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Potassium Channel–mediated Hyperpolarization of Mesenteric Vascular Smooth Muscle by Isoflurane

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Background: **A primary source of calcium (Ca^{2+}) necessary for excitation contraction in vascular smooth muscle (VSM) is influx *via* voltage-dependent Ca^{2+} channels. Thus, force generation in VSM is coupled closely to resting transmembrane potential, which itself is primarily a function of potassium conductance. Previously, the authors reported that volatile anesthetics hyperpolarize VSM of small mesenteric resistance arteries and capacitance veins. The current study was designed to determine whether isoflurane-mediated hyperpolarization is the result of specific effects on one or more of four types of potassium channels known to exist in VSM.**

Methods: **Transmembrane potentials (E_m) were recorded from *in situ* mesenteric capacitance and resistance vessels in Sprague-Dawley rats weighing 250–300 g. In separate experiments, selective inhibitors of each of four types of potassium channels known to exist in VSM were administered in the superfusate of the vessel preparations to assess their effects on isoflurane-mediated hyperpolarization.**

Results: **Resting VSM E_m ranged from −38 to −43 mV after local sympathetic denervation. Isoflurane produced a significant hyperpolarization (2.7–4.3 mV), whereas each potassium channel inhibitor significantly depolarized (2.8–8.5 mV) the VSM. Both 100 nM iberiotoxin (inhibitor of high conductance calcium-activated potassium channels) and 1 μM glybenclamide (inhibitor of adenosine triphosphatase–sensitive potassium channels) significantly inhibited VSM hyperpolarization induced by 1 MAC (minimum alveolar concentration) levels of inhaled isoflurane (0.1–0.9 mV E_m change, which was not significant). In contrast, isoflurane hyperpolarized the VSM significantly despite the presence of 3 mM 4 aminopyridine (inhibitor of voltage-dependent potassium channels) or 10 μM barium chloride (an inhibitor of inward rectifier potassium channels) (3.7–8.2 mV change in VSM E_m).**

Conclusions: **These results suggest that isoflurane-mediated hyperpolarization (and associated relaxation) of VSM can be attributed in part to an enhanced (or maintained) opening of calcium-activated and adenosine triphosphate-sensitive potassium channels but not voltage-dependent or inward rectifier potassium channels. (Key words: Membrane potential; mesenteric capacitance; mesenteric resistance; peripheral circulation; vascular control.)**

THE vasodilating properties of volatile anesthetics are well recognized.[1–3] Anticipation and management of this side effect has become a standard of clinical practice. Nevertheless, the mechanisms by which these agents attenuate vascular tone is not completely understood. With recent increases in the understanding of factors responsible for vascular smooth muscle (VSM) contraction and its regulation, many new mechanisms of control of VSM tone (including neural, endothelial, and intrinsic) have been reported. Each of these represents a potential specific site of anesthetic action that may contribute to vasorelaxation. One common pathway by which anesthetics produce VSM relaxation is a decrease in intracellular activator calcium (Ca^{2+}) available for excitation-contraction.[4–6] Under normal conditions, the transmembrane potential (E_m) of VSM cells is less negative (approximately −35 to −55 mV) than that typically observed in skeletal muscle and nerve tissue (−70 to −80 mV).[7] For

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this range, conductance through voltage-sensitive Ca^{2+} channels has been shown to be an exponential function of VSM E_m. Consequently, even small changes in VSM E_m can significantly affect Ca^{2+} influx.[8] The VSM E_m itself is determined principally by potassium conductance.[9] Thus, potassium conductance greatly influences Ca^{2+} influx and therefore VSM tone. We[10] and others[9,11] have shown such coupling of VSM E_m with active Ca^{2+}-dependent VSM force generation. Because Ca^{2+} influx *via* voltage-dependent channels represents such a principal source of activator Ca^{2+} available for excitation-contraction coupling,[9,11] changes in the regulation of potassium current, the major determinant of VSM E_m (and therefore of voltage-dependent Ca^{2+} channels) would be expected to affect vascular tone significantly.[9,11] We recently reported that volatile anesthetics produce VSM hyperpolarization in small mesenteric resistance arteries and capacitance veins by reducing neural and nonneural mechanisms involved in the regulation of VSM E_m.[12] Furthermore, in other tissues, volatile anesthetics have been shown to produce effects consistent with enhanced potassium channel activation.[13] Therefore, our hypothesis for the current study was that direct (*i.e.*, neurally independent) effects of volatile anesthetics on the peripheral vasculature include enhanced activation of VSM potassium channels leading to hyperpolarization, a consequent decrease in activator Ca^{2+}, and reduced VSM tone. To test this hypothesis, we used a preparation similar to that previously described[12] to measure the effects of 1 minimum alveolar concentration (MAC) inhaled isoflurane (as a representative volatile anesthetic) on *in situ* VSM E_m. Measurements were taken in the presence and absence of selective inhibitors of each of four major types of potassium channels: high conductance calcium-activated (K_{Ca}), voltage-dependent (K_V), ATP-sensitive (K_{ATP}), and inward rectifier (K_{IR}).[9]

