

Isoflurane-induced Dilation of Porcine Coronary Arterioles Is Mediated by ATP-sensitive Potassium Channels

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Background: Isoflurane causes increases in coronary blood flow *in vivo*, which are mediated by the adenosine triphosphate (ATP)-sensitive potassium channels, but the role of the arterioles (resistance vessels) in these responses is controversial.

Methods: Medium porcine coronary arterioles (internal diameter, 172 ± 51 [SD] μm) were placed in a chamber supplied with Krebs's buffer, pressurized (40 mmHg), and precontracted with acetylcholine (10^{-8} – 10^{-6} M). Vascular diameter (VD) was assessed using an optical density video-detection system. Isoflurane (in 95% oxygen and 5% carbon dioxide) was added to buffer using a membrane oxygenator supplied by a calibrated vaporizer. In series 1 ($n = 14$), 2% isoflurane was administered according to an abrupt (ISO-A) and gradual (ISO-G) protocol. In series 2 ($n = 13$) and 3 ($n = 6$), ISO-A (1.5%) was assessed before and after glibenclamide (an ATP-sensitive

potassium channel antagonist) or 8-phenyltheophylline (a nonselective adenosine receptor antagonist), respectively. In series 4 ($n = 5$), validation studies were performed using sodium nitroprusside and adenosine diphosphate to verify that the vascular smooth muscle and endothelium of the vessels were functionally intact. In series 5 ($n = 6$), ISO-A (0.75 and 1.5%) was compared during precontraction with acetylcholine and the thromboxane analog U46619 (10^{-6} M).

Results: ISO-G caused essentially concentration-dependent increases in VD. At 2% isoflurane, the increases in VD were greater during ISO-A than ISO-G. Glibenclamide, but not 8-phenyltheophylline, attenuated isoflurane-induced increases in VD. Both sodium nitroprusside and adenosine diphosphate caused dose-dependent increases in VD. Isoflurane caused equivalent concentration-dependent increases in VD during acetylcholine and U46619.

Conclusions: Isoflurane is a concentration-dependent dilator of porcine coronary arterioles precontracted with acetylcholine or U46619. This effect is blunted by gradual administration, suggesting that the vessels may adapt to the relaxing effects of isoflurane. Isoflurane-induced dilation of coronary arterioles is mediated by the ATP-sensitive potassium channels but not by the adenosine receptors. (Key words: Coronary microcirculation; coronary vasodilators; volatile anesthetics.)

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STUDIES conducted *in vivo* in dogs and swine¹⁻⁸ have shown that isoflurane is a dilator in the coronary circulation, as evidenced by its ability to cause increases in coronary blood flow (and decreases in coronary vascular resistance), accompanied by increases in coronary venous oxygen pressure (P_{O_2}). The magnitude of these effects suggested that isoflurane was relaxing vascular smooth muscle at the level of the coronary arterioles, where most of the resistance to flow resides.⁹ However, this remains to be verified by direct observation.

The current study was conducted using a video-imaging technique¹⁰ to assess *in vitro* the direct effects of isoflurane on porcine coronary arterioles precontracted with acetylcholine. Previous *in vivo* studies from our laboratory showed that the increases in coronary blood flow caused by isoflurane were blunted when it was administered gradually.¹¹ It was not deter-

ISOFLURANE AND THE CORONARY CIRCULATION

mined whether this phenomenon reflected an intrinsic tendency of coronary resistance vessels to adapt to the relaxing effects of isoflurane or was a result of the competitive influence of vasoconstrictor factor(s) released from the surrounding myocardium (e.g., in response to the depressive effect of isoflurane on myocardial contractility and oxygen demand). Thus in series 1 we evaluated the contribution of the former mechanism by comparing the effect of abrupt and gradual administrations of isoflurane on the coronary arterioles.

