Anesthesiology
1998; 89:132–42

Flow-induced Dilation of Rat Coronary Microvessels Is Attenuated by Isoflurane but Enhanced by Halothane

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Background: **Volatile anesthetics attenuate agonist-induced endothelium-dependent vasodilation of coronary arteries. This study considered the hypothesis that the anesthetics may also attenuate flow-induced endothelium-dependent vasodilation.**

Methods: **Rat subepicardial arteries of ~100 μm were monitored for diameter changes *in vitro* by a video detection system, with the midpoint luminal pressure held constant at 40 mmHg but the pressure gradient (and therefore flow) across each vessel increased from 0 to 80 mmHg, in the presence or absence of 1 or 2 minimum alveolar concentration (MAC) isoflurane or 1 or 2 MAC halothane, with or without 10 μM of the nitric oxide (NO) synthase inhibitor N^G-nitro-L-arginine (L-NNA) or 10 μM of the cyclooxygenase inhibitor indomethacin.**

Results: **Flow-induced dilation was attenuated by L-NNA or indomethacin ($P < 0.001$ each). It was attenuated by isoflurane in a concentration-dependent manner ($P < 0.001$). Attenuation by 2 MAC isoflurane persisted even in the presence of L-NNA ($P < 0.01$) or indomethacin ($P < 0.05$). On the other hand, flow-induced dilation was enhanced by 2 MAC halothane ($P < 0.05$). Halothane at 1 MAC had no significant effect. Enhancement by 2 MAC halothane was evident in the presence of indomethacin ($P < 0.05$) but not L-NNA ($P = 0.40$).**

Conclusions: **In rat subepicardial arteries, flow-induced dilation is endothelium-dependent and mediated by both NO and a prostanoid. Isoflurane attenuates flow-induced dilation, possibly by decreasing synthesis, the action of NO and a prostanoid, or both, whereas halothane enhances it, possibly by increasing synthesis, the action of NO, or both. (Key words: Endothelium; myocardial blood flow; nitric oxide.)**

FLOW-INDUCED dilation (FID) is one of the determinants of myocardial blood flow distribution, along with metabolic, myogenic, and neurohumoral influences.[1] When flow increases in a coronary vessel, the resulting increase in shear stress on the endothelium leads to the production of endothelium-derived relaxing factor(s).[2] Although metabolic control may be primarily responsible for the close matching of coronary blood flow to tissue needs, myogenic and endothelial regulatory mechanisms play synergistic roles *via* transduction of intravascular pressure and flow, respectively.[1]

Volatile anesthetics attenuate agonist-mediated endothelium-dependent vasodilation (EDD) of both conductance[3,4] and resistance arteries[5,6] *in vitro* and of pulmonary vessels *in vivo*.[7] Further, the hemodynamic effect of nitric oxide (NO) synthase inhibitors is less during halothane anesthesia than during the awake state, suggesting that the unstimulated EDD is less with halothane.[8] Although isoflurane and halothane both attenuate EDD, the sites of anesthetic action in the NO–cyclic guanosine monophosphate (NO-cGMP) pathway may be different, depending on the anesthetic, the vessel type, and perhaps experimental conditions.[4,5,9–12]

In this study, we examined the effect of volatile anesthetics on another type of EDD, namely FID. We studied changes in pressure gradient across isolated coronary resistance arteries while keeping the intravascular pressure constant to remove any myogenic effects. Changes in pressure gradient produce changes in flow in and shear stress on the vessel. We considered whether vasomotion produced by changes in pressure gradient and flow was endothelium dependent by studying the effect of endothelial denudation, the NO synthase inhibitor N^G-nitro-L-arginine (L-NNA), the NO scavenger hemoglobin, and the cyclooxygenase inhibitor indomethacin.

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Received from the Departments of Anesthesia and Critical Care and of Surgery, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts. Submitted for publication September 25, 1997. Accepted for publication February 25, 1998. Supported in part by United States Public Health Service grant HL-46716 and by a grant from Beth Israel Anesthesia Foundation. Presented in part at the 1997 Annual Meeting of the Association of University Anesthesiologists, Washington, DC, April 18–20, 1997.

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We examined the effect of the volatile anesthetics isoflurane and halothane on FID, in the presence of L-NNA, indomethacin, or neither one. Because, in our previous study with the NO-cGMP pathway in rat resistance coronary arteries,[5] halothane had no effect distal to the agonist receptor whereas isoflurane did, we hypothesized that isoflurane may have an attenuating effect on FID but halothane may not.

Methods

Vessel Preparation

In accordance with institutional animal care committee standards, Wistar rats of either sex that weighed 100–150 g were anesthetized by injecting 40 mg/kg ketamine and 5 mg/kg xylazine intraperitoneally. Subepicardial microvessels that were third- or fourth-order branches of the left anterior descending artery were prepared as described previously.[13] Each vessel was placed in a vessel chamber, cannulated with dual micropipettes (50–70 μm), and secured with 10-0 Ethilon (Ethicon Inc., Somerville, NJ) sutures. To ensure uniformity of size and thus resistance offered by the pipettes, each pipette was prepared using Narishige automatic pipette maker (Narishige Scientific Instrument Laboratory, Tokyo, Japan) at constant settings and matched for size under the microscope on both sides of a vessel. The pipettes were connected with silicone tubing to columns of fluids to provide a distending pressure in the vessel. The system was arranged symmetrically so that the vessel represented the midpoint. By varying the heights of the columns of fluids simultaneously and equally in opposite directions, we could vary the pressure gradient (ΔP) and, therefore, flow across the vessel, while maintaining the midpoint intravascular pressure constant.[14,15] In seven vessels the actual midpoint intravascular pressure was 40 ± 2 mmHg (mean ± SD), as the pressure gradient was varied from 0 to 80 mmHg and the mean of the upstream and downstream pressures was held constant at 40 mmHg. In another preliminary study, symmetry of the setup was further verified by measuring FID in four microvessels with flow in one direction and then in the reverse direction (in random order) and noting no significant difference in FID with changes in the direction of flow.

