

Increases in Coronary Collateral Blood Flow Produced by Sevoflurane Are Mediated by Calcium-activated Potassium (BK_{Ca}) Channels In Vivo

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Background: Sevoflurane enhances coronary collateral blood flow independent of adenosine triphosphate-regulated potassium channels. The authors tested the hypothesis that this volatile anesthetic increases coronary collateral blood flow by either opening calcium-activated potassium channels or by directly stimulating nitric oxide synthesis in the canine coronary collateral circulation.

Methods: Twelve weeks after left anterior descending coronary artery ameroid constrictor implantation, barbiturate-anesthetized dogs ($n = 22$) were instrumented for measurement of hemodynamics and retrograde coronary flow. Dogs received sevoflurane (0.5 and 1.0 minimum alveolar concentration [MAC]) during intracoronary infusions of drug vehicle (0.9% saline), the calcium-activated potassium channel antagonist iberiotoxin (13 $\mu\text{g}/\text{min}$), or the nitric oxide synthase inhibitor *N* ω -nitro-L-arginine methyl ester (L-NAME, 300 $\mu\text{g}/\text{min}$). Retrograde coronary collateral blood flow was measured under baseline conditions, during and after administration of sevoflurane, and during intracoronary infusion of bradykinin. Data are mean \pm SEM.

Results: Sevoflurane increased ($*P < 0.05$) retrograde coronary collateral blood flow (from 65 ± 11 during control to $67 \pm 12^*$ and $71 \pm 12^*$ ml/min during 0.5 and 1.0 MAC, respectively). Iberiotoxin but not L-NAME attenuated these sevoflurane-induced increases in retrograde flow ($6 \pm 1^*$, $7 \pm 2^*$, and 3 ± 2 ml/min during vehicle, L-NAME, and iberiotoxin, respectively). After discontinuation of sevoflurane, retrograde flow returned to baseline values in each group. Bradykinin increased retrograde flow in vehicle- (63 ± 12 to $69 \pm 12^*$ ml/min) but not in iberiotoxin- (61 ± 7 to 62 ± 5 ml/min) or L-NAME-treated dogs (64 ± 11 to 63 ± 10 ml/min).

Conclusions: The results demonstrate that sevoflurane increases coronary collateral blood flow, in part, through activation of calcium-activated potassium channels *in vivo*. This action occurs independent of nitric oxide synthesis.

CHRONIC reduction of myocardial oxygen supply produced by severe coronary artery stenoses leading to coronary occlusion stimulates growth of collateral vessels that increase blood flow to myocardium at risk for

ischemia. Coronary collateral perfusion is a major determinant of the degree of injury associated with an ischemic event.¹ The vasodilator response of coronary collaterals to physiologic and pharmacologic stimuli is an important factor that affects the extent of damage to ischemic myocardium. Coronary collateral vessels respond to both endothelium-dependent and -independent vasodilators, including bradykinin and nitroglycerin, respectively.^{2,3} Activation of large conductance calcium-activated potassium (BK_{Ca}) channels⁴ by endothelium-derived hyperpolarizing factor plays a major role in the relaxation of collateral vessels.⁵ Recent evidence also suggests that BK_{Ca} channels are an important mediator of volatile anesthetic-induced alterations in mesenteric vasomotor tone.⁶

Sevoflurane is a coronary vasodilator⁷⁻⁹ that may produce beneficial effects in ischemic myocardium by increasing coronary collateral blood flow. We have previously shown that increases in collateral perfusion produced by sevoflurane occur independent of activation of adenosine triphosphate-regulated potassium channels.¹⁰ In this investigation, we tested the hypothesis that sevoflurane increases retrograde collateral blood flow by activating BK_{Ca} channels in a canine ameroid constrictor model of enhanced collateral development.

Materials and Methods

All experimental procedures and protocols used in this investigation were reviewed and approved by the Institutional Animal Care and Use Committee of the Medical College of Wisconsin (Milwaukee, Wisconsin). All procedures conformed to the *Guiding Principles in the Care and Use of Animals* of the American Physiologic Society and were performed in accordance with the *Guide for the Care and Use of Laboratory Animals* (Washington, DC, National Academy Press, 1996).

