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Isoflurane-induced Dilation of Porcine Coronary Microvessels Is Endothelium Dependent and Inhibited by Glibenclamide

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Background: **Isoflurane has been reported to cause dose-dependent constriction in isolated coronary microvessels. However, these results are inconsistent with data from *in situ* and *in vivo* heart preparations which show that isoflurane dilates the coronary vasculature. To clarify the direct effects of isoflurane on coronary tone, we measured the response of isolated porcine resistance arterioles (ID, 75 ± 4.0 μm; range, 41–108 μm) to isoflurane in the presence and absence of adenosine triphosphate–sensitive and Ca^{2+}-activated potassium channel blockers and also after endothelial removal.**

Methods: **Subepicardial arterioles were isolated, cannulated, and pressurized to 45 mmHg without flow in a 37°C vessel chamber filled with MOPS buffer (pH = 7.4). After all vessels developed spontaneous (intrinsic) tone, dose-dependent (0.17–0.84 mM; approximately 0.5–2.5 minimum alveolar concentration) isoflurane-mediated effects on vessel ID were studied in the presence and absence of extraluminal glibenclamide (1 μm; an adenosine triphosphate–sensitive channel blocker) or iberiotoxin (100 nM; a Ca^{2+}-activated potassium channel blocker) or before and after endothelial denudation using the nonionic detergent CHAPS (0.4%). Vessel ID was measured using an inverted microscope and videomicrometer, and vasomotor responses were analyzed by normalizing changes in arteriole ID to the dilation observed after exposure to 10^{-4} M sodium nitroprusside, which causes maximal dilation.**

Results: **Isoflurane caused dose-dependent dilation of all coronary arterioles. This vasodilation was 6.0 ± 0.7 μm at an isoflurane concentration of 0.16 mM (approximately 0.5 minimum alveolar concentration) and 25.3 ± 2.1 μm at 0.75 mM (approximately 2.5 minimum alveolar concentration). These values represent 18.1 ± 1.7% and 74.1 ± 3.3%, respectively, of that observed with 10^{-4} sodium nitroprusside (34 ± 3 μm). Glibenclamide, but not iberiotoxin, exposure affected arteriolar dilation in response to isoflurane. Glibenclamide caused a downward displacement of the isoflurane dose–response curve, reducing isoflurane-mediated dilation by an average of 36%. Denuded arterioles showed a marked (approximately 70%) reduction in their ability to dilate in response to isoflurane.**

Conclusions: **The authors conclude that isoflurane dilates coronary resistance arterioles in a dose-dependent manner, and that this dilation is partially mediated by adenosine triphosphate–sensitive channels and is highly dependent on the presence of a functioning endothelium.**

ISOFLURANE has marked effects on blood flow and vascular resistance in many tissues, including the heart.[1–5] However, the primary mechanisms responsible for the direct effect of isoflurane on coronary vascular resistance, and indeed the nature of the vasomotor response of coronary microvessels to this volatile anesthetic, are still in question. *In vivo* and *in situ* studies that examined the effects of isoflurane on coronary perfusion indicate that isoflurane increases coronary blood flow[4–11] and dilates epicardial coronary vessels (20–450-μm ID),[12] strongly implying that resistance in the smallest coronary arterioles must therefore be decreased. Based on these data, isoflurane is widely regarded as a coronary vasodilator. Despite this evidence, several *in vitro* studies of cannulated microvessels[13–16] report that isoflurane is a potent vasoconstrictor of coronary resistance arterioles. These results are inconsistent with the preponderance of data from the aforementioned *in vivo* studies as well as a recent *in vitro* coronary microvessel study (Zhou *et al.*[17]), and have lead to the publication of several editorials and letters of correspondence.[18–20]

Because the response of coronary resistance arterioles to isoflurane is paramount to our understanding of how this volatile anesthetic affects myocardial blood flow, it is imperative to clarify whether isoflurane directly constricts or dilates these vessels. In this study we used small swine coronary arterioles (75 ± 4.0 μm) that developed spontaneous (intrinsic) tone and were not subject to preconstriction or predilation, to examine the dose-dependent effect of clinically relevant concentrations of isoflurane on coronary microvessel diameter. In addition, to address the question of mechanisms of isoflurane-induced vasodilation in these vessels, we: (1) measured the dose–response of coronary arterioles to isoflurane in the presence and absence of glibenclamide, a blocker of adenosine triphosphate–sensitive potassium (K_{ATP}) channels, and iberiotoxin, a blocker of Ca^{2+}-activated potassium (K_{Ca}) channels; and (2) examined the role of the endothelium in the pharmacologic response to isoflurane by measuring vasomotor responses in vessels before and after endothelial denudation. This study clearly demonstrates that isoflurane-mediated dilation of coronary microvessels is not dependent on pharmacologic preconstriction and provides further evi-

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dence that dilation is the predominant response of coronary arterioles to isoflurane.

