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Blockade of Adenosine Triphosphate-sensitive Potassium Channels Eliminates Isoflurane-induced Coronary Artery Vasodilation

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Background: **The mechanisms by which volatile anesthetics induce vasodilation are unknown. Recent studies of adenosine triphosphate–sensitive potassium channels (K_{ATP} channels) in the vascular smooth muscle of the coronary circulation suggest that these channels play a role in the coronary artery dilation produced by hypoxemia, the coronary blood flow (CBF) reactive hyperemic response, and in CBF auto regulation. We therefore conducted this study to determine the role of K_{ATP} channels in isoflurane-induced coronary vasodilation.**

Methods: **Studies were conducted in six open-chest, anesthetized swine. The left anterior descending coronary artery was cannulated and perfused by blood passed through a membrane oxygenator. This preparation allowed us to administer drugs and volatile anesthetics regionally to the perfused myocardium, minimizing systemic effects. Regional CBF response to 1.5% and 3.0% isoflurane administered *via* the membrane oxygenator was measured before and after blockade of K_{ATP} channels, and was compared to the vasodilation produced by regional administration of several doses of sodium nitroprusside and adenosine. Blockade of K_{ATP} channels was achieved by regional intracoronary administration of glibenclamide (1–22 $\mu g \cdot kg^{-1} \cdot min^{-1}$), a specific blocker of these channels.**

Results: **Administration of 1.5 and 3.0 percent isoflurane increased regional CBF by 29 ± 29% and by 62 ± 28%, respectively. Under control conditions, blockade of K_{ATP} channels decreased mean CBF by 18%, but did not cause ischemia. K_{ATP} channel blockade totally eliminated the vasodilator response to both doses of isoflurane. During K_{ATP} channel blockade the response to 3% isoflurane was converted to net vasoconstriction: mean $\Delta CBF = -5\% \pm 6\%$, $P = <0.05$ *versus* control. Negative inotropic effects of isoflurane were not eliminated by glibenclamide. Because K_{ATP} channel blockade was so effective in eliminating isoflurane-induced coronary vasodilation, the dose of glibenclamide was decreased in sequential experiments, but total blockade of isoflurane vasodilation was achieved even at the smallest dose of glibenclamide studied (1 $\mu g \cdot kg^{-1} \cdot min^{-1}$). The vasodilator response to nitroprusside was not affected, and the vasodilator response to adenosine was partially inhibited (consistent with their known mechanisms of action).**

Conclusions: **Blockade of K_{ATP} channels by glibenclamide completely inhibits isoflurane-induced coronary vasodilation in the regionally perfused swine myocardium. The response to sodium nitroprusside, a drug that induces vasodilation *via* a different mechanism, was unaffected. The response to adenosine, a drug whose vasodilation is partially mediated *via* K_{ATP} channels, was partially inhibited. These results suggest that *in vivo* isoflurane-induced coronary artery vasodilation is predominantly mediated by K_{ATP} channels. (Key words: Anesthetics, volatile: isoflurane. Potassium channels, ATP-sensitive: glibenclamide.)**

ALTHOUGH volatile anesthetics are known to be vasodilators in the coronary circulation[1–6] and in other vascular beds,[5,7–10] the mechanisms of their vasodilating action are not fully understood. Multiple effects of volatile anesthetics on vascular tone certainly have been described, including "direct" vasodilator effects on vascular smooth muscle,[3,11–14] and "indirect" vasodilator effects caused by the anesthetic state and concomitant interruption of neurally or humorally mediated vasoconstriction tone. In addition, competing vasoconstrictor effects of volatile anesthetics have been described, including flow adaptation to reduced metabolic requirements during anesthesia,[15,16] and mild indirect vasoconstrictor effects caused by attenuation of the release or action of an endothelium-derived relaxing factor.[17,18] In clinically used doses, the result of the competing effects is generally net vasodilation, and the volatile anesthetics are thus best regarded as dose-dependent vasodilators.

This article is accompanied by a Highlight. Please see this issue of ANESTHESIOLOGY, page 27A.

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Regarding the mechanisms of "direct" anesthetic effects on vascular smooth muscle, attention has appropriately focused on anesthetic-induced alterations in intracellular calcium concentrations and the processes which regulate intracellular calcium availability. Vascular smooth muscle tone is largely regulated by intracellular cytosolic calcium concentration and by factors that alter sensitivity to calcium. Intracellular cytosolic calcium concentration is, in turn, determined by many factors including calcium release from sites of intracellular sequestration, and calcium influx from extracellular sites *via* plasma membrane ion channels. Cellular ion channels are also regulated or modulated by many factors, including neural input, endothelial hormones, mechanical deformation,[19] and cellular transmembrane potential which affects the open probability of voltage-dependent channels.

