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Nitric Oxide Does Not Mediate Coronary Vasodilation by Isoflurane

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Background: **Isoflurane causes vasodilation in the coronary circulation. The current study employed a canine model permitting selective intracoronary administrations of isoflurane (1) to test the hypothesis that coronary vasodilation by isoflurane is mediated by nitric oxide and (2) to evaluate the persistence of coronary vasodilation during an extended exposure to isoflurane.**

Methods: **Open-chest dogs anesthetized with fentanyl and midazolam were studied. The left anterior descending coronary artery (LAD) was perfused *via* extracorporeal system with normal arterial blood or with arterial blood equilibrated with 1.4% (1 MAC) isoflurane. In the LAD bed, coronary blood flow (CBF) was measured with an electromagnetic flowmeter and used to calculate myocardial oxygen consumption ($M\dot{V}_{O_2}$). In series 1, performed at constant coronary perfusion pressure (CPP), the LAD was exposed to 3 h of isoflurane in two groups of eight dogs: control group, normal coronary endothelium; and experimental group, intracoronary infusion of the nitric oxide synthase inhibitor L-NAME (0.15 mg/min for 30 min). Series 2 was performed with CBF constant; thus, CPP varied directly with coronary vascular resistance. In this series, initial steady-state changes in CPP by isoflurane were evaluated in the same four dogs before and after L-NAME.**

Results: **In the control group of series 1, isoflurane caused a maximal, initial increase in CBF of 444%; however, CBF decreased progressively reaching a value not significantly different from baseline after 3 h of isoflurane. Isoflurane caused a significant (approximately 35%) decrease in $M\dot{V}_{O_2}$, which persisted during the 3-h administration. Findings after L-NAME (experimental group) were not significantly different from those in control group. In series 2, isoflurane caused significant decreases in CPP that were not affected by L-NAME.**

Conclusions: **The lack of effect of L-NAME on isoflurane-induced coronary vasodilation suggests that nitric oxide does not mediate this response. The increase in CBF during prolonged isoflurane waned over time, perhaps because of tachyphylaxis or emergence of a competitive vasoconstrictor mechanism, *e.g.*, metabolic factors secondary to reduced oxygen demands. (Key words: Anesthetics, volatile: isoflurane. Heart: coronary blood flow; myocardial contractility; myocardial oxygen consumption. Nitric oxide.)**

NITRIC oxide is produced in the vascular endothelium from the amino acid L-arginine in a reaction requiring the enzyme nitric oxide synthase (NOS).[1] Nitric oxide diffuses from the vascular endothelium to the vascular smooth muscle, where it stimulates guanylyl cyclase activity and production of 3′,5′-cyclic guanosine monophosphate (cGMP), which has vasorelaxing properties. Endothelium-derived nitric oxide has been demonstrated to make an important contribution to the coronary vasodilation elicited by a variety of physiologic and pharmacologic stimuli.[1] Although studies have investigated the role of nitric oxide in vascular relaxation induced by the volatile anesthetics, they have been limited primarily to *in vitro* preparations of large noncoronary vessels.[2–4] Only the study by Greenblatt *et al.*[5] evaluated the role of nitric oxide in volatile anesthetic-induced coronary vasodilation in the intact heart. Greenblatt *et al.* demonstrated that intravenous infusions of the NOS inhibitor N^G-monomethyl-L-arginine (L-NMMA) increased coronary vascular resistance

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in rats anesthetized with isoflurane, and thus concluded that nitric oxide was involved in isoflurane-induced coronary vasodilation. However, the study by Greenblatt *et al.* cannot be considered definitive because the systemic administrations of both isoflurane and L-NMMA caused changes in hemodynamic parameters, *e.g.*, aortic pressure, which themselves are major determinants of coronary blood flow (CBF).[6]

Time-dependent recovery of vascular tone during continued exposure to isoflurane was first described in the cerebral circulation.[7,8] Kenny *et al.*[9] demonstrated this phenomenon in the coronary circulation during inhalation induction in chronically instrumented dogs. Although the study by Kenny *et al.* provided new and important information, its interpretation was limited by factors inherent to the animal model and experimental approach used, including alterations in systemic hemodynamic parameters and global cardiac work requirements and progressively increasing arterial blood concentrations during the interval when CBF was resolving.

The main objective of the current study was to test the hypothesis that nitric oxide mediates coronary vasodilation by isoflurane, using a canine model that permitted selective intracoronary administrations of both isoflurane and the NOS inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) and tight control of the hemodynamic determinants of CBF.[10] A second objective of the study was to use this model to evaluate time-related changes in CBF during an extended exposure to isoflurane.

The current study consisted of two protocols. In series 1, changes in CBF were evaluated during a 3-h intracoronary administration of isoflurane with perfusion pressure constant in two groups of dogs: dogs with normal coronary endothelium (control group) and dogs in which L-NAME was infused selectively into the coronary circulation. Because of the tendency for flow-induced shear stress to stimulate release of nitric oxide from the vascular endothelium,[11] series 2 was performed with CBF maintained constant. In these studies, duplicate, short-term exposures to isoflurane were performed in the same coronary bed, first before and then after treatment with L-NAME.

Methods

Canine Preparation

The study was conducted in compliance with the Institutional Animal Research Committee. Experiments were performed on 24 conditioned, healthy mongrel dogs of either sex (weight range 20–29.5 kg). Anesthesia was induced with intravenous bolus injection of 15 mg/kg thiopental. Anesthesia was maintained by continuous intravenous infusion of fentanyl and midazolam at rates of 12 $\mu g \cdot kg^{-1} \cdot h^{-1}$ and 0.6 $mg \cdot kg^{-1} \cdot h^{-1}$, respectively. Adequacy of this anesthesia regimen was demonstrated by lack of muscle movement and hemodynamic responses during surgical preparation. After tracheal intubation and left thoracotomy in the fourth intercostal space, the lungs were mechanically ventilated (Air Shields, Hatboro, PA) with an inspired oxygen fraction equal to 1.0. The volume and rate of the ventilator were established to maintain arterial partial pressure of carbon dioxide (P_{CO_2}) at physiologic levels. Partial pressure of oxygen (P_{O_2}), P_{CO_2}, and *p*H of arterial blood samples as well as coronary perfusate and venous samples (see below) were measured electrometrically (model 413, Instrumentation Laboratories, Lexington, MS). After surgical preparation, 0.1 mg/kg vecuronium bromide, with supplements at 0.05 $mg \cdot kg^{-1} \cdot h^{-1}$, was administered to facilitate mechanical ventilation. Body temperature was maintained at 38°C with a heating pad. Lactated Ringer's solution was administered continuously at a rate of 5 $ml \cdot kg^{-1} \cdot h^{-1}$ intravenously to compensate for evaporative fluid losses. Heparin (400 U/kg with supplementation) was used for anticoagulation.