Materials and Methods

General Preparation

This experimental protocol was approved by the Laboratory Animal Care Committee at Texas A&M University (College Station, TX) and followed the guidelines for animal use published by the American Physiologic Society. Pigs (n = 22) of either sex that weighed 7–11 kg were sedated with an intramuscular injection of tiletamine and zolazepam (1:1, 4.4 mg/kg) and xylazine (2.2 mg/kg), anesthetized with pentobarbital (20 mg/kg), and injected with heparin (1,000 U/kg) *via* a marginal ear vein. After tracheotomy and intubation, the animals were ventilated with room air. A left thoracotomy was performed, and the heart was electrically fibrillated, excised, and placed in cold (5°C) saline.

Isolation and Cannulation of Microvessels

The techniques for identification and isolation of porcine coronary microvessels were described previously.[21] In brief, a mixture of india ink and gelatin in physiologic salt solution (PSS) containing 145.0 mM NaCl, 4.7 mM KCl, 2.0 mM $CaCl_2$, 1.17 mM $MgSO_4$, 1.2 mM NaH_2PO_4, 5.0 mM glucose, 2.0 mM pyruvate, 0.02 mM EDTA, and 3.0 mM MOPS was perfused into the left anterior descending artery (0.3 ml) and the circumflex artery (0.4 ml) to enable the visualization of coronary microvessels. Subepicardial arteriolar vessels (40–108-μm ID and 0.6–1.0 mm long without branches) from the left anterior descending or circumflex arteries were selected and carefully dissected from the surrounding cardiac tissue under cold (5°C) PSS containing bovine serum albumin (1%; Amersham, Arlington Heights, IL) at pH 7.4. Each isolated arteriole was then transferred for cannulation to a Lucite vessel chamber (2 ml in volume) containing PSS–albumin solution equilibrated with room air at ambient temperature. One end of the microvessel was cannulated with a glass micropipette (40 μm in tip diameter) filled with filtered PSS–albumin solution, and the microvessel was securely tied to the pipette with 11-O ophthalmic suture (Alcon, Forth Worth, TX). The ink–gelatin solution inside the vessel was flushed out at low perfusion pressure (< 15 mmHg), and the other end of the vessel was cannulated with a second micropipette and tied with a suture. We previously showed that the ink–gelatin solution has no detrimental effect on either endothelial or vascular smooth muscle function.[22,23]

Instrumentation

After microvessel cannulation, the chamber was transferred to the stage of an inverted microscope (model IM35; Zeiss, Thornwood, NY) coupled to a CCD camera (KP-161; Hitachi, Tokyo, Japan) and a videomicrometer (Cardiovascular Research Institute, Texas A&M University System Health Science Center, College Station, TX). Vessel ID was measured with a precision of ± 1 μm throughout the experiment using videomicroscopy (160× final magnification) and a calibrated video caliper (795 × 596 pixel resolution). The micropipettes were connected to independent reservoir systems, and intraluminal pressures were measured through sidearms of the two reservoir lines by low-volume displacement strain-gauge transducers (Statham P23 Db; Gould, Cleveland, OH). The isolated vessels were pressurized without flow by setting both reservoirs at the same hydrostatic level. Leaks were detected by differences between reservoir pressure and intraluminal pressure. Preparations with leaks were excluded from further study.