One important regulator of cellular transmembrane potential is the adenosine triphosphate–sensitive potassium channel (K_{ATP} channel). Several recent investigations have found a substantial role for K_{ATP} channels in the control of coronary blood flow (CBF). Studies have determined that blockade of these K_{ATP} channels abolishes hypoxia-induced coronary artery vasodilatation,[20] interferes with CBF autoregulation,[21] and limits coronary artery reactive hyperemia.[22] In addition, K_{ATP} channels participate in setting basal coronary artery tone.[23] Blockade of these channels can reduce resting CBF and can even induce myocardial ischemia.[23,24]

To date, there has been little direct study of the effects of volatile anesthetics on K_{ATP} channels of vascular smooth muscle, or of other smooth muscle. Larach and Schuler found that treatment with glyburide reduced halothane-induced vasodilatation in crystalloid-perfused, tetrodotoxin-arrested rat hearts[25] by 56%. In the same model, perfusion with a high concentration of potassium ion eliminated halothane vasodilation almost completely, suggesting that one of the mechanisms of halothane vasodilation is through opening of cellular potassium channels. In addition, Mehr and Lindeman recently reported that halothane-induced bronchodilation was completely inhibited by K_{ATP} channel blockade.[26] These reports led us to hypothesize that *in vivo* coronary artery vasodilatation induced by volatile anesthetics might be mediated through K_{ATP} channels. Therefore, we examined the effect of blockade of K_{ATP} channels on isoflurane induced *in vivo* coronary artery vasodilation. In the presence and absence of glibenclamide (a K_{ATP} channel blocker), we measured the dose-related vasodilating potency of isoflurane and two other vasodilators, adenosine and sodium nitroprusside, drugs whose mechanism of vasodilation is more fully known.

Experiments were performed in a swine model of *in vivo* isolated coronary perfusion in which isoflurane and other drugs could be administered regionally, thus minimizing systemic effects.

Materials and Methods

Anesthesia

This experimental protocol was approved by our Animal Welfare Committee and followed the guidelines for animal use by the American Physiological Society. Six domestic swine weighing 40–45 kg received ketamine hydrochloride (1.0 g intramuscularly) and then intravenous pentobarbital (20-mg · kg^{-1} loading dose) followed by a 60-$\mu g \cdot kg^{-1} \cdot min^{-1}$ infusion and fentanyl (50-$\mu g \cdot kg^{-1}$ bolus followed by 0.50-$\mu g \cdot kg^{-1} \cdot min^{-1}$ infusion). Under general anesthesia, a tracheostomy was performed and ventilation was controlled to maintain normal *p*H and arterial carbon dioxide tension. To minimize ventricular arrhythmias, lidocaine hydrochloride was given as an intravenous bolus (3 mg · kg^{-1}) followed by a constant intravenous infusion of 2 mg · min^{-1} for the duration of the study. Central body temperature was maintained at 36.5–38.5°C by warmed intravenous fluids and surface warming. Arterial blood gases were measured at frequent intervals throughout the experiment with a blood gas analyzer (ABL-II, Radiometer, Copenhagen, Denmark). Hemoglobin and oxyhemoglobin saturation were measured with a hemoximeter (OSM3, Radiometer) with internal correction made for swine hemoglobin absorption characteristics. After completion of the experiment the animals were killed by anesthetic overdose.

Surgical Preparation and Instrumentation

The heart was exposed through a median sternotomy and suspended in a pericardial cradle. A calibrated micromanometer (Millar Instruments, Houston, TX) was inserted through the left atrium into the left ventricle for measurement of the left ventricular pressure and its first derivative with respect to time. Epicardial pacing wires were sutured to the right atrium and electrical pacing was begun after completion of the preparation at a rate 20% higher than the intrinsic heart rate. Pacing then continued at the same rate throughout the experiment. Both carotid arteries were cannulated with 14-

G catheters to supply blood to the extracorporeal circuit, and to provide periodic arterial blood samples and continuous systemic pressure monitoring. All transduced signals were recorded on a Grass Instruments polygraph (Quincy, MA). Both internal jugular veins were cannulated to provide intravenous access for drug and saline infusions. Finally, the anterior interventricular coronary vein was cannulated with a 20-G catheter to allow intermittent sampling of the blood draining the left anterior descending coronary artery (LAD) perfusion bed.

Cannulation and Perfusion of the Left Anterior Descending Artery

Just before cannulation of the LAD, the pig's blood was anticoagulated with a 10,000-U intravenous bolus of heparin followed by a 5,000-U · h^{-1} continuous infusion. The LAD was dissected free of surrounding tissue for a 2-cm length near its origin at the base of the heart. The coronary artery was then cannulated with a 3 mm OD plastic cannula and perfused with oxygenated blood pumped from the carotid artery with a rotary pump calibrated by time collection for each experiment (Masterflex digital roller pump, Cole-Parmer, Chicago, IL). Before perfusing the heart, blood was passed through a 40-μm filter (Pall Biomedical, Glen Cove, NY). Systolic shortening was measured to ensure adequacy of CBF. If systolic shortening did not return to the precannulation level within 3 min, cannulation was deemed unsuccessful and the pig was excluded from the study. Two animals were excluded from the study for this reason. LAD pressure was measured at the tip of the perfusion cannula through a 25-G catheter inside the cannula. Flow was measured continuously by an in-line Doppler flow meter (Transonic, Ithaca, NY), calibrated by timed blood collection in a graduated cylinder at the beginning of each experiment.