The left anterior descending coronary artery (LAD) was perfused *via* an extracorporeal system, as described in detail previously.[1] In brief, a thin-wall stainless-steel cannula (ID 2.5 mm) was introduced into the LAD just distal to its first major diagonal branch. This cannula was connected *via* tubing to two pressurized reservoirs, which served as alternate sources of blood for the LAD. The normal blood reservoir was supplied with isoflurane-free blood withdrawn directly from the left femoral artery, and the isoflurane-equilibrated blood reservoir was supplied with blood from the right femoral artery that was first pumped into a bubble oxygenator (Bentley-5 pediatric blood oxygenator, Irvine, CA) supplied with a 95% O_2/5.0% CO_2 gas mixture, which passed through a calibrated Fortec vaporizer (Cyprane, Yorkshire, England) providing 1.4% (1 MAC) isoflurane.

The LAD perfusion tubing was equipped with (1) a heat exchanger to maintain the temperature of the coronary perfusate at 38°C, (2) an electromagnetic flow transducer to measure CBF, (3) ports for collecting samples of coronary perfusate, and (4) a mixing cham-

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ber for drugs infused into the LAD perfusion line. Coronary perfusion pressure (CPP) was sensed through a small-diameter tube positioned at the orifice of the perfusion cannula.

Measurements of arterial blood pressure, left ventricular pressure, left ventricular dP/dt_{max}, and heart rate were obtained using standard methods.[10] A continuous record of these variables and CBF was obtained on an eight-channel physiologic recorder (model 2800S, Gould, Cleveland, OH).

Experimental Measurements

Myocardial Oxygen Consumption. To distinguish direct vascular effects of isoflurane from those secondary to changes in oxygen demand, measurements of myocardial oxygen consumption ($M\dot{V}_{O_2}$) were obtained in the LAD-perfusion territory. The anterior interventricular vein was cannulated to obtain samples of venous effluent from the LAD-perfused myocardium.[10] The venous cannula was allowed to drain freely into a beaker to prevent venous stagnation and interstitial edema. This venous blood was returned intermittently to the dog to maintain isovolemic conditions. At specified times in the study, 1-ml blood samples were collected from the coronary venous cannula under mineral oil to maintain anaerobic conditions. These venous blood samples were paired with 1-ml arterial blood samples obtained from the LAD perfusion line, so that the arteriovenous oxygen difference for oxygen could be determined. Hemoglobin concentration and percent hemoglobin oxygen saturation of the coronary blood samples was measured with a CO-Oximeter (model 482, Instrumentation Laboratories) and used to calculate oxygen bound to hemoglobin assuming an oxygen-carrying capacity for hemoglobin of 1.39 ml O_2/g.[12] The oxygen dissolved in the blood was computed (oxygen dissolved = 0.003 ml $O_2 \cdot 100$ ml $blood^{-1} \cdot mmHg^{-1}$) and added to the bound component to compute total oxygen content. $M\dot{V}_{O_2}$ was computed from the product of the coronary arteriovenous oxygen difference and CBF at the time that blood samples were obtained.

Blood Isoflurane Concentration. In six dogs from series 1 (two control and four experimental animals), isoflurane concentration was determined using a modification of the equilibration method described in detail by Yamamura *et al.*[13] Briefly, serial 2-ml samples of arterial blood were obtained from the LAD perfusion tubing using an airtight glass syringe and introduced into a 5-ml glass vial. The vial was placed in a constant-temperature chamber at 38°C for 30 min. After equilibration, 100 μl of the gas in the vial was introduced into a gas chromatograph (model 5890, Hewlett Packard, Wilmington, DE) equipped with a flame ionization detector, and the area under the curve was measured. Anesthetic concentration in blood was determined by means of a calibration curve derived from appropriate standards. All analyses were performed in triplicate and mean values calculated.

Experimental Protocols

Series 1. Time-dependent Effects of Isoflurane on CBF in Absence and Presence of L-NAME with CPP Controlled. Series 1 consisted of three experimental groups, which were studied randomly. In a control group of eight dogs, time-dependent changes during intracoronary isoflurane were assessed in the coronary circulation with normal vascular endothelium. After cannulation, the LAD was allowed to recover for at least 45 min before measurements of CBF were obtained. During this recovery period, the LAD was perfused with isoflurane-free arterial blood with mean CPP set equal to mean aortic pressure (approximately 100 mmHg). CPP was maintained at this level throughout the study. After measurement of baseline CBF, adenosine in isotonic saline was infused directly into the perfusion line at a dose sufficient to cause steady-state, maximal vasodilation (8.1 ± 0.6 mg/min corresponding to an infusion rate of 1.1 ± 0.1 ml/min). Preliminary studies indicated that infusion of the saline vehicle itself at this rate had no effect on CBF or related parameters. Maximal vasodilation was indicated by failure of an increase in the rate of infusion of adenosine to cause a further increase in CBF. The coronary vasodilator reserve ratio was calculated by dividing peak CBF during adenosine infusion by the pre-adenosine baseline CBF. After terminating the adenosine infusion, new baseline measurements for CBF and related parameters were obtained, and perfusion was switched to the isoflurane-equilibrated blood reservoir. Perfusion with isoflurane-equilibrated blood was maintained for 3 h, after which time the LAD was returned to the isoflurane-free blood reservoir. During isoflurane administration, measurements were obtained when the value for CBF maximized (after approximately 10–15 min of isoflurane) and after 0.5, 1.0, 2.0, and 3.0 h of isoflurane. Recovery values were obtained 15 min after return to isoflurane-free blood. A second value for coronary vasodilator reserve ratio was obtained during the recovery period using the rate of adenosine infusion

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that was found to cause maximal vasodilation before isoflurane administration.