Methods

Experimental Preparation

After we received approval from our institutional animal care and use committee, Sprague-Dawley SD male rats weighing 250–350 g (Harlan Sprague-Dawley, Indianapolis, IN) were prepared for VSM E_m measurement as described before.[12] Briefly, the animals were sedated with a single dose of intraperitoneal ketamine (40 mg/kg) followed by anesthetic induction with intraperitoneal pentobarbital (20 mg/kg). Surgical preparation consisted of femoral arterial and venous cannulation, a midline laparotomy incision, and a tracheotomy through which ventilation was controlled using a model 680 rodent respirator (Harvard Apparatus Co., South Natick, MA). Respiratory rate and tidal volume were adjusted to maintain the end-tidal carbon dioxide level between 30 and 40 mmHg. Basal anesthesia was maintained throughout the experiments with 10-mg/kg intravenous boluses of pentobarbital at 1-h intervals.

After surgery, the animals were placed on a temperature-regulated movable microscope stage mounted on a Micro-G vibration-free table (Technical Manufacturing Co., Woburn, MA). Small (200–300 μm) paired mesenteric arteries and veins were exposed through the midline laparotomy. After gently dissecting perivascular fat (without disturbing luminal blood flow or adventitial innervation), the connective tissue surrounding these vessels was fastened to the silastic rubber floor of a temperature-regulated tissue chamber using 50 and 125-μm-diameter stainless steel pins. This technique minimized movement artifact. The vessels were superfused continuously with a physiologic salt solution (PSS) composed of 119 mM NaCl, 4.7 mM KCl, 1.17 mM $MgSO_4$, 1.6 mM $CaCl_2$, 24 mM $NaHCO_3$, 1.18 mM NaH_2PO_4, and 0.026 mM EDTA. The PSS was maintained at body temperature and aerated continuously using a gas mixture of nitrogen, oxygen, and carbon dioxide to maintain the *p*H level between 7.35 and 7.45, the carbon dioxide tension between 35 and 45 mmHg, and the oxygen tension between 75 and 100 mmHg.

Vascular Smooth Muscle Transmembrane Potential Measurements

Single-cell *in situ* VSM E_m values were measured by advancing glass micropipettes (with a tip diameter of approximately 0.1 μm and an impedance ranging between 40 and 60 MΩ) into the VSM layer using a Wells hydraulic micromanipulator (Trent Wells, Coulterville, CA). The microelectrodes were pulled from borosilicate glass using a model P-97 Flaming-Brown micropipette puller (Sutter Instrument Co., Novato, CA). The electrodes were connected to a model 8100 biological amplifier (Dagan Corp., Minneapolis, MN). VSM E_m values were recorded simultaneously with blood pressure on a Grass Model RPS7C polygraph (Astro-Med/Grass, West Warwick, RI) and digitally using a Superscope II (version 1.44) data acquisition system (GW Instruments Co., Som-

erville, MA). Before VSM E_m measurements were made, local sympathetic innervation of the vessel preparation was eliminated at the level of the neuromuscular junction by destroying norepinephrine-containing vesicles within the presynaptic terminal. This was accomplished by superfusing with 300 μg/ml 6-hydroxydopamine for 20 min. Aprigliano *et al.*[14] reported that this technique produces effective perivascular sympathetic denervation (*i.e.*, inhibition of contractile responses to field simulation, blockade of H^3 norepinephrine uptake by sympathetic nerve endings, and histologic changes consistent with adrenergic nerve terminal degeneration). Before denervation using this technique, the preparation was pretreated with PSS containing 10^{-6} M phentolamine for 5 min to inhibit the vasoconstriction by catecholamines locally released by the 6-hydroxydopamine treatment.[15]