Sonomicrometry

Myocardial contractile function was quantitated in the LAD perfusion zones using segmental systolic shortening. Systolic shortening was calculated as:

$$\text{Systolic shortening (\%)} = \frac{(\text{end-diastolic length} - \text{end-systolic length}}{\text{end-diastolic length}} \times 100$$

Diastolic and systolic segment lengths were measured with a sonomicrometer (Triton, San Diego, CA) and 2-mm lensed ultrasonic crystals (Dimension 3, La Jolla, CA) which were embedded in the subendocardial muscle through a small epicardial incision. The crystals were positioned approximately 11 mm apart, facing each other and parallel to the short axis of the heart. Electrical signals from the dimension crystals were continuously displayed on an oscilloscope. Crystal position and orientation were confirmed by examination of the function tracing and by direct inspection at necropsy. Systolic shortening readings were averaged over 5 heart beats. End-diastole was defined as the onset of a positive first derivative of left ventricular pressure with respect to time; end-systole was defined as the time of peak negative value of this derivative.[27]

Left Anterior Descending Artery Perfusion Bed

The myocardium perfused by the LAD was delineated by a dye infusion technique: blood stained with Evans blue dye was infused into the cannulated LAD at normal aortic pressures, while the remainder of the heart was perfused at the same pressures with unstained blood from the aortic root. The blue area, representing myocardium perfused by the LAD, was excised and weighed.

Perfusion Circuit and Membrane Oxygenator. The circuit diagram for the perfusion circuit and membrane oxygenator (Avecor Cardiovascular, Plymouth, MN) is shown in figure 1.

Blood Sampling. A T-connector was inserted into the LAD perfusion circuit immediately before the LAD cannula. Blood was withdrawn from this site at 12 ml/min by a calibrated rotary pump and re-infused into the venous circulation of the pig. This allowed us to sample arterial blood for blood gasses, hemoglobin oxygen saturation, and Hb concentration, without changing CBF.

Drugs

Glibenclamide (Sigma Chemical, St. Louis, MO) was prepared in stock solutions of 1.0 or 2.7 mM in a vehicle consisting of 1.5 ml of dimethyl sulfoxide and 30 mEq of sodium bicarbonate added to 1,000 ml of normal saline. A fresh solution of adenosine, 100 μg/ml, was made each day, dissolved in normal saline. Solutions of sodium nitroprusside, 100 and 10 μg/ml, were made each day, dissolved in normal saline. Isoflurane (Ohmeda, Madison, WI) was administered *via* bubble-through vaporizer, through the perfusion circuit membrane oxygenator.

Experimental Protocol

After LAD cannulation and a minimum of 60 min, stabilization measurements were made while infusing

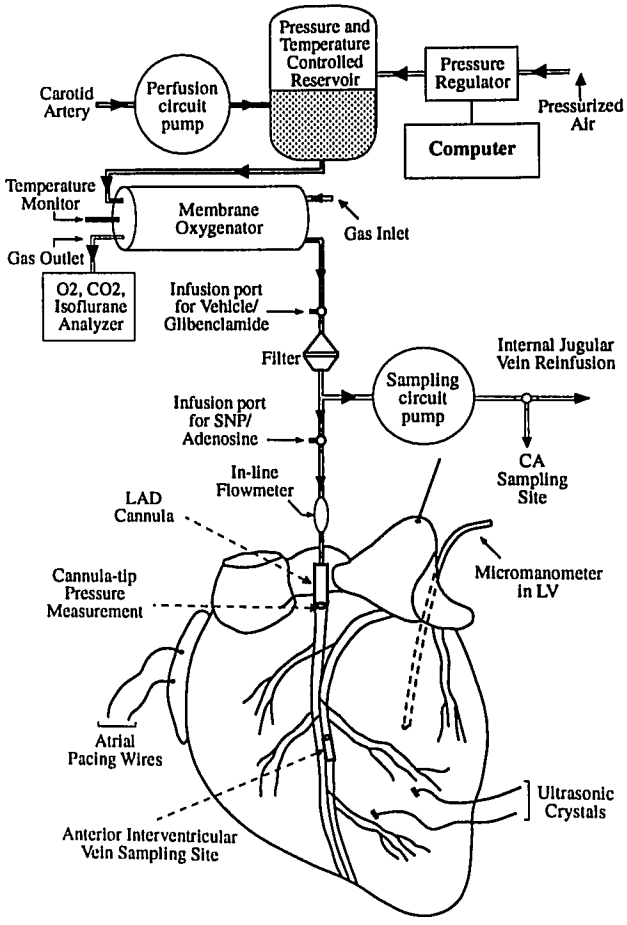

Fig. 1. Arterial blood is pumped from the carotid artery into a glass reservoir through the membrane oxygenator into the left anterior descending coronary artery (LAD). Reservoir pressure was servocontrolled by a Macintosh Quadra computer system (Cupertino, CA) running Labview II software, which provided control signals to a precision electropneumatic pressure regulator (10R, Fairchild, Winston-Salem, NC) connected to a laboratory compressed-air system. Reservoir pressure was maintained so that mean LAD pressure matched mean systemic pressure. The blood in the reservoir was continuously stirred and warmed to maintain normothermia. The day before each study, new silicone elastomere tubing, a sterile reservoir, and an unused membrane oxygenator were assembled; filled with sterile saline containing heparin 50 U/ml; and debubbled. The perfusion circuit and the membrane oxygenator was aerated by oxygen (2.0 l/min) and carbon dioxide (0.1–0.125 l/min) while effluent gas was continuously sampled to measure partial pressures of oxygen (OHIO analyzer, Madison, WI) and of carbon dioxide and isoflurane (Puritan Bennett analyzer, Wilmington, MA). Isoflurane was added to the circuit by the use of a bubble-through vaporizer. The membrane oxygenator was warmed by an external heat source and its temperature measured and adjusted to match central body temperature. Arterial blood gases measured from the membrane oxygenator were matched to systemic arterial values.

