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Effects of Volatile Anesthetic Agents on In Situ *Vascular Smooth Muscle Transmembrane Potential in Resistance- and Capacitance-regulating Blood Vessels*

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Introduction: **This study was designed to compare the inhibitory effect of inhaled volatile anesthetic agents on *in situ* sympathetic neural *versus* nonneural regulation of vascular smooth muscle transmembrane potentials as correlates of vascular smooth muscle tone in resistance- and capacitance-regulating blood vessels.**

Methods: **Vascular smooth muscle transmembrane potentials were measured *in situ* with glass microelectrodes in neurally intact, small (200–300 m OD) mesenteric arteries and veins of rats before, during, and after inhaled halothane, isoflurane, or sevoflurane (0.5 or 1.0 minimum alveolar concentration [MAC]). Such transmembrane potentials and their anesthetically induced changes were compared, respectively, with those measured in similar vessel preparations after local sympathetic neural denervation with 6-hydroxydopamine.**

Results: **In neurally intact vessels, transmembrane potentials (in millivolts, mean ± SD) before inhalation of the anesthetic agent were −39 ± 2.8 (artery) and −43 ± 4.6 (vein). At 1.0 MAC, halothane, isoflurane, and sevoflurane induced respective hyperpolarizations (in millivolts, mean ± SD) of 9 ± 3.1, 6 ± 2.7, and 9 ± 4.0 in arteries and 6 ± 4.4, 2.8 ± 3.0, and 8.7 ± 5.6 in veins. Sympathetic denervation significantly attenuated these hyperpolarizations (except for venous response to isoflurane). At 0.5 MAC, transmembrane potential responses to all three volatile anesthetic agents were small and not consistently significant in either the intact or denervated vessels.**

Conclusions: **In resistance-regulating arteries *in situ,* inhaled halothane, isoflurane, and sevoflurane (1.0 MAC) attenuate both sympathetic neural and nonneural regulation of vascular smooth muscle transmembrane potentials (and tone). In capacitance-regulating veins *in situ,* sevoflurane (1.0 MAC) also attenuates both regulatory mechanisms, whereas halothane and isoflurane primarily attenuate nonneural mechanisms. At 0.5 MAC, none of these agents significantly affected either mode of regulation of vascular smooth muscle transmembrane potentials in arteries or veins. (Key words: Halothane; isoflurane; peripheral vascular tone; sevoflurane; splanchnic capacitance; splanchnic resistance.)**

VOLATILE anesthetic agents cause peripheral vasodilation and hypotension, part of which is due to a reduction in both arterial and venous vascular smooth muscle (VSM) contractile force (*i.e.,* VSM tone).[1] It is not clear, however, which of the cellular structures and many control mechanisms shown to modulate VSM tone *in vitro* are most affected by volatile anesthetic agents *in vivo.* Long-standing evidence from *in situ* and *in vitro* studies suggests that a primary mechanism for anesthetically induced inhibition of VSM tone is an attenuation of both central and peripheral neural excitatory activity and, in particular, the release of excitatory neurotransmitters from efferent sympathetic nerve terminals.[2-8] With increasing knowledge of the molecular mechanisms involved in the regulation of VSM contractile force, however, more recent *in vitro* studies indicate that volatile anesthetic agents also can attenuate both neurally and nonneurally mediated excitatory control of VSM tone through an action at the level of VSM cell membrane receptors, ion channels, intracellular second messenger systems, intracellular free calcium $[Ca^{2+}]_i$

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and the contractile proteins themselves.[7,9–14] Relative to their action on endothelial regulation of VSM tone, volatile anesthetic agents have been reported to enhance[15,16] and to inhibit[17,18] the synthesis or action of nitric oxide or other vasodilatory agents generated by the vascular endothelium.

Because the physical properties of volatile anesthetic agents readily enable them to cross membrane structures,[19] it is not surprising that some type of anesthetically induced inhibition or facilitation has been demonstrated for nearly every mechanism described here, participating in control of VSM tone. Because most mechanistic studies have used isolated *in vitro* preparations, however, it is not clear which mechanisms participating in control of VSM tone are most significantly affected by inhaled anesthetic agents *in vivo*.

We hypothesize that clinically relevant *in situ* concentrations of volatile anesthetic agents exert an approximately equal inhibitory effect on both sympathetic and nonneural control of VSM tone in resistance- and capacitance-regulating blood vessels and that such inhibition is coupled to VSM hyperpolarization. Therefore, the objective of the current study was to measure VSM transmembrane potentials *in situ* to assess indirectly the relative importance of the inhibitory effects of three inhalational volatile anesthetic agents (halothane, isoflurane, and sevoflurane) on regulation of VSM tone *in vivo* in neurally intact *versus* denervated blood vessel preparations.

