LABORATORY INVESTIGATIONS

Anesthesiology
81:1190–1197, 1994

Heterogeneous Vasomotor Responses of Rabbit Coronary Microvessels to Isoflurane

Kyung W. Park, M.D., Hai B. Dai, M.D.,† Edward Lowenstein, M.D.,‡ Amir Darvish, M.A.,§ Frank W. Sellke, M.D.‖*

Background: **Previous *in vitro* studies on the mechanism of isoflurane-elicited vasodilation have examined conductance arteries and reported conflicting data on whether the vasomotor response is mediated through the release of endothelium-derived nitric oxide. The current study was undertaken to define the effect of isoflurane on both resistance and conductance coronary arteries in rabbits and to elucidate the mechanism of the effect.**

Methods: **Rabbit coronary arteries of varying sizes were dissected and each placed in a microvessel chamber. The arteries were studied in a pressurized (40 mmHg), no-flow state and were exposed to increasing concentrations of isoflurane, 0–3%, by an in-line bubble-through vaporizer. The vessel lumen diameter was monitored using an optical density video detection system. Selected experiments were performed on microvessels after preincubation with indomethacin, N^G-monomethyl-L-arginine, or methylene blue or after endothelial denudation.**

Results: **Isoflurane caused a dose-dependent constriction of small rabbit coronary arteries (internal diameter of 139 ± 34 μ, mean ± SD), whereas it caused dilation of large coronary arteries (371 ± 54 μ). The vasoconstriction of the small coronary arteries by isoflurane was abolished by endothelial denudation or after preincubation with indomethacin. The vasodilation of the large vessels by isoflurane was inhibited by endothelial denudation or after preincubation with N^G-monomethyl-L-arginine, methylene blue, or indomethacin.**

Conclusions: **Our data suggest that vessel size is a determinant of the vasomotor response to isoflurane. Exposure to isoflurane produces vasodilation of conductance coronary arteries, whereas it is associated with vasoconstriction of resistance coronary microvessels. The latter appears to be endothelium-dependent and mediated by cyclooxygenase product(s), whereas the former, also endothelium-dependent, is mediated by both product(s) of cyclooxygenase and endothelium-derived nitric oxide. (Key words: Anesthetics, volatile: isoflurane. Heart: coronary arteries; coronary vascular resistance; redistribution of resistance. Endothelium: endothelium-derived nitric oxide. Prostanoid: vasoconstrictive.)**

This article is accompanied by an editorial. Please see Merin RG, Johns RA: Does isoflurane produce coronary vasoconstriction? ANESTHESIOLOGY 81:1093–1096, 1994.

* Instructor of Anesthesiology.

† Research Fellow in Surgery.

‡ Professor and Chairman of Anesthesiology and Critical Care.

§ Research Assistant in Anesthesiology.

‖ Assistant Professor of Surgery.

Received from the Departments of Anesthesiology and Critical Care and Surgery, Beth Israel Hospital, Harvard Medical School, Boston, Massachusetts. Accepted for publication June 15, 1994.

Address reprint requests to Dr. Sellke: Division of Cardiothoracic Surgery, Beth Israel Hospital, Dan 905, 330 Brookline Avenue, Boston, Massachusetts 02215.

IT is generally accepted that isoflurane is a dilator of both systemic and coronary arterial blood vessels.[1] Most *in vitro* studies examining the mechanism of isoflurane-induced vasodilation have been performed using rings or strips of large arteries, generally considered to be conductance vessels. These studies report conflicting data on whether the observed vasodilation elicited by isoflurane is mediated by endothelium-derived nitric oxide (EDNO) or *via* endothelium-independent mechanism.[2–7] A previous study suggested that the response of vessels to an inhalational agent may differ according to the size.[8] Furthermore, the ability to convert organic nitrates to their active metabolites may be heterogeneous in vessels of different sizes.[9,10]

The current study was undertaken to define the effect of isoflurane on rabbit epicardial coronary arteries. We studied both small resistance microvessels and larger arteries that are more characteristic of conductance vessels to examine possible differences. Last, we attempted to elucidate the mechanisms for the observed effects of isoflurane on rabbit epicardial coronary microvessels.