The vessel was bathed continuously with modified Krebs buffer (120 mM NaCl, 5.9 mM KCl, 11.1 mM dextrose, 25 mM $NaHCO_3$, 1.2 mM NaH_2PO_4, 1.2 mM $MgSO_4$, and 2.5 mM $CaCl_2$), gassed with a 95% oxygen and 5% carbon dioxide$_e$ mixture, and maintained at 37°C and *p*H 7.4. The oxygen tension (P_{O_2}) in the vessel chamber exceeded 400 mmHg.

Direct proportionality between ΔP and flow in an *in vitro* preparation such as ours was shown before.[15] In a preliminary study, to confirm that production of ΔP was associated also with proportional changes in flow in our experimental preparation, we measured flow across six control vessels at ΔP values of 20, 40, 60, and 80 mmHg by collecting the effluent for a period of 1 h and measuring the weight of the fluid collected. (The density of Krebs solution at 37°C was 1.018 g/ml.) The flows were 77 ± 11 nl/s, 134 ± 17 nl/s, 194 ± 13 nl/s, and 233 ± 17 nl/s at ΔP values of 20, 40, 60, and 80 mmHg, respectively. There was a linear relation between ΔP and flow (correlation coefficient [r] = 0.97, $P < 0.001$). The flows in our preparation were greater than the flows in the preparation used by Kuo *et al.*[15] but of the same order of magnitude.

The vessel was visualized with an inverted phase-contrast 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).[13] Measurements of the lumen diameter were recorded with a Western Graphtec Multicorder (Irvine, CA). The stability of both endothelium-intact and endothelium-denuded vessels in our experimental preparation for at least 2.5 h was shown previously.[16] Schematics of our[13] and similar[14,15] preparations have been published already.

Measurement of Flow-induced Vasodilation

Each vessel was equilibrated in the vessel chamber at 37°C with an intraluminal pressure of 40 mmHg and ΔP of 0 mmHg for 30 min. After measurement of the baseline internal diameter ($D_{baseline}$), the vessel was preconstricted with 1 μM of the thromboxane analog U46619 for 5 min and the constricted diameter (D_{const}) was measured. ΔP was increased from 0 to 80 mmHg in 10-mmHg increments while the midpoint intraluminal pressure constant was maintained at 40 mmHg. In a preliminary experiment with six rat coronary arteries, no hysteresis was observed. We found that the ΔP-diameter relation obtained with ΔP increments from 0 to 80 mmHg could be superimposed on the relation obtained with ΔP decrements from 80 to 0 mmHg.

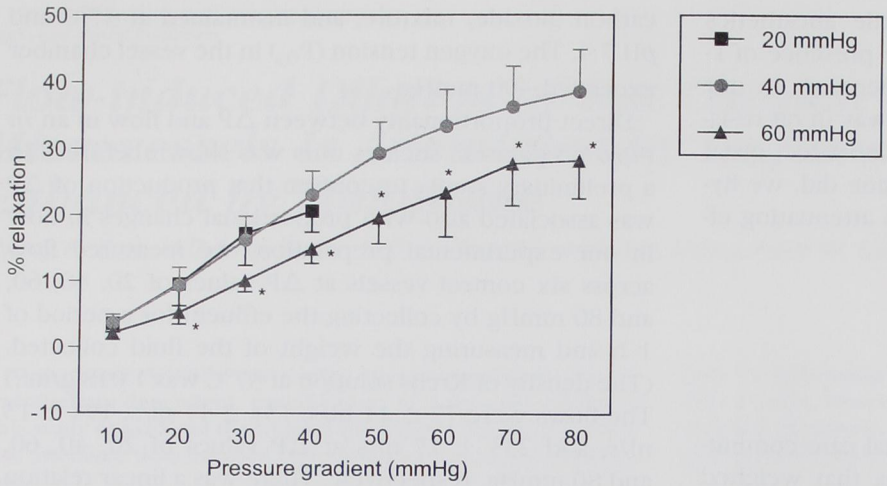

Fig. 1. Percentage relaxation of the U46619-preconstricted vessel in response to changes in pressure gradients (ΔP) across the vessel, at three different intraluminal pressures (20, 40, and 60 mmHg). Flow-induced dilation was significantly greater at 40 mmHg than at 60 mmHg ($P < 0.01$). Although flow-induced dilation at 20 mmHg was not significantly different than that at 40 mmHg ($P = 0.82$), a greater range of pressure gradients could be examined at a vessel intraluminal pressure of 40 mmHg than at 20 mmHg, because the lowest downstream pressure was 0 mmHg in our preparation.

Therefore, in the current study, all measurements were obtained during ΔP increments only.