Methods

Experimental Preparations

One hundred two male Sprague-Dawley rats (250–300 g body weight) were studied (after approval of the study by the Animal Care Committee at the Medical College of Wisconsin, Milwaukee, WI). The animals were sedated with ketamine (40 mg/kg) given intraperitoneally to facilitate measurement of weight and initial preparation. Subsequently, anesthesia was induced with 20 mg/kg pentobarbital given intraperitoneally followed by a 10-mg/kg intravenous bolus dose at ≈1-h intervals throughout the course of each experimental protocol. Surgical preparation included femoral venous and arterial cannulation for infusion of medication and direct measurement of arterial blood pressure, respectively. In addition, a tracheostomy tube was placed, and ventilation was controlled with a Model 680 rodent respirator (Harvard Apparatus Co., South Natick, MA) to maintain end-tidal CO_2 between 30 and 40 mmHg.

The animal was placed on a movable microscope stage, which was resting on a Micro-g vibration-free table (Technical Mfg. Co., Woburn, MA). A midline laparotomy was performed, and a loop of terminal ileum with its attached mesentery was externalized to expose paired small (200 μm OD) mesenteric arteries and (300 μm OD) veins. These vessels were carefully cleared of perivascular fat without disturbing luminal blood flow or perivascular innervation. Surrounding connective tissue was anchored to the Silastic rubber floor of a temperature-regulated tissue chamber that was part of the animal platform *via* short (75 and 125 μm in diameter) stainless steel pins. The vessel preparation was continuously superfused with physiologic salt solution composed of (in mM): NaCl 119, KCl 4.7, $MgSO_4$ 1.17, $CaCl_2$ 1.6, $NaHCO_3$ 24.0, NaH_2PO_4 1.18, and ethylenediaminetetraacetic acid 0.026. The physiologic salt solution was aerated with a gas mixture of N_2, O_2, and CO_2 to maintain *p*H between 7.35 and 7.45, PCO_2 between 35 and 45 mmHg, and PO_2 between 75 and 140 mmHg.

Measurements of VSM Transmembrane Potential (E_m)

A row of smaller diameter (50 μm) stainless steel pins was placed immediately alongside each artery and vein in the dissected mesentery to separate and stabilize them. This served to minimize arterial pulsations and respiratory movements that prevented acquisition of stable E_m measurements. Single cell *in situ* E_m values were measured by manually advancing glass micropipettes filled with 3 M KCl into VSM cells from the adventitial side of the vessel using a hydraulic micromanipulator (Trent Wells Inc., Coulterville, CA). Micropipettes were pulled from borosilicate glass with a Model P-97 Brown-Flaming micropipette puller (Sutter Instruments Co., Novato, CA). Microelectrode tip impedances ranged between 40 and 60 megohms.

In half of the experiments, sympathetic innervation to the vessel preparation was eliminated at the neuromuscular junction by destruction of norepinephrine-containing vesicles within the presynaptic terminal. This was accomplished by local superfusion with 300 μg/ml 6-hydroxydopamine for 20 min before measurements of E_m followed by a 1-h washout period with physiologic salt solution.[20] Before administration of 6-hydroxydopamine, the vessel preparation was superfused with physiologic salt solution containing 10^{-6} M phentolamine for 5 min to block the effect of catechol-

amines released by the treatment with 6-hydroxydopamine.[21,22]

For each experimental preparation, E_m was measured in the artery and vein together with blood pressure initially just before inhalation of volatile anesthetic agent. Measurements were then repeated after a 15-min inhalational equilibration period with either halothane, isoflurane, or sevoflurane at a minimum alveolar concentration (MAC) of 0.5 or 1.0 and again after a 15- to 30-min washout period during which the concentration of anesthetic agent in blood became negligible. Values at 1 MAC were 1.0% and 1.5% for halothane and isoflurane, respectively,[23] and 2.8% for sevoflurane.[24] A single experiment consisted of sequential arterial and venous measurements of E_m and blood pressure using an individual animal preparation subjected to the three-step protocol (preanesthetic period, anesthetic period, and washout period). Measurements were made using either 0.5 or 1.0 MAC inhaled volatile anesthetic agent in either a neurally intact or locally denervated vessel preparation. Therefore, the total study consisted of 12 experimental groups. A second series of measurements made during each of the two experimental conditions (intact and denervated vessels) served as time controls. The time course of the three-step protocol for measurements of E_m was the same as in the first series of measurements; however, no volatile anesthetic agent was administered during the anesthesia step. These time controls served to evaluate the stability of the experimental preparation during the course of each experimental protocol.