Methods and Materials

Vessel Preparation

In accordance with institutional Animal Care Committee standards, adult New Zealand White rabbits of either sex, weighing 2.5–3.0 kg, were anesthetized by

administering 8–10 ml of 3.2% 1,2-o-[2,2,2 Trichloroethylidine]-α-gluco-furanose (α-chloralose) into an ear vein. Their hearts were quickly harvested and placed in cold modified Krebs buffer (NaCl 120 mM, KCl 5.9 mM, dextrose 11.1 mM, $NaHCO_3$ 25 mM, NaH_2PO_4 1.2 mM, $MgSO_4$ 1.2 mM, $CaCl_2$ 2.5 mM). Epicardial microvessels were dissected carefully from the surrounding myocardial tissue. Each dissected vessel (0.5–1.5 mm in length) was placed in an isolated vessel chamber, cannulated with dual micropipettes measuring 50–100 μm in diameter, and secured with a 10-0 Ethilon suture. The vessel was continuously bathed with Krebs buffer, gassed with 95% O_2/5% CO_2 mixture, and maintained at 37°C and *p*H of 7.4. PO_2 in the vessel chamber exceeded 400 mmHg. As the vessel was studied in a no-flow state, the pressure in the micropipettes was maintained at 40 mmHg to provide distention. Microvessels were visualized with an inverted microscope (Olympus IMT-2, Tokyo, Japan) connected to a video camera. The vessel image was projected onto a television screen (Panasonic, Osaka, Japan). The vessel internal lumen diameter was measured using an optical density video detection system (Living Systems Instrumentation, Burlington, VT), as previously described.[11] Measurements of the lumen diameter were recorded with a Western Graphtec Multicorder (Irvine, CA) (fig. 1).

Stability of the Preparation

To test for the stability of the vessel preparation over time, the internal diameters of 13 microvessels (internal diameter range of 77–449 μ) were followed over 2.5 h. The vessels were found to reach an equilibration point within the first 5 min, and there was neither a vasodilatory nor vasoconstrictive tendency over the 2.5-h period.

To test for the stability of vasomotor responses over time, six small (internal diameter 96 ± 13 μ) and five large (410 ± 24) vessels were equilibrated in the vessel chamber for 30 min and then subjected successively to 100 mEq/l KCl, 1×10^{-5} M acetylcholine, and 1×10^{-6} M U46619, a synthetic thromboxane A2 mimetic, with rinsing and reequilibration for 5 min between interventions. The vessels were subjected to the same set of interventions after 2 h in the vessel chamber. Magnitudes and direction of vasomotor responses were compared.

Study Protocol

After a minimum of 15–30 min of equilibration in the vessel chamber, a baseline measurement of the vessel internal lumen diameter was obtained. With no preconstriction or predilatation, the vessels were subjected to increasing concentrations of isoflurane, 0.3%, 0.5%, 1%, 2%, and 3% for 10 min each, by adding the anesthetic to the 95% O_2/5% CO_2 mixture bubbling the Krebs buffer solution, using an in-line Vernitrol vaporizer (Ohio Medical Products, Madison, WI). In a preliminary experiment, it was determined by gas chromatography that it took less than 10 min for isoflurane to reach a steady-state concentration after it was introduced in the vessel chamber. The internal diameter of the vessel was measured at each concentration. The anesthetic content in the gas mixture was continuously monitored using a Rascal II Gas Analyzer (Ohmeda, Salt Lake City, UT), that had been calibrated with in-

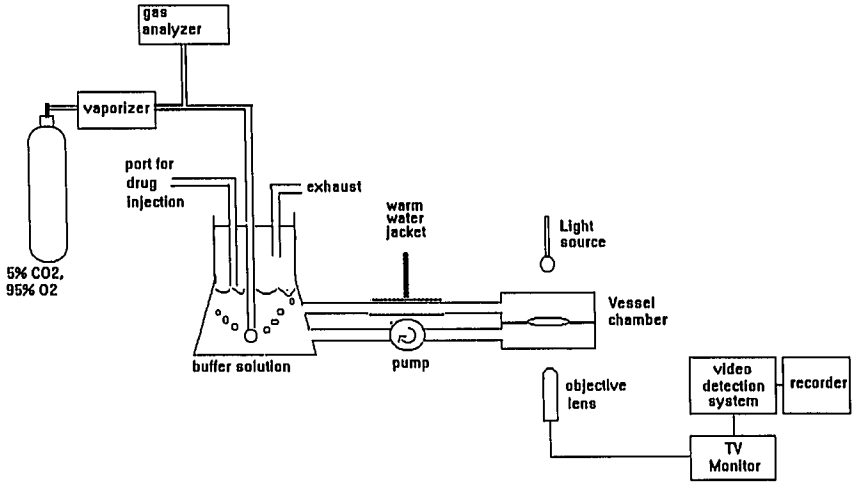

Fig. 1. Schematic for the experimental setup. Isoflurane was carried by 5% CO_2/95% O_2 mixture and bubbled into the buffer solution, which bathed the vessel chamber. In the chamber, the vessel was distended by connecting the micropipettes to columns of Krebs solution (not shown for clarity of presentation). The vessel was magnified and imaged on the television monitor, and its dimensions were measured by the video detection system and recorded.

dustrial standards. Selected samples also were taken from the vessel chamber to measure the concentration of isoflurane. It was found that the millimolar concentration and partial pressure of isoflurane in the vessel chamber (approximately 0.1–1.1 mM and 2–22 mmHg) remained consistently proportional to its concentration in the gas mixture bubbled into the buffer solution. At the end of each experiment, the anesthetic was discontinued. The vessel chamber was flushed with fresh Krebs buffer and the vessel reequilibrated at 37°C. KCl was then added in increments to final concentrations of 25, 50, 75, and 100 mEq/l and the internal lumen diameter measured at each concentration. Only those vessels that constricted by at least 10% to KCl at the end of each experiment were considered still viable and included for data analysis. This represented exclusion of any vessel that constricted less than the average by more than approximately 1 standard deviation. Sixty-nine vessels from 24 rabbits, not including the vessels used for the time control study, met this criterion and are the subject of this study.