vehicle and then repeated while infusing glibenclamide. Vehicle was administered to match the planned rate of glibenclamide infusion. Glibenclamide was administered in each experiment at a constant fraction of CBF. In an effort to define the minimal effective intracoronary dose of glibenclamide, we decreased the glibenclamide infusion rate in successive experiments. Rates of glibenclamide administration were specifically: 22.0, 13.8, 4.25, 3.75, 1.1, and 1.0 $\mu g \cdot kg^{-1} \cdot min^{-1}$ in the six experiments. Intracoronary infusion rates of vehicle, glibenclamide, adenosine and sodium nitroprusside typically represented 0.5–2% of total LAD CBF.

During infusion of vehicle, then subsequently during infusion of glibenclamide, we measured the dose-related coronary vasodilator responses to sodium nitroprusside, adenosine and isoflurane. Different vasodilators were administered in a randomized fashion but within each vasodilator doses were not randomized. Instead progressively increasing doses were given. First, control data was obtained before isoflurane and before intravenous vasodilator administration. The actual vasodilator measurement periods were 4.2, 22.5, and 225 $\mu g/min$ for nitroprusside; 30, 82, and 160 $\mu g/min$ for adenosine; and F1, F2 = 1.5% and 3.0% isoflurane. Within 2–4 min of the progressive dose administration of sodium nitroprusside or adenosine CBF stabilized. Measurements were made of CBF (LAD perfusion bed), systemic blood pressure, heart rate, and systolic shortening. After all doses of each vasodilator were administered, CBF was allowed to return to normal for 10 min before beginning infusion of the next vasodilator. Isoflurane was administered to achieve the desired effluent gas concentration and CBF stabilized 6–7 min thereafter. Measurements of CBF were made after 15 min of isoflurane administration.

Arterial and coronary venous hemoglobin concentrations, hemoglobin oxygen saturation, and blood gasses were measured during each experimental period to calculate myocardial oxygen consumption ($M\dot{V}_{O_2}$) by the Fick method.

Calculations

Left Anterior Descending Artery Blood Flow. Regional CBF was obtained from the in-line flow meter and expressed in $ml \cdot min^{-1} \cdot 100g^{-1}$, using the weight of the LAD perfusion bed.

Myocardial Oxygen Consumption. $M\dot{V}_{O_2}$ was calculated for the LAD-perfused zone using the Fick Principle, as the product of the LAD blood flow and the

difference between the coronary arterial and the anterior interventricular vein oxygen content.

Data Analysis

To compare the response to a given vasodilator dose in the presence and absence of K_{ATP} channel blockade, the paired *t* test was used. To compare different doses of a given vasodilator to the control period, repeated measures analysis of variance was used, and where indicated by analysis of variance, Scheffé's F test was used for multiple comparisons. Values reported are means ± SD. *P* values < 0.05 were considered significant.

Results

Hemodynamic data are summarized in table 1.

Control Periods

The paced heart rate of 117 ± 10.4 beats/min was constant throughout the study. Mean systemic blood pressure did not change throughout the study and varied from 51 ± 4 to 52 ± 4 mmHg in the four control period measurements (table 1). Coronary pressure was servocontrolled to match MAP, and was never more than 2.1 mmHg different from systemic pressure. The relatively low control values of MAP are accounted for by the nonhyperdynamic anesthetic state induced by pentobarbital and fentanyl. Mean arterial pressures remained constant throughout the experiment, and were not altered either by glibenclamide infusion or by regional administration of coronary vasodilators. Complete hemodynamic data for all measurement periods appears in table 1.

Systemic arterial blood gas values were oxygen tension 564 ± 55 mmHg, carbon dioxide tension 39 ± 7 mmHg, and *p*H 7.38 ± 0.06. Membrane oxygenator blood gas values were arterial oxygen tension 435 ± 30 mmHg, arterial carbon dioxide tension 43 ± 4 mmHg, and *p*H = 7.32 ± 0.03.

Baseline Effects of Glibenclamide

Administration of glibenclamide reduced mean CBF by 14–22% ($P < 0.05$, both) in the two control periods, with a mean reduction of 18%. CBF was reduced in each of the six swine studied, with no correlation found between glibenclamide dose and percent reduction in CBF. Glibenclamide infusion also reduced coronary venous oxyhemoglobin saturation from 29.6 ± 5.8 to 21.9 ± 7.9%. Although CBF and coronary venous oxyhemoglobin saturation were reduced, K_{ATP} blockade had no effect on systolic shortening, indicating that ischemia was not induced. Glibenclamide treatment increased oxygen extraction by the myocardium, but actual $M\dot{V}_{O_2}$ decreased from 8.2 ± 1.0 to 6.9 ± 1.1 $ml \cdot min^{-1} \cdot 100\ g^{-1}$ (table 2).