Experimental Design

The design of this study was to determine the effect of the following potassium channel antagonists on isoflurane-mediated VSM hyperpolarization[12] in separate experiments: 100 nM iberiotoxin for high-conductance K_{Ca} channel inhibition, 3 mM 4-aminopyridine for K_V channel inhibition, 10 μM $BaCl_2$ for K_{IR} channel inhibition, and 1 μM glybenclamide for K_{ATP} channel inhibition. These represent working concentrations in PSS and are all between 0.5 and 2 orders of magnitude greater than those reported to produce half block of each of the respective channels.[9] Iberiotoxin (Research Biochemicals International, Boston, MA), 4-aminopyridine (Sigma Chemical Co., St. Louis, MO), and $BaCl_2$ (Aldrich Chemical Co., Milwaukee, WI) all were prepared from 100 μM, 3 mM, and 10 mM stock solutions, respectively, in distilled water. Glybenclamide (Sigma Chemical Co.) was prepared from a 10^{-3} mM stock solution in 0.01 N NaOH. Each of the potassium channel inhibitors was administered to separate vessel preparations by local superfusion with PSS in the presence and absence of 1.5% inhaled isoflurane, which corresponds to 1 MAC.[16] Two different four-step experimental protocols were used with each potassium channel inhibitor to verify that the order in which the inhibitor and isoflurane were administered did not affect the results. A single experiment consisted of sequential arterial and venous E_m and blood pressure measurements made in an individual animal preparation during each of the four steps in a protocol. For protocol 1, the individual sequential steps (experimental conditions) consisted of sympathetically denervated control, locally superfused potassium channel inhibitor, locally superfused potassium channel inhibitor plus administration of 1 MAC inhaled isoflurane, and washout (*i.e.*, elimination of potassium channel inhibitor from the PSS superfusate and isoflurane from the circulation). Individual steps for protocol 2 were sympathetically denervated control, 1.5% inhaled isoflurane, local superfusion with potassium channel inhibitor (after elimination of isoflurane from the circulation), and local superfusion with potassium channel inhibitor in the presence of 1 MAC inhaled isoflurane administered again. A 20-min equilibration period was allowed between each successive step in each protocol. A group of eight experimental animals was used for each of the two protocols using each of the four channel inhibitors (for a total of 64 experimental animals).

Inhaled Isoflurane Administration

Throughout every experiment, animals breathed an oxygen–nitrogen mixture to keep the inspired oxygen concentration at 30% (thus avoiding any potential hypoxemia during controlled ventilation). Isoflurane was administered *via* an Ohio Medical Products vaporizer (AirCo, Madison, WI). End-tidal isoflurane and carbon dioxide concentrations were verified using a POET 2 infrared capnograph and end-tidal agent monitor (Criticare Systems, Waukesha, WI). Experimental blood concentrations of isoflurane were measured from samples taken immediately before and again after washout periods using a Sigma model 38 gas chromatograph (Perkin-Elmer Co., Norwalk, CT).

Statistical Analysis

For arteries and veins in an experimental preparation, each recorded E_m during each of the four protocol steps was the numeric average of five sequential, stable single VSM cell impalements that lasted at least 6 s. Similarly, the recorded mean arterial blood pressure during each of the protocol steps in an experimental preparation was the numeric average of simultaneously measured mean arterial blood pressure during each of the previously described E_m measurements. The E_m and mean arterial blood pressure values reported in the data tables are the means ± SD of these numeric average values, with a replication factor of eight animals for each of the four potassium channel inhibitors and each protocol. These average E_m values were also used to calculate mean changes in E_m (*i.e.*, Δ E_m values) in response to the administration of potassium channel inhibitors and

isoflurane. A one-way analysis of variance for repeated measures was performed on the means of the average E_m and mean arterial blood pressure values for each protocol step. The levels of the repeated (within) factor were defined by the experimental conditions for each of the four sequential protocol steps. Mean ΔE_m values were analyzed by simple *t* tests to determine their significance from 0. The changes that were significant were compared with each other in a simple one-way analysis of variance. For each of the four groups of potassium channel inhibitor experiments in protocol 2, the mean isoflurane concentration in blood was determined for the second, third, and fourth sequential protocol steps (*i.e.*, during inhaled isoflurane alone, during superfused potassium channel inhibitor after isoflurane washout, and during superfused potassium channel inhibitor plus inhaled isoflurane). Accordingly, a one-way analysis of variance was used to compare mean isoflurane concentrations in blood during these steps. In protocol 1, isoflurane concentrations were measured only in the third and fourth sequential steps (*i.e.*, potassium channel inhibitor plus inhaled isoflurane and washout). Therefore, for this protocol a simple *t* test was used to compare the mean isoflurane concentrations in blood for the two steps.

All analyses of variance in this study were calculated using the superANOVA program (Abacus Concepts, Berkeley, CA). The program determined significance of differences between mean values by comparing calculated "least square means" at a significance level of $P \leq 0.05$. The *t* tests in the current study were calculated using the Stat-View program (Abacus Concepts) and the significance level of $P \leq 0.05$ was used to define the significance of differences between mean values.

