Anesthesiology
1999; 91:207–14

Effect of Isoflurane on In Situ *Vascular Smooth Muscle Transmembrane Potential in Spontaneous Hypertension*

Thomas A. Stekiel, M.D., Naohiro Kokita, M.D., Ph.D.,† Mitsuaki Yamazaki, M.D., Ph.D.,‡ Zeljko J. Bosnjak, Ph.D.,§ John P. Kampine, M.D., Ph.D.,‖ William J. Stekiel, Ph.D.#*

Background: **Administration of general anesthetics to patients with chronic hypertension often causes hemodynamic instability that has been attributed in part to a poorly understood increased loss of control of peripheral vascular smooth muscle tone. The purpose of the current study was to determine if such an increased loss occurs in the spontaneously hypertensive (SH) rat neurogenic model of chronic hypertension, as reflected by a greater volatile anesthetic-induced *in situ* vascular smooth muscle hyperpolarization compared with normotensive Wistar-Kyoto (WKY) rat controls.**

Methods: **Vascular smooth muscle transmembrane potentials (E_m) were measured *in situ* using glass microelectrodes in externalized small mesenteric resistance- and capacitance-regulating blood vessels in 10- to 12-week-old SH and WKY rats before, during, and after administration of 1 minimum alveolar concentration levels (1.5%) of inhaled or 0.60 mM superfused isoflurane. Vascular smooth muscle E_ms were also measured in vessels after local sympathetic denervation with superfused 6-hydroxydopamine.**

Results: **Local sympathetic denervation caused a significant hyperpolarization of arterial and venous vascular smooth muscle in SH but not WKY rats. Hyperpolarization induced by either inhaled or superfused isoflurane was significantly greater in innervated than in denervated arterial and venous vascular smooth muscle, particularly in SH rats. In addition, for innervated (but not denervated) arterial and venous vascular smooth muscle, hyperpolarization induced by inhaled (but not superfused) isoflurane was significantly greater in SH than in WKY rats.**

Conclusions: **In the neurogenic SH rat model of human hypertension, a primary mechanism underlying elevated isoflurane-induced vascular smooth muscle hyperpolarization (and reduced vascular smooth muscle tone) in both resistance- and capacitance-regulating blood vessels is a central neural inhibition of excitatory sympathetic input. Peripheral neural and nonneurally mediated hyperpolarization by isoflurane is similar in SH and WKY rat vascular smooth muscles. (Key words: Central vascular control; membrane potential; peripheral vascular control.)**

PERIOPERATIVE management of hypertension continues to be an important clinical problem because the anesthetic course of patients with chronic hypertension is characterized by a poorly understood circulatory instability and increased perioperative complications.[1–3] In addition, wide or sustained fluctuations in hemodynamic control may occur when general anesthesia is administered. The incidence of such circulatory instability is increased in the presence of chronic hypertension.[4] It is well established that peripheral vascular smooth muscle (VSM) tone is regulated by a balanced input from many interacting control mechanisms. This balance is disrupted in the presence of chronic hypertension.[5–8]

Volatile anesthetics (which are well known to be vasodilators) also can disrupt the control of VSM tone at multiple levels. These include extrinsic (neural and humoral),[9–11] endothelial,[12,13] and intrinsic (membrane and intracellular)[14,15] mechanisms. However, the rela-

* Assistant Professor of Anesthesiology, The Medical College of Wisconsin.

† Visiting Professor of Anesthesiology, The Medical College of Wisconsin; Staff Anesthesiologist, Sapporo Medical University School of Medicine, Sapporo, Japan.

‡ Associate Professor of Anesthesiology, Toyama Medical and Pharmaceutical University, Toyama, Japan.

§ Professor of Anesthesiology and Physiology, The Medical College of Wisconsin.

‖ Professor and Chair of Anesthesiology and Professor of Physiology, The Medical College of Wisconsin.

Professor of Physiology, The Medical College of Wisconsin.

Received from the Departments of Anesthesiology and Physiology, Medical College of Wisconsin, and the Zablocki Veterans Affairs Medical Center, Milwaukee, Wisconsin. Submitted for publication September 14, 1998. Accepted for publication February 22, 1999. Supported by National Institutes of Health grant HL56290, Bethesda, Maryland. Presented in part at the annual meeting of the Federation of American Societies for Experimental Biology, Atlanta, Georgia, April 11, 1995, and at the annual meeting of the American Society of Anesthesiologists, New Orleans, Louisiana, October 22, 1996.

Address reprint requests to Dr. Stekiel: Anesthesia Research, MEB, 462C, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, Wisconsin 53226. Address electronic mail to: tstekiel@mcw.edu

tive importance of an anesthetic-mediated vasodilator effect at each of these levels remains to be established. In addition, little is known about differences in such anesthetic effects on the mechanisms of vascular control between persons with or without hypertension.