In a second group of eight dogs, the time-dependent effects of isoflurane on CBF were evaluated after intracoronary infusion of L-NAME. After adequate time for recovery from cannulation, steady-state increases in CBF were evaluated during intracoronary infusions of acetylcholine (20 μg/min), an endothelium-dependent vasodilator, and sodium nitroprusside (80 μg/min), an endothelium-independent vasodilator.[1] Acetylcholine and sodium nitroprusside were dissolved in isotonic saline to achieve concentrations of 20 and 80 μg, respectively, so that the agents could be infused at an identical rate, 1.0 ml/min. After obtaining baseline measurements for CBF and related parameters, L-NAME in isotonic saline was infused into the perfusion line at a rate of 150 μg/min (corresponding to 1.0 ml/min) for 30 min. Fifteen minutes after terminating the L-NAME infusion, a second set of infusions of acetylcholine and sodium nitroprusside was performed to confirm adequacy and specificity of the NOS inhibition. Post-L-NAME measurements were obtained, and intracoronary administration of isoflurane was initiated and continued, in accordance with 3-h protocol described above for the control group. After return to isoflurane-free blood and acquisition of recovery values, a third set of infusions of acetylcholine and sodium nitroprusside was performed to confirm persistence of the effect of L-NAME in the LAD bed.

In a third group of four dogs, time-control studies were performed to assess the intrinsic stability of the regional coronary perfusion preparation. In these studies, the LAD was perfused at constant pressure continuously for 3 h with isoflurane-free arterial blood, and measurements of CBF, $M\dot{V}_{O_2}$, and coronary venous oxygen tension (Pv_{O_2}) were obtained on an hourly basis.

At the completion of each experiment, Evans blue dye was injected into the perfusion line, with perfusion pressure maintained at the normal level, to identify the LAD perfusion field. After the heart was stopped with potassium chloride, it was removed, trimmed, and frozen to facilitate sampling. The dyed tissue was excised and weighed so that CBF could be expressed on a per-100 g basis. The average weight of the LAD perfusion field was 36 ± 1 g.

Series 2. Effect of L-NAME on Reductions in CPP by Isoflurane with CBF Controlled. The contribution of nitric oxide to isoflurane-induced coronary vasodilation was assessed with CBF, rather than CPP, held constant in four dogs. Under such constant-flow conditions, CPP varies directly with coronary vascular resistance. In these studies, CPP initially was set at 100 mmHg and was reduced manually as necessary to maintain CBF constant during the intracoronary administrations of isoflurane, acetylcholine, and sodium nitroprusside. After baseline measurements, perfusion was switched to arterial blood equilibrated with isoflurane and values were obtained when CPP achieved a steady-state decrease, which was within 5–10 min. After return to the normal blood reservoir and adequate time for recovery from isoflurane (at least 15 min), the steady-state decreases in CPP by acetylcholine and sodium nitroprusside were evaluated, using the doses described under series 1.

After sufficient time for recovery from responses to isoflurane, acetylcholine and sodium nitroprusside in the normal LAD (at least 30 min), L-NAME was infused directly into the perfusion line using the protocol described above, and the responses to isoflurane, acetylcholine, and sodium nitroprusside were reevaluated. The order of isoflurane, acetylcholine, and sodium nitroprusside was randomized throughout the study. In series 2, the anterior interventricular vein was not cannulated, and measurements of $M\dot{V}_{O_2}$ were not obtained.

Statistical Analyses

Experimental effects within treatment groups of series 1 were evaluated using a randomized block analysis of variance with repeated measurements in the same dogs, each dog serving as a block.[14] The Student-Newman-Keuls test was used to determine which means differed.[14] Differences between groups in series 1 were evaluated with Student's *t* test for unpaired samples. Student's *t* test for paired samples was used to compare effects of isoflurane and other coronary vasodilators in the absence and presence of L-NAME in series 2. $P < 0.05$ was considered significant throughout this study.

Results

Series 1. Time-dependent Effects of Isoflurane on CBF in Absence and Presence of L-NAME with CPP Controlled

Figure 1 is a representative tracing from the control group of series 1 showing initial changes in hemodynamic variables during perfusion of the LAD with isoflurane-equilibrated blood. The arrow indicates the point at which perfusion was switched to the isoflurane