Inhalational Administration of Anesthetic Agent

In all experiments, animals breathed an O_2/N_2 mixture to keep inspired oxygen concentration at 30%. Halothane and sevoflurane were administered *via* selective Draeger vaporizers (North American Draeger Co., Telford, PA). Isoflurane was administered *via* an Ohio Medical Products vaporizer (Airco Inc., Madison, WI). Verification of inhaled concentrations of anesthetic and end-tidal CO_2 was made with a POET II infrared capnograph and end tidal agent monitor (Criticare Systems Inc., Waukesha, WI). In addition, in each experiment concentrations of anesthetic agent in blood were measured from samples taken immediately before and at the end of the washout period using a Sigma Model 38 gas chromatograph (Perkins-Elmer Co., Norwalk, CT).

Statistical Analysis

For the artery and the vein in each experimental vessel/animal preparation, the recorded E_m value during each of the three protocol steps is the numerical average of 5–10 sequential single VSM cell impalements (each 6–10 s long). The typical range of E_m measurements for this sequence of cell impalements in a single vessel was 10 mV during each of the three protocol steps. The mean SE for this sequence in each of the three steps in the protocol (*i.e.,* preanesthetic and postanesthetic periods), calculated using the replicate number of vessel/animal preparations, ranged between 0.7 and 2.0 mV. This SE was not consistently larger during the volatile anesthetic inhalation step in each protocol. Each E_m value recorded in the data tables is the mean ± SD of these numerical average values, with a replication factor ranging from 6–13 for individual protocols. A repeated-measures analysis of variance was performed on the means of the average E_m values measured during each experimental condition using the preanesthetic control and the anesthetic and the postanesthetic periods as the repeat factor (tables 1 and 2). An analysis of variance without repeated measures was used to compare the mean hyperpolarization response of a vessel type (*i.e.,* change in E_m values in table 3) with each anesthetic agent during a specific experimental condition (*e.g.,* anesthetic type and dose, presence or absence of local innervation). One-group *t* tests were used to determine which anesthetically induced hyperpolarization responses were significantly different from zero.

All analyses of variance were calculated using the super ANOVA program (Abacus Concepts, Berkeley, CA). The significance of differences between mean values was determined by comparing calculated least-squares means at a significance level of $P \leq 0.05$. The *t* tests of the change in E_m values were calculated using the Stat-View program (Abacus Concepts).

Results

Results are presented as the mean ± SD unless otherwise indicated.

Mean Transmembrane Potential Values before, during, and after Inhalation of Volatile Anesthetic Agents

Figure 1 illustrates representative examples of individual *in situ* E_m recordings from VSM cells in a small mesenteric artery and vein respectively, before, during, and after inhalation of 1.0 MAC sevoflurane. Similar E_m recordings were obtained with the other two anesthetic agents during the various experimental conditions.

Table 1. Effect of Inhalational Anesthetics on E_m of *In Situ* Mesenteric Arterial VSM

Anesthetic Dose (MAC)	n	Vessel Condition	Preanesthetic	During Anesthetic	Postanesthetic
Halothane					
0.5	8	Innervated	−42 ± 2.0	−46 ± 5.4*	−44 ± 3.9
0.5	8	Denervated	−45 ± 0.8	−46 ± 1.0	−45 ± 2.9
1.0	8	Innervated	−42 ± 1.3	−51 ± 2.0*†	−43 ± 2.5
1.0	7	Denervated	−46 ± 2.1	−51 ± 3.4*†	−45 ± 2.6
Isoflurane					
0.5	6	Innervated	−38 ± 2.7	−39 ± 3.5	−37 ± 3.3
0.5	7	Denervated	−39 ± 2.4	−40 ± 3.8†	−37 ± 1.8
1.0	13	Innervated	−40 ± 1.9	−46 ± 2.0*†	−40 ± 3.0
1.0	8	Denervated	−41 ± 3.2	−44 ± 2.7*	−42 ± 2.5
Sevoflurane					
0.5	6	Innervated	−37 ± 1.3	−39 ± 2.3†	−36 ± 1.7
0.5	8	Denervated	−37 ± 3.0	−39 ± 0.8†	−36 ± 2.4
1.0	9	Innervated	−36 ± 2.2	−45 ± 3.4*†	−36 ± 2.2
1.0	6	Denervated	−38 ± 2.2	−42 ± 4.2*	−42 ± 5.0*

Values are mean E_m + SD (mV) in "n" vessel/animal preparations.

* Significantly hyperpolarized relative to "Pre."

† Significantly different from "Post."

Mean arterial blood pressure MAP (not illustrated) was measured simultaneously with E_m in each animal.

