delivery of Anesthetics

Desflurane, enflurane, halothane, isoflurane and sevoflurane were delivered from a calibrated vaporizer (Devapor, Vapor 19.3, Dräger, Lübeck, Germany) to give appropriate concentrations of 2–4% for enflurane, halothane, isoflurane, and sevoflurane or 8–16% for desflurane in the carbogen gas (95% O_2/5% CO_2) aerating the Krebs-Henseleit solution (400 ml/min). Because desflurane and sevoflurane were not available at the beginning of the study, the effects of enflurane, halothane and isoflurane were tested with a different batch of rabbits than those used for desflurane and sevoflurane.

Determination of Anesthetic Concentrations in the Krebs-Henseleit Solution

The concentrations of the anesthetics reflect the clinically relevant concentrations required to induce or maintain adequate anesthesia. The concentration in the carrier gas was monitored by infrared light spectroscopy (Capnomac-Ultima, Datex, Helsinki, Finland, from Hoyer, Bremen, Germany), which was calibrated daily with a standard calibration gas (Hoyer). In separate experiments, the concentration of desflurane, enflurane, halothane, isoflurane and sevoflurane in the Krebs-Henseleit solution was determined by gas chromatography–flame ionization detection (180°C) analysis with a gas chromatograph (series 8500, Perkin-Elmer, Überlingen, Germany) equipped with a HS6 head space injector (maintained at 130°C). The anesthetics were separated at 75–80°C on steel capillary columns (1 m long, 0.32 cm in diameter, filled with 60/80 mesh graphite/0.4% Carb 1500 for halothane, enflurane, isoflurane, and sevoflurane; 1.8 m long, 0.32 cm in diameter, filled with 100/120 mesh graphite/10% SP1000/1% H_3PO_4, for desflurane; Perkin-Elmer) with N_2 (120 kPa, 15 ml/min) as carrier gas. Tetrahydro-

furan and dichloromethane in ethylene glycol were used as internal standards.

After 16 min equilibration with the anesthetics at 37°C in the organ bath, the following Krebs-Henseleit–gas partition coefficients were determined (means ± SD): desflurane 0.242 ± 0.012 (n = 39), enflurane 0.584 ± 0.46 (n = 11), halothane 0.660 ± 0.013 (n = 12), isoflurane 0.356 ± 0.054 (n = 11) and sevoflurane 0.354 ± 0.040 (n = 11). On the basis of these partition coefficients, the final millimolar concentrations of the anesthetics in the organ bath was calculated as shown in table 1.

Table 1. Concentration of the Inhalation Anesthetics in Krebs-Henseleit Solution as Determined by Gas Chromatography

Anesthetic	% (vol/vol)	Concentration (mM)
Desflurane	8	0.714
Enflurane	2	0.431
	4	0.861
Halothane	2	0.488
	3.5	0.852
Isoflurane	2	0.263
	4	0.525
Sevoflurane	2	0.261

Experimental Protocol

Four rings of the same carotid artery were examined simultaneously, one ring was randomly used as a control to exclude any time-dependent changes in agonist sensitivity.

Nitric Oxide–Induced PGI_2-independent Relaxation. After washout of acetylcholine and phenylephrine, the rings were allowed to equilibrate for 20 min and then preconstricted again with phenylephrine (1 μM). When a stable constriction was obtained, the inhalation anesthetics were administered for 15–20 min and a cumulative concentration–response curve to acetylcholine (0.03–10 μM) was established in the presence of the anesthetics followed by a washout period of 30 min.

Nitric Oxide– and PGI_2-independent Relaxation. Thereafter the segments were treated with the NO synthase inhibitor, N^G-nitro-L-arginine (0.1 mM), for 30 min followed by the same experimental protocol as described before.

Sodium Nitroprusside–Induced Relaxation. After another 30-min washout period, the same segments were again constricted with phenylephrine and the effects of the test compounds on the endothelium-independent relaxation induced by sodium nitroprusside (0.01–10 μM) were investigated.