To determine the endothelial dependence of an observed vasomotor effect, vessels were denuded of the endothelium by repeatedly passing a human hair and then flushing air bubbles and Krebs solution through the lumen. Endothelial denudation was then verified by demonstrating absence of dilation with 1×10^{-5} M acetylcholine (ACh), an endothelium-dependent vasodilator, *previously shown to cause dilation of endothelium-intact vessels (see above),* but retention of vasodilation to 1×10^{-6} M sodium nitroprusside (SNP), an endothelium-independent vasodilator. The small resistance vessels after denudation constricted to ACh by about 20% but returned to baseline after administration of SNP. The larger conductance vessels constricted after denudation to ACh by about 30% of baseline diameter but recovered to about 90% of baseline after SNP. These vessels were rinsed, reequilibrated at 37°C, and then subjected to increasing concentrations of isoflurane as described above, and the lumen diameter was measured at each concentration. Similarly, other vessels were, before exposure to isoflurane, incubated for at least 15 min with either the cyclooxygenase inhibitor indomethacin (2.8×10^{-5} M), the nitric oxide synthase inhibitor N^G-monomethyl-L-arginine (L-NMMA, 3×10^{-4} M), or the soluble guanylate cyclase inhibitor methylene blue (1×10^{-5} M), to determine whether EDNO or a cyclooxygenase product may be implicated as the cause for the vasomotor effect. In our no-flow setup, there was no significant spontaneous change in vessel internal diameter after incubation with any of these three agents.

Statistical Analysis

Comparison of vessel sizes in different experimental groups was made by Student's *t* test. Comparison of the vasomotor responses to KCl, acetylcholine, or U46619 after 30 min of equilibration *versus* after 2 h also was by Student's *t* test. Dose response curves of rabbit coronary arteries to increasing concentrations of isoflurane were analyzed by a one-way analysis of variance (linear contrast), to test the null hypothesis that isoflurane had no dose-dependent effect on the vessel internal diameter. The effects of denuding the endothelium, preincubation with indomethacin, preincubation with L-NMMA, and preincubation with methylene blue were analyzed by multiway analysis of variance (blocked design) to test the null hypothesis that these interventions had no effect on how the vessels responded to isoflurane. Significance was taken at $P < 0.05$.

Results

Stability of Vessel Preparation

There was no significant difference in the magnitude of vasomotor responses of rabbit coronary arteries to KCl, acetylcholine, or U46619 after 2 h of equilibration *versus* after 30 min (fig. 2). The degree of maximum vasoconstriction in response to 1×10^{-6} M U46619 was greater than that in response to 100 mEq/l KCl,

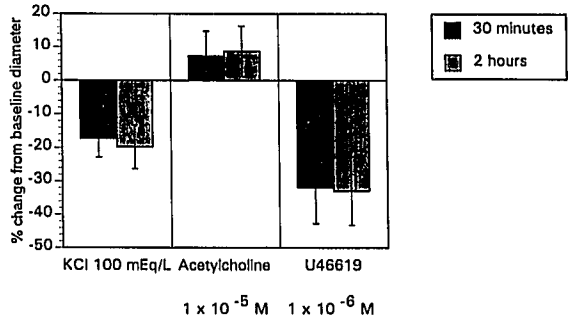

Fig. 2. Vasomotor responses after 30 min of equilibration *versus* after 2 h of equilibration. Data points in the graph represent mean ± SD. There was no significant difference in the vasomotor responses of rabbit coronary microvessels to KCl, acetylcholine, or U46619 between the two time points. The vessels constricted more in response to U46619 than to KCl after 30 min ($P < 0.0008$) and after 2 h ($P < 0.0006$).

suggesting that, in these vessels, receptor-mediated Ca^{++} channels produce greater constriction than voltage-operated Ca^{++} channels.