Recently, we reported that halothane, isoflurane, and sevoflurane produced a dose-dependent hyperpolarization in VSM of *in situ* mesenteric resistance arteries and capacitance veins.[16] Approximately one half of this hyperpolarization was attributed to attenuation of sympathetic vascular control. Because VSM transmembrane potential (E_m) is tightly coupled to active VSM force generation over the physiologic range of E_m (approximately −30 to −60 mV),[17-19] the observed hyperpolarization reflected an anesthetic-mediated vasorelaxation.[16]

The current study measured (previously established) isoflurane-mediated VSM hyperpolarization to compare neural and nonneural regulation of *in situ* E_m in VSM of small resistance- and capacitance-regulating blood vessels in an animal model of established human essential hypertension and its normotensive control.[20-22]

Materials and Methods

Animal and Vessel Preparations

After we received approval from our institution's animal care and use committee, *in situ* E_ms of VSM were measured in 10- to 12-week-old (weight, 250-300 g) spontaneously hypertensive (SH) rats and normotensive Wistar-Kyoto (WKY) control rats (Taconic Farms, Germantown, NY) using previously described methods.[16,23] The animals were anesthetized with 40 mg/kg intraperitoneal ketamine, followed by 20-30 mg/kg intraperitoneal sodium pentobarbital to facilitate initial surgical preparation. Subsequently, basal anesthesia was maintained during the course of each experimental protocol using intravenous pentobarbital administered as a constant infusion at 30 $mg \cdot kg^{-1} \cdot h^{-1}$. Femoral venous and arterial cannulae were introduced to infuse medication and to directly measure blood pressure, respectively. A endotracheotomy tube was placed, through which ventilation was controlled using a rodent respirator (model 680; Harvard Apparatus, South Natick, MA) to maintain the end-tidal carbon dioxide level between 30 and 40 mmHg. Inspired oxygen concentrations were maintained at 30%.

In each animal, a segment of terminal ileum (with its attached mesentery) was externalized through a midline laparotomy to expose small (approximately 200 μm OD) mesenteric arteries and paired (approximately 300 μm OD) corresponding veins. In each preparation, the vessels were gently freed of perivascular fat without disturbing luminal flow or adventitial innervation. The surrounding connective tissue was fastened to the silastic rubber floor of a temperature-regulated tissue chamber with 50- and 125-μm diameter stainless steel pins. The vessels were superfused continuously with physiologic salt solution (PSS) and maintained at 36.5-37.5°C. The PSS was composed of 119 mM NaCl, 4.7 mM KCl, 1.17 mM Mg SO_4, 1.6 mM $CaCl_2$, 24 mM $NaHCO_3$, 1.18 mM NaH_2PO_4, and 0.026 mM EDTA. The PSS was aerated with a gas mixture of nitrogen, oxygen, and carbon dioxide to maintain the pH between 7.35 and 7.45, the carbon dioxide tension between 35 and 45 mmHg, and the oxygen pressure between 100 and 150 mmHg. Smaller 50-μm diameter stainless steel pins were placed beside arteries and the veins to separate them and to minimize vessel wall pulsation and movements that interfere with E_m measurements.

Experimental Measurements

Single VSM cell E_ms were measured *in situ* by manually advancing 3 M potassium chloride-filled glass micropipettes (tip diameter, 0.1 μm and input impedance ranging between 40 and 60 MΩ) into the VSM layer from the adventitial side of the vessel using a Wells hydraulic micromanipulator (Trent Wells, Coulterville, CA). Electrodes were pulled from borosilicate glass using a Sutter model P-97 Flaming/Brown micropipette puller (Sutter Instrument, Novato, CA) and were connected to a model 8100 biological amplifier (Dagan Corp., Minneapolis, MN). E_m and femoral arterial blood pressure were recorded simultaneously using a Grass model RPS 7C polygraph (Astro-med/Grass, West Warwick, RI) and digitally using a Superscope II version 1.44 data acquisition system (GW Instruments, Somerville, MA). Measurements were made before, during, and after inhalation of 1.5% end-tidal isoflurane (equivalent to 1 minimum alveolar concentration levels)[24] or superfusion with 0.6 mM isoflurane in the PSS. The latter was equal to the average concentration of isoflurane measured in blood during inhalation of the 1.5% concentration. The 0.6 mM concentration in PSS corresponds to a calculated volume percentage of 2.77% compared with 1.1% for the same concentration in blood.[25] Although it is not clear exactly what effect protein-bound isoflurane could produce on the VSM, the "free" isoflurane concentration was greater when administered superfused (in the PSS) than inhaled

(in the blood). Because the vessels were superfused continuously with PSS in an open chamber, the greater "free" concentration of anesthetic in the superfusate at least partially compensated for atmospheric loss. The concentrations of both inhaled isoflurane and that dissolved in PSS were delivered using an Ohio Medical Products vaporizer (Airco, Madison, WI).

One half of the total vessel preparations studied were denervated sympathetically by local superfusion with 300 μg/ml of 6- hydroxydopamine for 20 min after initial blood pressure, and E_m measurements were made in neurally intact vessels. Local superfusion of blood vessels with 6-hydroxydopamine effectively eliminates perivascular sympathetic innervation, as evidenced by inhibition of contractile responses to local neural electric field stimulation, blockade of H^3-norepinephrine uptake by sympathetic nerve endings, and histologic changes consistent with adrenergic nerve degeneration.[26] Before 6-hydroxydopamine administration, each vessel preparation was superfused with 10^{-6} M phentolamine for 5 min to block the prolonged vasoconstriction resulting from 6- hydroxydopamine-initiated catecholamine release. These agents were washed out with a 1-h superfusion with PSS before E_m measurements were resumed.[16,23]

Four groups of vessel and animal preparations were studied under each of the two methods of exposure of the vessels to isoflurane (inhaled *vs.* superfused). The four groups included intact and denervated vessel preparations from SH rats and intact and denervated vessel preparations from WKY rats.