After we found a vasodilating action for isoflurane in series 1, we did other studies to clarify the mechanism underlying this effect. Recent *in vivo* findings have shown that the inhibitor of the adenosine triphosphate-sensitive potassium (K_{ATP}) channels, glibenclamide, attenuated the increases in coronary blood flow during isoflurane administration, thus implying a role for these channels in the responses.^{12,13} Accordingly, in series 2, we assessed the effect of glibenclamide on isoflurane-induced dilation of the coronary arterioles. Given the results of recent studies suggesting that adenosine receptors may be coupled with K_{ATP} channels *via* G proteins,¹⁴ in series 3 we evaluated the effect of the nonselective adenosine receptor antagonist, 8-phenyltheophylline (8-PT),¹⁵ on isoflurane-induced dilation of the coronary arterioles. In series 4, we conducted validation studies using the endothelium-independent vasodilator, sodium nitroprusside (SNP),¹⁶ and the endothelium-dependent vasodilator, adenosine diphosphate,¹⁶ to verify that the vascular smooth muscle and endothelium of our isolated coronary arterioles were functionally intact. Our findings in series 1-3 differed from those of Park *et al.*,¹⁷⁻¹⁹ which showed that isoflurane caused constriction of isolated coronary arterioles. To determine whether this discrepancy was because those authors used a different agent for precontraction (*i.e.*, the thromboxane analog U46619), we performed studies (series 5) in which we assessed the vasomotor effects of isoflurane in the same coronary arterioles precontracted sequentially with acetylcholine and U46619.

Methods

General Preparation

Forty-four porcine hearts were obtained from a slaughterhouse, immediately placed in iced Krebs's buffer, and transported to the laboratory. The composition of the Krebs's buffer was 99.5 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4 , 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$,

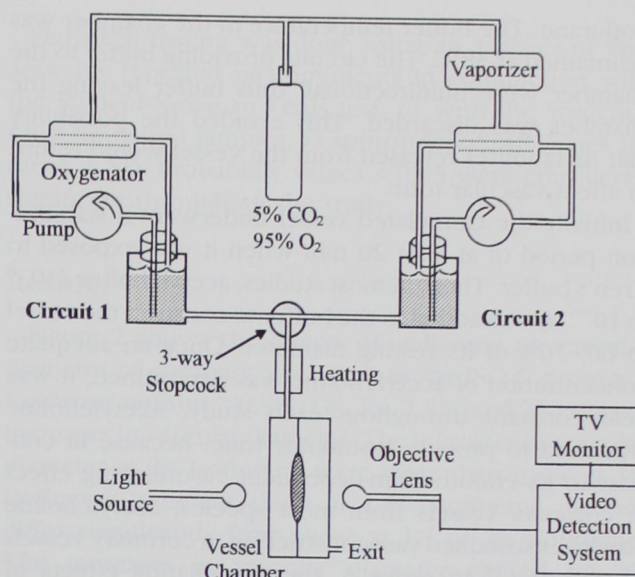


Fig. 1. The system used to expose isolated porcine coronary arterioles to isoflurane.

4.9 mM Na pyruvate, 5.4 mM Na fumarate, 1.1 mM $CaCl_2$, and 11.5 mM glucose.

A subepicardial microvessel with an internal diameter of 173 ± 46 (SD) μm and a length of 1-2 mm was dissected carefully from the distribution of left anterior descending coronary artery. The vessel was placed in a lucite chamber containing cold Krebs's buffer and cannulated at either end with a glass micropipette, which was secured with fine monofilament sutures. One cannula was connected to a pressure reservoir system that maintained intraluminal pressure (measured through a side arm) at 40 mmHg. The other cannula was closed, thus preventing flow through the vessel.

Figure 1 is a diagram of the microvessel preparation. The chamber containing the coronary arteriole was seated on a stage of an inverted microscope coupled to a video camera. The lumen diameter was determined using an optical density video-detection system.¹⁰ The chamber was supplied with Krebs's buffer *via* either of two circuits: Circuit 1 supplied isoflurane-free buffer and circuit 2 supplied buffer containing isoflurane. In both circuits, the buffer passed through an oxygenator, which was provided with 95% oxygen and 5% carbon dioxide (thus producing values for P_{O_2} , carbon dioxide pressure [P_{CO_2}], and pH in the buffer of 542 ± 54 mmHg, 40 ± 4 mmHg, and 7.41 ± 0.05 , respectively). However, only in circuit 2 was the gas supplied to the oxygenator passed through a calibrated vaporizer providing

isoflurane. The buffer temperature in the chamber was maintained at 38°C. The circuits providing buffer to the chamber were unidirectional; thus buffer leaving the chamber was discarded. This avoided the possibility that metabolites released from the vessel would return to affect vascular tone.