At each pressure gradient the vessel was allowed to reach a steady state diameter for 3 min and then the steady state diameter was recorded (D_{relax}). The percentage relaxation from preconstriction with U46619 was calculated as:

$$\% \text{ relaxation} = 100 \times (D_{relax} - D_{const})/(D_{baseline} - D_{const})$$

At the end of each vessel run, the ΔP was returned to 0 mmHg and the vessel was flushed with fresh Krebs buffer and reequilibrated at 37°C. Potassium chloride was added to a final concentration of 100 mM, followed by 10 μM of the endothelium-dependent dilator adenosine diphosphate. Only those vessels that constricted to potassium chloride by 15% or more were considered still viable[16] and included for data analysis. Preservation of endothelial function was assessed by the vessel response to adenosine diphosphate.

Similar studies were performed with the intraluminal pressures kept at 20 or 60 mmHg. Because flow-mediated dilation was maximal at 40 mmHg (fig. 1) and this is in the physiologically typical intraluminal pressure range of 40–50 mmHg,[15,17] the rest of our studies were performed with an intraluminal pressure of 40 mmHg.

Endothelial Dependence of Flow-induced Vasodilation

Additional vessels were equilibrated at 37°C in Krebs solution containing 10 μM of the NO synthase inhibitor L-NNA, 10 μM of the cyclooxygenase inhibitor indomethacin, or both. The ΔP–diameter relation was obtained as noted before and the percentage relaxation from constriction with U46619 was calculated. Viability of the vessels was tested as noted before.

Additional vessels were denuded of the endothelium by passing a piece of human hair through the lumen of the artery.[13] The ΔP–diameter relation was then obtained as noted above and the percentage relaxation from constriction with U46619 was calculated. Viability of the vessels was tested as above. These endothelium-denuded vessels showed no dilation in response to 10 μM adenosine diphosphate.

To determine the effect of NO scavenging by hemoglobin in blood, blood was aspirated from the apex of the heart into a syringe containing heparin (final concentration, 333 U/ml) before the heart was removed. The hemoglobin content of the blood collected was measured using a 482 Co-Oximeter (Instrument Laboratory Co., Lexington, MA). The lumen of the isolated vessel was filled with either heparinized Krebs solution, heparinized blood, or heparinized blood diluted with Krebs solution. In all these solutions, the concentration of heparin was 333 U/ml, which effectively prevented blood coagulation in micropipettes. Extraluminally the vessel was bathed in Krebs solution. After equilibration of each vessel at 37°C and preconstriction with U46619 1 μM, the ΔP–diameter relation was obtained as noted before. Viability of the vessel was tested as described here before.

Effect of Volatile Anesthetics

To determine the effect of volatile anesthetics, the ΔP–diameter relations of additional vessels were obtained in the presence of 1 or 2 MAC isoflurane (1 MAC

= approximately 1.2%)[18] or 1 or 2 MAC halothane (1 MAC = approximately 0.9%)[18] and compared with the relation obtained with endothelium-intact control. After each vessel was equilibrated at 37°C for 30 min in the vessel chamber and preconstricted with U46619 1 μM, the vessel was subjected to the anesthetic by adding the anesthetic to the 95% oxygen and 5% carbon dioxide mixture and bubbling the Krebs buffer solution using an in-line bubble-through vaporizer. In a preliminary experiment using gas chromatography, we determined that isoflurane and halothane reached steady state concentrations after introduction in the vessel chamber in <10 and 15 min, respectively. The anesthetic content in the gas mixture was monitored continuously using a Rascal II Gas Analyzer (Ohmeda, Salt Lake City, UT) that was previously calibrated to industrial standards. In a previous report[19] we showed by gas chromatographic analysis that in our experimental preparation the millimolar concentration and partial pressure of isoflurane or halothane in the vessel chamber reflected its concentration in the gas mixture bubbled into the buffer solution.

No significant change in internal diameter of the U46619-preconstricted vessel was noted after steady state concentrations of 1 or 2 MAC isoflurane were obtained. In contrast, there was mild (10–15%) dilation of the U46619-preconstricted vessel after steady state concentrations of halothane were obtained. The diameter obtained after U46619 and either isoflurane or halothane (or neither anesthetic in case of a control vessel) was considered as the constricted diameter.

At least 10 or 15 min after introduction of isoflurane or halothane, respectively, the ΔP–diameter relation was obtained as noted before. At the end of each vessel run, the anesthetic was discontinued and viability of the vessel was tested as noted before.

In addition, the ΔP–diameter relation was determined in the presence of either 2 MAC isoflurane or 2 MAC halothane for vessels pretreated with either 10 μM L-NNA or 10 μM indomethacin. Viability of these vessels was tested as noted before.

Statistical Analysis

Each animal contributed no more than one vessel to any one experimental group; therefore, n for each group represents the number of animals and the number of vessels. All data are presented as mean ± SD.

Whether there is a ΔP-dependent dilation of the vessels was tested by one-way analysis of variance (with Scheffé's linear contrast). The effect of an intervention such as endothelial denudation or pretreatment of the vessel with L-NNA on the ΔP-diameter relation was evaluated by two-way analysis of variance with a repeated measures factor, with the *post hoc* Neuman-Keuls test for between-groups comparison and stratified *z* tests to identify the gradients in which the differences in responses were significant. Similarly, the effect of anesthetics on the ΔP-diameter relationship was evaluated by a two-way analysis of variance with a repeated-measures factor. The correlation between the flows measured and the pressure gradients was assessed by simple linear regression. Significance was considered as $P < 0.05$. All statistics were calculated using True Epistat software (Epistat Services, Richardson, TX).