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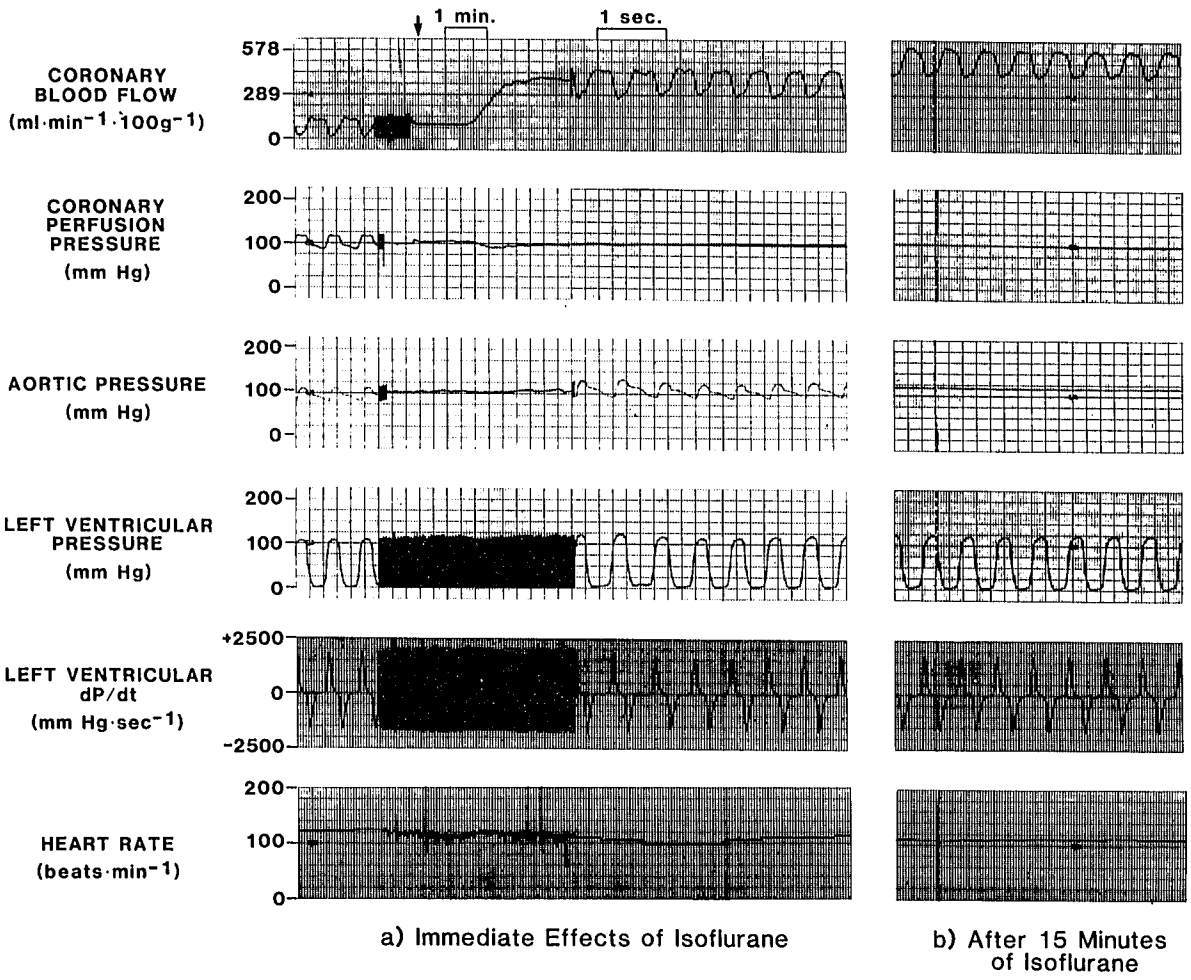

Fig. 1. Original representative tracing demonstrating ability of intracoronary administration of isoflurane to cause appreciable (fivefold) increase in blood flow in the LAD bed with normal vascular endothelium under constant-pressure conditions. Note that systemic hemodynamic variables remained stable during the intracoronary administration of isoflurane.

reservoir. After a short delay (corresponding to the time required for the isoflurane-equilibrated blood to reach the vascular bed), isoflurane caused a rapid increase in CBF, which maximized at approximately five times the control level after 15 min. Of note was that CPP was maintained constant and that monitored systemic hemodynamic parameters and determinants of global cardiac function, *e.g.*, aortic pressure, were not changed by the selective intracoronary administration of isoflurane.

Figure 2 shows that the initial and time-related changes in CBF, $M\dot{V}_{O_2}$, and coronary Pv_{O_2} caused by isoflurane were not significantly different in the absence (control) and presence of L-NAME. Under both conditions, isoflurane caused marked initial increases in CBF, which, at constant CPP, reflected proportional decreases in coronary vascular resistance. However, CBF remained at these maximal levels only transiently and decreased progressively to levels that were not significantly different from baseline after 3 h of isoflurane (fig. 2A). Because the increases in CBF by isoflurane were accompanied by significant decreases in $M\dot{V}_{O_2}$ (fig. 2B), the arteriovenous oxygen difference was reduced, as reflected in the pronounced significant increases in Pv_{O_2} (fig. 2C). Because $M\dot{V}_{O_2}$ remained relatively constant, albeit at a significantly reduced level, during en-

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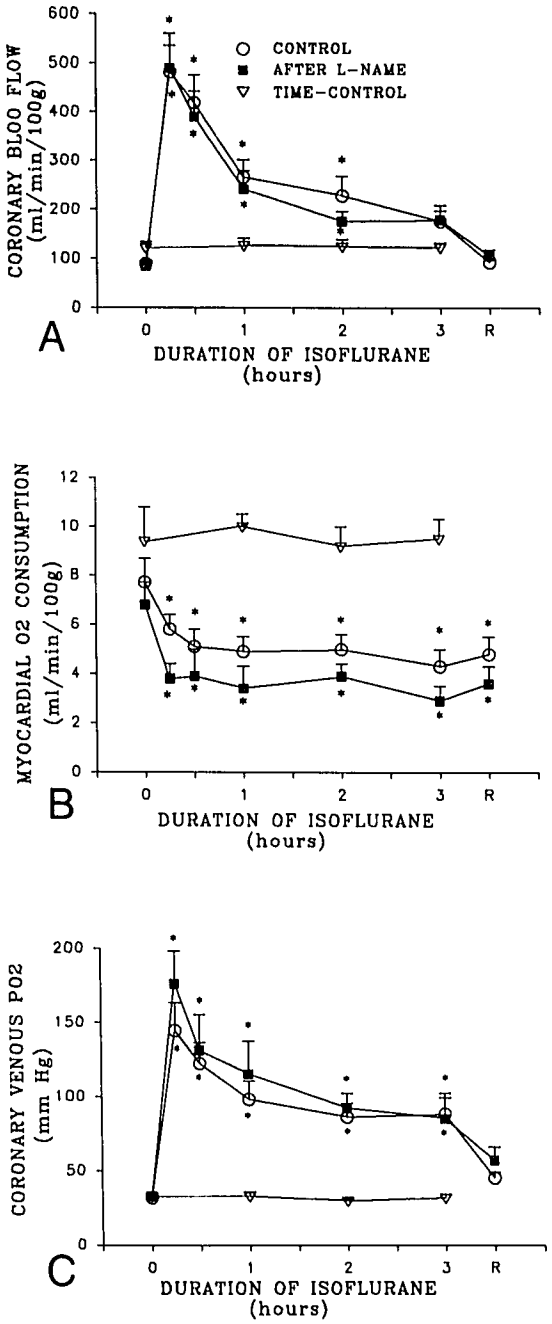