Implantation of Ameroid Constrictors

The methods have previously been described in detail.¹⁰ Conditioned mongrel dogs were fasted overnight. Anesthesia was induced with intravenous propofol (5 mg/kg). After tracheal intubation, anesthesia was maintained with sevoflurane (2.5–3%) in 100% oxygen *via* positive-pressure ventilation. A left thoracotomy was performed using sterile technique, and a 1.0- to 1.5-cm segment of the left anterior descending coronary artery (LAD) immediately distal to the first diagonal branch was

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isolated. An ameroid constrictor (Research Instruments and MFG, Corvallis, OR) was placed around the vessel. The diameter of the internal lumen of the constrictor was 2.0 to 3.0 mm, and its size was chosen for a snug fit around the vessel without producing visible stenosis. The chest was closed in layers and pneumothorax evacuated with a chest tube. Each dog received antibiotics (cefazolin [40 mg/kg] and gentamicin 4.5 mg/kg) and analgesics (epidural morphine [0.2 mg/kg] and fentanyl [2 μ g/kg]).

General Preparation

Twelve weeks after implantation of ameroid constrictors, dogs were anesthetized with sodium pentobarbital (15 mg/kg) and sodium barbital (200 mg/kg) and ventilated using positive pressure with oxygen-enriched air (fractional inspired oxygen concentration = 0.40) after tracheal intubation. Arterial blood acid-base status was maintained within a physiologic range by adjusting tidal volume and respiratory rate and by administering sodium bicarbonate as necessary. A dual micromanometer-tipped catheter (Millar) was inserted into the aorta and left ventricle (LV) through the left carotid artery to measure arterial and LV pressures, respectively. Heparin-filled catheters were inserted into the right femoral vein and artery for administration of intravenous fluids and withdrawal of reference arterial blood samples, respectively.

A thoracotomy was performed in the left fifth intercostal space, the lung was gently retracted, and the heart was suspended in a pericardial cradle. A heparin-filled catheter was inserted into the left atrium for injection of radioactive microspheres. Snares were placed around the descending thoracic aorta and inferior vena cava to facilitate control of arterial pressure, maintaining arterial pressure at baseline values throughout experimentation. Segments of the left circumflex coronary artery (LCCA; proximal to the first marginal branch) and LAD (distal to the ameroid constrictor) were dissected free from surrounding connective tissue and myocardium. A transit time flow probe (Transonic, Ithaca, NY) was positioned around the LCCA to measure coronary blood flow and a fluid-filled intracoronary catheter (24 gauge) was inserted into this vessel for drug infusion. Each dog was anticoagulated with heparin (500 U/kg). A large-bore polyethylene cannula attached to Silastic tubing was inserted in the right carotid artery. The proximal LAD was ligated, a large-bore metal cannula was positioned and secured in the distal arterial segment, and a carotid artery to LAD shunt was established to ensure patency of the distal LAD perfusion cannula between measurements of retrograde flow. Perfusion in the LAD was restored within 3 min after vessel ligation. A segment of tubing perpendicular to the metal cannula was used to measure retrograde coronary collateral blood flow during interruption of antegrade coronary flow through the carotid

artery to LAD shunt. Hemodynamics were monitored continuously on a polygraph and digitized using a computer interfaced with an analog-to-digital converter.

Measurement of Myocardial Perfusion

Carbonized plastic microspheres labeled with ^{141}Ce , ^{103}Ru , ^{51}Cr , or ^{95}Nb were used to measure myocardial perfusion as previously described.¹¹ At the conclusion of each experiment, 10 ml Patent blue dye was injected into the LCCA simultaneously with saline infused intracoronary into the LAD perfusion tubing at equal pressure to delineate the normal and collateral-dependent regions, respectively. Transmural tissue samples were selected from the collateral-dependent (distal to the ameroid constrictor) and normal (LCCA) regions and subdivided into subepicardial, mid myocardial, and subendocardial layers of approximately equal thickness and weight. Tissue blood flow ($\text{ml} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$) was calculated as $Q_r \cdot C_m \cdot C_r^{-1}$, where Q_r is the rate of withdrawal of the reference blood flow sample (ml/min); C_m is the activity (counts per minute per gram) of the myocardial tissue sample; and C_r is the activity (counts/min) of the reference blood flow sample. Transmural blood flow was considered as the average of subepicardial, mid myocardial and subendocardial blood flows. Injection of radioactive microspheres was performed with the retrograde flow cannula and the carotid artery to LAD shunt clamped. Thus, tissue blood flow to the collateral-dependent myocardium was a measure of total (e.g., microvascular and interarterial) collateral blood flow during steady state conditions.¹² Coronary perfusion pressure was determined as the difference between end-diastolic arterial pressure and LV end-diastolic pressure. Retrograde collateral conductance was calculated as the ratio of retrograde blood flow to coronary perfusion pressure.