Inspired and expired isoflurane and end-tidal carbon dioxide concentrations were measured using a POET II infrared capnograph and end-tidal agent monitor (Criticare Systems, Waukesha, WI). Blood anesthetic concentrations were measured using a Sigma 38 gas chromatography system (Perkin-Elmer, Norwalk, CT) in samples taken immediately before the washout period and again after all washout measurements were completed.

Statistical Analyses

In each experimental vessel and animal preparation, the arterial and venous VSM E_m measured during each of the sequential protocol steps (*i.e.*, before isoflurane, with isoflurane, and after isoflurane for neurally intact and locally denervated vessels) is the calculated numeric average of 5–10 single VSM cell impalements (each lasting 6–10 s). Each E_m value reported in the tables is the mean ± SD of these numeric average values, with a replication factor ranging from 7 to 9 vessel and animal preparations for each protocol. A repeated measures analysis of variance (Super ANOVA program; Abacus Concepts, Berkeley, CA) was performed on the mean E_m values with the sequential protocol steps as the repeated unknown factor (*i.e.*, innervated [or denervated] before, during, and after isoflurane). An identical repeated measures analysis of variance was used to compare the effects of isoflurane on the averaged mean arterial pressure measured together with E_m before, during, and after isoflurane administration in each of the experimental animal groups. Paired *t* tests (Stat-View program, Abacus Concepts) were used to verify that the mean isoflurane-induced hyperpolarization response (ΔE_m) in each experimental group was significantly greater than 0 ($P \leq 0.05$). An analysis of variance without repeated measures was performed to compare mean isoflurane-induced hyperpolarization of a vessel type between the innervated and denervated and the hypertensive and normotensive experimental groups. For all analyses of variance in the study, the significance of differences between mean values was determined by comparing calculated least-square means at a significance level of $P \leq 0.05$.

Results

Effect of Local Sympathetic Denervation on Transmembrane Potentials of Vascular Smooth Muscle in the WKY and SH Rat Models

Table 1 lists the mean values of *in situ* E_m of VSM in sympathetically innervated and locally denervated small mesenteric arteries and veins of WKY and SH rats before, during, and after inhalation of isoflurane, 1.5%. Before local denervation or exposure to volatile anesthetic, the mean E_m of both arteries and veins in the SH rats tended to be less negative (less polarized VSM) than that of the respective vessels in WKY rats. After local sympathetic denervation, mean E_ms of SH rats were not different from those of denervated WKY rats. However, mean E_ms of both vessel types in SH rats (but not WKY) became significantly more negative than those of respective neurally intact vessels.

Effect of Inhaled Isoflurane on Transmembrane Potentials of Vascular Smooth Muscle in WKY and SH Rats

Table 1 shows that during inhalation of isoflurane, 1.5%, mean E_ms in VSM of both innervated and denervated small arteries and veins were significantly more negative than respective preisoflurane controls in both WKY and SH rats. Table 2 compares the mean hyperpo-

Table 1. Effect of Inhaled Isoflurane on Transmembrane Potentials (E_m) across VSM of *In Situ* WKY and SHR Small Mesenteric Vessels

Experimental Group	Innervated Control	Denervated Control	ISO	POST
Artery				
WKY				
Innervated	−40 ± 1.8	—	−44 ± 1.6†	−39 ± 1.6
Denervated	−39 ± 2.3	−40 ± 2.6	−43 ± 2.9†‡	−39 ± 2.1
SHR				
Innervated	−37 ± 1.1	—	−44 ± 2.6†	−38 ± 1.1
Denervated	−37 ± 1.9	−40 ± 1.7*	−41 ± 1.1†	−38 ± 2.3
Vein				
WKY				
Innervated	−41 ± 2.5	—	−46 ± 1.8†	−39 ± 2.7
Denervated	−40 ± 1.6	−42 ± 2.1	−44 ± 3.0†‡	−40 ± 1.0
SHR				
Innervated	−40 ± 1.8	—	−48 ± 3.2†	−40 ± 2.5
Denervated	−39 ± 2.1	−43 ± 2.1*	−46 ± 3.1†‡	−41 ± 2.8

Innervated control = before denervation; denervated control = after local chemical sympathetic denervation with 6-OHDA; ISO = during 1.0 MAC (1.5%) inhaled isoflurane; POST = after washout of isoflurane from the circulation.

Values are mean ± SD VSM E_m (mV); n = 7–9 vessel/animal preparations.

* Denervated control E_m more negative than innervated control E_m ($P \leq 0.05$).

† ISO E_m more negative than innervated control or POST E_m ($P \leq 0.05$).