Vasomotor Response to Isoflurane

The response of rabbit epicardial coronary arteries to isoflurane was dependent upon their size (fig. 3): Initial studies of 19 vessels suggested that those larger than 290 μ in diameter (n = 7, internal diameter 371 ± 54 μ, range 296–460 μ) dilated in response to isoflurane; those smaller than 180 μ (n = 6, 139 ± 34 μ, range 72–174) constricted, and those in between (n = 6, 217 ± 16 μ, range 199–249) had no net response and were not studied further. In the New Zealand White rabbits used in this study, the coronary arteries 290–600 μ in diameter were large epicardial arteries such as the left anterior descending artery or its first order branches that would function mainly as conductance vessels, whereas those smaller than 180 μ were higher generation subepicardial branches that would provide significant resistance to coronary perfusion.

When the small arteries were denuded of endothelium (n = 6, 123 ± 17, range 103–151), they no longer showed constriction to isoflurane (fig. 4), *indicating that the effect was endothelium-dependent.* Similarly, preincubation with indomethacin (n = 8, 126 ± 31, range 70–172) abolished vasoconstriction to isoflurane (fig. 4), *suggesting that a vasoconstrictive prostaglandin substance or product of cyclooxygenase was involved in the vasoconstrictive effect of isoflurane on the small coronary microvessels.* Preincubation of the small microvessels with methylene blue (n = 6, 124 ± 21, range 86–152) or L-NMMA (n = 6, 126 ± 16, range 99–144) did not change the vasoconstrictive effect of isoflurane in these vessels, *suggesting that isoflurane did not have a concurrent EDNO-dependent vasodilatory effect on these vessels masked by a stronger vasoconstrictive effect* (fig. 4).

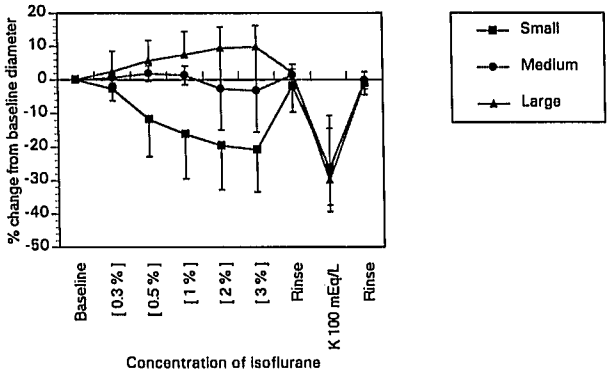

Fig. 3. Percent change from baseline internal diameter *versus* concentration of isoflurane for three different size groups of rabbit coronary microvessels. Data points in the graph represent mean ± SD. Isoflurane was associated with dose-dependent constriction of small resistance microvessels ($P < 0.002$) but dose-dependent dilation of larger conductance vessels ($P < 0.004$). The medium-sized vessels neither dilated nor constricted in response to isoflurane. The three groups were significantly different from one another in baseline internal diameter.

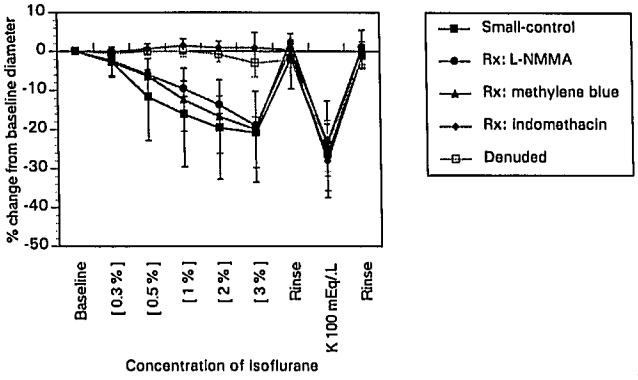

Fig. 4. Percent change from baseline internal diameter *versus* concentration of isoflurane for small resistance vessels. Data points in the graph represent mean ± SD. Vasoconstriction of the small vessels in response to isoflurane was abolished by endothelial denudation ($P < 0.000002$) or by pretreatment with indomethacin ($P < 0.000001$). However, pretreatment of the vessels with either L-NMMA ($P = 0.08$) or methylene blue ($P = 0.35$) had no effect on how the vessels responded to isoflurane. The five groups of small microvessels were not significantly different from one another in baseline internal diameter.

When the large coronary arteries were denuded of endothelium (n = 5, 400 ± 76, range 301–478), they no longer dilated in response to isoflurane (fig. 5), *showing that the effect was endothelium-dependent.* Preincubation of the large arteries with L-NMMA (n = 6, 401 ± 68, range 313–514), methylene blue (n = 7, 424 ± 95, range 322–568), or indomethacin (n = 6, 367 ± 76, range 298–605) reduced the vasodilation to isoflurane (fig. 5), *suggesting that the vasodilatory effect on the large vessels may be mediated through the release of both EDNO and a vasodilatory prostaglandin substance.*

Discussion

The most important findings of our study are: (1) isoflurane produces a concentration-dependent con-