Experimental Protocol

Hemodynamics were recorded, radioactive microspheres were injected, and LAD retrograde blood flow was measured 30 min after completion of the acute surgical preparation. Measurements of LCCA blood flow were performed during steady state conditions before measurement of retrograde flow. Retrograde blood flow, an index of large interarterial collateral flow, was assessed by clamping the carotid to LAD shunt and collecting blood from the LAD cannula flowing into a graduated cylinder for 90 s while the cannula tip was held at the level of the left atrium. Measurements were performed in triplicate, and the results were averaged. After measurement of retrograde flow, total collateral blood flow *via* large interarterial and microvascular anastomoses was determined with microsphere injections performed after the retrograde flow cannula and carotid artery to LAD shunt had been clamped. Dogs were randomly assigned to receive intracoronary vehicle (0.9% saline), the BK_{Ca} channel antagonist iberiotoxin (13 μ g/min),¹³

or the nitric oxide synthase inhibitor *N* ω -nitro-L-arginine methyl ester (L-NAME, 300 μ g/min)^{13,14} in three separate groups. Measurements of retrograde flow were repeated during administration of sevoflurane (0.5 and 1.0 minimum alveolar concentration [MAC]) after 10 min equilibration at each concentration, 10 min after discontinuation of the anesthetic (recovery), and during infusion of bradykinin (0.625 μ g/min).¹³ Bradykinin has previously been shown to increase coronary collateral blood flow through both BK_{Ca}- and nitric oxide-dependent mechanisms and was used as a positive control. End-tidal concentrations of sevoflurane were measured at the tip of the endotracheal tube by an infrared anesthetic gas analyzer that was calibrated with known standards before and during experimentation.

Drugs

Iberitoxin and L-NAME were purchased from Sigma (St. Louis, MO), and sevoflurane was purchased from Abbott Laboratories (North Chicago, IL).

Statistical Analysis

Statistical analysis of data within and between groups was performed with multiple analysis of variance for repeated measures followed by application of Student-Newman-Keuls test. Changes within and between groups were considered statistically significant when $P < 0.05$. Data are mean \pm SEM.

Results

Twenty-five dogs were instrumented with ameroid constrictors to obtain 22 successful experiments. Three dogs died after implantation of ameroid constrictors during the 12-week period of coronary collateral development.

Hemodynamic Effects of Sevoflurane

Sevoflurane produced significant ($P < 0.05$), dose-dependent decreases in heart rate and LV+dP/dt_{max} and increases in LV end-diastolic pressure in the presence or absence of iberitoxin or L-NAME (table 1). Mean arterial and LV systolic pressures were intentionally maintained at baseline values by partial constriction of the thoracic aorta or the inferior vena cava throughout each experiment. Retrograde blood flow varied considerably between dogs at baseline (25–114 ml/min). Sevoflurane consistently increased retrograde collateral blood flow (from 65 \pm 11 ml/min under baseline conditions to 67 \pm 12 and 71 \pm 12 ml/min during 0.5 and 1.0 MAC, respectively; table 1) and conductance in each dog receiving intracoronary drug vehicle (n = 7). This was a selective effect for coronary collaterals as LCCA coronary blood flow remained unchanged. Iberitoxin (n = 7) but not L-NAME (n = 8) attenuated sevoflurane-induced

increases in retrograde flow (6 \pm 1 and 7 \pm 2 ml/min in vehicle and L-NAME experiments as compared to 3 \pm 2 ml/min during iberitoxin; fig. 1). Retrograde collateral blood flow and conductance returned to baseline values after sevoflurane was discontinued. Transmural perfusion of normal and collateral-dependent myocardium (table 2) was similar between groups under baseline conditions. Sevoflurane did not alter transmural or regional myocardial perfusion to either the normal or the collateral-dependent regions in the presence or absence of iberitoxin or L-NAME.

Hemodynamic Effects of Bradykinin

Bradykinin increased retrograde collateral flow (63 \pm 12 ml/min after discontinuation of sevoflurane to 69 \pm 12 ml/min) in dogs receiving intracoronary drug vehicle (table 1). The magnitude of this bradykinin-induced increase in retrograde flow was similar to that observed during 1.0 MAC sevoflurane. Iberitoxin and L-NAME abolished increases in retrograde flow associated with administration of bradykinin. In contrast to the findings with sevoflurane, bradykinin increased perfusion to normal and collateral-dependent myocardium (table 2) in dogs receiving vehicle or iberitoxin but not L-NAME.