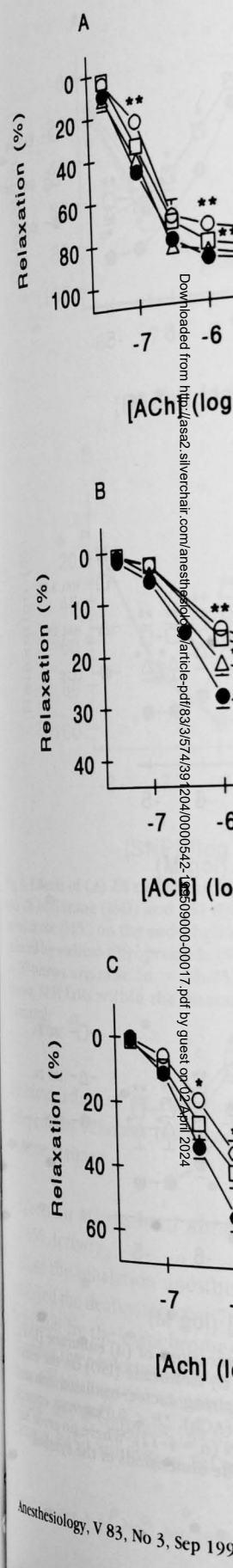
In some experiments, the NO synthase inhibitor was administered at the beginning of the experiment to directly assess the effects of the anesthetics on the NO- and PGI_2-independent relaxant response to acetylcholine. Results from these experiments, however, did not differ from those obtained with the other experimental protocol. In another series of experiments, the relaxant response to acetylcholine was investigated in the presence of the cytochrome P450 inhibitor clotrimazole (3 μM) or the K_{Ca}^+-channel inhibitor tetrabutylammonium (0.3 mM) alone or in combination with enflurane (2%).

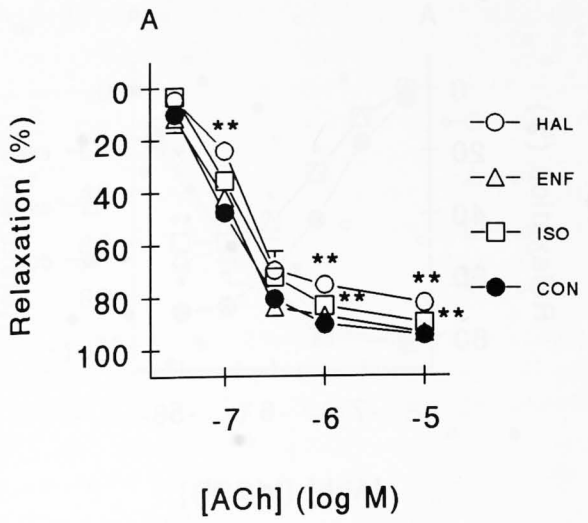
Cytochrome P450 Assay

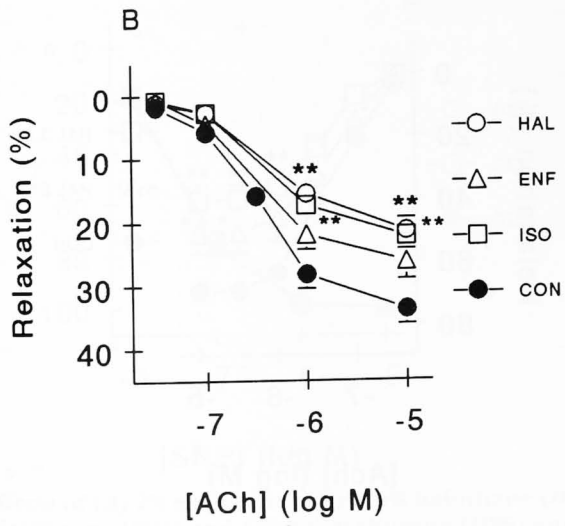
To elucidate the potential cytochrome P450–inhibitory effect of the anesthetics, we used a sensitive spectrofluorometric assay in which the O-dealkylation of 7-ethoxycoumarin to the highly fluorescent 7-hydroxycoumarin (umbelliferon) is monitored over time.[24,25] As a source for cytochrome P450 the microsomal fraction from the liver of noninduced New Zealand White rabbits was prepared essentially as previously described for NO synthase.[26] An aliquot of the microsomal protein (0.5 mg) was stirred in a quartz glass cuvette with 0.1 M tris(hydroxymethyl)aminomethane–HCl buffer (*p*H 7.6), containing 20 μM 7-ethoxycoumarin. After a 2-min equilibration at 37°C, in the presence or absence of the anesthetics, the reaction was initiated by the addition of reduced nicotinamide adenine dinucleotide phosphate (0.1 mM) and monitored over a period of 10 min in a dual wavelength spectrofluorometer (PTI, Wedel, Germany) with the excitation and emission wavelengths set to 370 and 455 nm, respectively. Calibration of the assay was performed by adding known concentrations of umbelliferon (0.1–10 μM) to a cuvette containing heat-denatured microsomal protein.