‡ ISO E_m more negative than denervated control E_m ($P \leq 0.05$).

larization responses (*i.e.*, ΔE_ms) with inhaled isoflurane, 1.5%, in innervated and denervated vessels. All hyperpolarization responses to isoflurane were significantly greater than zero, except for the WKY vein. Of particular interest is that the hyperpolarization in innervated arteries and veins was significantly greater than in respective denervated arteries and veins (except for the WKY artery). In addition, for the respective innervated (but not denervated) vessel, the hyperpolarization in SH rats was significantly greater than in WKY rats. In contrast, in denervated vessels, there was no difference in the hyperpolarization response between SH and WKY rats. However, in denervated veins, the hyperpolarization responses of WKY (in contrast to that of SH rats) was not significantly different from zero.

Table 2. Hyperpolarization Responses to 1 MAC (1.5%) Inhaled Isoflurane in VSM of *In Situ* WKY and SHR Small Mesenteric Vessels

	Artery Δ E_m		Vein Δ E_m	
	WKY	SHR	WKY	SHR
Innervated vessels	3.9 ± 2.1*	7.3 ± 2.6*†‡	4.5 ± 2.0*†	7.4 ± 3.2*†‡
Denervated vessels	3.2 ± 1.4*	1.4 ± 1.5*	2.0 ± 3.7	2.8 ± 3.1*

Data are mean ± SD (mV); n = 7–9 vessel/animal preparations. For innervated vessels, Δ E_m = mean E_m difference ± SD between ISO and innervated control. For denervated vessels, Δ E_m = mean E_m difference ± SD between ISO and denervated control.

* Hyperpolarization response significantly greater than zero ($P \leq 0.05$).

† Hyperpolarization response in innervated vessel greater than in denervated vessel ($P \leq 0.05$).

‡ Hyperpolarization response in SHR vessel greater than in WKY vessel ($P \leq 0.05$).

Table 3. Effect of Locally Superfused Isoflurane on Transmembrane Potentials (E_m) across VSM of *In Situ* WKY and SHR Small Mesenteric Vessels

Experimental Group	Innervated Control	Denervated Control	ISO	POST
Artery				
WKY				
Innervated	−39 ± 1.7	—	−43 ± 1.5*	−39 ± 1.9
Denervated	—	−40 ± 2.4	−43 ± 2.4†	—
SHR				
Innervated	−38 ± 2.1	—	−44 ± 3.3*	−38 ± 1.7
Denervated	—	−40 ± 2.4	−43 ± 2.4†	—
Vein				
WKY				
Innervated	−41 ± 4.3	—	−46 ± 4.4*	−40 ± 4.3
Denervated	—	−43 ± 2.2	−45 ± 2.7†	—
SHR				
Innervated	−39 ± 3.0	—	−45 ± 2.5*	−38 ± 1.5
Denervated	—	−44 ± 2.6	−47 ± 2.1†	—

Innervated control = before denervation; denervated control = after local chemical sympathetic denervation with 6-OHDA; ISO = during local superfusion of vessels with 0.6 mM isoflurane in PSS superfusate; POST = after washout of isoflurane by return to PSS superfusate.

Values are mean ± SD VSM E_m (mV); n = 6–8 vessel/animal preparations.

* ISO E_m more negative than innervated control or POST E_m ($P \leq 0.05$).

† ISO E_m more negative than denervated control E_m ($P \leq 0.05$).

Effect of Superfused Isoflurane on Transmembrane Potentials of Vascular Smooth Muscle in WKY and SH Rats

Table 3 shows that, similar to inhaled isoflurane, 1.5%, 0.6 mM superfused isoflurane caused mean E_ms of VSM in both innervated and denervated small arteries and veins to become significantly more negative than respective preisoflurane controls in both WKY and SH rats. Table 4 compares the mean hyperpolarization responses (*i.e.*, ΔE_ms) with superfused isoflurane in innervated and denervated vessels of WKY and SH rats. As with inhaled isoflurane, the hyperpolarization responses were significantly greater than zero. In addition, the hyperpolarization in the innervated arteries and veins were significantly greater than in the respective denervated vessels (except for the WKY arteries). However, unlike during inhaled isoflurane, the hyperpolarization response to superfused isoflurane in innervated SH rat arteries and veins was not significantly greater than in the respective WKY vessels.

Table 4. Hyperpolarization Responses to 1 MAC (0.6 mM) Locally Superfused Isoflurane in VSM of *In Situ* WKY and SHR Small Mesenteric Vessels

	Artery Δ E_m		Vein Δ E_m	
	WKY	SHR	WKY	SHR
Innervated vessels	4.8 ± 1.9*	6.0 ± 2.4*†	4.4 ± 2.5*†	5.5 ± 1.9*†
Denervated vessels	3.3 ± 1.6*	2.8 ± 1.2*	2.4 ± 0.9*	3.1 ± 1.3*

Data are mean ± SD (mV); n = 6–8 vessel/animal preparations. For innervated vessels, Δ E_m = mean E_m difference ± SD between ISO and innervated control. For denervated vessels, Δ E_m = mean E_m difference ± SD between ISO and denervated control.

* Hyperpolarization response significantly greater than zero ($P < 0.05$).

† Hyperpolarization response in innervated vessel greater than in denervated vessel ($P \leq 0.05$).

