

## Role of Adenosine Triphosphate-sensitive Potassium Channels in Coronary Vasodilation by Halothane, Isoflurane, and Enflurane

George J. Crystal, Ph.D.,\* Juozas Gurevicius, M.D.,† M. Ramez Salem, M.D.,‡ Xiping Zhou, M.D.§

**Background:** Halothane, isoflurane, and enflurane cause coronary vasodilation and cardiac depression. This study was performed to assess the role of adenosine triphosphate (ATP)-sensitive potassium channels ( $K_{ATP}$  channels) in these effects.

**Methods:** Twenty-five thoracotomized dogs were anesthetized with fentanyl and midazolam. The left anterior descending coronary artery was perfused *via* either of two pressurized (80 mmHg) reservoirs. One reservoir was supplied with arterial blood free of a volatile anesthetic, and the second reservoir was supplied with arterial blood equilibrated in an oxygenator with a 1 minimum alveolar concentration of either halothane (0.9%,  $n = 10$ ), isoflurane (1.4%,  $n = 8$ ), or enflurane (2.2%,  $n = 7$ ). Coronary blood flow (CBF) was measured using a Doppler flow transducer, and segmental shortening (SS) was measured with ultrasonic crystals. Responses to the volatile anesthetics were assessed under control conditions, during intracoronary infusion of the  $K_{ATP}$  channel inhibitor glibenclamide (100  $\mu\text{g}/\text{min}$ ), and after cessation of glibenclamide (recovery). The effectiveness of glibenclamide was verified from inhibition of coronary vasodilator responses to the  $K_{ATP}$  channel opener cromakalim without effect on those to the

$K_{ATP}$  channel-independent vasodilators, sodium nitroprusside and acetylcholine.

**Results:** Under control conditions, the volatile anesthetics caused pronounced increases in CBF (isoflurane > halothane = enflurane), and decreases in SS (enflurane > halothane = isoflurane). Glibenclamide blunted significantly (and reversibly) the increases in CBF, but it had no effect on the decreases in SS.

**Conclusions:** The  $K_{ATP}$  channels play an important role in coronary vasodilation but apparently are not involved in cardiac depression caused by halothane, isoflurane, and enflurane in canine hearts *in situ*. (Key words: Anesthetics, volatile: halothane; isoflurane; enflurane. Heart: coronary vascular tone; myocardial oxygen demand; myocardial contractility; adenosine triphosphate-sensitive potassium channels.)

In previous studies from this laboratory, selective intracoronary administration of the volatile anesthetics halothane, isoflurane, and enflurane in canine hearts *in situ* caused increases in coronary blood flow (CBF), accompanied by reductions in oxygen extraction.<sup>1-3</sup> These findings are classical signs of pharmacologic dilation of coronary resistance vessels.<sup>4</sup> Despite extensive investigation, the mechanism(s) responsible for the ability of the volatile anesthetics to cause coronary vasodilation have not been completely described.

Potassium channels closed by intracellular adenosine triphosphate (called ATP-sensitive potassium channels [ $K_{ATP}$  channels]) were first identified in cardiac muscle by Noma.<sup>5</sup> More recently, these channels also were found in vascular smooth muscle cells.<sup>6,7</sup> The  $K_{ATP}$  channels may provide a means whereby the metabolic status of the cell regulates its level of activity. Furthermore, it has been shown that various drugs, such as cromakalim, may modulate the opening of the  $K_{ATP}$  channels.<sup>8,9</sup> Opening of the  $K_{ATP}$  channels causes hyperpolarization of vascular smooth muscle cells by shifting the membrane potential closer to the  $K^+$  reversal potential. Hyperpolarization then inhibits calcium entry through voltage-dependent calcium channels, leading to vasodilation.<sup>10</sup> The  $K_{ATP}$  channels appear to play a role in maintaining basal coronary vascular tone<sup>11,12</sup> and in the

\*Director of Research Laboratory, Department of Anesthesiology, Illinois Masonic Medical Center; Associate Professor, Department of Anesthesiology and of Physiology and Biophysics, University of Illinois College of Medicine.