Results

Endothelial Dependence of Flow-induced Dilation

The control coronary microvessels (n = 7; baseline size, 103 ± 6 μm [mean ± SD]) demonstrated ΔP-dependent dilation ($P < 0.001$). This effect of ΔP was attenuated by either pretreatment of the vessels with L-NNA ($P < 0.001$; n = 8; size, 94 ± 8 μm) or indomethacin ($P < 0.001$; n = 5; size, 97 ± 11 μm) and completely abolished by pretreatment with both L-NNA and indomethacin ($P < 0.001$; n = 6; size, 111 ± 8 μm) or by endothelial denudation ($P < 0.001$; n = 5; size, 90 ± 10 μm). With endothelial denudation, there was actually mild constriction of the vessels at high pressure gradients (fig. 2).

The addition of heparin to the Krebs solution bathing the lumen of the vessels (n = 7; size, 99 ± 6 μm) mildly enhanced the dilatory response to increasing ΔP ($P < 0.01$; fig. 3A), consistent with the previously reported endothelium-dependent dilatory effect of heparin.[20] Blood in the lumen attenuated the dilatory response to increasing ΔP, in a hemoglobin concentration-dependent manner ($P < 0.001$; fig. 3B). (Blood in the lumen: n = 7; size, 94 ± 15 μm; hemoglobin, 11.1 ± 0.2 g/dl. Blood diluted with Krebs solution in the lumen: n = 7; size, 101 ± 5 μm; hemoglobin, 5.6 ± 0.1 g/dl).

Effect of Volatile Anesthetics on Flow-induced Dilation

Flow-induced dilation of rat coronary vessels was attenuated by isoflurane in a concentration-dependent manner ($P < 0.001$; fig. 4A). (1 MAC isoflurane: n = 7; size, 82 ± 13 μm. 2 MAC isoflurane: n = 6; size, 100 ± 5 μm). After pretreatment of the vessels

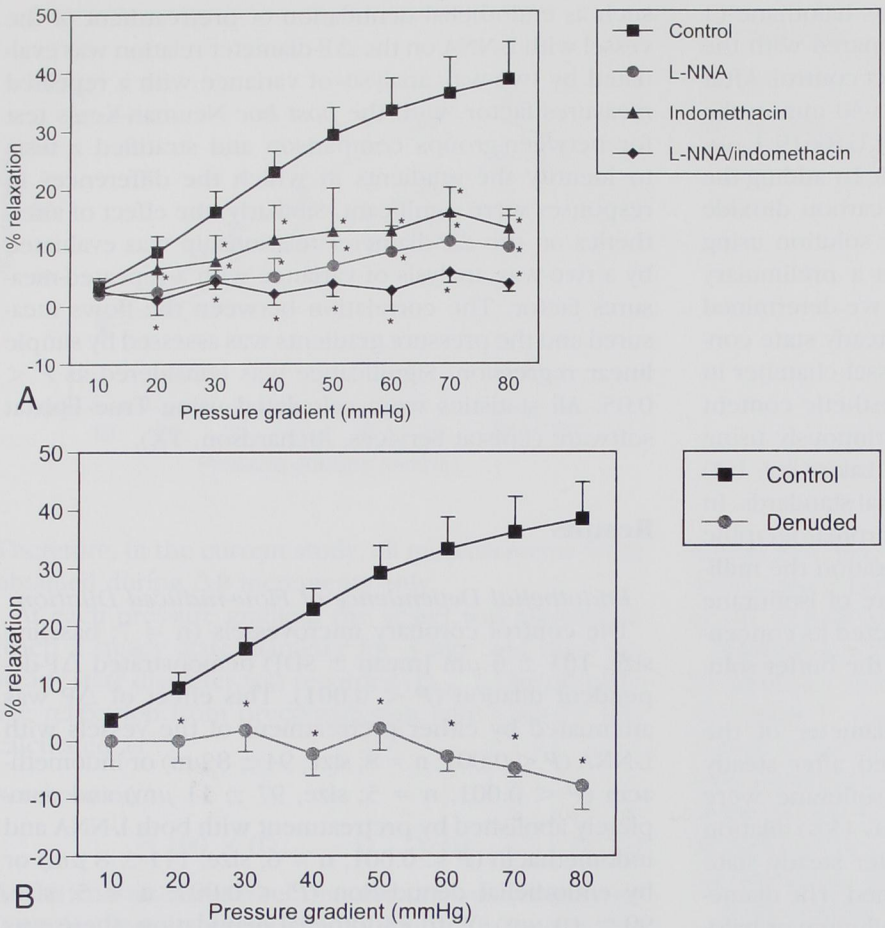

Fig. 2. Percentage relaxation of the U46619-preconstricted vessel in response to changes in pressure gradients (ΔP) across the vessel. Control vessels showed ΔP-dependent dilation ($P < 0.001$). (*A*) This effect was attenuated by either pretreatment of the vessels with the nitric oxide synthase inhibitor N^G-nitro-L-arginine (L-NNA) or the cyclooxygenase inhibitor indomethacin and completely abolished either in the presence of both L-NNA and indomethacin, or (*B*) with endothelial denudation ($P < 0.001$ each). *$P < 0.05$ *versus* control.

with 10 μM L-NNA, 2 MAC isoflurane attenuated FID even further ($P < 0.01$; n = 6; size, 104 ± 1 μm; fig. 4B), indicating that isoflurane was attenuating dilation as a result of an L-NNA-insensitive agent such as a prostanoid. After pretreatment of the vessels with 10 μM indomethacin, 2 MAC isoflurane attenuated FID even further ($P < 0.05$; n = 5; size, 107 ± 8 μm; fig. 4C), indicating that isoflurane was also attenuating dilation as a result of an indomethacin-insensitive agent such as NO.