Initially the cannulated vessel underwent a stabilization period of at least 20 min when it was exposed to Krebs's buffer. Then, in most studies, acetylcholine (10^{-8} to 10^{-6} M) was added to the buffer to constrict the vessel to 60–70% of its resting diameter. Once an adequate concentration of acetylcholine was determined, it was held constant throughout each study. Acetylcholine was used to provide vasomotor tone, because in contrast to its endothelium-dependent vasorelaxing effect in coronary vessels from most species, acetylcholine produces sustained vasoconstriction in coronary vessels from swine.²⁰ In series 5, the vasodilating effects of isoflurane were assessed in the same vessels precontracted sequentially with acetylcholine and U46619.

Experimental Protocols

Series 1 (n = 14): Abrupt versus Gradual Administration of Isoflurane. In nine vessels, buffer (and acetylcholine) was initially supplied through circuit 1. After adequate time for stabilization of the precontracted vessel (at least 30 min), the baseline diameter was determined and a sample of buffer was obtained for gas analysis. The vessel was exposed to isoflurane according to two protocols: abrupt (ISO-A) and gradual (ISO-G). In ISO-A, the vessel was switched abruptly from isoflurane-free buffer to buffer containing 2% isoflurane; that is, from circuit 1 to circuit 2. The buffer in circuit 2 was recirculated for at least 30 min through the oxygenator to ensure complete equilibration before it was supplied to the vessel. After vessel diameter was stable for 5 min during ISO-A, the value was recorded and samples of buffer were obtained for analysis of gas composition and isoflurane concentration. Then the vessel was returned to circuit 1, which contained buffer with the precontracting dose of acetylcholine but free of isoflurane. At least 20 min was allowed for recovery. During this period, the buffer in circuit 2 was recirculated three times through the oxygenator with the vaporizer setting at 0% to remove isoflurane. The vessel was switched to circuit 2 and a second set of baseline measurements was obtained. Then the ISO-G protocol was performed, whereby the vaporizer setting was increased stepwise (in 0.5% increments) to 2% during a period of 40 min. Each setting was maintained for 10

min before the vascular diameter was determined and samples of buffer were obtained for analysis. The total elapsed time for the ISO-A and ISO-G protocols was 2 to 2.5 h.

Two approaches were used to address the potential time-dependent effects on vessel reactivity. First, the order of the ISO-A and ISO-G protocols was randomized. Second, an additional set of five vessels, prepared as described before, was subjected to duplicate exposures to isoflurane using the ISO-A protocol.

Series 2 (n = 13): K_{ATP} Channels and Isoflurane-induced Coronary Vasodilation. Studies were conducted to determine the effect of the K_{ATP} channel inhibitor glibenclamide on isoflurane-induced coronary vasodilation. After obtaining an initial baseline measurement for vascular diameter, isoflurane in a concentration of 1.5% (approximating the 1 minimum alveolar concentration [MAC]) was added to the bathing solution using the abrupt protocol described before, and the measurements were repeated (control response). The vessel was then returned to isoflurane-free buffer. After at least 20 min for recovery from the effects of isoflurane, glibenclamide (10^{-5} M) was added to the buffer and a value for vascular diameter was obtained. While glibenclamide was continued, a second administration of isoflurane was performed. Then the glibenclamide stopped, and isoflurane was administered for a third time. The coronary vasodilating effects of cromakalim (10^{-6} M), a K_{ATP} channel opener,¹² and SNP (10^{-5} M), a K_{ATP} channel-independent vasodilator,¹² were assessed under each condition (before, during, and after glibenclamide) to verify the efficacy of glibenclamide. Measurements of isoflurane concentration in the buffer were obtained before and during, but not after, glibenclamide. The order of isoflurane, cromakalim, and SNP was randomized. Each condition in series 2 required approximately 1 h (about 20 min per dilator); thus the total duration of series 2 was approximately 3 h.