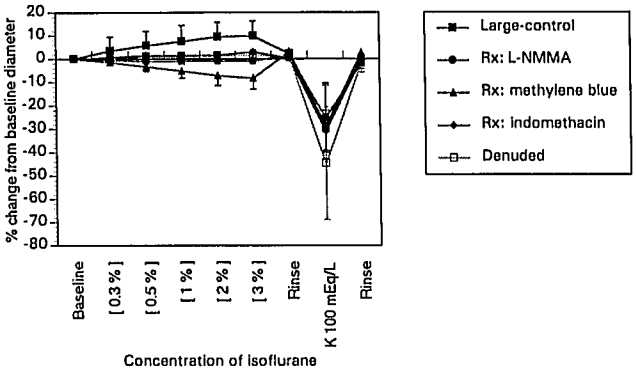

Fig. 5. Percent change from baseline internal diameter *versus* concentration of isoflurane for large conductance vessels. Data points in the graph represent mean ± SD. Vasodilation of the large microvessels in response to isoflurane was abolished by endothelial denudation ($P < 0.00006$), pretreatment with methylene blue ($P < 0.000001$), L-NMMA ($P < 0.000001$), or indomethacin ($P < 0.00003$). Pretreatment with methylene blue resulted in a significant dose-dependent vasoconstriction in response to isoflurane ($P < 0.000004$). The five groups of vessels were not significantly different from one another in baseline internal diameter.

striction of rabbit resistance coronary arteries (mean size about 140 μ), whereas it is associated with dilation of conductance vessels (mean size about 400 μ); (2) the constriction of the resistance vessels is mediated by cyclooxygenase product(s) released by the endothelium; and (3) the dilation of the conductance vessels is also endothelium-dependent but may have multiple mediators.

Previous studies examining a possible role of EDNO in isoflurane-induced vasodilation have yielded conflicting data.[2-7] Blaise *et al.*[2] demonstrated in ring preparations of canine epicardial coronary arteries that the ability of 2.3% isoflurane (1.5 MAC in dogs) to attenuate vasoconstriction produced by serotonin, phenylephrine, or prostaglandin $F_{2\alpha}$ depended on the presence of an intact endothelium. *However, in their preparation, vasoconstriction produced by KCl alone was not altered by isoflurane.* Further, Greenblatt *et al.*[3] demonstrated in indomethacin-treated rats anesthetized with equipotent concentrations (1 MAC) of isoflurane or halothane that the administration of L-NMMA, an inhibitor of nitric oxide synthase, significantly increased vascular resistance in various organs including the heart, kidneys, and the gastrointestinal tract and that the increase was greater with isoflurane than halothane. This suggested that EDNO-mediated vasodilation in the vascular beds studied was more prominent during isoflurane anesthesia than halothane anesthesia. *However, their study lacked a control group of rats without anesthesia in which the effect of L-NMMA was measured and, therefore, it could not be said whether isoflurane or halothane increased the basal release of EDNO or attenuated it.* In contrast, Jensen *et al.*[4] showed that isoflurane produced dose-dependent relaxation in vascular rings from potassium-constricted rabbit basilar arteries and that this effect was endothelium-independent. Flynn *et al.*[5] studied ring preparations from canine cerebral arteries preconstricted with either serotonin or prostaglandin $F_{2\alpha}$ and demonstrated that isoflurane produced dose-dependent relaxation that was endothelium-independent and was not inhibited by L-NMMA or indomethacin. Brendel and Johns[6] showed that isoflurane produced dose-dependent dilation of indomethacin-treated rat thoracic aortic rings preconstricted with either KCl or phenylephrine but that there was no concurrent increase in cyclic GMP (cGMP) content in the rings. Because EDNO causes vascular relaxation by a guanylate cyclase mechanism and thus increases cGMP, this suggested that the vasodilatory effect of isoflurane was not based on EDNO.

Adding to the complexity of the vasomotor effect of inhalational anesthetics, several studies demonstrated attenuation or inhibition of endothelium-mediated vasodilation by inhalational anesthetics. Muldoon *et al.*[12] demonstrated in isolated ring preparations of rabbit aorta and canine femoral and carotid arteries that halothane attenuated vasodilation produced by receptor-mediated, endothelium-dependent agonists acetylcholine and bradykinin but had no effect on vasodilation produced by nitroglycerin, which, in those vessels, acts by an endothelium-independent mechanism. Subsequent studies in different laboratories[13-16] confirmed that halothane,[14-16] isoflurane,[13,14,16] and enflurane[16] attenuate or inhibit EDNO-dependent vasodilation by receptor-mediated agonists, such as acetylcholine, but have no effect on vasodilation by endothelium-independent vasodilators, such as sodium nitroprusside and nitroglycerin. However, whether inhalational anesthetics attenuate EDNO-dependent vasodilation by nonreceptor-mediated agonist A23187 has been controversial.[14,16] A recent study from Muldoon's laboratory[17] reported that, in endothelium-denuded rat aortic rings, halothane attenuated vasodilation and cGMP production caused by nitric oxide, suggesting that the anesthetic might have multiple sites of action in the EDNO pathway.