†Resident, Illinois Masonic Medical Center.

‡Chair, Department of Anesthesiology, Illinois Masonic Medical Center; Clinical Professor, Department of Anesthesiology, University of Illinois College of Medicine.

§Research Fellow, Departments of Anesthesiology, Illinois Masonic Medical Center and the University of Illinois College of Medicine.

Received from the Department of Anesthesiology, Illinois Masonic Medical Center, and the Departments of Anesthesiology and of Physiology and Biophysics, University of Illinois College of Medicine, Chicago, Illinois. Submitted for publication July 2, 1996. Accepted for publication October 24, 1996. Supported by grant HL-47629 from the National Heart, Lung, and Blood Institute. Presented in part at the annual meetings of the American Society of Anesthesiologists, October 21-25, 1995, Atlanta, Georgia, and October 19-23, 1996, New Orleans, Louisiana.

Address reprint requests to Dr. Crystal: Department of Anesthesiology, Illinois Masonic Medical Center, University of Illinois College of Medicine, 836 West Wellington Avenue, Chicago, Illinois 60657-5193. Address electronic mail to: George.J.Crystal@uic.edu.



## VOLATILE ANESTHETICS AND POTASSIUM CHANNELS

coronary vasodilation associated with increased myocardial oxygen consumption ( $MVO_2$ )<sup>13</sup> and reduced coronary perfusion pressure.<sup>14,15</sup>

To date two studies,<sup>16,17</sup> using different experimental models and approaches, have assessed the role of the  $K_{ATP}$  channels in coronary vasodilation by volatile anesthetics. Although both these studies presented evidence to support involvement of the  $K_{ATP}$  channels in this response, the extent of this involvement varied. Larach and Schuler<sup>16</sup> showed that blockade of the  $K_{ATP}$  channels with glibenclamide attenuated (by 56%) the halothane-induced increase in coronary flow in crystalloid-perfused rat hearts arrested with tetrodotoxin, whereas Cason *et al.*<sup>17</sup> showed that glibenclamide completely prevented the increases in CBF (and converted them to decreases) during intracoronary administration of isoflurane in swine. Together the results from these diverse studies suggest that the volatile anesthetics may differ in their dependence on the  $K_{ATP}$  channels for coronary vasodilation. However, this remains to be confirmed systematically in the same experimental model under the same experimental conditions.

The primary objective of this study was to evaluate the relative effect of glibenclamide on the increases in CBF caused by intracoronary administration of halothane, isoflurane, and enflurane in canine hearts *in situ*. Potassium ATP channel-opening drugs have been shown to decrease the duration of the ventricular action potential and the strength of cardiac muscle contraction.<sup>18,19</sup> Thus another objective of the study was to evaluate the role of the cardiac  $K_{ATP}$  channels in the negative inotropic effects of the volatile anesthetics.

An extracorporeal controlled-pressure perfusion system was used to administer the drugs, including the volatile anesthetics, glibenclamide and cromakalim, into the left anterior descending coronary artery (LAD) of canine hearts *in situ*.<sup>1-3</sup> This approach avoided the systemic hemodynamic effects of the drugs, which simplified interpretation of the findings.

## Materials and Methods

### Preparation of Experiments

The study was conducted after approval from the institutional animal research committee. Experiments were performed on 25 healthy mongrel dogs of either sex (weight, 20.5–27.3 kg). Anesthesia was induced with intravenous bolus injections of 15 mg/kg thiopental. Anesthesia was maintained by continuous intrave-