Results

Vascular Smooth Muscle Transmembrane Potentials in Protocol 1

These data are summarized in tables 1 and 2. For both arteries and veins, superfusion of the vessel preparations with each of the four types of potassium channel inhibitors significantly depolarized VSM relative to the denervated control condition. However, despite such depolarization by K_V channel inhibition with 3 mM 4-aminopyridine or by K_{IR} channel inhibition with 10 μM barium chloride, administration of 1.5% inhaled isoflurane induced a significant VSM hyperpolarization in both arteries and veins when calculated as the mean E_m difference between isoflurane plus potassium channel inhibitor *versus* channel inhibitor alone (table 2). In addition, the VSM hyperpolarization was significant even when calculated (table 1) as the difference between E_m means during isoflurane plus potassium channel inhibitor *versus* E_m means during the control before potassium channel inhibition (except for 4-aminopyridine in the vein preparation). In sharp contrast to results obtained with channel inhibition by 4-aminopyridine or $BaCl_2$, the isoflurane-mediated hyperpolarization was abolished completely in artery and vein preparations during K_{Ca} channel inhibition with 100 nM iberiotoxin and during K_{ATP} channel inhibition with 10 μM glybenclamide (tables 1 and 2). In all groups in protocol 1, washout of potassium channel inhibitor, isoflurane, or both returned the average VSM E_m to preinhibitor and preisoflurane control values (table 1).

Table 1. Effects of Potassium Channel Inhibitors and Isoflurane on Vascular Smooth Muscle Transmembrane Potential in Protocol 1

K Channel Inhibitor	Control	K Channel Inhibitor	ISO and K Channel Inhibitor	Washout
Arteries				
Iberiotoxin	−40 ± 1.6	−32 ± 1.7*	−31 ± 1.9*	−39 ± 2.3
4-Aminopyridine	−38 ± 0.8	−36 ± 1.2*	−40 ± 0.7*†	−38 ± 0.8
$BaCl_2$	−39 ± 1.8	−34 ± 1.3*	−41 ± 2.0*†	−39 ± 0.7
Glibenclamide	−39 ± 1.1	−33 ± 1.2*	−34 ± 1.7*	−38 ± 1.1
Veins				
Iberiotoxin	−43 ± 2.1	−34 ± 1.7*	−35 ± 2.8*	−43 ± 2.1
4-Aminopyridine	−43 ± 6.6	−40 ± 6.9	−45 ± 6.3†	−40 ± 1.4
$BaCl_2$	−42 ± 1.8	−37 ± 3.0*	−45 ± 2.9*†	−41 ± 2.5
Glibenclamide	−43 ± 2.7	−38 ± 2.4*	−37 ± 2.4*	−42 ± 1.9

Values are mean ± SD (mV) of eight vessel/animal preparations for each experiment.

Control = after local chemical sympathetic denervation with 6-OHDA; K channel inhibitor = in the presence of 100 nM iberiotoxin, 3 mM 4-aminopyridine, 10 μM $BaCl_2$, or 1 μM glibenclamide; ISO = during 1.0 MAC (1.5%) inhaled isoflurane; Washout = after elimination of isoflurane from the circulation.

* Different from Control ($P \leq 0.05$).

† Different from K channel inhibitor ($P \leq 0.05$).

Vascular Smooth Muscle Transmembrane Potential Results from Protocol 2

These data are presented in tables 2 and 3. Consistent with our previous measurements,[12] in the absence of any potassium channel inhibitors, 1 MAC levels of inhaled isoflurane significantly hyperpolarized arterial and venous VSM in all four groups (tables 2 and 3). Also consistent with results from protocol 1 (tables 1 and 2), each of the potassium channel inhibitors significantly depolarized arterial and venous VSM

Table 2. Changes in Vascular Smooth Muscle Transmembrane Potential in Response to Potassium Channel Inhibitors and Isoflurane

	ΔE_m (ISO − Control)	ΔE_m (K channel inhibitor − Control)	ΔE_m {(ISO and K channel inhibitor) − K channel inhibitor}	
	[Protocol 2]	[Protocol 1]	Protocol 1	Protocol 2
Artery				
Iberiotoxin	−4.3 ± 1.7*	+8.5 ± 2.3*	+0.5 ± 1.9	+0.9 ± 1.0
4-Aminopyridine	−3.0 ± 1.6*	+2.3 ± 1.5*	−3.7 ± 1.0*	−4.8 ± 1.1*
$BaCl_2$	−3.4 ± 1.7*	+4.9 ± 2.7*	−7.0 ± 2.5*	−5.2 ± 1.0*
Glibenclamide	−3.3 ± 0.5*	+5.0 ± 1.1*	−0.5 ± 1.4	−0.1 ± 0.6
Vein				
Iberiotoxin	−3.4 ± 1.7*	+9.0 ± 2.1*	−0.3 ± 1.6	−0.0 ± 1.1
4-Aminopyridine	−3.9 ± 1.7*	+2.8 ± 2.1*	−4.7 ± 3.0*	−6.2 ± 2.1*
$BaCl_2$	−2.7 ± 0.8*	+5.0 ± 2.2*	−8.2 ± 2.1*	−6.4 ± 2.3*
Glibenclamide	−3.2 ± 2.3*	+5.5 ± 2.7*	+0.4 ± 1.2	−0.9 ± 1.1

Values are mean ΔE_m ± SD (mV) of eight animals for each experiment.