Discussion

The canine ameroid constrictor model of a well-developed coronary collateral circulation used in the present investigation allows distinction of coronary collateral blood flow on the basis of collateral size. Changes in large interarterial coronary collateral blood flow can be differentiated from that occurring in microvascular collaterals. Interarterial collateral blood flow (measured with retrograde flow) represents the major source of nutritive blood flow to collateral-dependent myocardium.^{12,15} Microvascular collateral blood flow is measured by injecting radioactive microspheres simultaneously with measurements of retrograde flow (retrograde flow cannula is open). Performed in this way, tissue blood flow to the collateral-dependent region represents microvascular collateral flow (\approx 50% of total collateral flow) that is measured during a period of relative hypoperfusion.¹⁵ This procedure was used in our previous study, and we demonstrated that sevoflurane did not increase microvascular collateral flow. Sevoflurane increased microvascular collateral flow only in the presence of glyburide, probably because glyburide caused a small degree of vasoconstriction that resulted in an increase in coronary collateral perfusion pressure (by decreasing the pressure drop across proximal resistances). Alternatively, tissue blood flow measured with the retrograde cannula closed represents total collateral flow (interarterial + microvascular).^{12,15} In the absence of retrograde flow diversion, this measurement is made

Table 1. Hemodynamic Effects of Sevoflurane and Bradykinin

	Sevoflurane				
	Baseline	0.5 MAC	1.0 MAC	Recovery	Bradykinin
HR (beats/min)					
Vehicle	135 ± 3	123 ± 3*	113 ± 2*‡	114 ± 3*	117 ± 4*
Iberiotoxin	137 ± 5	123 ± 3*	113 ± 3*	117 ± 3*	125 ± 4*
L-NAME	143 ± 7	131 ± 5	120 ± 3*	133 ± 7†	138 ± 6†
MAP (mmHg)					
Vehicle	86 ± 5	88 ± 4	89 ± 5	88 ± 5	89 ± 5
Iberiotoxin	83 ± 5	84 ± 5	83 ± 5	83 ± 5	83 ± 4
L-NAME	79 ± 5	79 ± 5	78 ± 4	78 ± 6	76 ± 5
LVSP (mmHg)					
Vehicle	93 ± 5	97 ± 5	99 ± 6	99 ± 5	99 ± 5
Iberiotoxin	91 ± 5	94 ± 5	95 ± 6	93 ± 7	92 ± 5
L-NAME	93 ± 5	92 ± 7	90 ± 6	85 ± 13	88 ± 6
LVEDP (mmHg)					
Vehicle	9 ± 1	11 ± 4*	13 ± 1*‡	11 ± 1*	11 ± 1*
Iberiotoxin	4 ± 1	6 ± 1*†	6 ± 1*†	5 ± 2†	3 ± 2*†§
L-NAME	7 ± 2	8 ± 1	11 ± 2*‡	7 ± 1	6 ± 1†
+dP/dt _{max} (mmHg/s)					
Vehicle	1,370 ± 70	1,130 ± 60*	960 ± 60*‡	1,200 ± 100	1,320 ± 110
Iberiotoxin	1,690 ± 110	1,320 ± 160*	1,217 ± 120*	1,510 ± 130	1,500 ± 130
L-NAME	1,740 ± 120	1,370 ± 80*	1,000 ± 40*‡	1,390 ± 90*	1,350 ± 80*
LCCA CBF (ml/min)					
Vehicle	66 ± 10	59 ± 9	58 ± 11	66 ± 10	140 ± 23*§
Iberiotoxin	77 ± 10	67 ± 10	72 ± 11	76 ± 9	124 ± 15*§
L-NAME	95 ± 17	83 ± 12	78 ± 11	92 ± 16	117 ± 20*§
RF (ml/min)					
Vehicle	65 ± 11	67 ± 12*	71 ± 12*‡	63 ± 12	69 ± 12*§
Iberiotoxin	63 ± 7	66 ± 7	66 ± 8	61 ± 7	62 ± 5
L-NAME	65 ± 11	68 ± 11	71 ± 12*	64 ± 11	63 ± 10
RC (mL · min ⁻¹ · mmHg ⁻¹)					
Vehicle	0.91 ± 0.19	0.96 ± 0.20	1.05 ± 0.23*‡	0.91 ± 0.20	0.99 ± 0.20*§
Iberiotoxin	0.91 ± 0.11	0.98 ± 0.12	0.97 ± 0.13	0.90 ± 0.13	0.88 ± 0.10
L-NAME	1.05 ± 0.19	1.13 ± 0.19	1.24 ± 0.21*‡	1.04 ± 0.18	1.01 ± 0.17

Data are expressed as mean ± SEM.

* Significantly ($P < 0.05$) different from baseline. † Significantly ($P < 0.05$) different from dogs receiving vehicle. ‡ Significantly ($P < 0.05$) different from 0.5 MAC sevoflurane. § Significantly ($P < 0.05$) different from recovery.