Citrulline Assay and Soluble Guanylyl Cyclase Assay

The activity of a semipurified rabbit cerebellar NO synthase preparation[27] was determined by monitoring the N^G-nitro-L-arginine-sensitive conversion of ^{3}H-labeled L-arginine to L-citrulline.[26] The activity of purified soluble guanylyl cyclase isolated from bovine lungs was







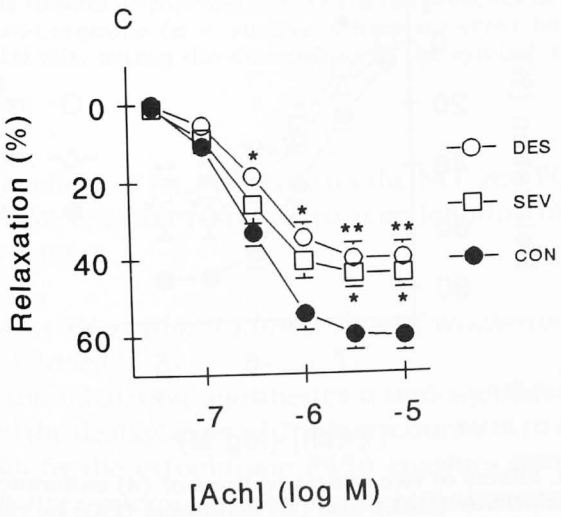


Fig. 1. (*A*) Effects of 2% enflurane (ENF), 2% halothane (HAL), and 2% isoflurane (ISO) on the acetylcholine (ACh)–induced nitric oxide—mediated relaxation of the rabbit carotid artery (*i.e.*, in the absence of N^G-nitro-L-arginine). (*B*) Effects of 2% ENF, 2% HAL, 2% ISO, and (*C*) 8% desflurane (DES) and 2% sevoflurane (SEV) on the ACh-induced endothelium-derived hyperpolarizing factor–mediated relaxation of the rabbit carotid artery (*i.e.*, in the presence of 100 μM N^G-nitro-L-arginine). *$P < 0.05$ *versus* control (CON); **$P < 0.01$ *versus* CON (n = 9–24). Where no error bar is shown, SEM falls within the dimensions of the symbol.

determined by monitoring the conversion of ^{32}P-labeled guanosine 5′-triphosphate to guanosine 3′,5′-phosphate.[26]

Data Analysis

Unless indicated otherwise, all data in the figures and text are expressed as means ± SEM of n experiments with ring segments from different arteries. Statistical evaluation was performed by two-sided Fisher-Pitman analysis between groups followed by a Bonferroni post hoc test for multiple comparisons. A P value of <0.05 was considered statistically significant.

Materials

Desflurane (Suprane) was obtained from Anaquest (Guayama, Puerto Rico); enflurane (Ethrane) and isoflurane (Forane) from Abbott (Wiesbaden, Germany); halothane (Fluothane) from ICI Pharma (Heidelberg, Germany); sevoflurane (Sevofrane) from Maruishi (Osaca, Japan); pentobarbital sodium (Nembutal) from Sanofi (München, Germany); diclofenac (Voltaren) from Ciba-Geigy (Wehr, Germany); N^G-nitro-L-arginine (free acid) from Serva (Heidelberg, Germany); acetylcholine, clotrimazole, metyrapone, phenylephrine, tetrabutylammonium chloride, and sodium nitroprusside from Sigma (Deisenhofen, Germany); and SKF525a (proadifen) from Calbiochem (Bad Soden, Germany).

Results

Acetylcholine-induced Relaxation Mediated by Nitric Oxide

The concentration-dependent relaxant response of the endothelium-intact carotid artery segments to acetylcholine was slightly (16 and 8% inhibition respectively) but significantly attenuated by halothane and isoflurane (fig. 1A), whereas enflurane (fig. 1A), des-

flurane, and sevoflurane had no such effect (data not shown).