Effects of Vasodilators on Coronary Blood Flow

To adjust the dose–response curves for the three vasodilators for the control state vasoconstriction caused by K_{ATP} blockade, data are plotted as percent change in CBF from control values (figs. 2, 3, 4). Absolute values of CBF are given in table 1. Isoflurane (1.5 and 3.0%) increased CBF by 29 ± 28.9% and 62 ± 27.9%, respectively during infusion of vehicle (fig. 2). During glibenclamide infusion, the vasodilator response to both doses isoflurane was eliminated, and the response to the 3.0% dose of isoflurane was converted to net vasoconstriction (fig. 2). K_{ATP} blockade did not change the dose–response of CBF to sodium nitroprusside (fig. 3), but did decrease this response to adenosine (fig. 4).

Effects of Glibenclamide and Vasodilators on Contractile Function

Baseline systolic shortening was 19.1 ± 2.9%. Systolic shortening was not changed by glibenclamide infusion (19.2 ± 3.1%). During infusion of vehicle, isoflurane reduced systolic shortening significantly, to 11.8 ± 4.1% and to 8.2 ± 4.2% for periods of 1.5% and 3.0% isoflurane, respectively. During infusion of glibenclamide, the same doses of isoflurane reduced regional systolic shortening by similar amounts (to 10.8 ± 3.9% and to 7.9 ± 3.6%, respectively). Neither nitroprusside nor adenosine had a significant effect on regional contractile function in the doses used.

Effects of Blockade of Adenosine Triphosphate–Sensitive Potassium Channels on Response to Three Vasodilators

The dose-related coronary vasodilator effects of isoflurane, sodium nitroprusside, and adenosine are illustrated in figures 2–4.

Discussion

The most important finding of this investigation is that *in vivo* isoflurane-mediated coronary vasodilation can be essentially eliminated by a selective inhibitor

Table 1. Dose-related Effects of Three Vasodilators

		Nitroprusside Dose			Adenosine Dose				Isoflurane Dose	
	Control 1	N1	N2	N3	A1	A2	A3	Control 2	F1	F2
MAP (mmHg)										
Vehicle	51 ± 4	51 ± 4	50 ± 4	50 ± 3	51 ± 3	51 ± 4	51 ± 4	52 ± 4	52 ± 4	51 ± 4
Glibenclamide	52 ± 4	51 ± 3	51 ± 4	49 ± 3	51 ± 3	52 ± 3	51 ± 3	52 ± 3	52 ± 3	51 ± 3
CAP (mmHg)										
Vehicle	52 ± 4	52 ± 4	51 ± 4	51 ± 4	52 ± 4	52 ± 4	52 ± 4	52 ± 4	52 ± 4	52 ± 4
Glibenclamide	51 ± 4	52 ± 4	52 ± 4	52 ± 4	52 ± 4	52 ± 4	51 ± 4	52 ± 4	52 ± 4	52 ± 4
HR (beats/min)										
Vehicle	117 ± 10	117 ± 10	117 ± 10	117 ± 10	117 ± 10	117 ± 10	117 ± 10	117 ± 10	117 ± 10	117 ± 10
Glibenclamide	117 ± 10	117 ± 10	117 ± 10	117 ± 10	117 ± 10	117 ± 10	117 ± 10	117 ± 10	117 ± 10	117 ± 10
SS (%)										
Vehicle	19.1 ± 2.9	19.1 ± 2.9	19.1 ± 3.0	19.0 ± 2.9	19.1 ± 2.9	19.1 ± 3.0	19.1 ± 3.0	19.1 ± 3.0	11.8 ± 4.1*	8.2 ± 4.2*
Glibenclamide	19.0 ± 3.0	19.0 ± 3.0	19.2 ± 3.0	19.1 ± 3.0	19.0 ± 3.0	19.1 ± 2.9	19.0 ± 2.8	19.3 ± 3.3	10.8 ± 3.9*	7.9 ± 3.6*
EDL (mm)										
Vehicle	13.3 ± 1.2	13.2 ± 1.2	13.1 ± 1.2	13.0 ± 1.2	13.1 ± 1.2	13.2 ± 1.2	13.1 ± 1.2	13.1 ± 1.2	14.3 ± 1.1*	14.7 ± 1.0*
Glibenclamide	12.9 ± 1.0	12.9 ± 1.1	12.9 ± 1.0	12.8 ± 1.1	12.9 ± 1.0	12.9 ± 1.1	12.9 ± 1.2	13.0 ± 1.2	14.0 ± 1.2*	14.6 ± 1.1*
ESL (mm)										
Vehicle	10.7 ± 0.8	10.6 ± 0.8	10.6 ± 0.8	10.5 ± 0.8	10.6 ± 0.8	10.6 ± 0.8	10.6 ± 0.8	10.6 ± 0.7	12.5 ± 0.5*	13.5 ± 0.5*
Glibenclamide	10.4 ± 0.6	10.4 ± 0.6	10.4 ± 0.6	10.4 ± 0.7	10.4 ± 0.6	10.4 ± 0.7	10.5 ± 0.7	10.4 ± 0.6	12.4 ± 0.5*	13.4 ± 0.5*
CBF (ml · 100 g^{-1} · min^{-1})										
Vehicle	64 ± 9	70 ± 10	75 ± 7*	105 ± 15*	81 ± 8*	118 ± 24*	141 ± 22*	60 ± 8	78 ± 22*	97 ± 22*
Glibenclamide	50 ± 7†	56 ± 10*	65 ± 7*	91 ± 11*	58 ± 5	71 ± 11*	92 ± 22*	51 ± 9†	49 ± 10	48 ± 9*

MAP = mean arterial pressure; CAP = coronary artery pressure; HR = heart rate; SS = systolic shortening; EDL = end-diastolic length; ESL = end-systolic length; CBF = coronary blood flow.