MAC = minimum alveolar concentration; HR = heart rate; L-NAME = N(ω)-nitro-L-arginine methyl ester; LVSP = left ventricular systolic pressure; LVEDP = left ventricular end-diastolic pressure; +dP/dt_{max} = maximal rate of increase of left ventricular pressure; LCCA CBF = left circumflex coronary artery blood flow; RF = retrograde coronary collateral blood flow; RC = retrograde coronary collateral conductance.

during conditions in which the collateral-dependent region is capable of autoregulation because hypoperfusion is absent. Thus, autoregulatory adjustments in resistance in the collateral-dependent microvasculature may offset changes in large interarterial conductance. The present findings indicate that sevoflurane increases blood flow through large interarterial coronary collateral vessels, in part, by activation of BK_{Ca} channels and is independent of nitric oxide synthesis. In contrast to the findings with sevoflurane, interarterial collateral blood flow responses to bradykinin were dependent on both nitric oxide and BK_{Ca} channels, while microvascular collateral flow appeared to be modulated in large part through nitric oxide alone.

The current results confirm our previous observations demonstrating that sevoflurane selectively increases coronary collateral blood flow independent of adenosine triphosphate-regulated potassium channels.¹⁰ Sevoflurane-induced increases in retrograde collateral blood

flow were attenuated by the BK_{Ca} channel antagonist iberiotoxin but not by the nitric oxide synthase inhibitor L-NAME. Sevoflurane increased retrograde flow by 11 ± 2% in well-developed collaterals 12 weeks after ameroid implantation. These results were less than those observed in our previous study (29 ± 7%) in dogs with moderately developed collaterals produced 8 weeks after ameroid implantation.¹⁰ However, small changes (3 ml/min) in retrograde blood flow are sufficient to markedly reduce myocardial infarct size.¹⁶ An increase in retrograde flow of 6 ml/min during sevoflurane would be expected to increase transmural coronary collateral perfusion by 0.10 ml/min/g and thus result in a doubling of collateral blood flow in a typical myocardial infarction experiment in the dog.¹⁷ Our results also support those of previous studies indicating that the vasodilator mechanisms present in the coronary collateral circulation are dependent on the degree of collateral development. Immature coronary collateral vessels exhibit redundant va-

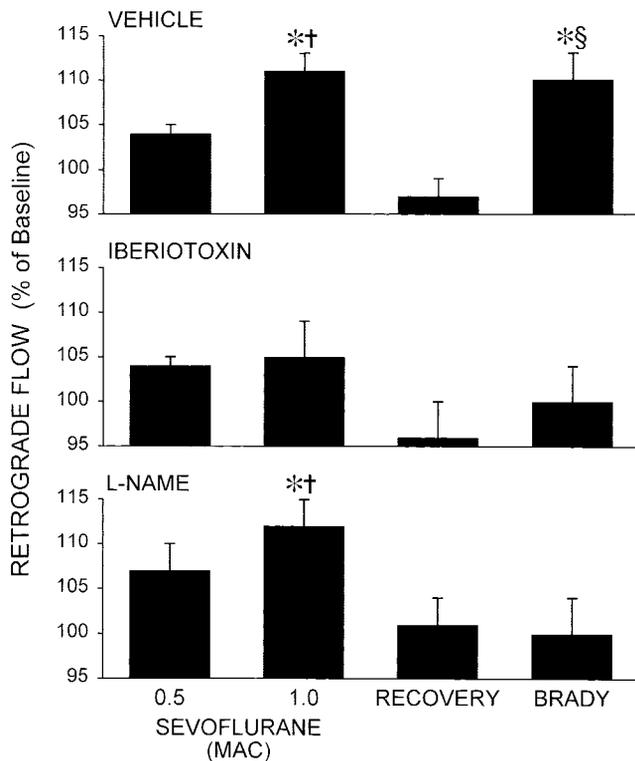


Fig. 1. Histograms depicting retrograde coronary collateral blood flow (expressed as a percentage of baseline) during administration of sevoflurane (0.5 and 1.0 minimum alveolar concentration [MAC]), after discontinuation of sevoflurane (recovery), and during administration of bradykinin in dogs receiving intracoronary drug vehicle (0.9% saline; *top*), iberiotoxin (13 $\mu\text{g}/\text{min}$; *middle*), or *N* ω -nitro-L-arginine methyl ester (L-NAME, 300 $\mu\text{g}/\text{min}$; *bottom*). *Significantly ($P < 0.05$) different from baseline; †significantly ($P < 0.05$) different from 0.5 MAC sevoflurane; §significantly ($P < 0.05$) different from recovery.