Experimental Protocols

The cannulated arterioles were bathed in PSS–albumin solution (pH 7.4 at 37°C) equilibrated with room air and maintained at 36–37°C by connecting the chamber's water jacket to an external heat exchanger. The microvessels were set to their *in situ* length and allowed to develop basal tone (< 70–75% of maximal ID) at 45 mmHg intraluminal pressure without flow. These pressures approximate the estimated intraluminal pressures for microvessels of this size *in vivo*.[24] After the vessels developed basal tone (30–40 min), the direct effect of isoflurane on microvascular tone was assessed by exposing each arteriole to increasing concentrations of isoflurane (0.16, 0.29, 0.43, 0.57, and 0.75 mM; 1 minimum alveolar concentration [MAC] = 0.3 mM). Isoflurane concentrations were achieved by sequentially injecting small quantities (20–45 μl) of isoflurane-saturated PSS–albumin solution into the well using a Hamilton syringe and mixing the isoflurane within the well by repeatedly (three to four times) drawing buffer into the Hamilton syringe and slowly reinjecting it. Measurements of vessel ID were taken approximately 1 min after each isoflurane injection was mixed within the well, and injections of isoflurane were separated by approximately 2 min. Vessel ID was very stable between 1 and 2 min, and changes in coronary vessel ID were never observed after single or repeated injections of vehicle (PSS–albumin) into the well.

The possible involvement of vascular smooth muscle K_{ATP} and K_{Ca} channels in isoflurane-induced vasodilation was examined by extraluminal incubation (30 min) of intact vessels with the specific inhibitors glibenclamide (1 μM) and iberiotoxin (100 nM), respectively.[25,26] The specificity of glibenclamide as a K_{ATP} channel inhibitor was verified by testing its ability to block vasodilation in response to pinacidil (10^{-6} M, a specific K_{ATP} channel opener[27]), and the efficacy of iberiotoxin was confirmed by inhibition of arachidonic-induced (10 μM) coronary arteriolar dilation.[26] In addition, to evaluate whether

glibenclamide had a nonspecific effect on vasodilatory function, the dose-dependent dilation of isolated vessels to sodium nitroprusside (SNP; 10^{-9} to 10^{-5} M) was examined in the absence and presence of extraluminal glibenclamide (1 μM for 30 min).

To assess the role of the endothelium in the vascular response to isoflurane, the diameter–isoflurane relation was studied before and after endothelial denudation. To remove the endothelial cells, a nonionic detergent, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS, 0.4%), was intraluminally perfused into the vessel for 1–2 min.[28] After removal of the endothelium, as verified by the absence of vasodilation to the endothelium-dependent vasodilator bradykinin (1 nM), the vessel was perfused with PSS–albumin for 5 min to remove the CHAPS. To ensure that vascular smooth muscle function was not compromised by CHAPS treatment, dose-dependent dilation in response to SNP (10^{-9} to 10^{-5} M) was also examined in vessels before and after denudation.

At the end of each experiment, vessels were relaxed completely with SNP (100 μM) to obtain the maximal diameter at 45 mmHg intraluminal pressure.

Isoflurane Measurements

The reported cumulative isoflurane concentrations were determined in separate experiments using gas chromatography. Briefly, after the injection of each small volume of isoflurane-saturated buffer and complete mixing within the well, the PSS–albumin solution within the well (approximately 2 ml) was drawn into a preweighed, volume-calibrated, gas-tight syringe. A volume of air was drawn into the syringe to achieve a total volume of 20 ml, and the air–PSS solution was equilibrated in a 37°C rotary bath for 2 h. After equilibration, a gas sample was injected into a precalibrated gas chromatograph.

In preliminary experiments using this albumin-containing MOPS buffer, we determined the buffer:gas partition coefficient for isoflurane at 37°C to be 0.80 ± 0.01, with a coefficient of variation of 1.25. The concentration of isoflurane in the PSS–albumin solution was calculated as:

$$[\text{ISO}]\ \text{mM/liter} = (\text{Partial Pressure (atm)} \times \text{PC} \times (273/310))/(22.4 \times 1000)$$

Chemicals

Drugs were obtained from Sigma Chemical Co. (St. Louis, MO), except as specifically stated. Isoflurane was obtained as Forane (Ohmeda, Liberty Corner, NJ). Bradykinin and SNP were dissolved in PSS. Glibenclamide was dissolved in dimethyl sulfoxide, and pinacidil and iberiotoxin were dissolved in ethanol as stock solutions (10 mM). Subsequent concentrations of these drugs were diluted in PSS. The final concentrations of dimethyl sulfoxide and ethanol in the vessel bath were 0.03 and 0.1%, respectively. Vehicle control studies indicated that these final concentrations of dimethyl sulfoxide and ethanol had no effect on arteriolar function.