On the other hand, 2 MAC halothane enhanced the FID of coronary vessels ($P < 0.05$; n = 5; size, 94 ± 14 μm). Halothane at 1 MAC had no significant effect on FID ($P = 0.52$; n = 6; size, 100 ± 14 μm; fig. 5A). After pretreatment of the vessels with 10 μM L-NNA, 2 MAC halothane did not enhance FID any further ($P = 0.40$; n = 6; size, 102 ± 10 μm; fig. 5B), indicating that halothane-mediated enhancement of FID was not the result of an L-NNA-insensitive agent such as a prostanoid. However, after pretreatment of the vessels with 10 μM indomethacin, 2 MAC halothane enhanced FID ($P < 0.05$; n = 6; size, 105 ± 7 μm; fig. 5C), indicating that the enhancement may involve an indomethacin-insensitive agent such as NO.

Discussion

The main findings of this study are as follows. First, the FID of rat coronary microvessels is endothelium dependent and appears mediated by both NO and a prostanoid(s). Second, as hypothesized, isoflurane attenuates FID of rat coronary microvessels. This attenuation appears to involve attenuation of both NO- and prostanoid-mediated dilation. Third, halothane does not have the same effect, but at a high concentration it enhances FID.

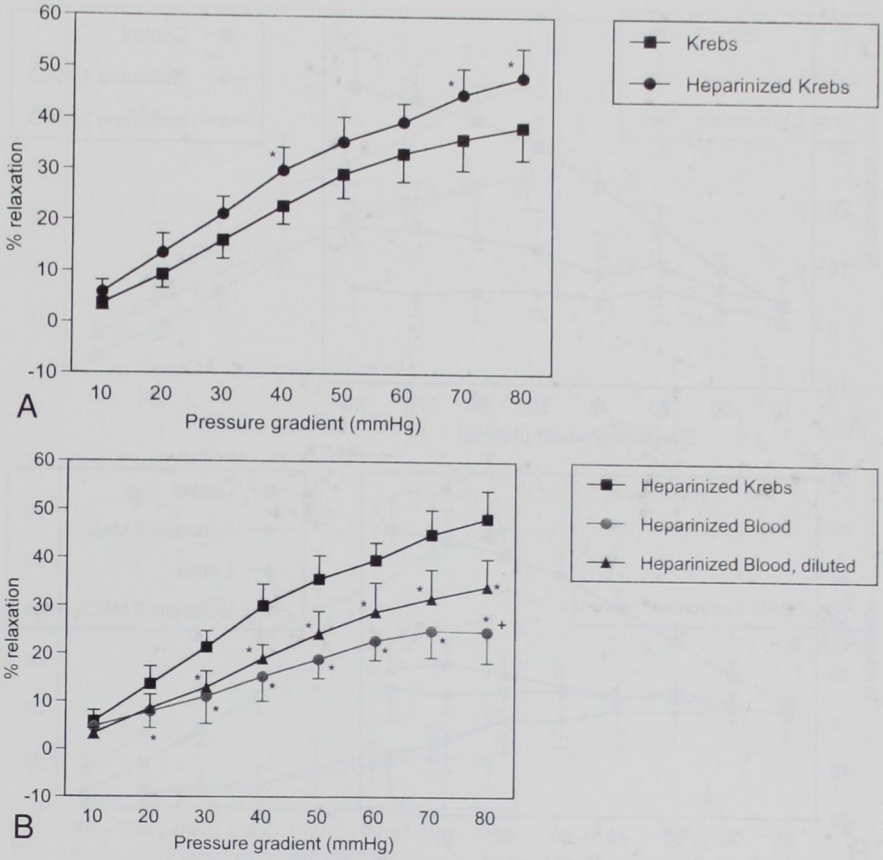

Fig. 3. (*A*) Percentage relaxation of the U46619-preconstricted vessel in response to changes in pressure gradients (ΔP) across the vessel when the perfusate was Krebs solution or heparinized Krebs solution. With heparinization, the ΔP-produced, flow-induced dilation was enhanced ($P < 0.01$). *$P < 0.05$ *versus* Krebs solution as the perfusate. (*B*) Percentage relaxation of the U46619-preconstricted vessel in response to changes in pressure gradients (ΔP) across the vessel with different perfusates. Flow-induced dilation was attenuated as the hemoglobin concentration of the perfusate was increased ($P < 0.001$). *$P < 0.05$ *versus* heparinized Krebs solution as the perfusate. +$P < 0.05$ *versus* diluted heparinized blood as the perfusate.

Critique of Our Vessel Preparation

Our preparation is a microvessel chamber video detection system[14,15] adapted for administration of volatile anesthetics into the chamber. By matching the pipettes for size and resistance and by altering the heights of the fluid reservoirs connected to the pipettes in opposite directions simultaneously, we could alter pressure gradients and thus flow without altering midpoint luminal pressure in the vessel, thus avoiding myogenic responses.[14,15] Functional symmetry of the setup was further proved by showing that FID did not depend on the direction of the flow.

The flows measured in our preparation were somewhat greater than those reported by Kuo *et al.*[15] Erythrocyte velocity in turtle and canine coronary arterioles (~10-40 μm diameter) approaches 3.5 mm/s during diastole but decreases to <2 mm/s during systole.[21] In rat skeletal muscle arterioles, erythrocyte velocity ranges from ~5-30 mm/s and blood flow from 1-170 nl/s, with the numbers increasing in the proximal arterioles.[22] Our vessels are distal resistance arteries in which the flow would be expected to be greater than in the distal arterioles (40-80 μm) examined by Kuo *et al.*[15] Therefore the flows we measured (~50-250 nl/s) appear to be within the physiologic range.