sodilator mechanisms mediated by endothelium-derived hyperpolarizing factor, nitric oxide, and prostaglandins. In contrast, mature coronary collateral vessels exhibit a greater dependence on nitric oxide with a diminished role for endothelium-derived hyperpolarizing factor or prostaglandins as mediators of endothelium-dependent relaxation.⁵ Coronary vasodilator reserve may also be

diminished in older hearts because the number of BK_{Ca} channels present in coronary vascular smooth muscle is reduced.¹⁸ Thus, less-pronounced increases in retrograde collateral blood flow observed during administration of sevoflurane in well-developed as compared to moderately developed collaterals may reflect a time-dependent decrease in the expression of BK_{Ca} channels during a period when the vasodilator response of interarterial collaterals is becoming more dependent on nitric oxide.

Sevoflurane had no appreciable effect on microvascular blood flow in normal or collateral-dependent myocardium in this or our previous¹⁰ investigations. We measured total collateral blood flow under steady state conditions during which arteriolar resistance adjustments in the collateral-dependent region would be expected to offset sevoflurane-induced increases in large collateral conductance. Under these circumstances, total collateral blood flow should increase only if sevoflurane produced a decrease in microvascular collateral or collateral-dependent arteriolar resistance. The absence of changes in perfusion to collateral-dependent myocardium suggests that sevoflurane did not substantially alter resistance in these small vessels. In contrast to findings during administration of sevoflurane, bradykinin increased both retrograde and microvascular collateral blood flow.² The potent vasodilator, bradykinin, produced increases in retrograde blood flow that were similar in magnitude to those observed during administration of sevoflurane. Iberiotoxin and L-NAME abolished the bradykinin-induced increases in retrograde blood flow. These findings are consistent with evidence suggesting that BK_{Ca} channels may be an end-effector for bradykinin,¹³ nitrovasodilators, including nitroglycerin,^{19,20} or endogenous nitric oxide.²¹⁻²³ Bradykinin also increased total normal and collateral-dependent zone blood flow, confirming previous observations that this endothelium-dependent vasodilator has a pronounced effect on the coronary microvascular circulation.^{2,24} The actions of bradykinin on large interarterial collaterals

Table 2. Transmural Myocardial Perfusion ($\text{ml} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$)

	Baseline	Sevoflurane		Bradykinin
		0.5 MAC	1.0 MAC	
Collateral-dependent region				
Vehicle	0.76 \pm 0.10	0.84 \pm 0.11	0.85 \pm 0.14	1.15 \pm 0.12*
Iberiotoxin	0.89 \pm 0.05	1.02 \pm 0.08	0.89 \pm 0.06	1.10 \pm 0.05*†
L-NAME	1.04 \pm 0.09	1.09 \pm 0.11	1.04 \pm 0.09	1.13 \pm 0.13
Normal region				
Vehicle	0.82 \pm 0.09	0.85 \pm 0.08	0.86 \pm 0.13	1.63 \pm 0.34*
Iberiotoxin	0.93 \pm 0.08	1.02 \pm 0.08	0.86 \pm 0.08	1.17 \pm 0.15†
L-NAME	1.03 \pm 0.09	1.01 \pm 0.10	0.97 \pm 0.07	1.17 \pm 0.16

Data are expressed as mean \pm SEM.

* Significantly ($P < 0.05$) different from baseline. † Significantly ($P < 0.05$) different from 1.0 MAC sevoflurane.

MAC = minimum alveolar concentration; L-NAME = N(ω)-nitro-L-arginine methyl ester.

were blocked by either iberiotoxin or L-NAME, but increases in normal and total collateral-dependent zone flow produced by bradykinin were inhibited by L-NAME but not iberiotoxin. These results suggest that small resistance vessels in normal and collateral-dependent myocardium exhibit greater dependence on nitric oxide during bradykinin-induced vasodilation as compared to large interarterial collaterals. Consistent with this interpretation, sevoflurane had no appreciable effect on microvascular blood flow in the normal or collateral-dependent region, suggesting that the actions of this volatile anesthetic are not dependent on nitric oxide.

Recent evidence suggests that bradykinin-induced vasodilation in immature coronary collateral vessels involves activation of multiple, redundant pathways mediated by endothelium-derived hyperpolarizing factor, nitric oxide, and prostaglandins.⁵ A role for prostaglandins in sevoflurane-induced coronary collateral vasodilation was not examined in the present investigation because the cyclooxygenase inhibitor indomethacin (50–300 $\mu\text{g}/\text{min}$) caused profound decreases in retrograde blood flow (from 74 ± 13 to 36 ± 8 ml/min) during pilot experiments. Nevertheless, a major role for prostaglandin-induced dilation of well-developed collaterals during administration of sevoflurane in our model appears unlikely because the effects of sevoflurane were significantly attenuated by the selective BK_{Ca} antagonist iberiotoxin.