The most surprising finding of our study is the isoflurane-induced vasoconstriction of coronary resistance microvessels. Stone and Johns[7] reported that rat thoracic aortic rings precontracted with phenylephrine showed further vasoconstriction to 0.5–2% (but not higher concentrations) of isoflurane, and this effect was abolished by endothelial denudation. However, in their study, indomethacin potentiated vasoconstriction rather than inhibited it. To our knowledge, there have been no other reports of isoflurane-associated active vasoconstriction. Rather, previous studies purporting to study the effect of inhalational anesthetics on coronary resistance vessels have reported a vasodilatory effect.[18,19] Differences between our finding and that of others may be, at least in part, due to differences in methodology and species studied.

In this study, we used an *in vitro* preparation of rabbit epicardial coronary arteries suspended in a vessel chamber in a no-flow state and distended by a constant pressure. Our preparation differs from that of most of the aforementioned studies in several important aspects. First, the vessels in our study were neither preconstricted nor predilated before being exposed to isoflurane to maximize the stability of the system and to ensure that both constrictive and dilatory responses could be examined. The net observed effect of the anesthetics depends on the pre-exposure vessel tone. Predilation of vessels might mask a vasodilatory effect. In contrast, preconstriction would tend to mask any vasoconstrictive effect of the anesthetic being tested. Second, we measured the internal diameter of the vessels rather than isometric tension. Vascular resistance is a function of the vessel diameter and only indirectly of the tension of the vascular smooth muscle. Therefore, we postulate that it is more physiologic to measure the vessel diameter rather than the tension of the smooth muscle in studies of vascular resistance. Third, we have studied vessels of varying sizes, including vessels small enough to be considered resistance vessels. Under normal conditions, the contribution of epicardial conductance vessels to overall coronary resistance is less than 10%.[20] The majority of vascular resistance resides in vessels comprising precapillary arterioles and small prearteriolar arteries.[20] In a study using New Zealand White rabbits, Nellis *et al.*[21] reported that arteries less than 140 μ account for 60–70% of coronary vascular resistance. Another study of rabbit coronary arteries reported the caliber of "resistance" arteries to be about 280 μ.[22] As conductance and resistance vessels may and often do respond differently to pharmacologic agents,[8,9,23–27] it is not valid to study the effect of an agent on conductance vessels and then extrapolate the data from conductance vessels to resistance vessels. Our experimental preparation allowed a direct study of resistance vessels as well as conductance vessels.

In addition, several limitations and advantages of our preparation may be noted. First, it is an *in vitro* preparation, which excludes the effect of the autonomic nervous system and blood-borne vasomotor mediators as well as autoregulatory and metabolic influences. While this allows a direct study of the effect of isoflurane, it may not be a faithful representation of what occurs *in vivo*. Second, our preparation suspends the vessel in a no-flow state. Basal release of endothelium-derived vasodilators such as EDNO has been shown to be flow-dependent[28] and would be minimized in a no-flow preparation. However, flow-mediated release of nitric oxide may complicate experimental findings. Thus, a no-flow preparation may be advantageous. Third, our studies were performed in a rabbit preparation. Application of the findings to other species including humans may not be justified. Obviously, further studies using other species are needed before any generalizations and recommendations for pharmacologic relevance can be made. In addition, other inhalational anesthetics need to be studied for their effects on coronary resistance vessels.

We have found that isoflurane produces a concentration-dependent vasodilation of conductance vessels and a vasoconstriction of resistance vessels. Vasodilation of the conductance vessels and vasoconstriction of the resistance vessels by isoflurane may result in redistribution of coronary vascular resistance so that the smaller arteries account for a greater portion of the total resistance. The resulting redistribution of resistance may lead to an increase in overall resistance and a decrease in coronary flow. Because there is a network of coronary arteriovenous anastomoses,[29] vasoconstriction of resistance vessels with dilation of anastomotic vessels proximal to resistance vessels may lead to a decrease in coronary resistance and an increase in flow. Indeed, Gelman *et al.*[30] found in dogs under 2 MAC isoflurane anesthesia that intramyocardial shunting was increased threefold compared to awake control, though the increase did not achieve statistical significance. In a study of the effect of isoflurane on coronary flow in isolated rabbit hearts, Tanguay *et al.*[31] found that 0.7% isoflurane decreased coronary flow, but 1.4% isoflurane transiently increased it, followed by a gradual decline to the baseline level. Our findings are in contrast to

two previous studies in dogs[8,18] in which isoflurane had little effect on epicardial conductance arteries but dilated small coronary arterioles. Nakamura *et al.*[8] measured changes in tension of epicardial artery rings precontracted with KCl. Sill *et al.*[18] measured epicardial conductance artery diameter and coronary flow, surmising arteriolar dimensions from the latter. Differences between our findings and theirs may be due to the different species studied and differences in methodology.