nous infusion of fentanyl and midazolam at rates of  $12 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  and  $0.6 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ , respectively. During surgical preparation, adequacy of this anesthesia regimen was demonstrated by lack of muscle movement and by stable systemic hemodynamic parameters, such as heart rate. After tracheal intubation and left thoracotomy in the fourth intercostal space, the lungs were mechanically ventilated (Air Shields Inc, Hatboro, PA) with the fractional inspired oxygen concentration equal to 1. The volume and rate of the ventilator were established to maintain arterial  $P_{\text{CO}_2}$  at physiologic levels.  $P_{\text{O}_2}$ ,  $P_{\text{CO}_2}$ , and pH of arterial and venous blood samples were measured electrometrically (model 413; Instrumentation Laboratories, Lexington, MS). After completion of surgery, muscle paralysis was obtained with an intravenous injection of 0.1 mg/kg vecuronium bromide, with supplements at  $0.05 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  to facilitate mechanical ventilation. For the rest of the study, hemodynamic parameters alone were used to assess the depth of anesthesia. Body temperature was maintained at  $38^\circ\text{C}$  with a heating pad. Lactated Ringer's solution was administered continuously at a rate of 5 ml  $\cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  intravenously to compensate for evaporative fluid losses. Heparin (400 U/kg with supplementation) was used for anticoagulation.

The LAD was perfused *via* an extracorporeal system, the details of which have been described previously.<sup>2</sup> Briefly, a thin-wall stainless-steel cannula (2.5-mm inside diameter) 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. One reservoir (normal blood reservoir) was supplied with volatile anesthetic-free blood withdrawn directly from the left femoral artery, whereas the other reservoir (anesthetic-equilibrated blood reservoir) was supplied with blood from the right femoral artery that was first pumped into a hollow-fiber membrane oxygenator (Capiiox 300 series; Terumo, Tokyo, Japan) supplied with a 95% oxygen–5% carbon dioxide gas mixture, which passed through a calibrated agent-specific vaporizer providing a 1 minimum alveolar concentration of either halothane (0.9%,  $n = 10$ ), isoflurane (1.4%,  $n = 8$ ), or enflurane (2.2%,  $n = 7$ ).<sup>20</sup> Blood supplied to the volatile anesthetic-equilibrated blood reservoir was recirculated at least three times through the extracorporeal oxygenator to ensure complete equilibration.

The LAD perfusion tubing was equipped with (1) a heat exchanger to maintain the temperature of the coronary perfusate at  $38^\circ\text{C}$ , (2) an electromagnetic flow



transducer to measure CBF, and (3) a port to collect samples of coronary perfusate. Coronary perfusion pressure was measured *via* a small-diameter tube positioned at the orifice of the perfusion cannula.

Measurements of aortic blood pressure, left ventricular pressure, left ventricular  $dP/dt_{\max}$ , and heart rate were obtained using standard methods.<sup>2</sup> A continuous record of hemodynamic variables was obtained on an eight-channel physiologic recorder (model 2800S; Gould, Cleveland, OH).

### Experimental Measurements

**Myocardial Oxygen Consumption.** Myocardial oxygen consumption was measured in the LAD-perfusion territory. The anterior interventricular vein was cannulated to provide samples of venous blood from the LAD-perfused myocardium.<sup>21,22</sup> The venous cannula was allowed to drain freely into a beaker to prevent venous stagnation and interstitial edema. The coronary venous blood was returned intermittently to the dog to maintain isovolemic conditions. At specified times in the study, a 1-ml sample of blood leaving the venous cannula was diverted into a test tube under mineral oil to prevent contamination with the ambient atmosphere. This venous sample was immediately drawn into a syringe, which was tightly sealed and stored on ice until analyses. The venous blood sample was paired with a 1-ml arterial blood sample obtained from the LAD perfusion tubing, so that the arteriovenous difference for oxygen could be determined. Hemoglobin concentration and percentage of hemoglobin oxygen saturation of the coronary blood samples were measured using a CO-Oximeter (model 482; Instrumentation Laboratories) to calculate oxygen bound to hemoglobin assuming an oxygen-carrying capacity for hemoglobin of 1.39 ml O<sub>2</sub>/g. The oxygen dissolved in the blood was computed (O<sub>2</sub> dissolved = 0.003 ml O<sub>2</sub> · 100 ml blood<sup>-1</sup> · mmHg<sup>-1</sup>) and added to the bound component to compute total oxygen content. Myocardial oxygen consumption was computed from the product of the coronary arteriovenous oxygen difference and CBF when blood samples were obtained. Oxygen extraction (in percentages) was calculated by dividing the arteriovenous oxygen difference by arterial oxygen content.