Control = after local chemical sympathetic denervation with 6-OHDA; K channel inhibitor = in the presence of 100 nM iberiotoxin, 3 mM 4-aminopyridine, 10 μM $BaCl_2$, or 1 μM glibenclamide; ISO = during 1.0 MAC (1.5%) inhaled isoflurane; − = hyperpolarization; + = depolarization.

* Different from zero ($P \leq 0.05$).

after washout of inhaled isoflurane from the circulation. Furthermore, in the fourth step of protocol 2, when inhaled isoflurane was readministered during superfusion of the vessel preparation with the potassium channel inhibitor, results were similar to those obtained during corresponding conditions in protocol 1 (*i.e.*, step 3). Specifically, in the presence of 3 mM 4-aminopyridine or 10 μM $BaCl_2$, readministration of inhaled isoflurane induced significant VSM hyperpolarization in both arteries and veins, respectively, which was similar to the hyperpolarization induced by isoflurane in the absence of any potassium channel inhibitor. As in protocol 1, in arteries and veins the isoflurane-mediated hyperpolarization was abolished completely in the presence of 100 nM iberiotoxin or 1 μM glybenclamide. Thus, the VSM in these groups under these conditions remained significantly depolarized compared with initial control conditions (and unchanged from the VSM E_m in the presence of either potassium channel inhibitor before isoflurane was administered again).

Mean Arterial Blood Pressure and Measured Isoflurane Concentrations in Blood

Table 4 lists the pooled mean arterial blood pressure measurements that were obtained simultaneously with

Table 3. Effects of Potassium Channel Inhibitors and Isoflurane on Vascular Smooth Muscle Transmembrane Potential in Protocol 2

K Channel Inhibitor	Control	ISO	K Channel Inhibitor	ISO and K Channel Inhibitor
Arteries				
Iberiotoxin	−38 ± 1.1	−43 ± 1.5*	−30 ± 1.8*	−29 ± 2.2*
4-Aminopyridine	−39 ± 1.0	−42 ± 1.1*	−36 ± 0.7*	−40 ± 0.9*†
$BaCl_2$	−39 ± 0.7	−42 ± 1.9*	−36 ± 1.1*	−41 ± 1.3*†
Glibenclamide	−39 ± 1.0	−42 ± 1.1*	−34 ± 0.6*	−34 ± 0.9*
Veins				
Iberiotoxin	−42 ± 3.0	−45 ± 3.1*	−32 ± 2.2*	−32 ± 2.7*
4-Aminopyridine	−42 ± 2.5	−46 ± 3.2*	−37 ± 2.7*	−43 ± 2.8†
$BaCl_2$	−42 ± 1.6	−45 ± 1.3*	−38 ± 2.3*	−45 ± 1.8*†
Glibenclamide	−42 ± 1.9	−46 ± 2.2*	−35 ± 1.4*	−36 ± 0.9*

Values are mean ± SD (mV) of eight vessel/animal preparations for each experiment.

Control = after local chemical sympathetic denervation with 6-OHDA; K channel inhibitor = in the presence of 100 nM iberiotoxin, 3 mM 4-aminopyridine, 10 μM $BaCl_2$, or 1 μM glibenclamide; ISO = during 1.0 MAC (1.5%) inhaled isoflurane.

* Different from Control ($P \leq 0.05$).

† Different from K channel inhibitor ($P \leq 0.05$).

Table 4. Effects of Isoflurane on Mean Arterial Blood Pressure

	Protocol 1				Protocol 2			
	Control	K Channel Inhibitor	ISO and K Channel Inhibitor	Washout	Control	ISO	K Channel Inhibitor	ISO and K Channel Inhibitor
Iberiotoxin	117 ± 5	120 ± 5	87 ± 4*	116 ± 3	119 ± 6	75 ± 5*	121 ± 5	75 ± 6*
4-Aminopyridine	114 ± 7	127 ± 5	95 ± 4*	118 ± 4	111 ± 8	74 ± 5*	118 ± 6	91 ± 5*
$BaCl_2$	144 ± 5	145 ± 5	91 ± 6*	136 ± 4	139 ± 3	91 ± 3*	135 ± 3	93 ± 5*
Glibenclamide	134 ± 6	130 ± 4	92 ± 3*	129 ± 5	139 ± 3	89 ± 3*	135 ± 5	90 ± 1*

Values are mean ± SD (mmHg) of eight animals for each experiment.