Tables 1 and 2 list mean VSM E_m values in the *in situ* small mesenteric artery and vein, before, during, and after inhalation of 0.5 and 1.0 MAC halothane, isoflurane, or sevoflurane. The combined mean VSM E_m values for all experimental groups before inhalation of any volatile agent were 39 ± 2.8 and 43 ± 4.6 mV for the innervated artery and vein, respectively (n = 50), and 41 ± 4.1 and 44 ± 5.3 mV for the denervated artery and vein, respectively (n = 44). In the artery (table 1), mean VSM E_m values were significantly more negative

Table 2. Effect of Inhalational Anesthetics on E_m of *In Situ* Mesenteric Venous VSM

Anesthetic Dose (MAC)	n	Vessel Condition	Preanesthetic	During Anesthetic	Postanesthetic
Halothane					
0.5	8	Innervated	−47 ± 3.3	−51 ± 3.9*	−49 ± 3.3
0.5	8	Denervated	−49 ± 1.5	−50 ± 2.5†	−49 ± 1.5
1.0	8	Innervated	−46 ± 1.8	−52 ± 3.3*†	−45 ± 3.9
1.0	7	Denervated	−51 ± 2.5	−54 ± 2.9*†	−49 ± 3.1
Isoflurane					
0.5	6	Innervated	−41 ± 2.1	−42 ± 2.6†	−40 ± 3.3
0.5	7	Denervated	−39 ± 3.8	−41 ± 4.7	−39 ± 3.2
1.0	13	Innervated	−45 ± 4.4	−48 ± 3.5*†	−43 ± 4.1
1.0	8	Denervated	−42 ± 4.1	−44 ± 3.4†	−42 ± 3.8
Sevoflurane					
0.5	6	Innervated	−39 ± 3.5	−42 ± 2.5*†	−35 ± 0.9
0.5	8	Denervated	−39 ± 3.0	−41 ± 2.4†	−38 ± 2.1
1.0	9	Innervated	−40 ± 3.8	−48 ± 2.4*†	−40 ± 5.8
1.0	6	Denervated	−43 ± 3.9	−44 ± 4.8†	−41 ± 3.2

Values are mean E_m ± SD (mV) in "n" vessel/animal preparations.

* Significantly hyperpolarized relative to "Pre."

† Significantly different from "Post."

Table 3. Hyperpolarization Responses of *In Situ* Small Mesenteric Artery and Vein VSM by Inhalational Anesthetics

Anesthetic Dose (MAC)	n	Vessel Condition	Hyperpolarization	
			Artery	Vein
Halothane				
0.5	8	Innervated	3.2 ± 5.2	4.2 ± 1.8*†
0.5	8	Denervated	0.9 ± 1.1	1.0 ± 2.6
1.0	7	Innervated	9.0 ± 3.1*†	6.4 ± 4.4*
1.0	7	Denervated	4.9 ± 3.0*	3.3 ± 1.8*
Isoflurane				
0.5	6	Innervated	1.2 ± 1.9	1.7 ± 1.8
0.5	7	Denervated	0.9 ± 1.8	1.9 ± 1.8*
1.0	13	Innervated	5.5 ± 2.7*†‡	2.8 ± 3.0*‡
1.0	8	Denervated	2.8 ± 2.4*	1.8 ± 1.9*
Sevoflurane				
0.5	6	Innervated	2.3 ± 2.6	3.3 ± 4.0
0.5	8	Denervated	2.2 ± 2.8	1.8 ± 1.9*
1.0	9	Innervated	9.1 ± 4.0*†	8.7 ± 5.6*†
1.0	6	Denervated	4.3 ± 3.3*	1.5 ± 3.7

Values are mean ΔE_m ± SD (mV) anesthetic-induced hyperpolarization in "n" vessel/animal preparations.

* Hyperpolarization significantly greater than zero.

† Hyperpolarization in innervated vessel significantly greater than in respective denervated vessel.

‡ 1.0 MAC isoflurane-induced hyperpolarization significantly less than respective halothane or sevoflurane-induced hyperpolarization.

relative to preanesthetic levels during inhalation of 1.0 MAC (but not 0.5 MAC) of each of the three anesthetic agents, indicating hyperpolarization of VSM. This was true for both the innervated and locally denervated small mesenteric artery. After washout of the 1.0 MAC volatile anesthetic agent, the mean VSM E_m values were not significantly different from preanesthetic levels (except in the 1.0 MAC sevoflurane denervated artery protocol). In the innervated vein (table 2), mean VSM E_m values also were more negative relative to preanesthetic levels during inhalation of 1.0 MAC of each of the three anesthetic agents and during inhalation of 0.5 MAC of halothane and sevoflurane. In the denervated vein, mean VSM E_m values were significantly more negative relative to preanesthetic levels only during inhalation of 1.0 MAC halothane. Similar to the small artery, postanesthetic venous VSM E_m values were not significantly different from preanesthetic levels.