The current findings must be interpreted within the constraints of several potential limitations. First, the effects of sevoflurane on coronary collateral blood flow may not be solely attributable to direct actions on coronary vascular smooth muscle tone. Sevoflurane caused dose-related reductions in heart rate and global myocardial contractility (e.g., LV+dP/dt_{max}). Thus, sevoflurane may have indirectly reduced coronary collateral blood flow *via* metabolic autoregulation secondary to a decrease in myocardial oxygen consumption. Such reductions in the determinants of myocardial oxygen consumption may also have masked the actions of sevoflurane on the conductance of resistance arterioles in normal myocardium through flow-metabolism coupling.²⁵ Crystal *et al.*⁹ have previously demonstrated that sevoflurane causes direct coronary vasodilation through activation of adenosine triphosphate-regulated potassium channels in an *in situ* canine heart model. However, we previously demonstrated that the nonselective adenosine triphosphate-regulated potassium channel antagonist glyburide does not alter sevoflurane-induced increases in retrograde coronary collateral blood flow.¹⁰ Increases in LCCA conductance produced by sevoflurane could also contribute to changes in retrograde blood flow by increasing collateral perfusion pressure. However, total left circumflex coronary blood flow and transmural perfusion to normal myocardium measured using radioactive microspheres were unchanged by

sevoflurane, confirming our previous findings that sevoflurane selectively increases coronary collateral blood flow in this model. Decreases in heart rate could also contribute to enhanced collateral blood flow during sevoflurane. However, attenuation of sevoflurane effects by iberiotoxin suggests that activation of BK_{Ca} channels by sevoflurane was most likely responsible for the increases in retrograde flow observed in this study. Sevoflurane did not increase retrograde flow in the presence of iberiotoxin ($P = 0.24$); however, the power of the study to detect small differences in retrograde flow under these conditions was limited. Bradykinin actions were also blocked by iberiotoxin (nonsignificant [$P = 0.56$] increases in retrograde flow during iberiotoxin), thus indirectly supporting a role for BK_{Ca} channel activation by sevoflurane.

In summary, the present results indicate that sevoflurane-induced increases in blood flow through large coronary collateral anastomoses are mediated, in part, by BK_{Ca} channel activation. Sevoflurane may exert beneficial effects in ischemic myocardium by not only activating cardioprotective signal transduction²⁶ but also by selectively increasing coronary collateral blood flow.

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References

1. Kersten JR, Toller WG, Gross ER, Pagel PS, Warltier DC: Diabetes abolishes ischemic preconditioning: role of glucose, insulin, and osmolality. *Am J Physiol Heart Circ Physiol* 2000; 278:H1218–24
2. Altman J, Dulas D, Pavek T, Laxson DD, Homans DC, Bache RJ: Endothelial function in well-developed canine coronary collateral vessels. *Am J Physiol Heart Circ Physiol* 1993; 264:H567–72
3. Flynn NM, Kenny D, Pelc LR, Warltier DC, Bosnjak ZJ, Kampine JP: Endothelium-dependent vasodilation of canine coronary collateral vessels. *Am J Physiol Heart Circ Physiol* 1991; 261:H1797–801
4. Hayabuchi Y, Nakaya Y, Matsuoka S, Kuroda Y: Endothelium-derived hyperpolarizing factor activates Ca²⁺-activated K⁺ channels in porcine coronary artery smooth muscle cells. *J Cardiovasc Pharmacol* 1998; 32:642–9
5. Rapps JA, Myers PR, Zhong Q, Parker JL: Development of endothelium-dependent relaxation in canine coronary collateral arteries. *Circulation* 1998; 98:1675–83
6. Kokita N, Stekiel TA, Yamazaki M, Bosnjak ZJ, Kampine JP, Stekiel WJ: Potassium channel-mediated hyperpolarization of mesenteric vascular smooth muscle by isoflurane. *ANESTHESIOLOGY* 1999; 90:779–88
7. Larach DR, Schuler HG: Direct vasodilation by sevoflurane, isoflurane, and halothane alters coronary flow reserve in the isolated rat heart. *ANESTHESIOLOGY* 1991; 75:268–78
8. Park KW, Dai HB, Lowenstein E, Sellke FW: Effect of sevoflurane and desflurane on the myogenic constriction and flow-induced dilation in rat coronary arterioles. *ANESTHESIOLOGY* 1999; 90:1422–7
9. Crystal GJ, Zhou X, Gurevicius J, Czinn EA, Salem MR, Alam S, Piotrowski A, Hu G: Direct coronary vasomotor effects of sevoflurane and desflurane in *in situ* canine hearts. *ANESTHESIOLOGY* 2000; 92:1103–13
10. Kersten JR, Schmeling T, Tessmer J, Hettrick DA, Pagel PS, Warltier DC: Sevoflurane selectively increases coronary collateral blood flow independent of KATP channels *in vivo*. *ANESTHESIOLOGY* 1999; 90:246–56
11. Kersten JR, Schmeling TJ, Hettrick DA, Pagel PS, Gross GJ, Warltier DC: Mechanism of myocardial protection by isoflurane: Role of adenosine triphosphate-regulated potassium (KATP) channels. *ANESTHESIOLOGY* 1996; 85:794–807
12. Hautamaa PV, Dai XZ, Homans DC, Bache RJ: Vasomotor activity of moderately well-developed canine coronary collateral circulation. *Am J Physiol Heart Circ Physiol* 1989; 256:H890–7
13. Node K, Kitakaze M, Kosaka H, Minamino T, Hori M: Bradykinin mediation of Ca²⁺-activated K⁺ channels regulates coronary blood flow in ischemic myocardium. *Circulation* 1997; 95:1560–7