Comparison with Previous Studies

Flow-induced dilation has been described in many species, including cats,[23] pigs,[15] and humans.[24] The first demonstration in resistance coronary arteries was by Kuo *et al.*[15] Our findings of FID in rats corroborates Kuo *et al.*'s findings in pigs. The importance of endothelium in mediating FID has been recognized nearly uniformly. Removal of endothelium has either abolished the response[15,25] or converted it to one of mild constriction.[26] Even Bevan *et al.*,[27] who reported that FID of rabbit ear arteries persisted after endothelium removal, noted that the response was enhanced in the presence of the endothelium. Our finding of abolished FID in the endothelium-denuded vessels or vessels pretreated with L-NNA and indomethacin are consistent with the results of the previous studies.

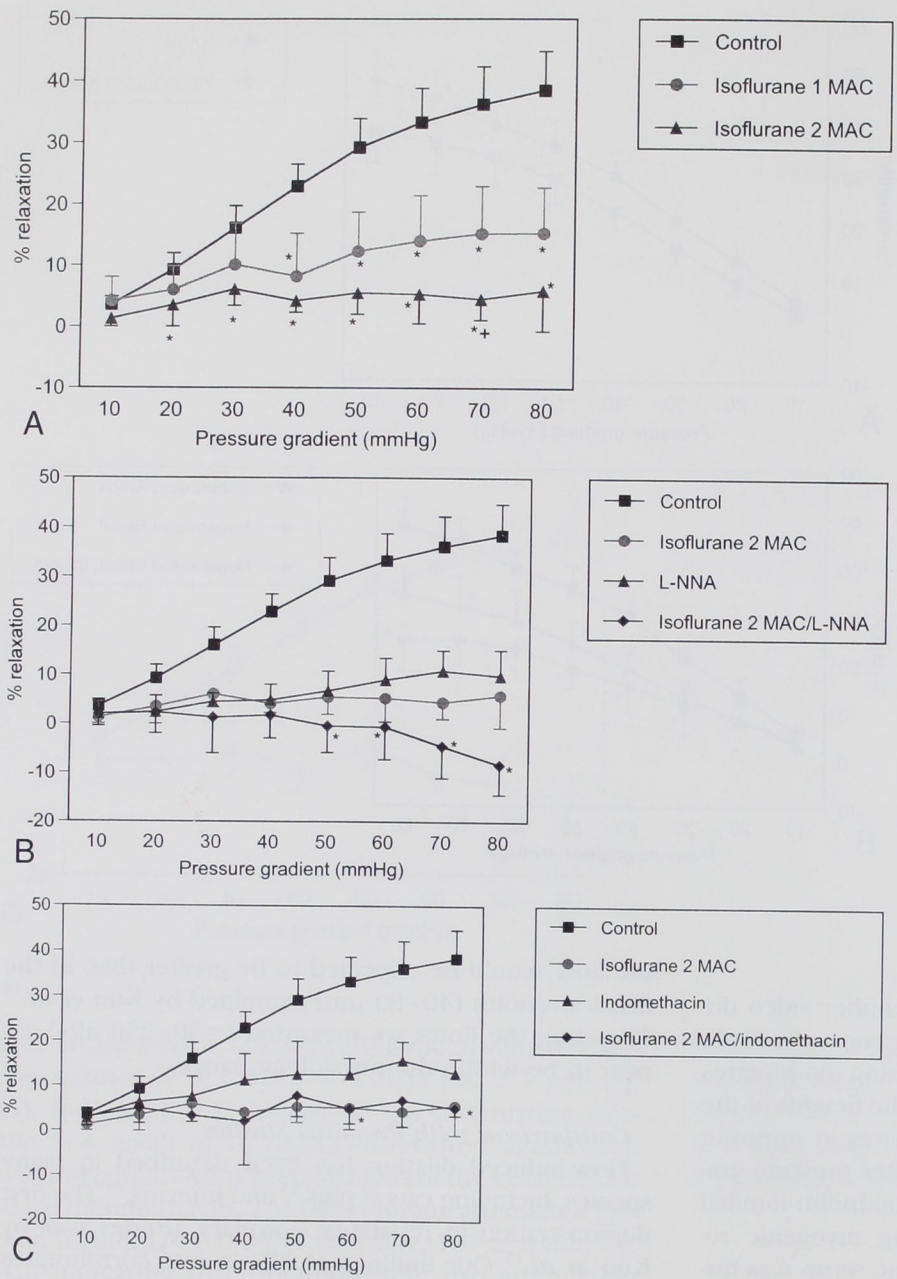

Fig. 4. (*A*) Percentage relaxation of the U46619-preconstricted vessel in response to changes in pressure gradients (ΔP) across the vessel in the presence of different concentrations of isoflurane. Isoflurane attenuated ΔP-produced, flow-induced dilation in a concentration-dependent manner ($P < 0.001$). *$P < 0.05$ *versus* control. +$P < 0.05$ *versus* 1 minimum alveolar concentration (MAC) isoflurane. (*B*) Even after pretreatment of vessels with N^G-nitro-L-arginine (L-NNA), 2 MAC isoflurane attenuated flow-induced dilation even further ($P < 0.01$), indicating that isoflurane attenuated dilation as a result of an L-NNA–insensitive agent, most likely a prostanoid. *$P < 0.05$ between vessels pretreated with L-NNA, whose flow-induced dilation was measured in the presence or absence of 2 MAC isoflurane. (*C*) Even after pretreatment of vessels with indomethacin, 2 MAC isoflurane attenuated flow-induced dilation even further ($P < 0.05$), indicating that isoflurane attenuated dilation because of an indomethacin-insensitive agent, most likely nitric oxide. *$P < 0.05$ between vessels pretreated with indomethacin. The flow-induced dilation of indomethacin was measured in the presence or absence of 2 MAC isoflurane.