Mean Arterial Blood Pressure Responses to Inhaled and Superfused Isoflurane

As expected, mean arterial blood pressure was significantly greater in the each of the SH rat experimental groups when compared with the WKY groups (table 5). Local chemical denervation of the *in situ* vessel preparation caused a small (approximately 10 mmHg) decrease in mean arterial blood pressure in the SH rats but no significant decrease in the WKY rats. The decrease in mean arterial blood pressure in response to 1 minimum alveolar concentration inhaled isoflurane was approximately twice as great in the SH rat groups as in the respective WKY control groups (41–42% for SH rats *vs.* 27–29% for WKY rats). This was also true when expressed as a percentage change. After elimination of the inhaled isoflurane, mean arterial blood pressure recovered completely to preisoflurane levels in all four experimental groups. No change in mean arterial blood pressure was observed in any of the four experimental groups in which vessel preparations were superfused with 0.6 mM isoflurane.

Blood Concentrations of Inhaled Isoflurane

Inhalation of 1.5% isoflurane produced blood isoflurane concentrations after equilibration in each of the four animal groups that ranged from 0.52 to 0.58 mM. In all of the groups, the elimination (post) period effectively and significantly washed out the isoflurane to negligible levels. No measurable concentrations were observed in the blood of animal preparations in which vessels were superfused with 0.6 mM isoflurane.

Discussion

The purpose of the current study was to assess the effects of volatile anesthetics on *in situ* neural and nonneural control of VSM tone in a neurogenic SH rat model of human essential hypertension. This was done indirectly by measurement of *in situ* responses of E_m of VSM in sympathetic neurally intact and denervated mesenteric resistance- and capacitance-regulating blood vessels to 1 minimum alveolar concentration inhaled and to an equivalent 0.6 mM locally superfused concentration of isoflurane. Regardless of how isoflurane was administered, hyperpolarization was greater in innervated than in denervated vessels in both WKY and SH rats. In innervated (but not denervated) vessels, the hyperpolarization resulting from inhaled (but not superfused) isoflurane was greater in SH than in WKY rats. Thus, differences in anesthetic effects on vascular control between hypertensive and normotensive (using the SH-WKY rat model) animals appear to be proximal to the peripheral vasculature (*e.g.*, in the central nervous system).

It could be argued that changes in VSM E_m are not necessarily accurate indicators of changes in VSM tone in the vessels used in the current study because of poor or nonexistent electromechanical coupling (*i.e.*, lack of correlation between the absolute magnitude of E_m and reduction of VSM contractile force). However, in a previous study using similar vessels,[17] we observed a 3–4% change in active force generation per millivolt change in VSM E_m. Similarly, Siegel *et al.*[18] observed a 50% relaxation that was coupled to a 2.5-mV hyperpolarization. The regulation of sustained VSM tone in these vessels depends on influx of extracellular Ca^{2+} through voltage-dependent Ca^{2+} channels, which in turn depends on E_m. Nelson and Quayle[19] have shown as much as a twofold

Table 5. Effect of 1.0 MAC Inhaled Isoflurane on Mean Arterial Blood Pressure

Experimental Group	Innervated Control	Denervated Control	ISO	POST
WKY				
Innervated	80 ± 11	—	57 ± 6†	88 ± 14
Denervated	90 ± 13	91 ± 15	66 ± 7†	93 ± 19
SHR				
Innervated	133 ± 14	—	79 ± 8†	133 ± 25
Denervated	148 ± 17	137 ± 15*	86 ± 18†	133 ± 22*

Innervated control = control (before denervation or isoflurane); denervated control = after local chemical sympathetic denervation with 6-OHDA; ISO = during 1.0 MAC (1.5%) inhaled isoflurane; POST = after elimination of isoflurane.

Values are MAP ± SD (mmHg); n = 8 or 9 vessel/animal preparations.

* Different from innervated control ($P \leq 0.05$).

† Different from innervated and denervated control and POST conditions ($P \leq 0.05$).

change in Ca^{2+} influx with a 3-mV change in VSM E_m. Thus, even relatively small E_m responses to isoflurane (tables 2 and 4) can be coupled to significant changes in VSM tone in small resistance- and capacitance-regulating vessels.

Earlier studies[23] indicate that a decrease in the magnitude of VSM E_m depends on age in small mesenteric vessels of SH rats compared with WKY rats. This has been attributed to an age-dependent increase in sympathetic neural tone in the SH rat vessels that is significantly greater when the animals are aged 12 weeks. In the current study, VSM E_m in innervated SH rats tended to be less polarized than in innervated WKY rats. However, the difference was not statistically significant. This may be related to smaller differences in VSM E_ms in the younger animals used in the current study. Nevertheless, in the current study, *in situ* local chemical sympathetic denervation with 6-hydroxydopamine caused a significant hyperpolarization of VSM in both small mesenteric resistance- and capacitance-regulating blood vessels in the neurogenic SH rat model but not in its normotensive WKY control (table 1). Such relatively greater VSM hyperpolarization in SH rats after sympathetic denervation corresponds with our earlier E_m measurements made in several vascular beds.[17,23] These results correspond with the large amount of existing evidence to support an enhanced sympathetic neural input to both resistance- and capacitance-regulating vessels in the SH rats and in humans with essential hypertension.[22]