There are several possibilities as to the nature of the endothelium-derived mediator that produced vasoconstriction in the resistance vessels in response to isoflurane. First, it may be a vasoconstrictor prostaglandin substance. Shayevitz *et al.*[32] demonstrated, in isolated rabbit lung preparations, that halothane augmented the pulmonary vasopressor response to t-butyl-hydroperoxide and theorized that thromboxane A_2 production in response to t-butyl-hydroperoxide was increased because halothane increased substrate (arachidonic acid) availability. Second, it may be an oxygen-derived free radical produced as a "byproduct" in the cyclooxygenase pathway. Third, it may be an endothelium-derived contractile factor, such as endothelin. Further studies are needed to elucidate the nature of the vasoconstrictive mediator.

In our preparation, isoflurane-mediated dilation of large conductance vessels was inhibited by not only endothelial denudation but also pretreatment with L-NMMA, methylene blue, or indomethacin. In addition, pretreatment with methylene blue but not pretreatment with L-NMMA resulted in dose-dependent vasoconstriction of the large vessels in response to isoflurane. Methylene blue is capable of oxidizing the ferrous heme moiety of the soluble guanylate cyclase[33] and can block not only the stimulated but also the unstimulated basal guanylate cyclase activity.[33] One possibility for the difference in effect of L-NMMA and methylene blue upon the large vessels is that methylene blue may have afforded a more complete inhibition of cGMP-mediated vasodilation than L-NMMA, an inhibitor of new production of nitric oxide, and unmasked a concomitant vasoconstrictive effect of isoflurane even on the large vessels. Another possibility is that methylene blue is associated with nonspecific oxidation and/or inhibition of other types of heme proteins.

In summary, we have reported the isoflurane-induced vasoconstriction of coronary resistance microvessels in rabbits. This effect was endothelium-dependent and mediated by cyclooxygenase product(s). Further studies are needed to identify the nature of the vasoconstrictive mediator(s) and to test whether a similar effect may be seen in other species and with other inhalational agents.

The authors express their gratitude to John Zeind, of Beth Israel Hospital, for assistance with gas chromatography of isoflurane; Dr. Emery Brown, of Massachusetts General Hospital, for comments and suggestions on statistical analysis; and Dr. David Feinstein, Leo Hannenberg, and Linda Robertson, of Beth Israel Hospital, for assistance with computer programs.

References

1. Lowenstein E, Park KW, Reiz S: Effects of inhalational anesthetics on systemic hemodynamics and the coronary circulation, Cardiac Anesthesia. Edited by Kaplan JA. Philadelphia, WB Saunders, 1993, pp 441–466

2. Blaise G, Sill JC, Nugent M, Van Dyke RA, Vanhoutte PM: Isoflurane causes endothelium-dependent inhibition of contractile responses of canine coronary arteries. ANESTHESIOLOGY 67:513–517, 1987

3. Greenblatt EP, Loeb AL, Longnecker DE: Endothelium-dependent circulatory control: A mechanism for the differing peripheral vascular effects of isoflurane *versus* halothane. ANESTHESIOLOGY 77: 1178–1185, 1992

4. Jensen NF, Todd MM, Kramer DJ, Leonard PA, Warner DS, Bollen BA: The role of the endothelium in modulating the vasodilation induced by isoflurane in the rabbit basilar artery (abstract). ANESTHESIOLOGY 73:A648, 1990

5. Flynn NM, Buljubasic N, Bosnjak ZJ, Kampine JP: Isoflurane produces endothelium-independent relaxation in canine middle cerebral arteries. ANESTHESIOLOGY 76:461–467, 1992

6. Brendel JK, Johns RA: Isoflurane does not vasodilate rat thoracic aortic rings by endothelium-derived relaxing factor or other cyclic GMP-mediated mechanisms. ANESTHESIOLOGY 77:126–131, 1992

7. Stone DJ, Johns RA: Endothelium-dependent effects of halothane, enflurane, and isoflurane on isolated rat aortic vascular rings. ANESTHESIOLOGY 71:126–132, 1989

8. Nakamura K, Toda H, Hatano Y, Mori K: Comparison of the direct effects of sevoflurane, isoflurane, and halothane on isolated canine coronary arteries. Can J Anaesth 40:257–261, 1993

9. Sellke FW, Myers PR, Bates JN, Harrison DG: Influence of vessel size on the sensitivity of porcine coronary microvessels to nitroglycerin. Am J Physiol 258:H515–H520, 1990

10. Sellke FW, Tomanek RJ, Harrison DG: L-cysteine selectively potentiates nitroglycerin-induced dilation of small coronary microvessels. J Pharmacol Exp Ther 258:365–369, 1991