* Significantly different at 95% from corresponding "Control" measurement period.

† Significantly different at 95% from "Control" measurement period with vehicle.

of K_{ATP} channels, glibenclamide. This finding strongly suggests that the predominant mechanism of isoflurane-induced coronary vasodilation is by either direct or indirect modulation of the activity of K_{ATP} channels.

In order to exclude the possibility of a nonspecific vasoconstrictor effect, we also tested the coronary vasodilator response to two other vasodilators, both in the presence and absence of glibenclamide. The coronary vasodilator response to sodium nitroprusside was not diminished by glibenclamide in the doses used. This result was expected because nitroprusside acts on vascular smooth muscle by liberation of nitric oxide, which subsequently activates cellular guanylate cyclase, thus increasing cytosolic cyclic guanosine monophosphate.[28,29] The actions of sodium nitroprusside are not known to be mediated by K_{ATP} channels. The vasodilator response to adenosine was, however, diminished in the presence of glibenclamide infusion. This, too, was predictable, as adenosine has been shown to act in part by opening K_{ATP} channels.[22,30–32] The adenosine A_1 receptor has recently been found to be linked to the K_{ATP} channel by a G-protein–dependent mechanism.[32] Therefore, administration of inhibitors of K_{ATP} channel opening has been found to block a portion of the coronary vasodilator response to exogenous adenosine.[30]

Table 2. Effects of K_{ATP} Channel Blockade on Baseline Coronary Flow, Function, and Myocardial Oxygen Consumption (Control Period 1)

	CBF (ml · min · 100 g)	Systolic Shortening (%)	Regional MVO_2 (ml · min · 100 g)	CVO_2-Hgb Saturation (%)
Vehicle	64 ± 9	19.1 ± 2.9	8.2 ± 1.0	29.6 ± 5.8
Glibenclamide	51 ± 7*	19.0 ± 3.0	6.9 ± 1.1*	21.9 ± 7.9*

* Different from corresponding values during infusion of vehicle ($P < 0.05$).

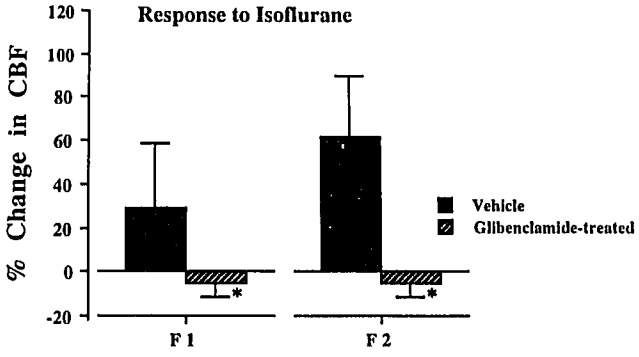

Fig. 2. Blockade of the adenosine triphosphate–sensitive potassium channel with glibenclamide eliminated the coronary vasodilator response to isoflurane. Isoflurane measured in oxygenator effluent: F1 = 1.5%; F2 = 3.0%. *Different from corresponding values during infusion of vehicle; $P < 0.05$.

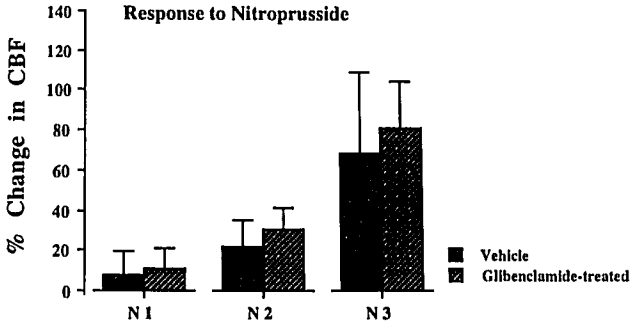

Fig. 3. Blockade of the adenosine triphosphate–sensitive potassium channel had no significant effect on the coronary vasodilator response to nitroprusside. Because of the baseline vasoconstriction induced by glibenclamide, there was a apparent trend toward greater percent change when nitroprusside was subsequently given. The trend did not reach statistical significance. Intracoronary sodium nitroprusside: N1 = 4.2 μg · min; N2 = 22.5 μg · min; N3 = 225 μg · min.

In this study we decreased the dose of glibenclamide in successive experiments in an attempt to find a minimal effective dose for blocking the vasodilator effects of isoflurane. We have not yet determined the minimal effective dose, as the lowest infusion rate used (1 $\mu g \cdot kg^{-1} \cdot min^{-1}$) still caused complete blockade of isoflurane vasodilation. In addition, we did not find a correlation between glibenclamide dose and reduction in basal CBF. Other investigators have, however, reported that higher doses of glibenclamide can induce myocardial ischemia, indicating that a correlation should exist.[23,24]