Data Analysis

In this study, only vessels that met the criteria for reactive isolated arterioles were included for data analysis. These criteria were as follows: (1) the development of spontaneous tone (*i.e.*, contraction to < 75% of maximal diameter at 37°C); (2) myogenic responsiveness when the 37°C PSS–albumin solution in the vessel chamber was replaced with fresh room-temperature solution; and (3) nonsignificant differences in dose-dependent vasodilation to SNP (10^{-9} to 10^{-5} M) before and after experimental treatment.[26]

Vasodilatory responses to isoflurane, in the absence and presence of K^+ channel blockers and after endothelial removal, were analyzed by normalizing changes in arteriolar diameter to the dilation observed for each vessel after exposure to 10^{-4} M SNP, which induced maximal vasodilation. In this formula, resting diameter refers to vessel diameter after the development of spontaneous tone.

$$\%\ \text{Maximal Dilation} = \frac{\text{diameter after isoflurane} - \text{resting diameter}}{\text{diameter after } 10^{-4}\ \text{SNP} - \text{resting diameter}} \times 100$$

Statistics

Statistical comparisons between groups (isoflurane *vs.* isoflurane + glyburide, isoflurane *vs.* isoflurane + iberiotoxin, and isoflurane before and after denudation) were made at each isoflurane concentration using paired student *t* tests. Repeated-measures analysis of variance, followed by multiple contrasts, were used to determine the concentrations of isoflurane and SNP that caused significant vasodilation. Student *t* tests were also used to examine whether glibenclamide or denudation reduced the vasodilatory response of isolated arterioles to SNP. All statistical analyses were performed using Statview and SuperAnova statistical software (Abacus Concepts, Berkeley, CA). Differences were considered significant at $P < 0.05$. Values presented in figures and throughout the text are mean ± SEM.

Results

The isolated coronary arterioles (n = 22) had a resting ID of 75 ± 4 μm (range, 41–108 μm) 30–40 min after being pressurized to 45 mmHg. Thus, the basal tone developed by these vessels was 69 ± 2% of maximal diameter (109 ± 5 μm). Isoflurane caused significant dose-dependent vasodilation in all of the control (untreated) coronary microvessels tested (fig. 1). This vasodilation was 6.0 ± 0.7 μm at an isoflurane concentration of 0.16 mM (approximately 0.5 MAC) and 25.3 ± 2.1 μm at the highest concentration of isoflurane tested (0.75 mM;

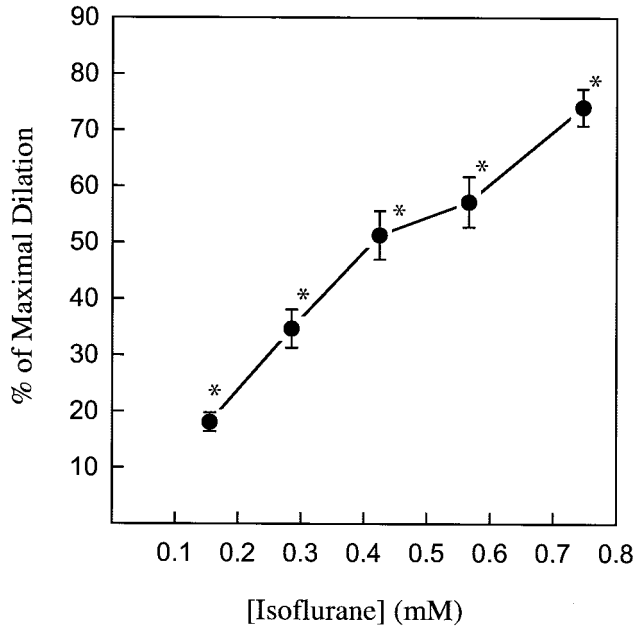

Fig. 1. Increases in porcine coronary arteriolar diameter (n = 22; baseline ID, 75 ± 4 μm) after administration of varying concentrations of isoflurane (0.16–0.75 mM; 0.5–2.5 MAC). Maximal dilation (34 ± 3 μm) was determined by exposing each vessel to 10^{-4} M sodium nitroprusside. Vertical bars represent 1 ± SEM. Asterisks indicate a significant ($P < 0.05$) increase in arteriolar ID as compared with vessels before isoflurane exposure.