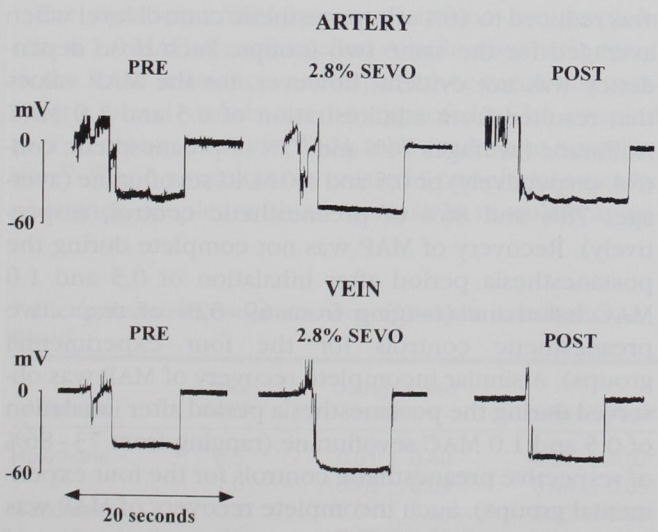

Fig. 1. Examples of sequential *in situ* vascular smooth muscle transmembrane potential recordings from a small mesenteric artery (*top*) and vein (*bottom*) made in the same experimental preparation before (Pre), during (2.8% sevo), and after (post) inhalation of 1.0 MAC sevoflurane, illustrating the hyperpolarization in response to the anesthetic agent.

Hyperpolarization (Change in Transmembrane Potential) Responses to Inhaled Anesthetic Agents in Mesenteric Vessels with Intact Sympathetic Innervation

To evaluate the effect of volatile anesthetic agents on *in situ* VSM E_m values more reliably in the innervated *versus* denervated vessel preparation, the mean hyperpolarization (*i.e.*, change in E_m) responses were compared for each agent (table 3). The enhanced reliability of such comparisons results from the use of each vessel

Table 4. Time Control Measurements of VSM E_m in Innervated Small Mesenteric Artery and Vein during Simulated Anesthesia

Vessel Type	n	E_m*			ΔE_m‡	
		Pre	During†	Post	(During − Pre)	(Post − Pre)
Artery	5	−39 ± 1.1	−38 ± 0.6	−37 ± 0.8	+1.7 ± 1.7	+2.0 ± 1.9
Vein	5	−40 ± 2.6	−39 ± 4.3	−39 ± 4.3	+0.5 ± 2.9	+1.0 ± 2.3

* Mean E_m ± SD (mV) in "n" vessel/animal preparations.

† Time period of simulated anesthetic administration.

‡ (+) = depolarization.

preparation as its own preanesthetic control. This eliminates the variability inherent in mean VSM E_m values attributable to factors such as surgical preparation and small variations in local environment (*e.g.,* temperature, blood and superfusate respiratory gas concentrations, levels of basal anesthetic agent in tissue). Table 3 illustrates that inhalation of 1.0 MAC halothane, isoflurane, or sevoflurane caused a significant hyperpolarization of both arterial and venous VSM. For both vessel types, isoflurane-induced hyperpolarization was less than that produced by halothane or sevoflurane. At 0.5 MAC, the respective hyperpolarization response of VSM in both types of innervated vessel to each of the three anesthetic agents was less than at 1.0 MAC and, in most cases, not significantly different from zero (table 3).

Hyperpolarization (Change in Transmembrane Potential) Responses to Inhaled Anesthetic Agents in Sympathetically Denervated Mesenteric Vessels

Table 3 also illustrates that inhalation of 1.0 MAC halothane, isoflurane, or sevoflurane still caused significant hyperpolarization of both arterial and venous VSM in denervated vessels (except for the insignificant venous VSM hyperpolarization response to sevoflurane). In addition, except for the venous response to 1.0 MAC halothane and isoflurane, the hyperpolarization in the denervated vessel was significantly less (by ≈50% or more) relative to the response in the innervated vessel. At 0.5 MAC, the VSM hyperpolarization response of denervated artery or vein to each type of anesthetic agent was variable and significantly different from zero only for the venous VSM response to isoflurane and sevoflurane.

Mean Transmembrane Potential Values during Time Control Measurements

Table 4 illustrates mean E_m time control measurements made in the innervated and denervated artery and vein. No significant hyperpolarization was observed over the period during which the volatile agent would have been inhaled (simulated anesthetic period) or after its washout (postanesthetic period). A small depolarization was observed in the denervated artery during the simulated anesthetic period.