We found that in rat subepicardial arteries both NO and prostanoids are effective in mediating EDD in response to flow increases. Although one or both of these mediators may be important in FID, the exact nature of the mediator(s) may depend on the species, the tissue type, and the vessel size and function. Whereas in porcine coronary arterioles FID appears to occur *via* NO and is not affected by the cyclooxygenase inhibitor indomethacin,[28] inhibitors of NO synthesis had no effect on FID of epicardial coronary arteries in dogs[29] and humans.[30] In canine femoral segments, both prostanoids and NO played roles in FID,[31] whereas in rat cremaster muscle arterioles prostanoids, but not NO, may be important.[14]

Changing the perfusate from Krebs solution to blood in our experiment not only increased the viscosity of the solution as a result of various components, such as proteins and cells, but also introduced hemoglobin in

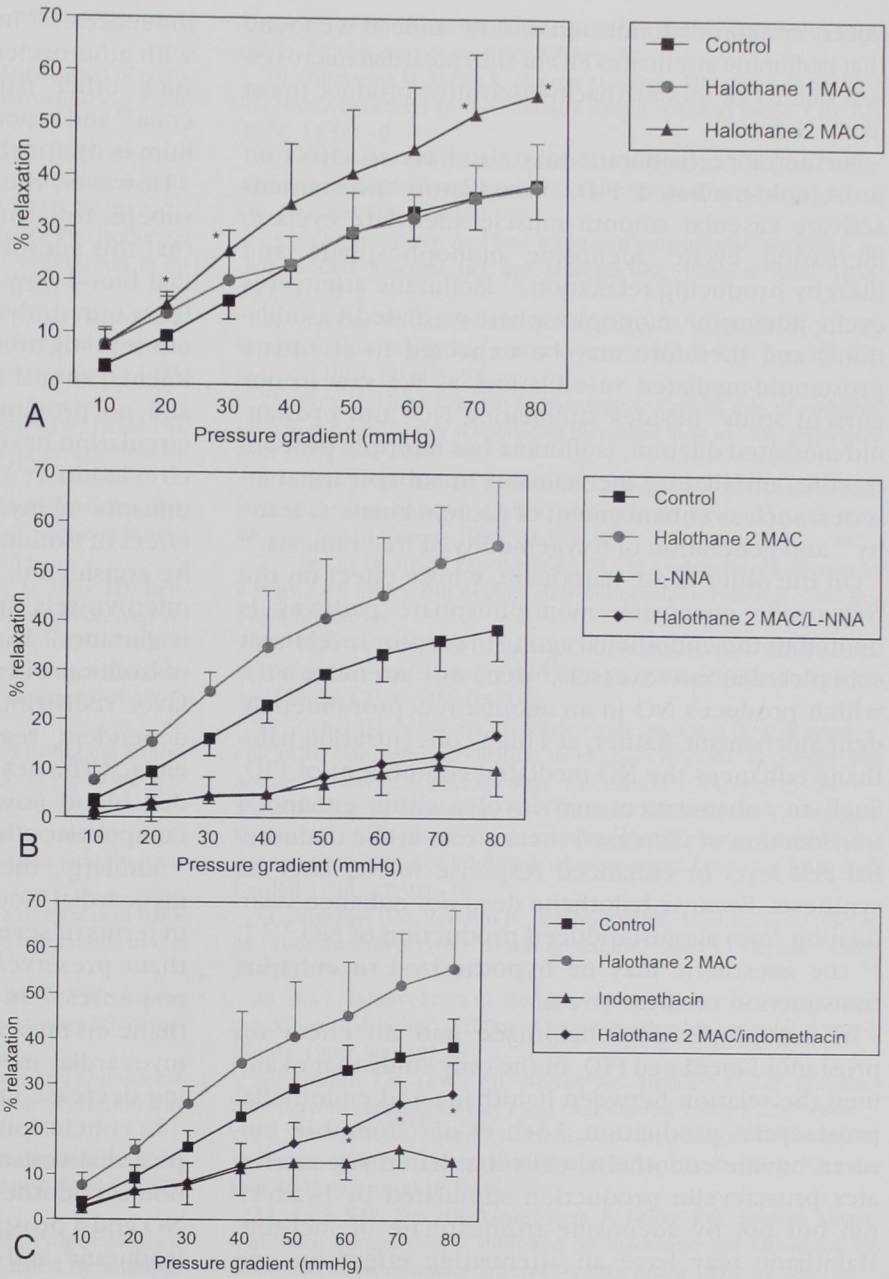

Fig. 5. (*A*) Percentage relaxation of the U46619-preconstricted vessel in response to changes in pressure gradients (ΔP) across the vessel in the presence of different concentrations of halothane. Two minimum alveolar concentration (MAC) halothane enhanced flow-induced dilation ($P < 0.05$), whereas 1 MAC halothane had no significant effect ($P = 0.52$). *$P < 0.05$ *versus* control. (*B*) After pretreatment of vessels with N^G-nitro-L-arginine (L-NNA), 2 MAC halothane did not enhance flow-induced dilation ($P = 0.40$), indicating that halothane-mediated enhancement of flow-mediated dilation was not caused by an L-NNA–insensitive agent such as a prostanoid. (*C*) After pretreatment of vessels with indomethacin, 2 MAC halothane enhanced flow-induced dilation even further ($P < 0.05$), indicating that halothane enhanced dilation as a result of an indomethacin-insensitive agent, most likely nitric oxide. *$P < 0.05$ between vessels pretreated with indomethacin, whose flow-induced dilation was measured in the presence or absence of 2 MAC halothane.