Series 3 (n = 6): Adenosine Receptors and Isoflurane-induced Coronary Vasodilation. The role the coronary adenosine receptors in isoflurane-induced coronary vasodilation was evaluated using 8-PT to block the adenosine receptors. The studies followed the protocol described for series 2, except no post-blockade responses were assessed. The effects of isoflurane (1.5%) were evaluated only under control conditions and during 8-PT (10^{-5} M). Responses to adenosine (10^{-5} M) and SNP (10^{-5} M) were used to evaluate the efficacy of the treatment with 8-PT.

Series 4 (n = 5): Validation Studies Demonstra-

ISOFLURANE AND THE CORONARY CIRCULATION

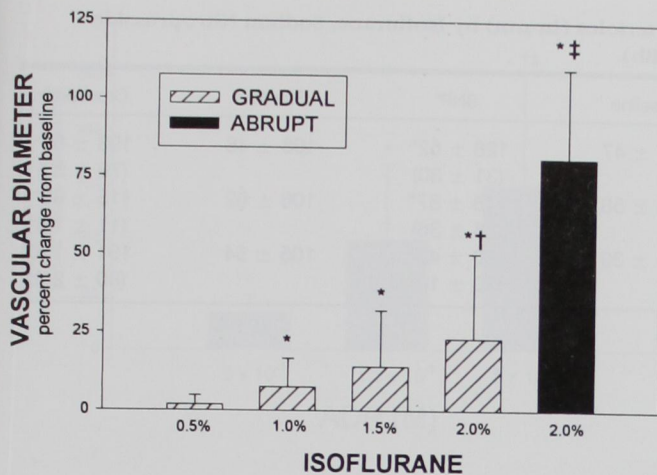


Fig. 2. Effects of gradual and abrupt administrations of isoflurane on the diameter of porcine coronary arterioles. $P < 0.05$, *versus baseline value; †versus 1% isoflurane; ‡versus 2% isoflurane gradual.

ting the Functional Integrity of Vascular Smooth Muscle and Endothelium. Studies were performed to verify that both the vascular endothelium and the vascular smooth muscle remained functionally intact in the isolated coronary arterioles. Vascular endothelial reactivity was evaluated using the endothelium-dependent vasodilator adenosine diphosphate (5×10^{-6} M, 1.5×10^{-5} M, and 5×10^{-5} M); whereas vascular smooth muscle reactivity was evaluated using the endothelium-independent vasodilator SNP (10^{-5} M, 3×10^{-5} M, and 10^{-4} M).

Series 5 (n = 6): Isoflurane-induced Coronary Vasodilation: Acetylcholine versus U46619 Precontraction. Studies were conducted to determine if isoflurane-induced coronary vasodilation is different if U46619 is substituted for acetylcholine as the precontractor. Responses to 0.75% and 1.5% isoflurane were assessed in the same vessel precontracted sequentially with acetylcholine and U46619. The protocol in these studies followed that described for series 2. The order of the precontractors was randomized. Care was taken to ensure that the initial precontractor was washed from the vessel before the second precontractor was applied.

The concentration of isoflurane in the buffer solution was determined in series 1–3 using a modification of the gas chromatographic technique of Yamamura *et al.*²¹ The data are presented in both units of milligrams per 100 ml and millimolar to facilitate comparisons with previous studies.^{17–19}

Statistical testing was done using an analysis of variance for repeated measurements in conjunction with the Student-Newman-Keuls test, or with the Student's *t* test for paired samples, as appropriate.²² All values are mean \pm SD. Probability values < 0.05 were considered significant throughout the study.

Results

Figure 2 shows the effects of isoflurane on vascular diameter of coronary arterioles. In the ISO-G protocol, vaporizer settings $> 0.5\%$ (i.e., 1%, 1.5%, and 2%) caused increases in vascular diameter. The increases in vascular diameter at 2% isoflurane were more than those at 1% isoflurane, whereas those at 1.5% isoflurane did not differ significantly from those at 1% or 2% isoflurane. The increases in vascular diameter caused by 2% isoflurane using the ISO-A protocol were nearly four times those using the ISO-G protocol. This difference in response was evident regardless of the sequence of the protocols.