Control = after local chemical sympathetic denervation with 6-OHDA; K channel inhibitor = in the presence of 100 nM iberiotoxin, 3 mM 4-aminopyridine, 10 μM $BaCl_2$, or 1 μM glibenclamide; ISO = during 1.0 MAC (1.5%) inhaled isoflurane; Washout = after elimination of isoflurane from the circulation.

* Different from Control, K channel inhibitor, and (for protocol 1) washout conditions ($P \leq 0.05$).

arterial and venous VSM E_m measurements. In each of the four groups in protocol 1, the mean arterial blood pressure was reduced significantly compared with control during inhalation of 1 MAC levels of isoflurane. After washout of isoflurane from the circulation in each of the four groups, the mean arterial blood pressure returned to control levels. Similarly, in each of the four groups in protocol 2, inhaled isoflurane significantly reduced the mean arterial blood pressure by an amount similar to that observed in protocol 1, and washout of isoflurane returned mean arterial pressure to baseline control levels. Blood concentrations of isoflurane during inhalation of 1 MAC levels of this agent (mean ± SD) ranged from 0.60 ± 0.155 mM to 0.67 ± 0.071 mM. After washout, blood concentrations decreased to insignificant values ranging from 0.02 ± 0.010 mM to 0.04 ± 0.003 mM. Blood concentrations of isoflurane were consistent among the four experimental groups during inhalation and after washout from the circulation. They also correspond with values measured in our previous study.[12]

Discussion

In this study, hyperpolarization of VSM by 1 MAC inhaled isoflurane was abolished by selective inhibition of K_{Ca} and K_{ATP} channels but not K_V and K_{IR} channels. Changes in VSM E_m closely regulate Ca^{2+} influx and Ca^{2+} release from the sarcoplasmic reticulum. Both sources of Ca^{2+} are important determinants of contractility.[9,10] A reduction of intracellular activator Ca^{2+} resulting from inhaled anesthetic-induced hyperpolarization is a likely mechanism that contributes to the hypotension induced by these agents (and exists in addition to direct depression[17,18] of Ca^{2+} channels). The data from the current study suggest that the isoflurane-induced hyperpolarization (and vasorelaxation) of small mesenteric vessels are coupled to activation of VSM K_{Ca} and K_{ATP} channels (or mechanisms that regulate them) but not to K_V and K_{IR} channels. One possible explanation for this observation is that K_V and K_{IR} channels do not exist or are present in substantially reduced numbers compared with K_{Ca} and K_{ATP} channels in the VSM of the vessels used in this study. However, substantial evidence supports the existence of all four of these major types of potassium channels in VSM.[9,11,19] Although the relative proportions of these channels in VSM cells of various blood vessels is not well defined, the current study shows clearly that selective blockade of each of them leads to significant depolarization. This suggests that a sufficient density of each is present in the VSM membrane of the small mesenteric blood vessels of the rat to contribute significantly to the magnitude of the VSM membrane potentials. Nevertheless, an analysis of variance (potassium channel inhibition − control) of data from table 2 (not reported) indicated that in both arteries and veins, iberiotoxin produced greater depolarization than either glybenclamide or $BaCl_2$, both of which produced greater depolarization than 4-aminopyridine. This implies that K_{Ca} channels are of greater importance and K_V channels are of lesser importance in regulating VSM E_m and therefore VSM tone. Confirmation of this would require a more direct comparison of different types of potassium channels in the same preparation. Although they are present in VSM in many tissues,[9] precise data on the exact distribution of potassium channels are not available. Nevertheless, the relative contribution of the different potassium channels on VSM tone and the anesthetic effects on VSM probably varies considerably with vessel size and vascular bed. For example, K_{ATP} channels have been shown to be particularly prominent in coronary VSM.[20]

Accordingly, the putative K_{ATP}-mediated hyperpolar-

ization (and presumably vasodilation) resulting from isoflurane in the current study corresponds with results from several other studies and suggests similar effects in other types of blood vessels. In canine coronary arteries, the administration of glybenclamide attenuated increases in flow produced by isoflurane (measured by radioactive microspheres)[13,21] and by halothane, isoflurane, and enflurane (measured by a Doppler flow probe).[22] Glybenclamide also significantly attenuates halothane-mediated relaxation of *in vitro* rat coronary artery rings.[23] In contrast, substantially fewer data exist to support the K_{Ca}-mediated hyperpolarization by isoflurane that was observed in the current study. In concentrations reportedly low enough to selectively inhibit K_{Ca}, but not other potassium channels, tetraethylammonium tended to reduce halothane-mediated hyperpolarization of rat coronary arteries.[23] In addition, using a whole-cell patch-clamp method, isoflurane directly potentiated K_{Ca} conductance in the pig portal vein.[24] Interestingly, either iberiotoxin or glybenclamide (when administered individually) completely inhibited isoflurane-mediated hyperpolarization. If K_{Ca} and K_{ATP} channels are truly distinct and if the inhibitors are truly selective as reported,[9] then this observation suggests that isoflurane-mediated hyperpolarization involves some interdependence in the regulation of these channels. A more distinct study of just these two channels (involving more specific methods) is needed to clarify these mechanisms.