Changes in Mean Arterial Blood Pressure

Inhalation of each of the three volatile anesthetic agents significantly decreased MAP at the 0.5 and 1.0 MAC concentrations to values ranging from 43–82% of respective preanesthetic controls (table 5). The mean MAP was reduced to 76% of preanesthetic control when averaged for the innervated and locally denervated experimental groups during inhalation of 0.5 MAC halothane. During inhalation of 1.0 MAC halothane, the MAP was reduced to 46% of preanesthetic control level when averaged for the same two groups. Such dose dependency was not evident, however, for the MAP values that resulted from administration of 0.5 and 1.0 MAC isoflurane (averages 74% and 77% of preanesthetic control, respectively) or 0.5 and 1.0 MAC sevoflurane (averages 78% and 86% of preanesthetic control, respectively). Recovery of MAP was not complete during the postanesthesia period after inhalation of 0.5 and 1.0 MAC halothane (ranging from 69–82% of respective preanesthetic controls for the four experimental groups). A similar incomplete recovery of MAP was observed during the postanesthesia period after inhalation of 0.5 and 1.0 MAC sevoflurane (ranging from 73–86% of respective preanesthetic controls for the four experimental groups). Such incomplete recovery of MAP was observed despite essentially complete elimination of each volatile anesthetic agent during the postanesthetic washout period (table 6). Recovery was complete for three of the four experimental groups during the postanesthesia period after inhalation of 0.5 and 1.0 MAC

Table 5. Mean Arterial Blood Pressure Measurements

Anesthetic Dose (MAC)	n	Condition	Preanesthetic	During Anesthetic	Postanesthetic
Halothane					
0.5	8	Innervated	135 ± 14	105 ± 23*	111 ± 23*
0.5	8	Denervated	124 ± 12	91 ± 13*	100 ± 23*
1.0	8	Innervated	131 ± 13	56 ± 16†	91 ± 33*
1.0	7	Denervated	105 ± 18	53 ± 9†	83 ± 21*
Isoflurane					
0.5	6	Innervated	109 ± 21	79 ± 17*	85 ± 17*
0.5	7	Denervated	103 ± 13	77 ± 22†	95 ± 28
1.0	13	Innervated	116 ± 15	83 ± 14†	108 ± 14
1.0	8	Denervated	103 ± 14	85 ± 11†	98 ± 24
Sevoflurane					
0.5	6	Innervated	124 ± 20	68 ± 16†	91 ± 20*
0.5	8	Denervated	103 ± 22	72 ± 15†	87 ± 22*
1.0	9	Innervated	120 ± 21	72 ± 18†	103 ± 23*
1.0	6	Denervated	110 ± 12	66 ± 4†	95 ± 20*
Time control	5	Innervated	111 ± 24	99 ± 18*	106 ± 18
	4	Denervated	88 ± 25	86 ± 28	99 ± 14*

Values are mean ± SD (mmHg) in "n" animals.

* Significantly lower than corresponding "Pre."

† Significantly lower than corresponding "Pre" and "Post."

isoflurane. Mean arterial pressure did not vary significantly during the time control protocol.

Discussion

The significant VSM hyperpolarization measured *in situ* at 1.0 MAC of each inhaled volatile anesthetic agent in the small resistance- and capacitance-regulating mesenteric blood vessels can be expected to be coupled to a decrease in active VSM tone leading to vasodilation[25-28] and a resultant decrease in peripheral vascular resistance coupled with an increase in vascular capacitance. Compared with innervated vessels, the respective *in situ* VSM hyperpolarization produced by 1.0 MAC of each volatile anesthetic agent were significantly less in locally denervated vessels (except for the venous hyperpolarization by halothane or isoflurane; table 3). Therefore, at 1.0 MAC, each of the three volatile anesthetic agents exerts a significant *in situ* inhibitory effect on E_m and tone both at the level of the sympathetic neuromuscular junction and at the level of the vascular cell or vessel endothelium. This dual effect is particularly evident in the resistance-regulating small arteries. The data from the current study also suggest that, at 1.0 MAC, the hyperpolarizing action of isoflurane is significantly less than that of halothane or sevoflurane. Table 3 indicates that, in the innervated small arteries and veins, the VSM hyperpolarization induced by 1.0 MAC isoflurane was significantly less than that induced either by halothane or sevoflurane. In the respective types of denervated vessel, however, such a difference was not evident. In addition, 1.0 MAC halothane and isoflurane produced similar respective VSM hyperpolarization in innervated *versus* denervated veins. Assuming that a coupling exists between VSM membrane hyperpolarization and reduction of tone, these measurements suggest that halothane and isoflurane increase mesenteric venous capacitance more by inhibition of nonneu-

Table 6. Measured Concentrations of Volatile Anesthetics in Blood

Anesthetic	Dose (MAC)	During Inhaled Anesthetic	After Washout
Halothane	0.5	0.31 ± 0.40	0.01 ± 0.02*
	1.0	0.70 ± 0.09	0.06 ± 0.05*
Isoflurane	0.5	0.34 ± 0.05	0.02 ± 0.02*
	1.0	0.65 ± 0.06	0.05 ± 0.05*
Sevoflurane	0.5	0.22 ± 0.03	0.01 ± 0.01*
	1.0	0.66 ± 0.19	0.02 ± 0.01*

Values are Mean ± SD (mM).