The concentration for isoflurane in the buffer solution varied directly with the percentage provided by the vaporizer (table 1). The anesthetic concentrations in the buffer at 2% were similar during the gradual and abrupt administrations (table 1).

Duplicate abrupt administrations of 2% isoflurane caused increases in vascular diameter that did not differ ($83 \pm 26\%$ and $92 \pm 19\%$, respectively).

Table 2 presents the actually measured changes in vascular diameter during isoflurane, SNP, and cromakalim before, during, and after glibenclamide. It shows (1) that glibenclamide itself did not affect vascular diameter; (2) that the vasodilators, including isoflurane,

Table 1. Isoflurane Concentration in Buffer Solution Delivered to Porcine Coronary Arterioles as a Function of Vaporizer Setting

Vaporizer Setting (%)	Isoflurane Concentration	
	mg/100 ml	mM
Gradual		
0.5	7.1 ± 1.2	0.39 ± 0.07
1.0	12.2 ± 2.1	0.66 ± 0.11
1.5	17.0 ± 2.4	0.92 ± 0.13
2.0	21.9 ± 2.7	1.19 ± 0.15
Abrupt		
2.0	23.5 ± 1.8	1.28 ± 0.10

Values are mean \pm SD.

Table 2. Actual Changes in Diameter of Precontracted Coronary Arterioles (in μm) by Isoflurane, Sodium Nitroprusside (SNP), and Cromakalim before, during, and after Glibenclamide (Glib)

	Baseline	Isoflurane	Baseline	SNP	Baseline	Cromakalim
Before Glib (control)	99 \pm 43	140 \pm 49* (46 \pm 30)	102 \pm 47	128 \pm 52* (31 \pm 30)	108 \pm 46	193 \pm 89* (79 \pm 33)
During Glib	104 \pm 44	112 \pm 42* (11 \pm 11)†	104 \pm 58	128 \pm 67* (28 \pm 36)	106 \pm 82	115 \pm 83* (11 \pm 10)†
After Glib (recovery)	102 \pm 25	143 \pm 27* (45 \pm 30)‡	101 \pm 39	130 \pm 47* (30 \pm 19)	105 \pm 54	191 \pm 110* (80 \pm 24)‡

Values are mean \pm SD. Values in parentheses are percent change from baseline.

* $P < 0.05$ versus baseline.

† $P < 0.05$ versus Before Glib.

‡ $P < 0.05$ versus During Glib.

caused responses that were reversible (*i.e.*, vascular diameter returned to baseline); and (3) that glibenclamide blunted considerably (but reversibly) the increases in vascular diameter by isoflurane and cromakalim, but it had no effect on increases caused by SNP. The buffer concentrations for isoflurane were 14.7 ± 0.8 mg/100 ml (0.80 ± 0.04 mM) and 14.9 ± 0.8 mg/100 ml (0.81 ± 0.04 mM) before and during glibenclamide, respectively.

Figure 3 shows that 8-PT blunted the increases in vascular diameter by adenosine but it had no effect on those by isoflurane and SNP. 8-PT had no effect on baseline vascular diameter (123 ± 14 vs. 119 ± 12 μm).

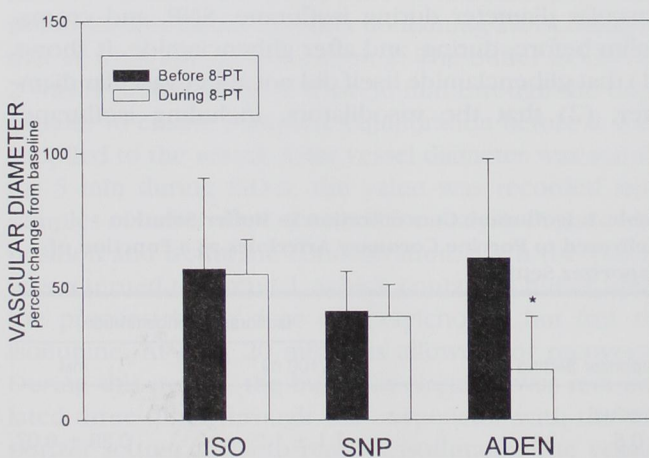


Fig. 3. Effects of isoflurane (ISO), sodium nitroprusside (SNP), and adenosine (ADEN) on the diameter of porcine coronary arterioles before and during administration of 8-phenyltheophylline (8-PT). Values for isoflurane concentration in the buffer solution were 14.9 ± 0.5 and 15 ± 0.6 mg/100 ml (0.81 ± 0.03 and 0.82 ± 0.03 mM) before and during 8-PT administration, respectively. * $P < 0.05$ versus before 8-PT.