Because the current study involved an *in situ* vessel preparation in whole animals, the potential influence of the endothelium must not be excluded when the results are interpreted. It is possible that anesthetic effects of the endothelium could contribute to the potassium channel–mediated hyperpolarization of the VSM. Several vasoactive factors are known to be released from the endothelium, including the endothelins, the prostaglandins, nitric oxide, and endothelially derived hyperpolarizing factor. Most of these substances are associated directly or indirectly with alterations in ion channel function.[25] However, the anesthetic effects on these mechanisms have not been clarified. Most evidence indicates that endothelially derived hyperpolarizing factor and nitric oxide release is inhibited by isoflurane,[26,27] thus suggesting that these substances are less likely to account for the effects observed in this study. Other data, however, support isoflurane-mediated enhancement of nitric oxide,[28] and some data suggest that volatile anesthetics may stimulate release of a vasodilating prostaglandin.[29] If such anesthetic effects on endothelial factors lead to an indirect enhancement of potassium channels in the VSM, they would need to be selective for K_{Ca} and K_{ATP} channels to explain the results of the current study. We cannot exclude the possibility of such influences in an *in situ* preparation. However, in other *in vitro* preparations in which the endothelium was eliminated, significant (endothelium-independent) vasorelaxing effects of isoflurane have been shown.[30]

As indicated before, the depolarizations observed with specific potassium channel inhibitors in the current study suggest that each of the four major types of potassium channels is present and participates in the regulation of VSM E_m magnitude in small mesenteric vessels (tables 1–3). An obvious question to address is the specificity of each type of potassium channel inhibitor at the concentration used in the current study. This question was addressed in a recent review by Nelson and Quayle,[9] who summarized the properties of selective inhibitors of the four major types of potassium channels found in VSM at various sites in the peripheral circulation. Concentrations of specific potassium channel inhibitors used in the current study were 0.5 to 2 orders of magnitude greater than those required for half block of a particular type of potassium channel over the physiologic range of VSM E_m.[9] Only iberiotoxin (K_{Ca} channel inhibitor) and glybenclamide (K_{ATP} channel inhibitor) are reported to be very selective. Iberiotoxin in particular is highly selective for high-conductance K_{Ca} channels, in contrast to other types of K_{Ca} channels that reportedly exist in some tissues (but the role of which is less well defined).[31] In contrast, 4-aminopyridine (K_V channel inhibitor) and barium chloride (K_{IR} channel inhibitor) also reportedly have effects on other potassium channels. In the current study, the 10 μM barium chloride concentration used is five times greater than that required for half block of K_{IR} channels. However, this concentration is still 10 times less than that observed to affect other potassium channels.[9] Therefore, a spillover effect from this agent would be unlikely. In contrast, the 3 mM 4-aminopyridine used to block K_V channels reportedly also is sufficient to have significant effects on K_{ATP} channels.[9] However, in the current study, neither the K_V nor the K_{IR} channel blocker had any affect on the isoflurane-mediated hyperpolarization despite the fact that both of these agents reportedly can block K_{ATP} channels. Thus, at the concentrations used in the current study, these agents did not exhibit significant K_{ATP} channel cross-reactivity in the VSM of the mesenteric vessels. If such cross-reactivity with K_{ATP} channels were present, an attenuation of the isoflurane-mediated hyperpolarization would be expected because inhibition of K_{ATP} channels with glyben-

clamide significantly reduced isoflurane-mediated hyperpolarization.