Acetylcholine-induced Relaxation Mediated by Endothelium-derived Hyperpolarizing Factor

After inhibition of NO synthesis with N^G-nitro-L-arginine, the maximum relaxant response to acetylcholine was significantly reduced from 94.5 ± 0.2% to 33.8 ± 2.3% and from 98.3 ± 0.7% to 60.5 ± 3.8% in the two control groups (figs. 1B and 1C). In the presence of the inhalation anesthetics, this NO- and PGI_2-independent relaxation was further attenuated (figs. 1B and 1C and figs. 2A–2C). Increasing the concentration of halothane from 2% to 3.5% (fig. 2B) or isoflurane from 2% to 4% (fig. 2C) led to a more pronounced inhibition of the acetylcholine-induced relaxation. In contrast, the inhibitory effect of 4% enflurane was not greater than that of 2% enflurane (fig. 2A). On a molar basis (table 1), isoflurane and sevoflurane appeared to be the most potent inhibitors of EDHF release followed by halothane, enflurane and desflurane.

Endothelium-independent Relaxation

In contrast to the acetylcholine-induced endothelium-dependent relaxation, none of the anesthetics used had a significant effect on the endothelium-independent relaxant response to sodium nitroprusside (figs. 3A and 3B).

Role of Cytochrome P450 and K_{Ca}^+ Channels

The NO- and PGI_2-independent relaxant response to acetylcholine was virtually abolished (fig. 4) in the presence of three different, structurally unrelated cytochrome P450 inhibitors, clotrimazole (0.1 mM), metyrapone (1 mM), and SKF525a (0.1 mM). Moreover, in the presence of the K_{Ca}^+-channel inhibitor, tetrabutylammonium, the EDHF-mediated relaxation was significantly affected at 0.3 mM (fig. 5A) and abolished at 1–3 mM (data not shown).

At 0.3 mM, tetrabutylammonium caused a shift to the right of the concentration–response curve of acetylcholine (50% effective concentration increased from 0.2 to 0.7 μM), but only weakly attenuated its maximum relaxing effect (fig. 5A). Clotrimazole at a concentration of 3 μM, on the other hand, reduced the maximum relaxant response to acetylcholine, but did not cause a shift to the right of the concentration–response curve (50% effective concentration 0.3 μM) (fig. 5A). Both the K_{Ca}^+-channel antagonist and the cytochrome P450 inhibitor (figs. 5B and 5C) significantly enhanced the

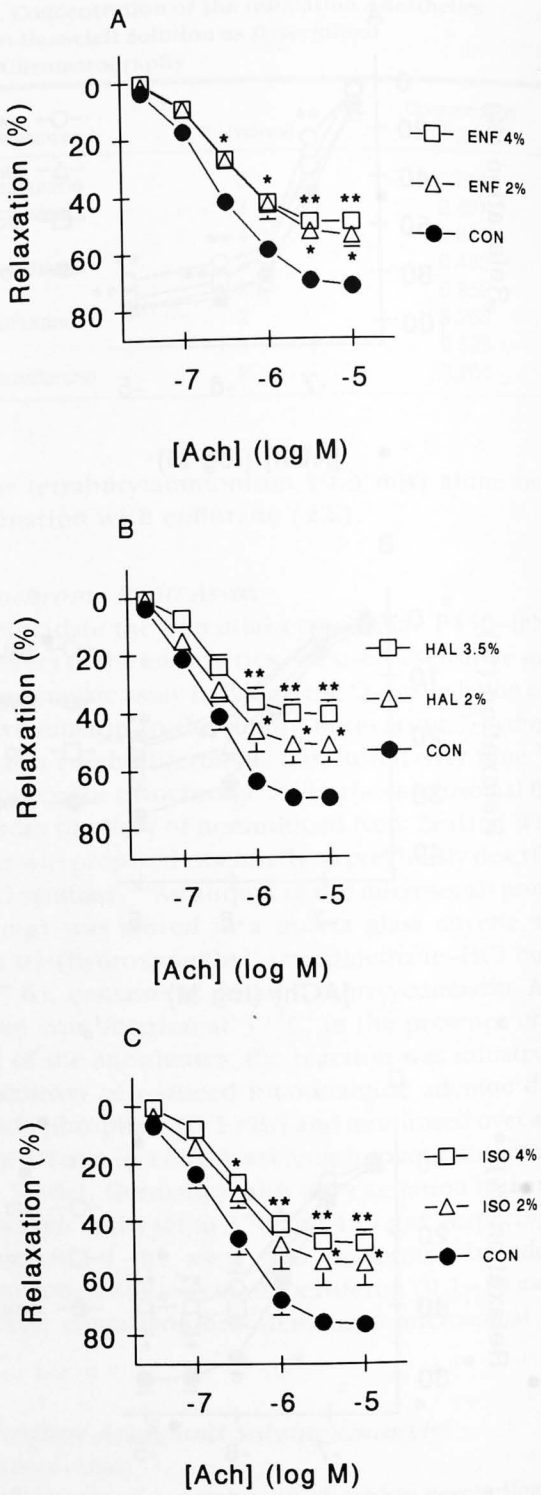


Fig. 2. Effects of two concentrations of (*A*) enflurane (ENF), (*B*) halothane (HAL), and (*C*) isoflurane (ISO) on the endothelium-derived hyperpolarizing factor–mediated relaxant response to acetylcholine (ACh). *$P < 0.05$ *versus* control (CON); **$P < 0.01$ *versus* CON (n = 4–12). Where no error bar is shown, SEM falls within the dimensions of the symbol.