* Different from "During Inhaled Anesthetic" ($P \leq 0.05$).

ral (or nonsympathetic neural) mechanisms regulating venous VSM E_m and tone. The relatively small anesthetically induced hyperpolarizations in the denervated veins, however, indicate the need for additional studies to establish the validity of this suggestion.

At 0.5 MAC, the *in situ* hyperpolarization produced by each of the inhaled volatile anesthetic agents was small and not clearly evident in either the intact or denervated vessels. The stability of the vessel preparation and the reliability of *in situ* VSM E_m measurements over the time course of each of the protocols were demonstrated by the lack of significant VSM hyperpolarization in the time control groups (table 4).

A variety of evidence exists to support an attenuation of VSM tone by volatile anesthetic agents through their actions on neural and nonneural regulatory mechanisms. It is well known that these agents can attenuate sympathetic neural control of VSM tone in small arteries and veins through inhibitory actions at both central[29,30] and peripheral[31,32] neural loci. Direct inhibitory effects at the VSM cell level include attenuation of inositol triphosphate-mediated Ca^{2+} release from the sarcoplasmic reticulum[33,34] and influx of Ca^{2+} across the sarcolemmal membrane in cultured VSM-like cells.[35] *In vitro* patch clamp studies indicate that these anesthetic agents can inhibit both inward Ca^{2+} and outward potassium (K^+) currents across the sarcolemmal membrane of VSM from canine coronary[36] and cerebral[12] arteries. Current initial studies in our laboratory, however, indicate that *in situ* hyperpolarization of denervated mesenteric vessels by isoflurane is eliminated by superfusion with specific K_{Ca} and K_{ATP} channel blockers,[37] strongly suggesting their activation by the volatile anesthetic agents.

Of central importance to the conclusions reached in this study is the validity of the assumed relationship between the anesthetically induced VSM hyperpolarization and inhibition of VSM tone. The *in situ* hyperpolarizations induced by volatile anesthetic agents observed in the current study coupled with their reported inhibitory effect on neural and nonneural regulation of VSM tone[29–35] lends support to the relationship between VSM hyperpolarization and reduction of active contractile force (*i.e.,* electromechanical coupling). Substantial evidence indicates that electromechanical coupling occurs over the *in vivo* physiologic range of VSM E_m values (approximately −30 – −50 mV) in a variety of vessels.[25,26,28,38] It is generally recognized that smaller (resistance) arteries are more sensitive to the action of Ca^{2+} entry blockers than are larger arteries, presumably because the former have smaller intracellular stores of Ca^{2+} available for excitation-contraction coupling. Hence, regulation of small artery VSM tone is more dependent on influx of extracellular Ca^{2+}, which, in turn, is dependent on E_m. Siegel *et al.*[27,39] have shown that vasoactive agents and changes in physiologic state (*e.g.,* acidosis or hypoxia) that cause VSM hyperpolarization also produce vasodilation. Both are the result of K^+ channel activation and consequent closure of voltage-sensitive Ca^{2+} channels.[39,40] Siegel *et al.*[39] also have shown that most vasodilators are K^+ channel openers and produce a tight electromechanical coupling (*e.g.,* a 50% relaxation for a 2.5-mV hyperpolarization). It is important to note, however, that inhibition of VSM tone by volatile anesthetic agents also may include actions at regulatory sites that do not affect E_m (*e.g.,* attenuation of intracellular second messenger enzymatic activity, sensitivity of VSM myosin light chain kinase activation by Ca^{2+}-calmodulin, phosphorylation of VSM myosin[9,11]).

Another controversial question concerning possible nonneural mechanisms of control of VSM E_m and tone is the effect of volatile anesthetic agents on production or release of endothelium-derived nitric oxide vasodilator and hyperpolarizing factors from the vascular endothelial cell. *In vitro* evidence supports an indirectly augmented release of endothelium-derived nitric oxide by volatile anesthetic agents[16] and an inhibition of endothelium-derived nitric oxide-mediated vasodilation *via* inhibition of its synthesis.[41] Endothelium-derived hyperpolarizing factor also can be inhibited *in vitro* by intravenous and volatile anesthetic agents.[42] In the current study, however, volatile anesthetic agents hyperpolarized VSM *in situ.* Therefore, if they do inhibit endothelium-derived nitric oxide or endothelium-derived hyperpolarizing factor activity *in vivo,*[43] it appears that such actions would be subordinate to the more potent anesthetic actions leading to VSM hyperpolarization and vasodilation.