**Myocardial Segmental Shortening.** In a subset of the dogs (halothane [n = 7], isoflurane [n = 4], and enflurane [n = 7]), measurements of myocardial segmental shortening were obtained in the LAD bed by sonomicrometry.<sup>23,24</sup> A pair of ultrasonic crystals was implanted

into the LAD-perfused myocardium to a depth approximating the subendocardium. Location in the LAD perfusion field and functionality of the crystals were verified by segmental lengthening during a brief (30-s) period of occlusion. Furthermore, the crystals were oriented so that they were parallel to the anticipated direction of myocardial fibers in the subendocardium.<sup>25</sup>

Changes in distance between the crystals were recorded from measurements of the ultrasonic transit time between the crystals (Triton Technology, San Diego, CA). The end-diastolic and end-systolic lengths were identified by the beginning of the rapid increase in the left ventricular pressure just before isovolumetric contraction and the maximum rate of decrease of left ventricular systolic pressure ( $dP/dt_{\max}$ ), respectively.<sup>26</sup> Percentage of segmental shortening, an index of local myocardial contractility, was calculated from the formula:

$$ss = [(EDL - ESL)/EDL] \times 100$$

where SS (%) = segmental shortening; EDL (mm) = end-diastolic length; and ESL (mm) = end-systolic length.

### Experimental Protocols

After at least 45 min for recovery from surgical preparation, initial control measurements of CBF and other parameters, including MVO<sub>2</sub> and SS, were obtained during perfusion from the normal blood reservoir with coronary perfusion pressure set equal to mean aortic pressure. With coronary perfusion pressure maintained at this level, the LAD was switched to the blood reservoir equilibrated with a volatile anesthetic, either halothane, isoflurane, or enflurane. Values during the volatile anesthetic were obtained when CBF stabilized at a peak level, which was usually approximately 3–5 min after the switch in reservoirs. The LAD was then returned to the normal blood reservoir, and at least 30 min was permitted for recovery.

The increases in CBF were assessed during intracoronary infusions of the K<sub>ATP</sub> channel-independent vasodilators, sodium nitroprusside (80 µg/min), and acetylcholine (20 µg/min), the K<sub>ATP</sub> channel-opener cromakalim (2.5 and 5 µg/min), and adenosine (8 mg/min). Each exposure of the LAD to a vasodilating drug was immediately preceded by a drug-free control period. The rate of infusion of the coronary vasodilators corresponded to 1–2 ml/min. The doses for sodium nitroprusside, acetylcholine, and cromakalim were chosen because they caused the maximal increase in CBF possible without decreases in aortic pressure. The dose for adenosine



## VOLATILE ANESTHETICS AND POTASSIUM CHANNELS

has been shown to cause steady-state, maximal coronary vasodilation.<sup>2</sup> The rate of CBF during adenosine infusion served as a measure of the vasodilator reserve of each preparation and also as a reference to assess the extent of coronary vasodilation by the volatile anesthetics. The intracoronary infusions of sodium nitroprusside, acetylcholine, cromakalim, and adenosine were continued as long as required to achieve steady-state increases in CBF, which was usually 2–3 min. After sufficient time for recovery from the effects of the vasodilators (>30 min), new baseline measurements were obtained and an intracoronary infusion of the  $K_{ATP}$  channel inhibitor glibenclamide (100  $\mu\text{g}/\text{min}$ ) was begun and maintained for 10 min before CBF and other parameters were measured.