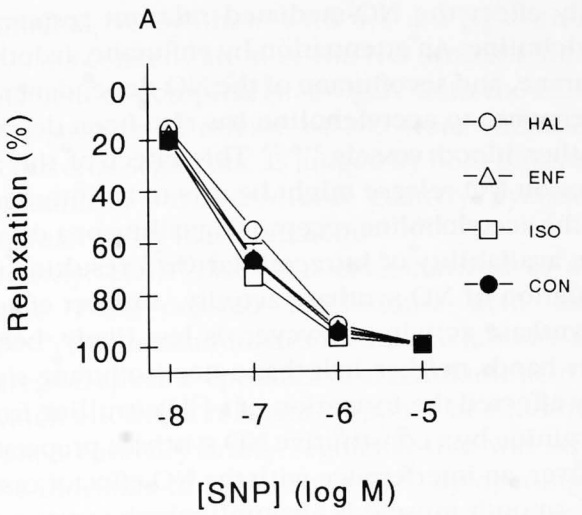


Fig. 3. Effects of (*A*) 2% enflurane (ENF), 2% halothane (HAL), and 2% isoflurane (ISO) and (*B*) 8% desflurane (DES) and 2% sevoflurane (SEV) on the endothelium-independent relaxation induced by sodium nitroprusside (SNP) in the presence of 100 μM N^G-nitro-L-arginine (n = 10–25). Where no error bar is shown, SEM falls within the dimensions of the symbol. CON = control.

inhibitory effect of 2% enflurane on the NO- and PGI_2-independent relaxant response to acetylcholine in an additive manner.

Effects on Microsomal Rabbit Liver Cytochrome P450 Activity

All of the inhalation anesthetics tested significantly inhibited the dealkylation of 7-ethoxycoumarin to umbelliferon by the cytochrome P450 enzymes present in rabbit liver microsomes in a concentration-dependent manner (fig. 6). On a molar basis (table 1), isoflurane and sevoflurane were the most potent cytochrome P450 inhibitors followed by halothane, enflurane and desflurane. Umbelliferon formation by these microsomes was abolished in the presence of metyrapone (93.3 ± 1.4% inhibition at 1 mM, n = 6) or after heating the microsomes to 95°C for 10 min (data not shown).

Effects on Nitric Oxide Synthase and Guanylyl Soluble Cyclase Activity

Neither 2% halothane (10.7 ± 2.3 pmol · mg^{-1} · min^{-1}, n = 4) nor 2% isoflurane (8.1 ± 0.6 pmol · mg^{-1} · min^{-1}, n = 4) significantly affected the activity of a constitutive NO synthase preparation from rabbit cerebellum (12.0 ± 1.2 pmol · mg^{-1} · min^{-1}, n = 4). Moreover, neither 2.5% halothane (9.6 ± 1.7–fold stimulation, n = 3), 2.5% isoflurane (8.9 ± 1.4–fold stimulation, n = 4), or 2.5% enflurane (12.6 ± 1.5–fold stimulation, n = 4) significantly attenuated the activity of a purified soluble guanylyl cyclase preparation from bovine lung stimulated with 10 μM sodium nitroprusside (10.1 ± 1.5–fold stimulation, n = 4).