Figure 4 shows that both adenosine diphosphate and SNP caused concentration-dependent increases in vascular diameter. Figure 5 shows that isoflurane caused equivalent concentration-dependent increases in vascular diameter during precontraction with acetylcholine and U46619. The findings were not affected by the order in which the agents were assessed. The pre-isoflurane vascular diameters were similar during acetylcholine and U46619, 150 ± 23 μm and 152 ± 23 μm , respectively.

Discussion

The study yielded several main findings. (1) Isoflurane caused concentration-dependent dilation of porcine coronary arterioles *in vitro*. (2) The coronary dilating effects of isoflurane were less during a gradual administration than during an abrupt administration. (3) Glibenclamide inhibited isoflurane-induced dilation of coronary arterioles, whereas 8-PT had no effect. (4) Isoflurane caused an equivalent dilation of coronary arterioles that were precontracted with acetylcholine or U46619.

We used an *in vitro* microvessel technique developed by Halpern *et al.*¹⁰ This technique is well established and has been used to study vessels obtained from various vascular beds and species.^{17-20,23-26} It had several advantages for our study. (1) The vasomotor responses of coronary arterioles (*i.e.*, resistance vessels) could be studied selectively. (2) The coronary vasomotor responses were independent of the associated changes in myocardial oxygen demand and extravascular compressive forces that are inherent to *in vivo* models. (3) Because the vessels were studied in a nonperfused state, their responses were assessed in the absence of shear

ISOFLURANE AND THE CORONARY CIRCULATION

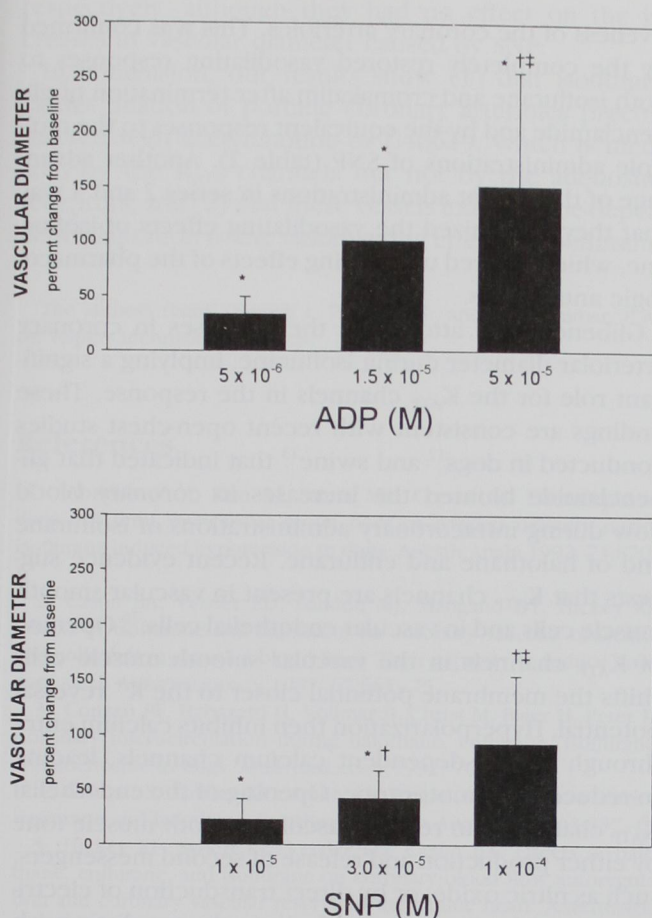


Fig. 4. Concentration-dependent effects of adenosine diphosphate (ADP), an endothelium-dependent dilator, and sodium nitroprusside (SNP), an endothelium-independent dilator, on the diameter of porcine coronary arterioles. $P < 0.05$, *versus baseline value; †versus low concentration; ‡versus intermediate concentration.

induced release of nitric oxide. The main disadvantage of the microvessel technique was that, like any *in vitro* technique, it had artificial and nonphysiologic features, including the use of a drug to establish "background" vascular tone and delivery of drugs *via* the adventitia. The increases in vascular diameter by adenosine diphosphate and SNP attested to the functional integrity of the vascular endothelium and underlying smooth muscle of the coronary arteriole preparations.