11. Halpern WG, Osol G, Coy G: Mechanical behavior of pressurized in vitro prearteriolar vessels determined with a video system. Ann Biomed Eng 121:463–479, 1984

12. Muldoon SM, Hart JL, Bowen KA, Freas W: Attenuation of endothelium-mediated vasodilation by halothane. ANESTHESIOLOGY 68: 31–37, 1988

13. Nakamura K, Hatano Y, Toda H, Mori K: Isoflurane inhibits endothelium-dependent relaxation and cyclic GMP formation in rat aorta (abstract). ANESTHESIOLOGY 75:A530, 1991

14. Toda H, Nakamura K, Hatano Y, Nishiwada M, Kakuyama M, Mori K: Halothane and isoflurane inhibit endothelium-dependent relaxation elicited by acetylcholine. Anesth Analg 75:198–203, 1992

15. Blaise G, Quy T, Parent M, Asenjo F: Does halothane inhibit the release or the action of EDRF (abstract)? ANESTHESIOLOGY 77:A692, 1992

16. Uggeri MJ, Proctor GJ, Johns RA: Halothane, enflurane, and isoflurane attenuate both receptor- and non-receptor-mediated EDRF production in rat thoracic aorta. ANESTHESIOLOGY 76:1012–1017, 1992

17. Hart JL, Jing M, Bina S, Freas W, Van Dyke RA, Muldoon SM: Effects of halothane on EDRF/cGMP-mediated vascular smooth muscle relaxations. ANESTHESIOLOGY 79:323–331, 1993

18. Sill JC, Bove AA, Nugent M, Blaise GA, Dewey JD, Grabau C: Effects of isoflurane on coronary arteries and coronary arterioles in the intact dog. ANESTHESIOLOGY 66:273–279, 1987

19. Longnecker DE, Harris PD: Dilatation of small arteries and veins in the bat during halothane anesthesia. ANESTHESIOLOGY 37:423–429, 1972

20. Marcus ML, Chilian WM, Kanatsuka H, Dellsperger KC, Eastham CL, Lamping KG: Understanding the coronary circulation through studies at the microvascular level. Circulation 82:1–7, 1990

21. Nellis SH, Liedtke AJ, Whitesell L: Small coronary vessel pressure and diameter in an intact beating rabbit heart using fixed-position and free-motion techniques. Circ Res 49:342–353, 1981

22. Osborne JA, Siegman MJ, Sedar AW, Mooers SU, Lefer AM: Lack of endothelium-dependent relaxation in coronary resistance arteries of cholesterol-fed rabbits. Am J Physiol 256:C591–C597, 1989

23. Lamping KG, Kanatsuka H, Eastham CL, Chilian WM, Marcus ML: Nonuniform vasomotor responses of the coronary microcirculation to serotonin and vasopressin. Circ Res 65:343–351, 1989

24. Nakamura Y, Parent R, Lavallee M: Opposite effects of substance P on conductance and resistance coronary vessels in conscious dogs. Am J Physiol 258:H565–H573, 1990

25. Zhang J, Somers M, Cobb FR: Heterogeneous effects of nitroglycerin on the conductance and resistance coronary arterial vasculature. Am J Physiol 264:H1960–H1968, 1993

26. Chilian WM, Layne SM, Klausner EC, Eastham CL, Marcus ML: Redistribution of coronary microvascular resistance produced by dipyridamole. Am J Physiol 256:H383–H390, 1989

27. Chilian WM, Layne SM, Eastham CL, Marcus ML: Heterogeneous microvascular coronary alpha-adrenergic vasoconstriction. Circ Res 64:376–388, 1989

28. Griffith TM, Edwards DH, Davies RL, Harrison TJ, Evans KT: EDRF coordinates the behaviour of vascular resistance vessels. Nature 329:442–445, 1987

29. Lazar HL: Coronary sinus interventions during cardiac surgery. Ann Thorac Surg 46:475–482, 1988

30. Gelman S, Fowler KC, Smith LR: Regional blood flow during isoflurane and halothane anesthesia. Anesth Analg 63:557–565, 1984

31. Tanguay M, Blaise G, Dumont L, Beique G, Hollmann C: Beneficial effects of volatile anesthetics on decrease in coronary flow and myocardial contractility induced by oxygen-derived free radicals in isolated rabbit hearts. J Cardiovasc Pharm 18: 863–870, 1991

32. Shayevitz JR, Traystman RJ, Adkinson NF, Sciuto AM, Gurtner GH: Inhalation anesthetics augment oxidant-induced pulmonary vasoconstriction: Evidence for a membrane effect. ANESTHESIOLOGY 63: 624–632, 1985

33. Martin W, Villani GM, Jothianandan D, Furchgott RF: Selective blockade of endothelium-dependent and glyceryl trinitrate-induced relaxation by hemoglobin and by methylene blue in the rabbit aorta. J Pharmacol Exp Ther 232:708–716, 1985