Discussion

The current findings demonstrate that the inhalation anesthetics tested mainly interfere with the synthesis or action of EDHF. Halothane and isoflurane also mar-

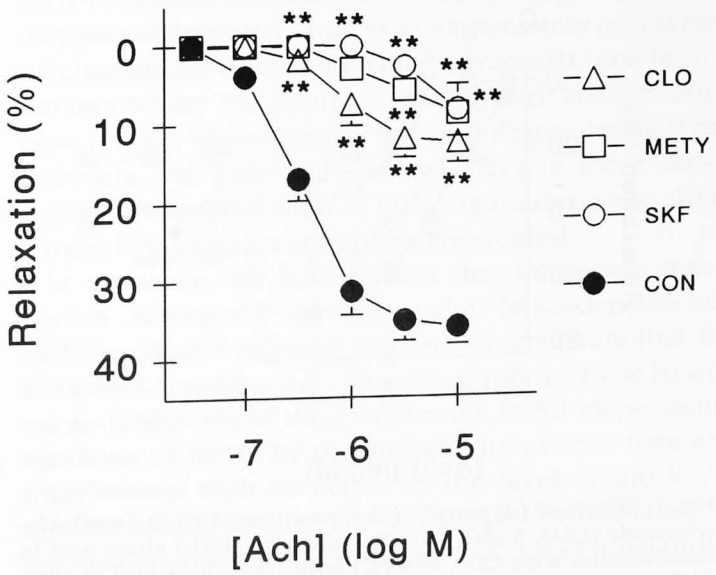


Fig. 4. Effects of clotrimazole (CLO, 0.1 mM), metyrapone (METY, 1 mM), and SKF525a (SKF, 0.1 mM) on the endothelium-derived hyperpolarizing factor–mediated relaxant response to acetylcholine (ACh). $^{**}P < 0.01$ *versus* control (n = 6–12). Where no error bar is shown, SEM falls within the dimensions of the symbol. CON = control.

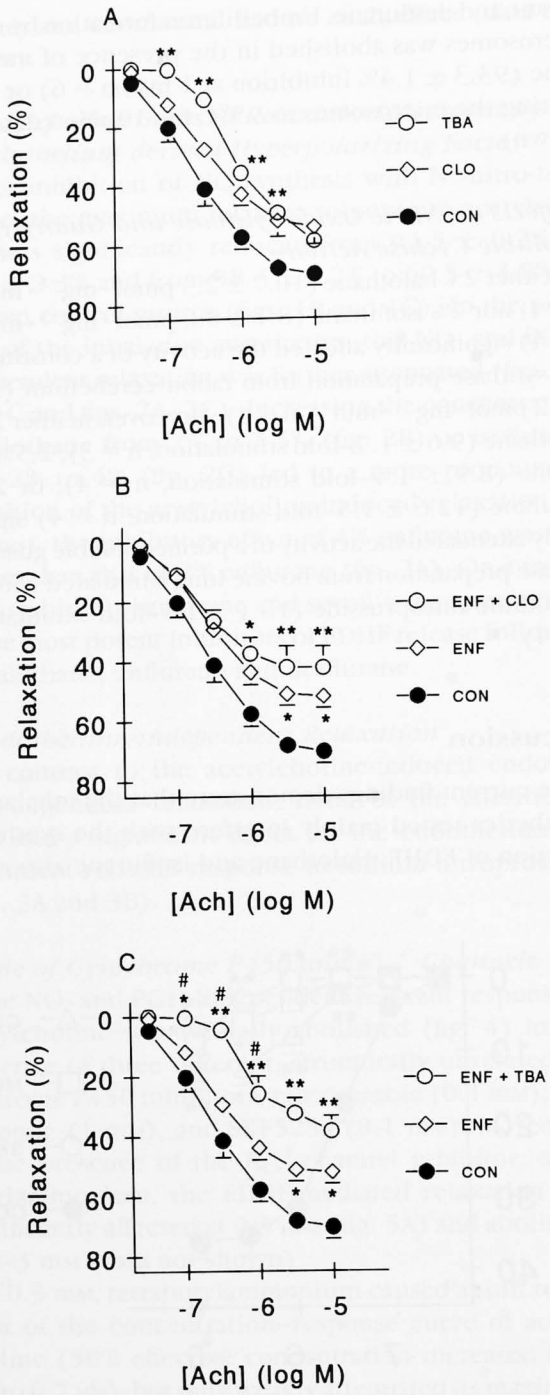


Fig. 5. Effects of (*A*) tetrabutylammonium (TBA, 0.3 mM), clotrimazole (CLO, 3 μM), (*B*) 2% enflurane (ENF) alone and in combination with CLO, and (*C*) enflurane alone and in combination with TBA on the acetylcholine (ACh)–induced endothelium-derived hyperpolarizing factor–mediated relaxation of the rabbit carotid artery. *$P < 0.05$ *versus* control (CON); **$P < 0.01$ *versus* CON; #$P < 0.05$ *versus* 2% ENF (n = 6–12). Where no error bar is shown, SEM falls within the dimensions of the symbol.