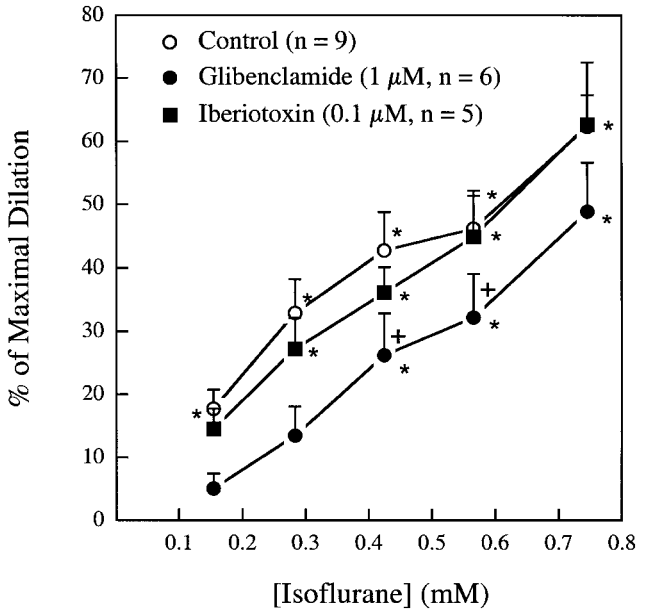

Fig. 2. Effect of potassium-channel blockade on isoflurane-mediated vasodilation in porcine coronary arterioles. Maximal dilation (36 ± 4 μm) was determined by exposing each vessel to 10^{-4} M sodium nitroprusside. Asterisks indicate a significant ($P < 0.05$) increase in arteriolar internal diameter as compared with vessels before isoflurane exposure. Plus signs indicate a significant difference in isoflurane-mediated vasodilation between control and glibenclamide-treated vessels at each isoflurane concentration. Baseline values for vessel ID in the control, glibenclamide, and iberiotoxin groups were 77 ± 7, 74 ± 6, and 82 ± 10 μm, respectively. Vertical bars represent 1 ± SEM.

approximately 2.5 MAC). These values represent 18.1 ± 1.7% and 74.1 ± 3.3%, respectively, of that observed with 10^{-4} SNP (34 ± 3 μm).

Incubation of microvessels with the K_{Ca} blocker iberiotoxin (0.1 μM) did not affect isoflurane-mediated vasodilation. Coronary arteriole ID was significantly increased at all isoflurane concentrations, and there was no difference in the degree of dilation between untreated and iberiotoxin-exposed vessels at any isoflurane concentration (fig. 2). The failure of iberiotoxin to influence the isoflurane dose–response curve was not caused by ineffective K_{Ca} blockade. Coronary dilation in response to arachidonic acid (10 μM) was reduced from 75 ± 2% to 8 ± 2% of maximal dilation after iberiotoxin treatment.

In contrast, blockade of K_{ATP} channels with glibenclamide (1.0 μM) greatly diminished the degree of vasodilation in response to isoflurane (fig. 2). Isoflurane failed to increase microvessel ID at both 0.15 and 0.29 mM. In addition, glibenclamide exposure caused a downward displacement of the isoflurane dose–response curve. K_{ATP} channel blockade significantly ($P < 0.05$) reduced isoflurane-mediated dilation as compared with control vessels at 0.43 and 0.59 mM, and the decreases observed at 0.29 and 0.75 mM just failed to reach significance ($P = 0.06$ for both). These reductions in isoflurane-mediated dilation were not likely the result of the indirect affects of glibenclamide on smooth muscle function. Glibenclamide almost completely blocked pinacidil-mediated vasodilation (control: 75 ± 4%, glibenclamide: 11 ± 5% of maximal dilation, n = 6), and glibenclamide pretreatment (1.0 μM) failed to affect the dose response of coronary microvessels to SNP (fig. 3).