Of further note, during the administration of glibenclamide, the higher dose of isoflurane (3%) produced a small but significant decrease in CBF (−5%). At the 1.5% dose of isoflurane, the decrement in CBF approached but did not reach statistical significance ($P = 0.10$). Two explanations for the finding that isoflurane reduces CBF during K_{ATP} channel blockade are possible: the decreased CBF may well be metabolically mediated and related to a reduction in $M\dot{V}_{O_2}$ produced by isoflurane. Alternatively, it is possible that K_{ATP} channel blockade, by eliminating the vasodilator effects of isoflurane, may have unmasked vasoconstrictor effects caused by the inhibition of release or action of an endothelial-derived dilator substance.[17]

Mechanisms of Isoflurane Modulation of Adenosine Triphosphate–Sensitive Potassium Channels

Although the current studies suggest strongly that isoflurane-induced coronary vasodilation is mediated by opening of K_{ATP} channels, these studies do not allow us to deduce the site at which isoflurane modulates these channels. It may be that isoflurane interacts with the channel protein directly, increasing the open probability, or it may be that isoflurane influences one or more other modulators of the K_{ATP} channel.

Regarding the possibility that isoflurane opens K_{ATP} channels by decreasing intracellular adenosine triphosphate (ATP) concentration, this possibility is unlikely for two reasons. First, studies have found that the intracellular levels of ATP required to cause channel opening are quite low when compared to normal in-

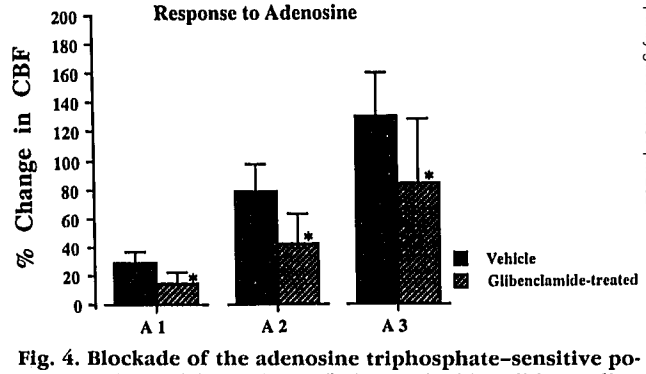

Fig. 4. Blockade of the adenosine triphosphate–sensitive potassium channel (K_{ATP} channel) diminished but did not eliminate the dose-dependent vasodilation induced by adenosine. This result is consistent with a previous finding that the adenosine receptor is linked to K_{ATP} channels *via* a G-protein–dependent mechanism.[32] Adenosine: A1 = 30 μg · min; A2 = 82 μg · min; A3 = 160 μg · min. *Different from corresponding values during infusion of vehicle; $P < 0.05$.

tracellular levels.[33] Intracellular levels of ATP must decrease to the range of 80–200 μM in order to reduce inhibition of these channels to half-maximal. Physiologic intracellular ATP concentration is typically in the 3–4 mM range. To our knowledge, the effects of isoflurane on intracellular ATP concentration in vascular smooth muscle have not been measured. However, isoflurane has been found to cause a small decrement (30% reduction at 2.8% isoflurane)[34] or no reduction[35] in intracellular ATP concentration (at 2.3% isoflurane) in ventricular myocytes. Given the small or minimal effects of isoflurane on intracellular ATP, isoflurane's mechanism of action in modulating K_{ATP} channels is probably by another route. Other factors have been described which also may modulate these channels, including the intracellular concentration of other nucleosides such as adenosine diphosphate, and probably intracellular lactate and *p*H. In addition, the adenosine A_1 receptor has been found to be coupled to K_{ATP} channels *via* a G-protein mechanism.[32] This raises the possibility that the vasodilation action of isoflurane is mediated *via* the adenosine receptor–G-protein pathway.

Our finding that the administration of glibenclamide was associated with a 16% reduction in $M\dot{V}_{O_2}$, even though systemic pressure, heart rate and left ventricular systolic shortening remained constant, was unexpected. We think it unlikely that glibenclamide truly increased myocardial contractile efficiency, allowing the heart to perform constant work at a reduced $M\dot{V}_{O_2}$. Based on the stable, normal subendocardial systolic shortening which we found during control glibenclamide infusion, we do not believe that the glibenclamide-induced reduction in $M\dot{V}_{O_2}$ was caused by ischemia. It should be noted, however, that studies utilizing higher doses of intracoronary glibenclamide have shown that K_{ATP} blockade can cause profound coronary vasoconstriction, thereby inducing myocardial ischemia and reducing $M\dot{V}_{O_2}$.[24,36]