The current studies were conducted with the animals maintained under a basal anesthetic. It is unlikely that the initial single dose of intraperitoneal ketamine administered at the start of each experiment had any significant persistent effect on the results, because this dose preceded any data measurements by 90 to 120 min and most likely was cleared from the circulation. Because basal anesthesia was maintained with a regular 1-h interval dose of 10 mg/kg intravenous pentobarbital, all VSM E_m and blood pressure measurements were taken within a relatively constant basal anesthetic background. Consequently, all measured changes in these dependent variables are attributed primarily to changes in the two independent variables (*i.e.*, inhaled isoflurane or potassium channel inhibitors, or both). However, barbiturates can hyperpolarize excitable membranes and produce vasorelaxation.[32] Thus, in the presence of pentobarbital anesthesia, the VSM already may have become partially hyperpolarized compared with E_m levels in the awake state. If so, the results of the current study indicate that inhaled isoflurane induced significant additional hyperpolarization in all experimental groups. Conversely, a background hyperpolarization caused by pentobarbital may have augmented the depolarization produced by the different potassium channel inhibitors (particularly if it produced hyperpolarization *via* enhanced potassium channel conductance). Recently, however (in cardiac tissue), barbiturates have been shown to attenuate rather than augment K_{IR} conductance.[33,34] If this effect also is present in VSM, it is at least possible that isoflurane-mediated activation of K_{IR} channels would be blocked in the current study by the pentobarbital, thus masking the effects of $BaCl_2$. We do not believe that this is likely, however, because all inhibitors, including $BaCl_2$, produced a significant depolarization in this study. Clearly, it is impossible to rule out completely the potentially confounding effects of a basal anesthetic on VSM E_m values measured *in situ*. However, regardless of any possible augmenting effect of pentobarbital anesthesia on the depolarization induced by potassium channel inhibitors in the current study, specific differences were observed in the effects of these inhibitors when administered concurrently with isoflurane.

The measured E_m responses in the current study provide evidence of the existence of potassium channels in VSM of small mesenteric arteries and veins that are affected by volatile anesthetics. However, additional studies will be necessary to determine the VSM membrane and intracellular mechanisms by which such volatile anesthetics enhance the activity of specific potassium channels. Several mechanisms of the regulation of K_{ATP} and K_{Ca} channels in VSM have been described and represent potential targets for anesthetic action. One that has been identified in myocytes[35] (and also may exist in VSM cells) is a G protein–mediated coupling to K_{ATP} channel activity. Cason *et al.*[36] suggested that such a mechanism may be involved in isoflurane-mediated vasodilation of coronary arteries. Volatile anesthetics have been shown to inhibit transmembrane Ca^{2+} current directly in cerebral[17] and coronary[18] VSM. This is a potential mechanism for the reduction of the intracellular activator Ca^{2+} that regulates excitation contraction coupling and, in turn, vasorelaxation. Reduction of intracellular Ca^{2+} also would be expected to reduce K_{Ca} channel activity. Direct measurements of the frequency of opening (using the patch-clamp technique) support a reduction in Ca^{2+}-dependent potassium channel activity by volatile anesthetics.[17,18] These data stand in contrast to the results of the current study, which suggest that K_{Ca} channel activity is enhanced by isoflurane. A possible cause for this discrepancy may lie in the differences in measuring techniques under *in vitro versus in situ* conditions. Other potential regulatory mechanisms of K_{Ca} channels have been described, including G protein–coupled adenylyl cyclase pathways, guanylate cyclase–mediated pathways, and direct effects of nitric oxide on the K_{Ca} channel.[9] Indirect effects of volatile anesthetics on these regulatory mechanisms in intact blood vessel preparations (compared with *in vitro* single VSM cell patch-clamp preparations in which indirect effects are reduced) also may account for such differential results.

Finally, it is possible that factors other than altered potassium channel function may contribute to the isoflurane-mediated hyperpolarization observed in the current study. Decreased blood pressure from VSM-independent effects of volatile anesthetics, such as myocardial depression, might produce stretch-dependent hyperpolarization (and relaxation) in the vasculature.[37] Similarly, potassium channel function might be enhanced indirectly by volatile anesthetics by inhibition of mechanisms that ordinarily feed back to inhibit potassium channel opening (*e.g.*, sheer stress on nitric oxide release). However, none of these potential concurrent mechanisms precludes the interpretation that a significant portion of the observed hyperpolarization results from specific actions affecting VSM K_{Ca} and K_{ATP} channels. A purely myogenic effect would not be blocked completely by individual selective potassium channel inhibitors. Similarly, if the

observed isoflurane-mediated hyperpolarization in the current study was only the result of attenuation of a negative feedback mechanism affecting potassium channel activity, it would need to be selective only for K_{Ca} and K_{ATP} channels because only iberiotoxin and glybenclamide inhibited the observed isoflurane-mediated hyperpolarization. Although not impossible, such a mechanism (specific for only two of four types of potassium channels) would be unlikely.

Regardless of whether the anesthetic effect is direct (on channel proteins) or indirect (on mechanisms regulating channel function), the results of the current study suggest that inhaled isoflurane (and presumably other volatile anesthetics) hyperpolarize (and therefore vasodilate) by the enhanced (or maintained) opening of K_{Ca} and K_{ATP} channels but not K_V and K_{IR} channels in the peripheral VSM.

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