Compared with the action of isoflurane on VSM, both 1.5% inhaled and 0.6 mM superfused concentrations significantly hyperpolarized VSM of mesenteric resistance- and capacitance-regulating blood vessels in both the SH rat model and in its normotensive WKY control. Such hyperpolarization occurred in both innervated and locally denervated vessels (tables 2 and 4). These results are consistent with the well-demonstrated vasodilator properties of volatile anesthetics.[14]

The significantly greater hyperpolarization responses to inhaled isoflurane in innervated vessels compared with respective denervated vessels (except for the WKY artery; tables 2 and 4) correspond with our previous *in situ* measurements in these types of vessels in normotensive Sprague–Dawley rats.[16] These results suggest sympathetic neural and other neural or nonneural sites for the vasodilator action of the inhaled volatile anesthetic.[16] In addition, the significantly greater hyperpolarization response to superfused isoflurane in sympathetically innervated *versus* denervated vessels (table 4) indicates an inhibition of sympathetic neural control of VSM tone at the level of the neuromuscular junction. It is well known that volatile anesthetics can attenuate sympathetic neural control of VSM tone through inhibitory actions at central[10] and peripheral[27,28] neural loci.

Of particular interest in the current study is that the VSM hyperpolarization response to inhaled isoflurane is significantly greater in the sympathetically innervated small mesenteric arteries and veins of SH rats when compared with the hyperpolarization response in respective WKY vessels (table 2). Such differences in hyperpolarization were not observed in the respective SH and WKY rat vessels when they were superfused with isoflurane (table 4).

These results can be interpreted in at least two ways. First, the greater hyperpolarization response to inhaled isoflurane in innervated SH rat vessels could result from the greater reduction in the transmural pressure gradient (*i.e.*, a reduction in a myogenic stimulus). The 1.5% inhaled isoflurane produced a significantly greater depressor effect in SH compared with WKY rats (table 5). Superfused isoflurane had no significant effect on blood pressure in either SH or WKY rats. However, such a myogenic mechanism could not explain the equality of the hyperpolarization response to inhaled isoflurane in denervated SH and WKY rat vessels (table 2). In these studies, a greater depressor effect was still evident in SH rats. In addition, such a proposed mechanism could not account for the generation of significant hyperpolarization responses to superfused isoflurane (table 4) in the absence of an anesthetic-induced depressor effect in either SH or WKY rats.

A second, more plausible explanation for the greater hyperpolarization response to inhaled isoflurane in innervated SH rat small vessels is a greater neurally mediated attenuation of VSM tone (compared with the WKY control) through a primary action at a central rather than peripheral neural site. This suggestion is also supported by the tendency for a greater hyperpolarization response to inhaled versus superfused isoflurane in respective sympathetically innervated SH rat vessels. These results correspond with the findings of many studies by Chalmers *et al.*,[29] who have shown that SH rats exhibit increased neural activity in a bulbospinal sympathoexcitatory pathway originating in the rostral ventrolateral medulla. This is of interest because there is strong evidence that an elevated sympathetic nervous input contributes to essential human hypertension.[30,31]

We acknowledge that there is disagreement about the validity of the SH rat as a model of human essential hypertension and about which strain of rat is an appro-

priate control. We clearly recognize that other models exist. However, although there may be no single best rat model for hypertension studies, the genetic hypertension that develops in the SH rat has many similarities to human essential hypertension, and there is also considerable support for its use as an appropriate model of the human condition.[21] Other investigators have shown that hemodynamic responses to halothane, enflurane,[20] and isoflurane[32] are different in SH animals compared with the WKY controls.

In a separate study, we found that specific K^+_{Ca} or K^+_{ATP} channel blockers could inhibit isoflurane-mediated hyperpolarization of VSM in small mesenteric vessels of Sprague–Dawley rats.[33] These results correspond with others showing that most vasodilator agents are VSM membrane K^+ channel openers that inhibit Ca^{2+} influx and produce a tight electromechanical coupling.[18,34,35] They also correspond with the demonstrated direct effect of volatile anesthetics to produce VSM relaxation by decreasing intracellular free Ca^{2+}.[36,37]

The similar isoflurane-induced *in situ* VSM hyperpolarization response observed in respective sympathetically denervated SH and WKY rat small mesenteric vessels suggests similar sites and mechanisms that may involve activation of VSM K^+ channels by volatile anesthetics. Using whole-cell and patch-clamp measurements, Rusch *et al.*[38] have shown a greater K^+ current in VSM cells from aortic, renal, and femoral arteries of SH rats compared with WKY animals. They attributed this difference to a compensatory increase in membrane density and Ca^{2+} sensitivity of the K^+_{Ca} channel in the VSM membranes of these SH rat arteries to blunt a myogenic response to an elevated arterial pressure. If VSM in SH rats in the current study exhibited such an increased K^+_{Ca} channel membrane density and Ca^{2+}-sensitivity, greater isoflurane-mediated hyperpolarization might be expected. The observed lack of such an effect in the mesenteric arteries may result from the minimal myogenic sensitivity of the VSM rats in these vessels to an increased transmural pressure.[39] Equal hyperpolarizing responses to isoflurane also may be due to the relatively small voltage sensitivity of the K_{Ca} channel at the *in situ* E_m levels that exist in the denervated VSM of SH and WKY rat vessels.