Bradykinin-induced vasodilation was completely eliminated (control: 85 ± 4% *vs.* denudation: 3 ± 1% of maximal dilation, n = 6) after CHAPS treatment, confirming that the endothelium of the vessel had been removed. Endothelial removal significantly reduced the capacity of isoflurane to dilate coronary microvessels (fig. 4). Isoflurane only increased microvessel ID significantly at the two highest concentrations (0.59 and 0.75 mM). In addition, with the exception of 0.29 mM isoflurane, the degree of isoflurane-mediated vasodilation was significantly reduced after vessel denudation. For example, denudation reduced the amount of dilation in response to 0.75 mM isoflurane by 70%. Again, the observed decrease in isoflurane-mediated vasodilation was not the result of indirect effects on smooth muscle function. Endothelium-denuded microvessels had similar dose-dependent responses to SNP as vessels with an intact endothelium (fig. 3).

Discussion

Dose-related Coronary Arteriolar Vasodilation

The most important findings of our study are that isoflurane caused significant dose-related vasodilation

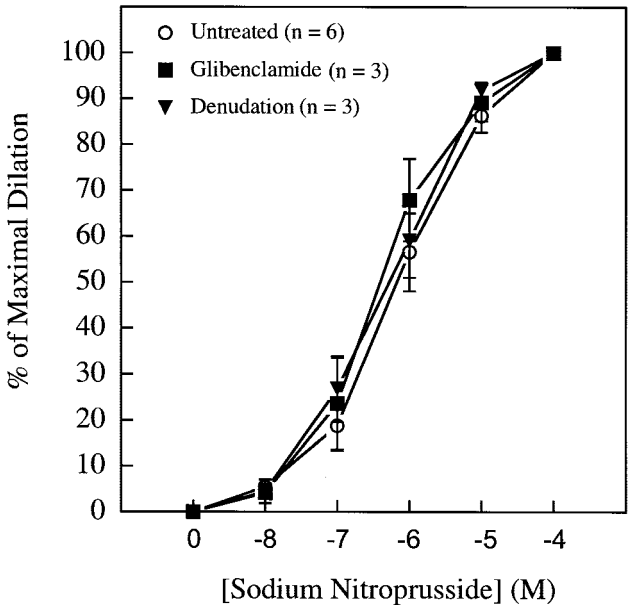

Fig. 3. Effect of glibenclamide (1 μM) treatment and endothelial removal with the nonionic detergent CHAPS (0.4%) on arteriolar responses to increasing concentrations of sodium nitroprusside. Neither glibenclamide nor endothelial denudation affected the dose response of vessels to sodium nitroprusside. Baseline ID in control vessels was 76 ± 3 μm and was not affected by either treatment. Vertical bars represent 1 ± SEM.

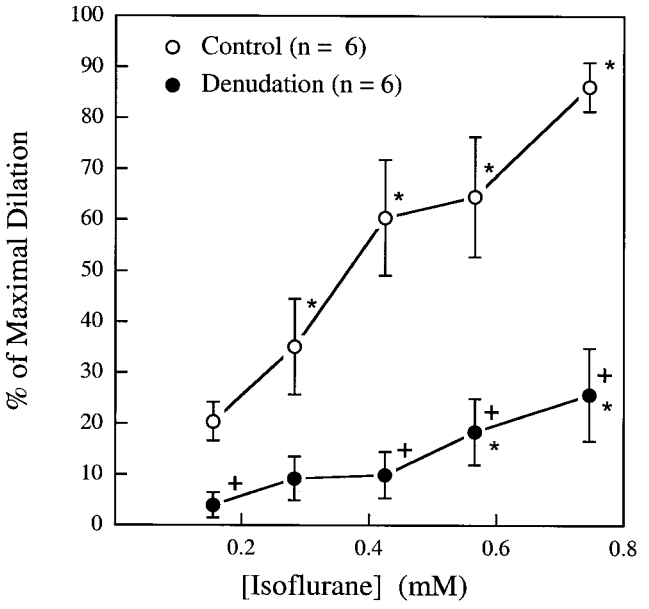

Fig. 4. Effect of endothelial denudation (0.4% CHAPS) on isoflurane-mediated vasodilation in porcine coronary microvessels. Asterisks indicate a significant ($P < 0.05$) increase in arteriolar ID as compared with vessels before isoflurane exposure. Plus signs indicate a significant difference in isoflurane-mediated vasodilation between control and denuded arterioles at each isoflurane concentration. Vertical bars represent 1 ± SEM. Baseline values for ID in the control and denuded groups were 80 ± 6 and 76 ± 6 μm, respectively.