ginally affect the NO-mediated relaxant response to acetylcholine. An attenuation by enflurane, halothane, isoflurane, and sevoflurane of the NO-dependent relaxant response to acetylcholine has also been described for other blood vessels.[3–8,23] This effect of the anesthetics on NO release might be due to an interference with the acetylcholine receptor signalling or a decrease in the availability of intracellular Ca^{2+} resulting in an attenuation of NO synthase activity. A direct effect on NO synthase activity, however, is less likely, because in our hands neither halothane nor isoflurane significantly affected the formation of L-[^{3}H]citrulline from L-[^{3}H]arginine by a constitutive NO synthase preparation. Moreover, an interference with the NO effector cascade in the smooth muscle is also unlikely, because no anesthetic had any effect on the endothelium-independent dilator response to sodium nitroprusside. In addition, neither enflurane, halothane, or isoflurane affected the stimulation of a purified soluble guanylyl cyclase preparation by sodium nitroprusside.

In addition to NO, 30–60% of the acetylcholine-induced relaxation in the rabbit carotid artery, similar to findings obtained with other vascular preparations,[12–14] appears to be mediated by the release of EDHF. This EDHF release was unmasked by the blockade of the

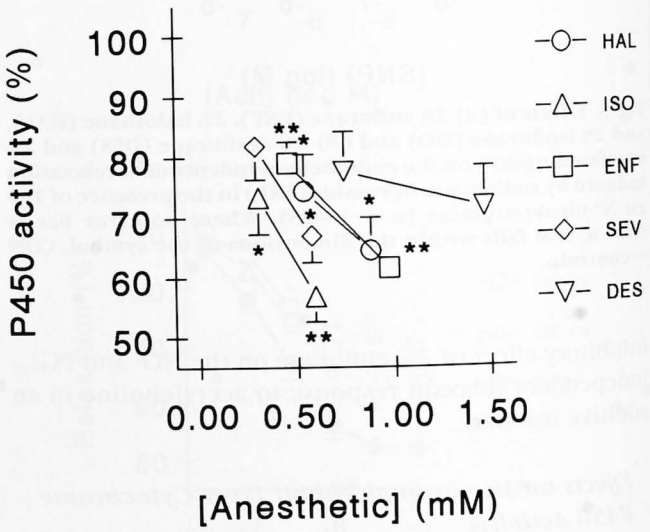


Fig. 6. Effects of desflurane (DES), enflurane (ENF), halothane (HAL), isoflurane (ISO), and sevoflurane (SEV) on the cytochrome P450 activity of rabbit liver microsomes. *$P < 0.05$ *versus* control; **$P < 0.01$ *versus* control (n = 3 or 4). The microsomal cytochrome P450 activity in the absence of the anesthetics (0.434 ± 0.031 nmol · mg^{-1} · min^{-1}) was taken as 100%, and their inhibitory effect expressed as percentage of the control activity. Where no error bar is shown, SEM falls within the dimensions of the symbol.

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endothelial NO synthase with 0.1 mM N^G-nitro-L-arginine. This concentration of the NO synthase inhibitor is sufficient to completely abrogate both the basal and agonist-stimulated release of NO from endothelium-intact arterial segments, as judged by both bioassay and stimulation of purified soluble guanylyl cyclase with the effluate from these segments.[13,28,29]

Recently, EDHF has been characterized as a cytochrome P450–derived arachidonic acid metabolite.[13,16,17] Prime candidates for this metabolite are the four region-specific epoxides of arachidonic acid,[19] one of which elicited a relaxant response in endothelium-denuded coronary artery segments that was sensitive to the blockade of K_{Ca}^+ channels.[13] By opening these K^+ channels, EDHF has been shown to hyperpolarize, hence relax different types of vascular smooth muscle.[9–12] In contrast to K_{Ca}^+-channel inhibitors such as apamin or charybdotoxin, the inhibitor of K^+_{ATP}-dependent channels, glibenclamide has no effect on the EDHF-mediated relaxation.[9,13] There are differences, however, in the type of K_{Ca}^+ channels mediating the effect of EDHF in different vascular beds.[12–14,16,17]