Finally, a potential limitation of the current investigation is the study of the volatile anesthetic (isoflurane) in animals in which basal anesthesia was induced using pentobarbital–ketamine. Although it is unlikely that the single intraperitoneal injection of ketamine at the beginning of each experiment would have any lasting effect, all VSM E_m measurements were attained in the presence of a basal anesthetic maintained with intravenous sodium pentobarbital. Nevertheless, this basal anesthetic has been used extensively in the past because it does not eliminate neural regulation of VSM tone or the VSM responses to agonists.[17,22,23,40] Furthermore, a minimal and relatively constant level of the basal anesthetic was present during all steps in each experimental protocol. Thus, any E_m changes attributable to the pentobarbital–ketamine basal anesthesia should have been consistent during the study.

In conclusion, an important factor contributing to the increased hypotensive effect and circulatory instability of volatile anesthetics in the SH rat model of hypertension is a VSM hyperpolarization (coupled to a reduced VSM tone) in both small resistance-regulating arteries and capacitance-regulating veins. Although similar differences may exist in the regulation of other vessel types as well, this remains to be established. A primary mechanism underlying such hyperpolarization in resistance and capacitance vessels is a central inhibition of excitatory sympathetic neural input. Peripheral neural and nonneurally mediated hyperpolarization by isoflurane is similar in VSM of respective SH and WKY rat vessels.

The authors thank Anita Tredeau for help in preparing the manuscript.

References

1. Longnecker DE: Alpine anesthesia: Can pretreatment with clonidine decrease the peaks and valleys? ANESTHESIOLOGY 1987; 67:1–2

2. Stone JG, Foex P, Sear JW, Johnson LL, Khambatta HJ, Triner L: Myocardial ischemia in untreated hypertensive patients: Effect of a single small oral dose of a beta-adrenergic blocking agent. ANESTHESIOLOGY 1988; 68:495–500

3. Estafanous FG: Hypertension in the surgical patient: Management of blood pressure and anesthesia. Cleve Clin J Med 1989; 56:385–93

4. Goldman L, Caldera DL: Risks of general anesthesia and elective operation in the hypertensive patient. ANESTHESIOLOGY 1979; 50:285–92

5. Nilsson H, Folkow B: Vasoconstrictor nerve influence on isolated mesenteric resistance vessels from normotensive and spontaneously hypertensive rats. Acta Physiol Scand 1982; 116:205–8

6. Luscher TF, Vanhoutte PM: Endothelium-dependent contractions to acetylcholine in the aorta of the spontaneously hypertensive rat. Hypertension 1986; 8:344–8

7. England SK, Wooldridge TA, Stekiel WJ, Rusch NJ: Enhanced single-channel K+ current in arterial membranes from genetically hypertensive rats. Am J Physiol 1993; 264:H1337–45

8. Marin J: Mechanisms involved in the increased vascular resistance in hypertension. J Auton Pharmacol 1993; 13:127–76

9. Seagard JL, Hopp FA, Donegan JH, Kalbfleisch JH, Kampine JP: Halothane and the carotid sinus reflex: Evidence for multiple sites of action. ANESTHESIOLOGY 1982; 57:191–202

10. Poterack KA, Kampine JP, Schmeling WT: Effects of isoflurane, midazolam, and etomidate on cardiovascular responses to stimulation of central nervous system pressor sites in chronically instrumented cats. Anesth Analg 1991; 73:64-75

11. Schmeling WT, Farber NE: Anesthetic actions on cardiovascular control mechanisms in the central nervous system. Adv Pharmacol 1994; 31:617-42

12. Meyer J, Lentz CW, Herndon DN, Nelson S, Traber LD, Traber DL: Effects of halothane anesthesia on vasoconstrictor response to NG-nitro-L-arginine methyl ester, an inhibitor of nitric oxide synthesis, in sheep. Anesth Analg 1993; 77:1215-21

13. Nakamura K, Terasako K, Toda H, Miyawaki I, Kakuyama M, Nishiwada M, Hatano Y, Mori K: Mechanisms of inhibition of endothelium-dependent relaxation by halothane, isoflurane, and sevoflurane. Can J Anaesth 1994; 41:340-6

14. Longnecker DE: Effects of general anesthetics on the microcirculation. Microcirc Endothelium Lymphatics 1984; 1:129-50

15. Bosnjak ZJ: Ion channels in vascular smooth muscle. Physiology and pharmacology. ANESTHESIOLOGY 1993; 79:1392-401

16. Yamazaki M, Stekiel TA, Bosnjak ZJ, Kampine JP, Stekiel WJ: Effects of volatile anesthetic agents on in situ vascular smooth muscle transmembrane potential in resistance- and capacitance-regulating blood vessels. ANESTHESIOLOGY 1998; 88:1085-95