(6–25 μm) in isolated coronary resistance arterioles, and that this vasodilation was near-maximal at the highest dose of isoflurane (0.75 mM; approximately 2.5 MAC). These results are in agreement with the findings of Zhou *et al.*[17] and numerous *in vivo* studies,[4–7,9–12] and add significantly to the weight of evidence which indicates that the predominant effect of isoflurane on the coronary vasculature is vasodilation.

Why our results contrast with the studies of Park *et al.*,[13–16] which report isoflurane-mediated coronary vasoconstriction, is unknown. However, it is apparent that neither differences in the species studied or in vessel size can account for the discrepancy in results. In the current study, we used swine coronary arterioles with an ID of 75 ± 4 μm (range, 41–108 μm). These microvessels were smaller than the arterioles studied by Park *et al.* in rabbits (139 ± 34 μm),[13] but similar to the size of vessels studied by that group in rats (99 ± 15 μm)[15] and swine (range, 60–150 μm).[16] Furthermore, our coronary arterioles were clearly "resistance" vessels, because the greatest fraction of the resistance in the coronary circulation of arrested swine hearts resides in arterial vessels less than 100 μm.[29]

There is no simple mechanistic explanation as to why isoflurane caused vasoconstriction of *in vitro* vessels in the studies by Park *et al.*,[13–16] but vasodilation in this study and those of Zhou *et al.*[17] and Park *et al.*[30] However, methodologic differences between studies cannot be ruled out as a potential contributor to these conflicting findings. One potentially important methodologic difference between the current study and the studies of Park *et al.*[13–16] was the criteria used as the primary indicator of the success of the arterial preparations, and thus the physiologic state of the arterioles. In the studies by Park *et al.*,[13–16] microvessel viability was determined by exposing microvessels to potassium chloride (maximum concentration, 100 mEq/l) at the end of the experiments and by ensuring that vessels constricted by at least 10%.[13] In contrast, we used three criteria to establish viability of coronary microvessels: the development of intrinsic tone within 30 min of being stretched to *in situ* length, clear myogenic responsiveness to a 10–12°C decrease in bath temperature, and consistent and significant dose-dependent vasodilation in response to SNP before and after treatments. We do not suggest that only isolated vessels with intrinsic tone should be studied. However, the development of intrinsic tone is an excellent indicator of the relative integrity of the physiologic systems of the microvessel, and results obtained with such vessels are most likely to represent those *in vivo*.

Other methodologic differences between this study and those by Park *et al.*[13–16] include oxygen concentrations in the bathing solution and the method of anesthetic delivery. In the studies by Park *et al.*, the vessels were continuously bathed in a Kreb's buffer solution gassed with 95% O_2–5% CO_2 (oxygen tension > 400 mmHg), and isoflurane was added to the buffer solution using an in-line vaporizer. In the current study, the PSS–albumin solution was equilibrated with room air, and isoflurane

concentrations were achieved by sequentially injecting small quantities (20–45 μl) of isoflurane-saturated PSS–albumin solution into the well. However, it is unlikely that either of these factors can explain the contradictory results obtained in the two studies. Park *et al.*[14] demonstrated that vasoconstrictive responses to isoflurane were similar in microvessels bathed in hyperoxic (95% O_2–5% CO_2) and nonhyperoxic buffer. In addition, Zhou *et al.*[17] demonstrated that, although abrupt *versus* gradual administration of isoflurane *via* a vaporizer caused differences in the magnitude of *in vitro* coronary arteriolar responses, isoflurane always produced significant vasodilation.

Isoflurane-induced Vasodilation Inhibited by Glibenclamide

Isoflurane-induced coronary vasodilation in this model was inhibited by glibenclamide, a blocker of K_{ATP} channels, but not by iberiotoxin, an inhibitor of K_{Ca} channels. This supports the idea that isoflurane-induced vasodilation of resistance coronary arterioles (< 100 μm) is mediated through cellular hyperpolarization caused by the opening of K_{ATP} channels. These findings agree with and support the *in vitro* study of Zhou *et al.*,[17] which looked at medium-sized coronary arterioles (172 ± 51 μm), and confirm previous *in vivo* work from our laboratory[6] and from Crystal *et al.*[8] that showed glibenclamide diminished isoflurane-mediated increases in coronary blood flow. Whether the K_{ATP} channels that participate in the vasodilatory response of coronary microvessels to isoflurane are located in coronary vascular smooth muscle or the endothelium is still unknown. Neither this study or that of Zhou *et al.*[17] performed glibenclamide experiments on denuded vessels, and the opening of endothelial K_{ATP} channels can reduce vascular smooth muscle tone by either the production of second messengers or by the direct transduction of electrical potentials. K_{Ca} channels, on the other hand, do not appear to be involved in isoflurane-induced coronary vasodilation. This result is concordant with the findings of Larach and Schuler,[31] who showed that TEA^+ (a blocker of K_{Ca}) did not significantly reduce halothane-mediated vasodilation in isolated rat hearts.