We suggest two factors which may help explain our finding of reduced $M\dot{V}_{O_2}$ during glibenclamide infusion. The first is the Gregg effect[37]: a decrement in CBF may produce a corresponding decrement in $M\dot{V}_{O_2}$, even though ischemia is not induced. This effect is relatively small and would probably not account for the significant reduction in $M\dot{V}_{O_2}$ we found. This concept has been reviewed in detail by Feigl and coworkers.[38] A second possible explanation would be that K_{ATP} blockade, by reducing hydrostatic pressure in the arterioles of the LAD perfusion bed, caused either an increase in collateral blood flow to the LAD region or an increase in contamination of anterior interventricular vein blood by blood from an adjacent perfusion bed with greater oxygen content. Either of these changes could lead to an erroneous measurement of $M\dot{V}_{O_2}$ by the Fick method. We think it unlikely, however, that either increased unmeasured collateral blood flow or increased venous admixture led to our finding of reduced $M\dot{V}_{O_2}$ during glibenclamide infusion. Collateral blood flow in swine is quite limited, and when measured after coronary occlusion is only 0.06–0.07 $ml \cdot g^{-1} \cdot min^{-1}$.[39] Regarding the possibility of venous admixture, the origin of interventricular vein blood in swine has recently been examined by Bier *et al.*[40] Using a dye-tracer method, these investigators determined the percentage of anterior interventricular vein blood which derived from the LAD perfusion bed *versus* from adjacent arterial perfusion beds, both when perfusion pressure was normal and when LAD perfusion pressure was decreased 50–60 mmHg by a coronary artery stenosis. They found that, normally, the interventricular vein was less then 7% "contaminated" with blood of non-LAD origin. Even when the LAD was constricted by a severe coronary stenosis, anterior interventricular vein contamination remained below 10%. This study suggests that contamination of the interventricular vein should, at most, account for only a small portion of the measured decrease in $M\dot{V}_{O_2}$. Although neither the Gregg effect nor a small error in $M\dot{V}_{O_2}$ measurement seems likely to account for the measured reduction in $M\dot{V}_{O_2}$ during glibenclamide infusion, we suggest that perhaps together they may explain our finding.

Potential Limitations

Interpretation of our results depends in part on the potency and specificity of glibenclamide as a blocker of K_{ATP} channels. Glibenclamide is certainly a potent blocker of ATP channels in vascular smooth muscle, and has been found to prevent the opening of these channels in response to processes which deplete intracellular ATP, such as hypoxia.[20] Glibenclamide has been found to be both a potent and specific blocker of K_{ATP} channels isolated from aortic smooth muscle.[41] On the other hand, glibenclamide has also been reported to have vasodilator effects which were not mediated by action on K_{ATP} channels.[42] We observed no such vasodilator effects in the current study. Although the published literature suggests that glibenclamide is a highly specific blocker of K_{ATP} channels, we cannot completely exclude the possibility that it may also have effects on other ion channels.

In this study we used sodium pentobarbital as part of our basal anesthetic based on work indicating that pentobarbital anesthesia does not alter coronary vascular tone significantly.[43] Kozlowski and Ashford reported that sodium pentobarbital is a K_{ATP} antagonist in cultured cells of the rat pancreas (CRI-G1 cell line).[44] This cell line is notably more sensitive to inhibition by ATP than either cardiac myocytes[33] or vascular smooth muscle cells.[41] These investigators reported that 360 μM pentobarbital inhibited 50% of whole-cell K_{ATP} channel currents. We think it unlikely, however, that sodium pentobarbital was a confounding factor in the current study, because sodium pentobarbital administration was equivalent in all measurement periods, and because the concentration of sodium pentobarbital that produces anesthesia is much less then that needed to inhibit K_{ATP} channels. The median effective concentration of sodium pentobarbital for producing lack of response to painful stimuli has recently been calculated to be approximately 50 μM,[45] a concentration about one-seventh that required to produce 50% inhibition of K_{ATP} channels in the CRI-G1 cell line. It is therefore unlikely that the anesthetizing doses of sodium pentobarbital used in the current experiment caused any relevant blockade of K_{ATP} channels of coronary vascular smooth muscle.

Although we have identified an important mechanism, and perhaps the predominant mechanism of isoflurane-induced coronary vasodilation, it should not be concluded that this is the only mechanism. Volatile anesthetics can potentially affect vascular smooth muscle tone at several sites and by several mechanisms. Probable mechanisms include interactions with the vascular endothelium and endothelium-derived vasoactive factors,[17,18] direct modulation of other ion channels such as calcium channels,[46,47] and alterations in myocyte cyclic guanosine monophosphate.[48,49] These mechanisms have been the subject of excellent recent reviews by Johns[50] and by Bosnjak.[51]

Implications for Future Studies

Our finding that the vasodilator effects of isoflurane are predominantly mediated by K_{ATP} channels suggests that the role of these channels should be pursued regarding possible molecular mechanisms of general anesthesia. In this regard, however, Zucker has studied the effects of two other K_{ATP} channel agonists on isoflurane MAC in rats.[52] Neither cromakalim nor pinacidil, administered intrathecally, decreased isoflurane MAC. This suggests that, either K_{ATP} channel opening is not an important mechanism of isoflurane anesthesia, or that the CNS sites important for general anesthesia were not readily accessible to these two intrathecally administered K_{ATP} agonists. On the other hand, interesting evidence has been presented by Vergoni *et al.*,[53] indicating that intrathecally administered pinacidil significantly increases and prolongs the effect of morphine on thermal pain threshold in rats. The role of K_{ATP} channels in general anesthesia and in pain clearly needs further investigation.

Also of special note are findings from several laboratories suggesting that K_{ATP} channel agonists can be protective against transient myocardial ischemia.[31,54,55] Based on such findings, and the results of the current investigation, we would suggest that the volatile anesthetics, and isoflurane in particular, should be further studied with regard to significant cardioprotective effects which may be specifically related to their modulation of K_{ATP} channels.

To the best of our knowledge this is the first report that blockade of K_{ATP} channels essentially eliminates isoflurane-induced coronary artery vasodilation. To further determine the nature of the interaction between isoflurane and K_{ATP} channels, studies of single-channel pharmacological effects will be necessary, using patch-clamp methodology. We are currently pursuing these studies.

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