Fig. 2. Coronary blood flow (*A*), myocardial oxygen consumption (*B*), and coronary venous P_{O_2} (*C*) during 3-h intracoronary administration of isoflurane in the absence (control group) and presence of L-NAME. Findings for time-control studies are provided for reference. Values are mean ± SE. *$P < 0.05$ *versus* pre-isoflurane administration baseline value. Isoflurane caused significant changes in coronary blood flow, myocardial oxygen consumption, and coronary venous P_{O_2}, which were not altered by pretreatment with L-NAME. No statistically significant effects were noted in time-control studies.

tire 3-h exposure to isoflurane, the changes in CBF and Pv_{O_2} were essentially parallel. Values for $M\dot{V}_{O_2}$ obtained during the recovery period remained significantly below baseline. Figure 2 also shows that CBF, $M\dot{V}_{O_2}$, and coronary Pv_{O_2} did not change significantly during a 3-h continuous perfusion of the LAD with isoflurane-free arterial blood (time-control group).

Figure 3 indicates that the coronary vasodilator reserve ratio in the control group was appreciable before isoflurane (approximately 5.0) and that this ratio was not altered by the prolonged intracoronary administration of isoflurane.

Figure 4 shows that, in the experimental group, L-NAME caused a significant and persistent attenuation of the acetylcholine-induced increases in CBF, whereas it had no effect on the sodium nitroprusside-induced increases in CBF.

Table 1 presents effects of intracoronary isoflurane on systemic hemodynamic and coronary blood variables for the control group. These results were similar to those found in the L-NAME group. Intracoronary isoflurane caused no changes in systemic hemodynamic parameters, with the exception that left ventricular dP/dt_{max} was decreased significantly after 30 min of isoflurane. Coronary arterial P_{O_2} was significantly greater during isoflurane administration (resulting in higher values for coronary arterial oxygen content), but other coronary arterial variables were not changed. The measured coronary arterial blood concentration for isoflurane was constant throughout the 3-h administration.

Baseline values for CBF, $M\dot{V}_{O_2}$, and systemic hemodynamic variables in the L-NAME group were comparable to those in the control group (table 1 *vs.* table 2). The intracoronary infusion of L-NAME itself had no effect on values for CBF, $M\dot{V}_{O_2}$, and systemic hemodynamic parameters (table 2).

Series 2. Effect of L-NAME on Reductions in CPP by Isoflurane with CBF Controlled

Figure 5 demonstrates (1) that acetylcholine, sodium nitroprusside, and isoflurane all caused significant decreases in CPP prior to L-NAME, and (2) that L-NAME attenuated significantly the decrease in CPP by acetylcholine, whereas it had no significant effect on the decreases in CPP caused by sodium nitroprusside or isoflurane. A power calculation[14] indicated that series 2 had a power of 95% in detecting a difference of ±8 mmHg in the decrease in CPP caused by isoflurane before and after L-NAME.

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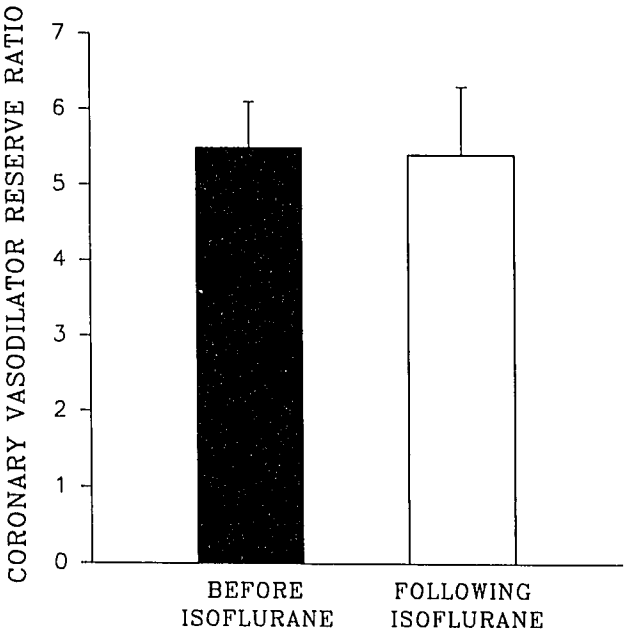

Fig. 3. Lack of change in coronary vasodilator reserve ratio in control hearts after 3-h intracoronary administration of isoflurane. Values are mean ± SE.

Discussion

Critique of Methods

Intravenous infusions of NOS inhibitors cause increases in systemic vascular resistance (and concomitant increases in arterial pressure and left ventricular afterload),[15] suggesting that tonic release of nitric oxide may play an important role in modulating basal vascular tone in the peripheral circulation. The use of selective intracoronary administrations of L-NAME (as well as of isoflurane) avoided their systemic effects, which simplified interpretation of the findings.

An important consideration in series 1 was that the NOS inhibitor remained effective throughout the 3-h administration of isoflurane. In light of the report by Smith and Canty[16] that acetylcholine-induced coronary vasodilation remained markedly attenuated for 24 h after intravenous administration of L-NAME in chronically instrumented, conscious dogs, we chose L-NAME to inhibit NOS. The intracoronary dose of L-NAME was extrapolated from the intravenous dose reported by Smith and Canty.[16] Adequacy and specificity of this dose were confirmed by observing the ability of L-NAME to cause a persistent inhibition of the coronary vasodilator responses to acetylcholine, while preserving the coronary vasodilator responses to sodium nitroprusside (fig. 4). The challenging doses of acetylcholine and sodium nitroprusside were chosen on the basis of preliminary studies demonstrating that these doses caused significant increases in CBF without affecting systemic hemodynamic parameters.