14. Node K, Kitakaze M, Kosaka H, Minamino T, Funaya H, Hori M: Amelioration of ischemia- and reperfusion-induced myocardial injury by 17beta-estradiol: Role of nitric oxide and calcium-activated potassium channels. *Circulation* 1997; 96:1953-63
15. Downey HF, Crystal GJ, Bashour FA: Functional significance of microvascular collateral anastomoses after chronic coronary artery occlusion. *Microvasc Res* 1981; 21:212-22
16. Warltier DC, Zyvoloski MG, Gross GJ, Brooks HL: Importance of retrograde coronary flow in the prediction of experimental myocardial infarct size. *Cardiology* 1986; 73:333-46
17. Kehl F, Krolikowski JG, Mraovic B, Pagel PS, Warltier DC, Kersten JR: Is Isoflurane-induced preconditioning dose related? *ANESTHESIOLOGY* 2002; 96:675-80
18. Marijic J, Li Q, Song M, Nishimaru K, Stefani E, Toro L: Decreased expression of voltage- and Ca(2+)-activated K(+) channels in coronary smooth muscle during aging. *Circ Res* 2001; 88:210-6
19. Khan SA, Higdon NR, Meisheri KD: Coronary vasorelaxation by nitroglycerin: Involvement of plasmalemmal calcium-activated K+ channels and intracellular Ca++ stores. *J Pharmacol Exp Ther* 1998; 284:838-46
20. Bang L, Boesgaard S, Nielsen-Kudsk JE, Vejlsstrup NG, Aldershvile J: Nitroglycerin-mediated vasorelaxation is modulated by endothelial calcium-activated potassium channels. *Cardiovasc Res* 1999; 43:772-8
21. Khan SA, Mathews WR, Meisheri KD: Role of calcium-activated K+ channels in vasodilation induced by nitroglycerine, acetylcholine and nitric oxide. *J Pharmacol Exp Ther* 1993; 267:1327-35
22. Fujino K, Nakaya S, Wakatsuki T, Miyoshi Y, Nakaya Y, Mori H, Inoue I: Effects of nitroglycerin on ATP-induced Ca(++)-mobilization, Ca(++)-activated K channels and contraction of cultured smooth muscle cells of porcine coronary artery. *J Pharmacol Exp Ther* 1991; 256:371-7
23. Bychkov R, Gollasch M, Steinke T, Ried C, Luft FC, Haller H: Calcium-activated potassium channels and nitrate-induced vasodilation in human coronary arteries. *J Pharmacol Exp Ther* 1998; 285:293-8
24. Oltman CL, Kane NL, Fudge JL, Weintraub NL, Dellsperger KC: Endothelium-derived hyperpolarizing factor in coronary microcirculation: Responses to arachidonic acid. *Am J Physiol Heart Circ Physiol* 2001; 281:H1553-60
25. Jones CJ, Kuo L, Davis MJ, Chilian WM: Myogenic and flow-dependent control mechanisms in the coronary microcirculation. *Basic Res Cardiol* 1993; 88:2-10
26. Toller WG, Kersten JR, Pagel PS, Hettrick DA, Warltier DC: Sevoflurane reduces myocardial infarct size and decreases the time threshold for ischemic preconditioning in dogs. *ANESTHESIOLOGY* 1999; 91:1437-46