17. Stekiel WJ: Electrophysiological mechanisms of force development by vascular smooth muscle membrane in hypertension, Blood Vessel Changes in Hypertension: Structure and Function. Edited by RMKW Lee. Boca Raton, FL, CRC Press, 1989, pp 127-70

18. Siegel G, Emden J, Wenzel K, Mironneau J, Stock G: Potassium channel activation in vascular smooth muscle. Adv Exp Med Biol 1992; 311:53-72

19. Nelson MT, Quayle JM: Physiological roles and properties of potassium channels in arterial smooth muscle. Am J Physiol 1995; 268:C799-822

20. Miller ED Jr, Beckman JJ, Althaus JS: Hormonal and hemodynamic responses to halothane and enflurane in spontaneously hypertensive rats. Anesth Analg 1985; 64:136-42

21. Frohlich ED: Is the spontaneously hypertensive rat a model for human hypertension? J Hypertens Suppl 1986; 4:S15-9

22. Arnolda L, Wang HH, Minson J, Llewellyn-Smith I, Suzuki S, Pilowsky P, Chalmers J: Central control mechanisms in hypertension. Aust N Z J Med 1997; 27:474-8

23. Stekiel WJ, Contney SJ, Lombard JH: Small vessel membrane potential, sympathetic input, and electrogenic pump rate in SHR. Am J Physiol 1986; 250:C547-56

24. Drummond JC: MAC for halothane, enflurane, and isoflurane in the New Zealand white rabbit: And a test for the validity of MAC determinations. ANESTHESIOLOGY 1985; 62:336-8

25. Honemann CW, Washington J, Honemann MC, Nietgen GW, Durieux ME: Partition coefficients of volatile anesthetics in aqueous electrolyte solutions at various temperatures. ANESTHESIOLOGY 1998; 89:1032-5

26. Aprigliano O, Rybarczyk KE, Hermsmeyer K, Van Orden LS: Adrenergic neuronal degeneration induced in portal vein and caudal artery by 6-hydroxydopamine in vitro. J Pharmacol Exp Ther 1976; 198:578-88

27. 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 1984; 63:1059-64

28. Boban N, McCallum JB, Schedewie HK, Boban M, Kampine JP, Bosnjak ZJ: Direct comparative effects of isoflurane and desflurane on sympathetic ganglionic transmission. Anesth Analg 1995; 80:127-34

29. Chalmers J, Arnolda L, Llewellyn-Smith I, Minson J, Pilowsky P: Central control of blood pressure, Textbook of Hypertension. Edited by JD Swales. Oxford, Blackwell Scientific Publications, 1994, pp 409-26

30. Esler MD: Catecholamines and essential hypertension. Baillieres Clin Endocrinol Metab 1993; 7:415-38

31. Floras JS, Hara K: Sympathoneural and haemodynamic characteristics of young subjects with mild essential hypertension. J Hypertens 1993; 11:647-55

32. Seyde WC, Durieux ME, Longnecker DE: The hemodynamic response to isoflurane is altered in genetically hypertensive (SHR), as compared with normotensive (WKY), rats. ANESTHESIOLOGY 1987; 66: 798-804

33. Kokita N, Stekiel TA, Yamazaki M, Bosnjak ZJ, Kampine JP, Stekiel WJ: Potassium channel-mediated hyperpolarization of mesenteric vascular smooth muscle by isoflurane. ANESTHESIOLOGY 1999 90: 779-88

34. Siegel G: Vascular smooth muscle, Comprehensive Human Physiology: From Cellular Mechanisms to Integration. Edited by R Greger, U Windhorst. Berlin, Springer-Verlag, 1996, pp 1941-64

35. Karaki H, Ozaki H, Hori M, Mitsui-Saito M, Amano K, Harada K, Miyamoto S, Nakazawa H, Won KJ, Sato K: Calcium movements, distribution, and functions in smooth muscle. Pharmacol Rev 1997; 49:157-230

36. Sill JC, Ozhan M, Nelson R, Uhl C: Isoflurane-, halothane-, and agonist-evoked responses in pig coronary arteries and vascular smooth muscle cells, Mechanisms of Anesthetic Actions in Skeletal, Cardiac and Smooth Muscle. Edited by TJJ Blanck, DM Wheeler. New York, Plenum, 1991, pp 257-69

37. Sill JC, Eskuri S, Nelson R, Tarara J, Van Dyke RA: The volatile anesthetic isoflurane attenuates Ca++ mobilization in cultured vascular smooth muscle cells. J Pharmacol Exp Ther 1993; 265:74-80

38. Rusch NJ, Liu Y, Pleyte KA: Mechanisms for regulation of arterial tone by Ca2+-dependent K+ channels in hypertension. Clin Exp Pharmacol Physiol 1996; 23:1077-81

39. Stekiel WJ, Meyers K, Monos E, Lombard J: Stretch-dependent tone in small mesenteric and gracilis muscle arteries from spontaneous (SHR) and volume-expended hypertensive rats, Resistance Arteries. Edited by W Halpern. New York, Perinatology Press, 1988, pp 342-50

40. Stekiel WJ, Contney SJ, Lombard JH: Sympathetic neural control of vascular muscle in reduced renal mass hypertension. Hypertension 1991; 17:1185-91