Although L-NAME greatly attenuated the coronary vasodilator responses caused by acetylcholine, a relatively small vasodilator effect remained (fig. 4). The inability for NOS inhibitors to abolish acetylcholine-induced coronary vascular relaxation has been reported widely.[17-19] This finding suggests that, although acetylcholine acted primarily *via* the nitric oxide-cGMP pathway, it also caused coronary vascular relaxation *via* an additional mechanism(s). A candidate for this mechanism is a hyperpolarization factor, which has been demonstrated in *in vitro* studies to be released from the vascular endothelium by acetylcholine.[20]

Use of a 95% O_2/5% CO_2 gas mixture ensured that coronary arterial P_{CO_2} and *p*H remained at normal values when perfusion was switched to the isoflurane-equilibrated blood reservoir. However, coronary arterial P_{O_2} was higher during administration of isoflurane be-

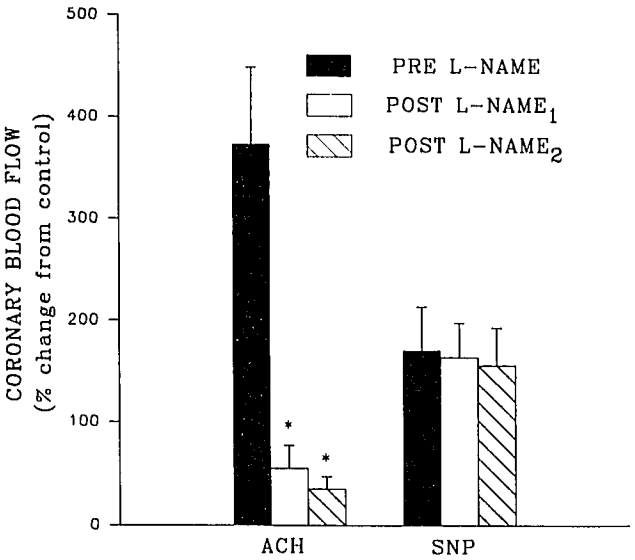

Fig. 4. Increases in coronary blood flow by acetylcholine (ACH) and sodium nitroprusside (SNP) before infusion of L-NAME (Pre L-NAME), 15 min after completing infusion of L-NAME (Post L-NAME$_1$), and after 3-h administration of isoflurane (Post L-NAME$_2$). Values are mean ± SE. *$P < 0.05$ *versus* Pre L-NAME value. L-NAME caused a significant attenuation of the acetylcholine-induced increase in CBF, which persisted for the duration of isoflurane administration, whereas it had no effect on the sodium nitroprusside-induced increase in CBF.

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Table 1. Hemodynamic and Coronary Arterial Blood Variables during 3-h Intracoronary Administration of Isoflurane in the Control Group of Series 1

		Isoflurane					
	Baseline	15 min	30 min	1 h	2 h	3 h	Recovery
Mean aortic pressure (mmHg)	98 ± 5	99 ± 5	95 ± 4	96 ± 7	100 ± 7	96 ± 8	97 ± 9
Heart rate (beats/min)	139 ± 6	136 ± 9	134 ± 8	130 ± 9	132 ± 10	129 ± 13	127 ± 12
Mean LVEDP (mmHg)	5.0 ± 1.1	7.2 ± 2.2	6.7 ± 1.7	6.7 ± 2.1	6.5 ± 1.9	6.3 ± 1.8	4.7 ± 1.2
LV dP/dt_{max} (mmHg/min)	1,975 ± 213	1,800 ± 186	1,663 ± 179*	1,650 ± 163*	1,700 ± 204*	1,542 ± 157*	1,614 ± 137*
Mean coronary perfusion pressure (mmHg)	93 ± 3	91 ± 3	93 ± 2	94 ± 3	92 ± 3	94 ± 3	93 ± 3
Coronary artery values							
P_{O_2} (mmHg)	193 ± 72	436 ± 51*	418 ± 58*	415 ± 41*	433 ± 38*	475 ± 42*	130 ± 18
P_{CO_2} (mmHg)	36 ± 1	32 ± 2	31 ± 2	33 ± 2	34 ± 2	32 ± 2	30 ± 2
*p*H	7.38 ± 0.01	7.40 ± 0.03	7.41 ± 0.02	7.38 ± 0.01	7.36 ± 0.01	7.38 ± 0.01	7.40 ± 0.01
Oxygen saturation (%)	95 ± 2	98 ± 3	98 ± 1	97 ± 1	97 ± 1	97 ± 1	92 ± 2
Oxygen content (vol %)	16.1 ± 0.5	18.2 ± 0.9	18.6 ± 0.8	19.0 ± 0.7*	19.9 ± 0.6*	20.0 ± 0.7*	18.0 ± 1.1
Hemoglobin ($g \cdot 100 \cdot ml^{-1}$)	13.0 ± 0.8	12.8 ± 0.5	13.1 ± 0.5	13.4 ± 0.5	14.0 ± 0.4	14.1 ± 0.5	14.1 ± 0.7
Isoflurane ($mg \cdot 100 \cdot ml^{-1}$)	0	11.7 ± 0.7	11.7 ± 0.9	11.1 ± 1.3	11.3 ± 1.3	10.7 ± 1.4	0

Values are mean ± SE in eight dogs in the control group, except for isoflurane blood concentration, which was obtained from two dogs in the control group and four dogs in the experimental group.

LVEDP = left ventricular end-diastolic pressure; LV = left ventricular.

Recovery values were obtained 15 min after return to isoflurane-free blood.

* $P < 0.05$ *versus* Control.

cause of more efficient gas exchange in the extracorporeal oxygenator compared to the lungs of experimental animal. Although hyperoxia has been shown to cause coronary vasoconstriction,[6] it is highly unlikely that this factor would have influenced our conclusions. First, coronary arterial P_{O_2} was sufficient under control conditions for essentially complete saturation of hemoglobin, so that the increase in coronary arterial oxygen content during isoflurane administration resulted primarily from an increased quantity of dissolved oxygen and was small (table 1). Second, the variations in coronary arterial oxygenation during isoflurane administration were similar in the absence and presence of L-NAME.