The fidelity of the isoflurane delivery system was demonstrated by buffer concentrations that remained proportional to the percentage supplied by the vaporizer and that did not vary when evaluated twice under different conditions in the same preparation (*e.g.*, during the abrupt and gradual protocols). Our buffer concentra-

tions for isoflurane were in close agreement with those reported by previous investigators using a similar experimental approach.^{17,18}

The coronary microvessels that we studied had diameters in the range of medium coronary arterioles.²⁷ *In vivo*, with coronary vasomotor tone intact, approximately 20–25% of total coronary vascular resistance is proximal to vessels of this size.²⁷ Further, studies *in vivo* have shown that the effects of vasodilators (*e.g.*, adenosine and dipyridamole) may differ in these vessels compared with coronary arterioles that are smaller or larger.²⁸ Thus it would not be appropriate to extrapolate our findings to other segments of the coronary microcirculation.

We observed that the increases in arteriolar diameter during gradual administrations of isoflurane were less than those during abrupt administrations, which suggested that the vessels adapted over time to the relaxing effects of isoflurane. This ability of the coronary arterioles likely explains the blunted increases in coronary blood flow during gradual administrations of isoflurane¹¹ and the recovery of coronary vascular tone during prolonged administrations of isoflurane²⁹ observed *in vivo*.

The specific mechanism responsible for the ability of the coronary arterioles to adapt to isoflurane is unknown, but it may involve downregulation of specific receptors or of the second messenger system (*e.g.*, K_{ATP} channels). In theory, one approach to assess the role of the K_{ATP} channels in this phenomenon would be to

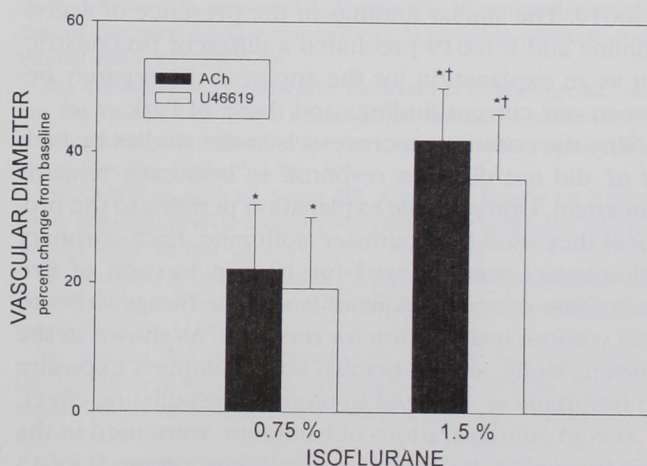


Fig. 5. Concentration-dependent effects of isoflurane (ISO) on porcine coronary arterioles preconstricted with acetylcholine (ACh) or U46619. * $P < 0.05$ versus baseline; †versus 0.75% ISO.

repeat the abrupt *versus* gradual comparison in the presence of glibenclamide. However, because glibenclamide nearly abolished the vasodilating effect of the abrupt administrations of isoflurane (table 2), it would be difficult, if not impossible, to detect a blunting of this effect during the gradual administrations. Further studies using sophisticated molecular approaches to study signal-transduction pathways would appear necessary to clarify the mechanism(s) underlying the ability of the coronary arterioles to adapt to isoflurane.

The validity of our findings in series 1 depends on the duplicate responses in the same vessel being independent of one another; that is, it was necessary to rule out that the blunted response during the gradual administration was due to tachyphylaxis after the abrupt administration. Two lines of evidence suggest that this was so. First, the gradual administrations had less of a vasodilating action even when they were placed before the abrupt administrations. Second, when two abrupt administrations were performed in succession, the vasodilating effects were similar.