Role of the Endothelium in Isoflurane-induced Coronary Vasodilation

Isoflurane-induced coronary vasodilation was inhibited by approximately 70% after endothelial denudation in these experiments, but dose-dependent and maximal vasodilations in response to the endothelium-independent vasodilator nitroprusside were not affected. These findings provide the first evidence that isoflurane-induced vasodilation of coronary microvessels (< 100 μm) is largely endothelium dependent. However, at present, no mechanism(s) has been clearly identified that can explain this result. Crystal *et al.*,[32] who used a dog model of isolated coronary perfusion, found that the nitric oxide synthase inhibitor *N*-nitro-L-arginine methylester did not reduce isoflurane-induced coronary vasodilation even though it significantly reduced the endothelium-dependent vasodilation caused by acetylcholine. Although species differences must be considered (dogs in the study by Crystal *et al. vs.* swine in the current study), these data suggest that the endothelium-dependent vasodilation measured in this study was not the result of enhanced nitric oxide production. Lischke *et al.*[33] showed that bradykinin-stimulated dilation of the rat coronary circulation after nitric oxide–prostaglandin GI_2 blockade was significantly attenuated by isoflurane (2 vol%). This attenuation suggests that isoflurane impairs the cytochrome P450-dependent synthesis of endothelium-derived hyperpolarizing factor in the coronary microcirculation. In addition, there are conflicting reports about the role of prostanoids in isoflurane-mediated vascular dilation. Stone and Johns[34] demonstrated that isoflurane dilates rat aortic rings at higher concentrations and suggested that this vasodilatory effect involved the release of a prostanoid from the endothelium. In contrast, Loeb *et al.*[35] showed that halothane inhibits bradykinin-stimulated prostacyclin production in cultured bovine aortic endothelial cells.

Methodologic Critique and Limitations of the Current Study

The most important limitation of the current study was that it was performed *in vitro*, using vessels in a no-flow state. The pharmacologic effects of isoflurane on *in vivo* vessels may be different and more complex, particularly because of the potential contribution and amplifying effect of flow-mediated vasodilation. For example, evidence suggests that volatile anesthetics, including isoflurane, inhibit flow-mediated coronary vasodilation.[36] Furthermore, coronary arterioles *in vivo* are surrounded by myocardium, and coronary blood flow is normally regulated so that it matches myocardial oxygen demand. Isoflurane, by altering metabolic demands of myocardial tissue, may cause indirect changes in coronary blood flow (*i.e.*, metabolically mediated vasoconstriction) that partially offset isoflurane-induced vasodilation. Although experiments such as the current one facilitate the study of direct pharmacologic effects, extrapolation of these results to the intact, blood-perfused organ must be made with caution.

Another potential drawback of this model is that the main pharmacologic agent to be tested, isoflurane, was applied extraluminally. Vessel adventitia and smooth muscle would thus be more directly exposed to isoflurane than would the vessel endothelium. However, the vessels tested are extremely small, and the fact that extraluminal administration mimics the physiologic effects of intraluminal administration (*via* the bloodstream)[6,8] suggests that this was not a limiting factor.

In conclusion, using swine coronary resistance arterioles with innate tone, we found that isoflurane caused dose-dependent vasodilation, and this marked vasodilation was inhibited by the K_{ATP} channel blocker glibenclamide and was predominantly endothelium-dependent. Our finding that isoflurane dilates coronary microvessels agrees with numerous *in vivo* studies which show that isoflurane reduces coronary vascular resistance and uncouples myocardial oxygen supply from demand, and provides further experimental evidence that vasodilation, not vasoconstriction, is the predominant action of isoflurane in the coronary circulation.

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