Table 2. Lack of Effect of Intracoronary Infusion of L-NAME Itself on Local Coronary Blood Flow and Myocardial Oxygen Consumption and on Systemic Hemodynamic Parameters

	Before L-NAME	After L-NAME
Coronary blood flow ($ml \cdot min^{-1} \cdot 100\ g^{-1}$)	90 ± 9	87 ± 10
Myocardial oxygen consumption ($ml \cdot min^{-1} \cdot 100\ g^{-1}$)	6.4 ± 0.8	6.4 ± 0.9
Mean coronary perfusion pressure (mmHg)	97 ± 3	98 ± 2
Mean aortic pressure (mmHg)	96 ± 4	93 ± 5
Heart rate (beats/min)	132 ± 7	129 ± 9
Mean LVEDP (mmHg)	4.2 ± 0.8	4.2 ± 0.8
LV dP/dt_{max} (mmHg/min)	1,838 ± 78	1,725 ± 82

Values are mean ± SE in eight dogs.

LVEPD = left ventricular end-diastolic pressure; LV = left ventricular.

General anesthesia was required in this open-chest canine preparation. A balanced anesthetic technique comprised of fentanyl and midazolam was used for several reasons. First, such techniques have been demonstrated by clinical investigators to be free of significant effects on cardiac function.[21] Second, Flacke *et al.*[22] reported that the combination of fentanyl and diazepam (another benzodiazepine) caused no additional cardiac depression in dogs anesthetized with enflurane after elimination of cardiac sympathetic drive with a spinal block. Finally, Reves *et al.*[23] found that excessive doses of fentanyl and diazepam together were required to depress isolated, perfused rat hearts, and that this effect occurred only in a strictly additive fashion.

Coronary Effects of Isoflurane

CBF normally is matched to the prevailing myocardial oxygen demands by local adjustments in coronary vasomotor tone mediated by metabolic control mecha-

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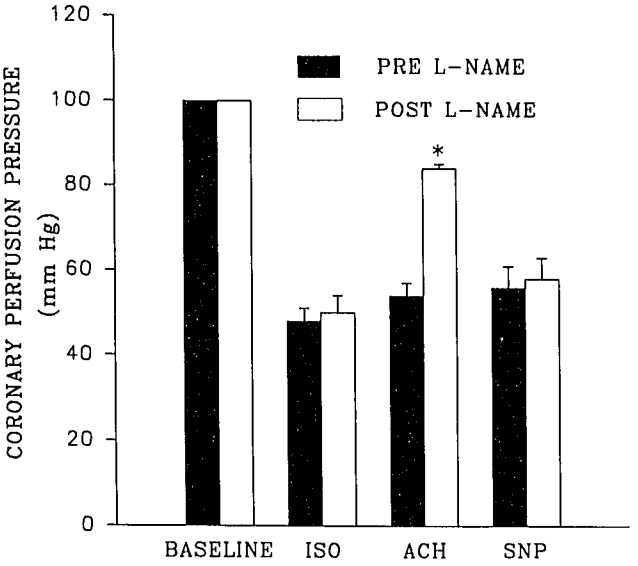

Fig. 5. Decreases in CPP caused by isoflurane (ISO), acetylcholine (ACH), and sodium nitroprusside (SNP) before and after L-NAME under constant-flow conditions. Values are mean ± SE. *$P < 0.05$ *versus* Pre L-NAME decrease in CPP. L-NAME significantly blunted decrease in CPP by acetylcholine, whereas it had no effect on the decrease in CPP caused by isoflurane or sodium nitroprusside.

nisms.[6] This local control of CBF functions to maintain coronary Pv_{O_2} essentially constant. An increase in coronary Pv_{O_2} indicates uncoupling of coronary oxygen supply from the myocardial oxygen demands and is the hallmark of a coronary vasodilating drug.[6] Under constant-pressure conditions (series 1), intracoronary isoflurane caused an initial fivefold increase in CBF. Because isoflurane also reduced $M\dot{V}_{O_2}$, coronary Pv_{O_2} values increased remarkably, providing evidence for a potent direct vasodilating action. The ability of isoflurane to cause marked dilation in the coronary vascular bed is consistent with previous findings obtained *in vivo* in canine hearts[10,24] and with those obtained *in vitro* in isolated, perfused rat hearts.[25,26]

Continued administration of isoflurane resulted in a gradual return of CBF toward baseline. Because measured values for coronary arterial isoflurane were constant, this progressive recovery of vascular tone was not due to a decline in the amount of isoflurane delivered to the LAD bed. Furthermore, the unchanged coronary vasodilator reserve ratio, as assessed with adenosine, ruled out the possibility that the decline in CBF was due to reduced functional area within the microcirculation, *e.g.*, due to microemboli or myocardial edema, or to generalized tolerance of coronary vascular smooth muscle to vasodilating stimuli. We can postulate two mechanisms that may have accounted for the progressive recovery of coronary vascular tone during isoflurane administration: (1) Coronary vascular smooth muscle became tachyphylaxic to the relaxing action of isoflurane. (2) A vasoconstrictor mechanism (perhaps metabolic factors secondary to a reduced local oxygen demand[6]) emerged gradually to antagonize the direct coronary vasorelaxing effect of isoflurane. Further studies are required to clarify the contributions of these mechanisms.

The current findings are qualitatively consistent with the work of Kenny *et al.*, but those investigators reported significantly smaller values for time to peak flow, magnitude of peak flow, and time to maximal recovery of coronary vascular tone when time-dependent changes in CBF were evaluated during inhalation induction in chronically instrumented dogs.[9] An explanation for this quantitative difference is uncertain. One possible explanation was that, in the study by Kenny *et al.* isoflurane concentrations were elevated in the systemic circulation, which caused reductions in aortic pressure and global cardiac work demand, thus providing a greater stimulus for coronary vasoconstriction *via* metabolic mechanisms. Another explanation pertains to the rapidity with which the coronary circulation was exposed to an elevated isoflurane concentration. In the study by Kenny *et al.*, the coronary circulation would have been exposed to isoflurane gradually, because its arterial concentration would rise in accordance with its pharmacokinetic profile in the alveoli and pulmonary capillary bed,[27] whereas in the current study, the coronary circulation was exposed abruptly to arterial blood previously equilibrated with isoflurane. It is possible that the gradual rise in isoflurane blood concentration may have provided an opportunity for the coronary vascular smooth muscle to become desensitized to the vasorelaxing actions of isoflurane, so that at comparable blood concentrations, the extent of vasodilation was less.