Our findings in series 1-3 conflicted with those of Park *et al.*¹⁷⁻¹⁹ that showed that isoflurane, in a similar range of concentrations, caused constriction of isolated coronary arterioles obtained from normal rabbit or rat hearts, or from the normal region of porcine hearts with a chronic coronary occlusion. To determine whether this difference in results occurred because we used acetylcholine whereas Park *et al.* used U46619 for precontraction, we did additional studies that compared the vasodilating effects of isoflurane in the same microvessels precontracted sequentially with acetylcholine and U46619. The similar findings in the presence of acetylcholine and U46619 precluded a different precontractor as an explanation for the apparent discrepancy between our current findings and those of Park *et al.*

Why the coronary microvessels in the studies by Park *et al.* did not dilate in response to isoflurane remains uncertain. One possible explanation pertains to the protocol they used to administer isoflurane. Each coronary microvessel was exposed for 10 min to each of five increasing concentrations of isoflurane (range, 0.3% to 3%) without interruption for recovery. As shown in the current study, such a gradual and prolonged exposure to isoflurane would tend to blunt its vasodilating effect.

Abrupt administrations of isoflurane were used in the glibenclamide, 8-PT, and acetylcholine *versus* U46613 studies (series 2, 3, and 5) to minimize the possibility that time-dependent factors (*i.e.*, vascular adaptation and vessel deterioration) would reduce the respon-

siveness of the coronary arterioles. This was confirmed by the completely restored vasodilating responses to both isoflurane and cromakalim after termination of glibenclamide and by the equivalent responses to the multiple administrations of SNP (table 2). Another advantage of the abrupt administrations in series 2 and 3 was that they maximized the vasodilating effects of isoflurane, which favored uncovering effects of the pharmacologic antagonists.

Glibenclamide attenuated the increases in coronary arteriolar diameter during isoflurane, implying a significant role for the K_{ATP} channels in the response. These findings are consistent with recent open-chest studies conducted in dogs¹² and swine¹³ that indicated that glibenclamide blunted the increases in coronary blood flow during intracoronary administrations of isoflurane and of halothane and enflurane. Recent evidence suggests that K_{ATP} channels are present in vascular smooth muscle cells and in vascular endothelial cells.²⁶ Opening of K_{ATP} channels in the vascular smooth muscle cells shifts the membrane potential closer to the K^+ reversal potential. Hyperpolarization then inhibits calcium entry through voltage-dependent calcium channels, leading to reduced vasomotor tone. Opening of the endothelial K_{ATP} channels can reduce vascular smooth muscle tone by either production and release of second messengers, such as nitric oxide, or by direct transduction of electrical potentials. Our current findings do not distinguish between the contribution of the K_{ATP} channels in vascular smooth muscle and in the endothelial cells to isoflurane-induced coronary vascular relaxation.

Kersten *et al.*³⁰ reported that the selective adenosine type 1 (A_1) receptor antagonist (8-cyclopentyl-1,3-dipropyl-xanthine), like glibenclamide in their previous studies,^{31,32} inhibited isoflurane-induced cardioprotection in stunned myocardium of dogs. These findings suggested that the myocardial protective effect of isoflurane may involve sequential activation of the A_1 receptor and the K_{ATP} channels in cardiac cells. We observed no effect of the nonselective adenosine receptor antagonist 8-PT on isoflurane-induced coronary vasodilation, which suggests that isoflurane may act *via* a different pathway in the coronary vasculature than it does in the cardiomyocytes. The mechanism underlying isoflurane-induced activation of the K_{ATP} channels in the coronary arterioles remains to be clarified.

The efficacy of our doses for glibenclamide and 8-PT was evident from their ability to obtund the increases in vascular diameter caused by cromakalim and adenosine,

ISOFLURANE AND THE CORONARY CIRCULATION

respectively, although they had no effect on the increases in vascular diameter caused by SNP.

In conclusion, our results show (1) that isoflurane causes dilation of porcine coronary arterioles precontracted with acetylcholine or U46619, which is mediated by the K_{ATP} channels but not by the adenosine receptors, and (2) that these vessels exhibit time-dependent adaptation to the vasorelaxing effects of isoflurane.

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