the erythrocytes. Increased viscosity would tend to increase shear stress at any given flow and enhance FID.[2] However, under conditions of high oxygen tension such as in our preparation, hemoglobin scavenges NO[32] and would be expected to attenuate the NO-mediated component of FID. The net effect of using blood in our preparation was to attenuate FID. Under conditions of lower oxygen tension, when hemoglobin does not also scavenge NO, blood might not attenuate FID as much.

The Effect of Volatile Anesthetics

Using rat subepicardial resistance arteries, we showed previously that isoflurane and halothane attenuate agonist-induced EDD and that whereas the attenuating action of halothane appears limited to the endothelial receptor of the agonist, the effect of isoflurane may include an action on the smooth muscle guanylate cyclase.[5] Based on these findings, isoflurane was hypothesized to attenuate the effect of NO, whether pro-

duced by agonist stimulation or flow. Indeed we found that isoflurane attenuates FID of subepicardial microvessels due to an indomethacin-insensitive product (most likely NO).

Furthermore, isoflurane may also have an effect on prostanoid-mediated FID. Vasodilatory prostanoids activate vascular smooth muscle adenylate cyclase, increasing cyclic adenosine monophosphate and thereby producing relaxation.[33] Isoflurane attenuates cyclic adenosine monophosphate-mediated vasodilation[34] and therefore may be expected to attenuate prostanoid-mediated vasodilation, as we saw in our current study. Besides attenuating NO- and prostanoid-mediated dilation, isoflurane has multiple procontractile, antidilatory mechanisms in subepicardial arteries, such as enhancement of protein kinase C activity[35] and generation of oxygen-derived free radicals.[36]

On the other hand, halothane, whose effect on the NO-cyclic guanosine monophosphate pathway is limited to the endothelial agonist-receptor level in rat subepicardial microvessels,[5] does not attenuate FID, which produces NO in an agonist receptor-independent mechanism. Rather, at a high concentration halothane enhances the NO-mediated component of FID. Such an enhancement may involve either enhanced transduction of increased shear stress at the endothelial cell level or enhanced response to NO after its synthesis. Because halothane does not enhance vasodilation from agonist-induced production of NO,[3-5,9-12] the anesthetic may be hypothesized to enhance transduction of shear stress.

In our preparation, halothane had no effect on prostanoid-mediated FID. In the only study that examined the relation between halothane and endothelial prostacyclin production, Loeb *et al.*[37] found in cultured bovine endothelial cells that halothane attenuates prostacyclin production stimulated by bradykinin but not by adenosine triphosphate or melittin. Halothane may have an attenuating effect on one pathway of endothelial prostacyclin production, but not others, depending on the animal species, vessel type, and type of stimulation.

Possible Implications of These Data

Flow-induced dilation is an important determinant of myocardial blood flow distribution, helping to match blood flow to tissue needs.[1] It may also play a role in the initial phase of collateral circulation development, adaptation of the arterial bed to altered blood viscosity, and modulation of vasoconstrictive influences.[3,38] Impairment of FID has been associated with atherosclerosis and coronary artery disease.[39] In such other pathologic states as hypercholesterolemia[40] and reperfusion injury,[41] in which the endothelium is dysfunctional, FID might be impaired as well.

However, the fact that isoflurane attenuates FID of subepicardial microvessels does not necessarily imply that this anesthetic has an adverse effect on myocardial blood flow distribution. First, any extrapolation from our study should be tempered by the fact that our investigation is but one *in vitro* study in an experimental animal species under conditions of hyperoxia and no proteins. However, rat models of coronary circulation have been useful extrapolations of human circulation.[42,43] Second, FID is only one of the determinants of myocardial blood flow distribution. The effect of isoflurane on other determinants should also be considered. Myogenic responses in subepicardial microvessels are well preserved or enhanced with isoflurane.[44] In addition, the direct vasomotor effect of isoflurane in a collateral-dependent circulation may favor redistribution of blood flow to the collateral-dependent region, thus tending to prevent ischemia.[45] The net *in vivo* effect of isoflurane on myocardial blood flow distribution may result from many component effects.

Similarly, the net *in vivo* effect of halothane on myocardial blood flow distribution should be assessed in terms of several component effects. Although halothane preserves or enhances FID, it inhibits myogenic responses.[44] *In vivo*, the predominant effect of halothane on myocardial blood flow may be secondary to myocardial metabolic depression and a corresponding decrease in flow.[19]

In conclusion, we found that FID occurs in rat subepicardial resistance arteries and that this phenomenon is endothelium-dependent and mediated by both NO and a prostanoid. Further, the volatile anesthetics isoflurane and halothane have opposite effects on FID, with the former attenuating it and the latter enhancing it. Our results may have implications for the effect of the anesthetics on myocardial blood flow distribution.

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