The major determinants of myocardial oxygen demand are myocardial contractility, wall tension, and heart rate.[28] Because mean aortic pressure, mean left ventricular end-diastolic pressure, and heart rate were constant, the isoflurane-induced decreases in $M\dot{V}_{O_2}$ in the current study can be ascribed to reduced myocardial contractility by isoflurane. Findings in the time-control studies ruled out the possibility that this reduced contractility was due to time-dependent deterioration of the regional coronary perfusion preparation (fig. 2).

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The ability of isoflurane to cause direct cardiac depression is consistent with *in vitro* studies conducted in isolated papillary muscles[29–31] and in isolated, paced, working rat hearts[32,33] as well as with studies conducted *in vivo* using relatively load-independent indexes of contractility.[3,4] Studies conducted *in vitro* in isolated ventricular myocardium of ferret have suggested that the negative inotropic effects of isoflurane are primarily the consequence of a reduction in intracellular calcium availability, and that anesthetic-induced decreases in myofibrillar responsiveness play only a minor role.[30]

Mechanical forces in the left ventricular wall compress the coronary arteries during systole, causing a mechanical impediment to blood flow.[6] This effect varies across the left ventricular wall, with the greatest influence being in the subendocardium. In studies in which coronary vascular tone was fixed by a maximally dilating infusion of adenosine and peak systolic left ventricular pressure was constant, some investigators have demonstrated that CBF varies inversely with the level of myocardial contractility,[6] and others have reported minimal influence of this factor.[6] Whether reduced extravascular compressive forces contributed to the isoflurane-induced increases in CBF was not addressed in the current study. However, our previous finding that myocardial blood flow, assessed with radioactive microspheres, remained transmurally uniform during a relatively brief (10 min) intracoronary administration of isoflurane, suggests that this mechanism played little or no role.[10]

A persistent reduction in $M\dot{V}_{O_2}$ was observed after termination of intracoronary administration of isoflurane, implying a delayed recovery of contractile activity. One possible explanation for this finding was that residual isoflurane remained in the myocardium despite 15 min of reperfusion with isoflurane-free blood. However, this appears unlikely, because a high rate of blood flow combined with a low tissue solubility favors rapid elimination of isoflurane from the myocardium.[27] A more likely explanation was that the myocardium required time to recover from an extended period of being forced to shorten against a normal afterload, *i.e.*, aortic pressure, while depressed by isoflurane. It should be kept in mind that this condition was artificial and peculiar to the experimental preparation. When isoflurane is inhaled, its concentration rises in the systemic circulation causing decreases in both myocardial contractility and afterload.[9]

The lack of effect of L-NAME on isoflurane-induced increases in CBF (series 1) and decreases in CPP (series 2) suggests no involvement of the nitric oxide-cGMP pathway in the coronary vasodilation caused by isoflurane. Greenblatt *et al.* presented findings that are in direct conflict with this conclusion.[5] They observed that an intravenous administration of L-NMMA in isoflurane-anesthetized rats caused an increase in coronary vascular resistance and thus concluded that nitric oxide played a role in isoflurane-induced coronary vasodilation. The explanation for the difference between the current findings and those of Greenblatt *et al.* is uncertain, but it may be related to methodologic differences, including those involving species, experimental design and protocols, and nitric oxide synthase inhibitor. One important difference in the studies was the route of administration of isoflurane and the NOS inhibitor. Greenblatt *et al.* administered both agents systemically, which caused in itself significant changes in hemodynamic conditions and global cardiac work requirements, whereas the use of local intracoronary administrations in the current study avoided these potentially complicating factors.

The current findings in the coronary circulation are consistent with previous findings obtained *in vitro* in vessels from other body locations also suggesting that nitric oxide plays no role in isoflurane-induced vascular relaxation. First, Flynn *et al.* observed well preserved isoflurane-induced relaxation of cerebral arterial rings after denuding of the vascular endothelium or pretreatment with L-NMMA,[2] whereas Stone and Johns[3] and Brendel and Johns[4] demonstrated comparable findings using rat aortic rings. Furthermore, Brendel and Johns reported that isoflurane did not increase cGMP content in the aortic rings.

In the normal heart, all but a small percentage of the total resistance to blood flow (approximately 3–5%) resides in the arteriolar segments of the microcirculation.[35] Thus the significant decreases in coronary vascular resistance caused by isoflurane (sufficient to increase CBF four-fold) probably are predominantly due to the effects of isoflurane on arterioles, although this remains to be verified by direct microscopic examination.

The mechanism by which isoflurane relaxes the smooth muscle of coronary resistance vessels remains uncertain. In light of the finding of Stone and Johns that isoflurane caused release of prostacyclin from isolated rat aortic rings,[3] a role for prostacyclin must be considered. Another possibility is that isoflurane has a direct inhibitory effect on vascular smooth muscle, perhaps by causing a reduction in calcium availability

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at the cell membrane, sarcoplasmic reticulum, and contractile proteins.[36]

In keeping with previous investigations,[17,18] we observed that inhibition of NOS with an arginine analog had no effect on baseline CBF. This finding is consistent with either of two interpretations: (1) nitric oxide is not released tonically and thus plays no role in regulation of basal coronary vascular tone, or (2) nitric oxide is released tonically but an alternate metabolic mechanism, *e.g.*, endogenous adenosine,[6] preserves myocardial oxygen supply/demand balance when the tonic influence of nitric oxide is blocked. In support of the second interpretation, Kostic and Schrader[37] reported increased basal release of adenosine from isolated guinea pig hearts after inhibition of NOS with L-NAME.

In summary, the results of the current study demonstrate (1) that isoflurane-induced coronary vasodilation is not diminished by L-NAME, and thus that nitric oxide does not mediate this response, and (2) that coronary vasodilation by isoflurane wanes over time, perhaps because of tachyphylaxis or emergence of a competing vasoconstrictor mechanism, *e.g.*, metabolic factors in response to depressed myocardial oxygen demands.

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