What is the mechanism underlying this partial inhibitory effect of the inhalation anesthetics on the EDHF-mediated relaxation? One possibility is that they interfere with the K_{Ca}^+ channel(s) mediating the effect of EDHF on the smooth muscle. In human red blood cells for example, halothane has been shown to decrease the conductance of K_{Ca}^+ channels with consequent inhibition of membrane hyperpolarization.[30] In rat glioma cells, as well as in rat neurons, enflurane, halothane and isoflurane also attenuated the open probability of charybdotoxin-sensitive K_{Ca}^+ channels at clinically relevant concentrations.[31,32] These effects on K^+-channel conductance may be due to an increase in membrane fluidity caused by the anesthetics or an increase in the lateral membrane pressure,[33,34] which could inhibit the opening or accelerate the closure of these channels.[35]

On the other hand, inhalation anesthetics are known to be metabolized by several cytochrome P450 isoenzymes.[36] It was not clear, however, whether the anesthetics can also inhibit the activity of these enzymes and therefore potentially also the cytochrome P450–dependent synthesis of EDHF. This notion is strongly supported by our finding that all five inhalation anesthetics inhibited the cytochrome P450 activity of rabbit liver microsomes in a concentration-dependent manner. Moreover, the rank order of potency established for this inhibitory effect on a molar basis matched that established for the inhibition by these anesthetics of the EDHF-mediated relaxant response to acetylcholine. Linear regression analysis thus revealed a correlation coefficient of $r = 0.7755$ with a P value of 0.04.

The hypothesis that the inhibition by the inhalation anesthetics of the release of EDHF is based on their cytochrome P450–inhibitory properties is further supported by the characteristics of the inhibitory action of enflurane in the presence of either clotrimazole or tetrabutylammonium. Thus, the pharmacologic profiles of enflurane and clotrimazole were very similar: both compounds attenuated the maximum relaxant response to acetylcholine but did not shift the concentration–response curve to the right. In contrast, tetrabutylammonium caused a rightward shift of the concentration–response curve, but did not significantly affect the maximum response. Moreover, the combination of enflurane with clotrimazole or tetrabutylammonium produced an additive effect. An increase in the concentration of isoflurane or halothane, on the other hand, resulted in an inhibitory effect that closely resembled that of the combination of enflurane and clotrimazole.

Taken together, these findings suggest that the inhalation anesthetics most likely interfere with the synthesis of EDHF by the endothelium rather than with its effect on K_{Ca}^+-channel activity in the smooth muscle. It should be emphasized, however, that on the basis of the current experiments we cannot entirely rule out an additional effect of the inhalation anesthetics on the activity of these channels. Because the release of EDHF may play an important role as a compensatory or reserve mechanism for the maintenance of vascular tone in situations where NO synthesis is reduced, such as atherosclerosis, hypertension, hypercholesterolemia,[14] or ischemia, the potential adverse effect of these anesthetics on the perfusion of vital organs during or after surgical procedures should be considered.

In summary, we have shown that inhalation anesthetics selectively attenuate the NO-independent, EDHF-mediated relaxant response to acetylcholine in the rabbit carotid artery. This effect appears to be based on an inhibition of the cytochrome P450–dependent synthesis of EDHF by the endothelium rather than an interference with its action at the level of the K_{Ca}^+ channels in the vascular smooth muscle. Further studies are needed to verify the inhibitory effects of inhalation anesthetics on EDHF formation in the human vasculature.

The authors thank Dr. Agnieszka T. Bara and Harald Rosenberg for help with the organ bath studies and gas chromatography analysis, respectively, and Gabriele Hoch for expert technical assistance.

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Anesthesiology
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Relation between Intraneural Loca Nerve Block

A Study in the Rat Scia

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Background: During peripheral ner (LA) penetrates within and along th observed functional deficits. Althou the kinetics and steady-state relation pulse activity *in vitro* in isolated ner the relation between functional loss tent *in vivo*. This study was underta lation of functional change to intra

Methods: A sciatic nerve block wa 0.1 ml 1% lidocaine radiolabeled with uptake of LA was determined at diffe and the distribution of lidocaine alo at different stages of functional blo compared with equilibrium lidocai rat sciatic nerve.

Results: Total intraneural lidocain steady-state in about 3 min, stabiliz nmol/mg wet tissue for about 12 zero at 70 min after injection. Altho was 1.6% of the injected dose durin left when deep pain sensation retu detected when functions fully reco

This article is accompanied by a issue of ANESTHESIOLOGY, page 27A.

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Received from the Pain Research Gr Research Laboratories, Harvard Medical Hospital, Boston, Massachusetts. Sub 10, 1995. Accepted for publication M by United States Public Health Servic in preliminary form at the annual me of Regional Anesthesia, Chicago, Illin

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