It could be argued that *in situ* change in blood vessel diameter is a more direct indicator of change in VSM tone (*i.e.,* active contractile force) than VSM E_m. Vessel diameter is also a function of intraluminal pressure and passive wall tension, however (as is evident from the La Place relationship).[44] Hence, these variables also must be assessed *in situ* to establish an accurate relation between VSM tone and vessel diameter. Therefore, we believe that the VSM hyperpolarization induced by volatile anesthetic agents observed in the current study, taken together with the demonstrated tight (and steep)

electromechanical coupling in small blood vessels,[25,26,28,38] allows the use of VSM E_m measurements to assess the effects of volatile anesthetic agents indirectly on *in situ* regulation of VSM tone.

As has been reported previously,[45,46] in the current study, each of the volatile anesthetic agents significantly reduced MAP. Therefore, the possibility exists that the *in situ* hyperpolarization induced by volatile anesthetic agents in the small artery is a result, rather than a cause, of the accompanying hypotension produced by these agents (*i.e.,* a myogenic effect[47]), particularly in the absence of local sympathetic innervation. In previous studies, we have measured a slope of 0.05 mV/mmHg intraluminal pressure in *in vitro* isolated, perfused segments of rat small mesenteric artery (unpublished observations). Thus, a myogenic mechanism may contribute to the hyperpolarization of arterial VSM induced by anesthetic agents. Definitive evidence for such a contribution can be obtained only by correlating a range of *in situ* intraluminal pressure measurements with VSM E_m. Indirect evidence suggests, however, that the myogenic response cannot be the sole cause of the VSM hyperpolarization. In the current study, the arterial and especially the venous hyperpolarization do not consistently correlate with the magnitude of reduction in arterial pressure. In addition, in preliminary studies designed to eliminate systemic hypotension by superfusing *in situ* mesenteric vessels with a concentration of isoflurane equal to that attained in blood at 1.0 MAC, inhaled (0.65 mM), we have observed VSM hyperpolarizations similar to those reported in table 3.

As indicated previously, sympathetic denervation was produced by superfusion of the *in situ* vessels with 6-hydroxydopamine.[20] The data in table 5 indicate that the mean value for MAP before administration of volatile anesthetic agents of the six denervated groups of animals tended to be lower than that of the innervated groups (14 ± 17 mmHg). A small reduction in MAP may have occurred during the local denervation because of some absorption of 6-hydroxydopamine into the circulation with redistribution to other sympathetic nerve terminals. Other factors, however, also may have contributed to the small reduction in MAP (*e.g.,* longer duration of the experimental protocol for VSM E_m measurements in denervated vessels).

It should be recognized that the MAC dose designated for each of the volatile anesthetic agents in the current study refer only to inspired concentrations and not to total level of depth of anesthesia. The hyperpolarization induced by volatile anesthetic agents measured in this study were superimposed on any effects that the necessary basal pentobarbital anesthetic agent may have had on background neural activity or the peripheral vasculature. Some early evidence indicates that high concentrations of barbiturates can hyperpolarize VSM in *in situ* small mesenteric arteries.[48] In the current study *in situ,* however, VSM E_m values were measured under a light to moderate level of basal anesthesia. Previous studies have shown that, with similar levels of pentobarbital-induced anesthesia, VSM in the small mesenteric arteries and veins responds to vasoconstrictor agonists with depolarization and to denervation with hyperpolarization.[22,26,49] Thus, although hyperpolarization induced by volatile anesthetic agents may have been attenuated by the basal anesthetic agent in the current study, it was still evident. Finally, it is also important to note that the potential exists for the ketamine used for initial sedation to attenuate hyperpolarization induced by volatile anesthetic agents (*e.g.,* by blocking K_{ATP} channels).[50] This effect should be slight, however, because measurement of E_m values in each protocol did not begin before a minimum of 2 h after administration of the single ketamine bolus dose.

Inhalation of 1.0 MAC, but not 0.5 MAC, halothane, isoflurane, or sevoflurane produced a consistent and significant *in situ* hyperpolarization of VSM in small mesenteric arteries and veins. Local sympathetic denervation attenuated the arterial VSM hyperpolarization induced by each volatile anesthetic agent and the venous hyperpolarization induced by sevoflurane. Venous VSM hyperpolarization induced by halothane or isoflurane, however, was not significantly attenuated by local sympathetic denervation. We conclude that at 1.0 MAC these inhaled volatile anesthetic agents attenuate sympathetic neural and nonneural mechanisms involved in the *in situ* regulation of E_m and tone in resistance-regulating arteries. In capacitance-regulating veins *in situ,* 1.0 MAC halothane and isoflurane primarily attenuate nonneural mechanisms regulating E_m and tone.

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