While continuing glibenclamide, the intracoronary administrations of the volatile anesthetic, sodium nitroprusside, acetylcholine, cromakalim, and adenosine were repeated. The infusion of glibenclamide was stopped and at least 30 min was allowed for recovery. After new baseline values were obtained, a third series of intracoronary administrations of the coronary vasodilators, including the volatile anesthetic, was performed. The order of the intracoronary administrations was randomized under all conditions to avoid introducing bias to the results.

### Drugs

Glibenclamide was dissolved with 0.01 N NaOH under gentle heat and diluted to a concentration of 100  $\mu\text{g}/\text{ml}$  with isotonic saline. Preliminary studies showed that infusion of the vehicle for glibenclamide alone had no effect on hemodynamic variables or on the response of the coronary circulation to the vasodilators used in this study. Sodium nitroprusside, acetylcholine, cromakalim, and adenosine were dissolved in isotonic saline to achieve concentrations of 80  $\mu\text{g}/\text{ml}$ , 20  $\mu\text{g}/\text{ml}$ , 2.5  $\mu\text{g}/\text{ml}$ , and 8  $\text{mg}/\text{ml}$ , respectively.

### Statistical Analysis

Student's *t* test for paired samples was used to assess effects of the volatile anesthetics, cromakalim, sodium nitroprusside, acetylcholine, adenosine, and glibenclamide relative to the predrug control value.<sup>27</sup> An analysis of variance in combination with the Student-Newman-Keuls test was used to evaluate effects of the vasodilating drugs before, during, and after administration of glibenclamide and to compare baseline responses to the various volatile anesthetics.<sup>27</sup> A probability value less than 0.05 was considered significant.

## Results

Figure 1 is a representative tracing showing effects of intracoronary halothane on CBF. Responses before and during infusion of glibenclamide in the same dog are compared. The main finding shown is that halothane caused an approximately 100% increase in CBF before glibenclamide but only a 40% increase in CBF during glibenclamide. Halothane reduced left ventricular  $dP/dt$ , and it had no effect on either aortic pressure or heart rate in the absence and presence of glibenclamide. These systemic hemodynamic effects of intracoronary halothane were a consistent finding during the study (table 1). Coronary arterial  $P_{O_2}$  was increased during halothane, although this effect was statistically significant only in the presence of glibenclamide (table 1). The changes in systemic hemodynamic parameters and coronary arterial blood gases in the isoflurane and enflurane studies were comparable to those presented for the halothane studies.

Figure 2 summarizes the changes in CBF by halothane, isoflurane, and enflurane, before, during, and after glibenclamide. The three volatile anesthetics caused pronounced increases in CBF (isoflurane > halothane = enflurane), which, at constant coronary perfusion pressure, reflected proportional decreases in coronary vascular resistance. The figure also shows that glibenclamide attenuated, in a reversible manner, the volatile anesthetic-induced increases in CBF.

Figure 3 summarizes the increases in CBF by cromakalim, sodium nitroprusside, acetylcholine, and adenosine before, during, and after glibenclamide. Before glibenclamide, all of the drugs caused pronounced increases in CBF. The increases in CBF by cromakalim were dose dependent. Glibenclamide blunted the increases in CBF caused by cromakalim and adenosine, but it had no effect on those caused by sodium nitroprusside and acetylcholine. Neither infusion of the coronary vasodilators nor of glibenclamide affected systemic hemodynamic parameters; these values remained at values comparable to those presented under control in table 1.

The volatile anesthetics caused reductions in SS (and in  $MVO_2$ ) (tables 2–4); the decreases in SS were greater for enflurane than for halothane and isoflurane. The combination of decreased  $MVO_2$  and increased CBF during administration of the volatile anesthetics resulted in pronounced reductions in oxygen extraction. Glibenclamide did not affect the decreases in SS and  $MVO_2$  by the volatile anesthetics, except that it abolished the decreases in  $MVO_2$  during isoflurane.



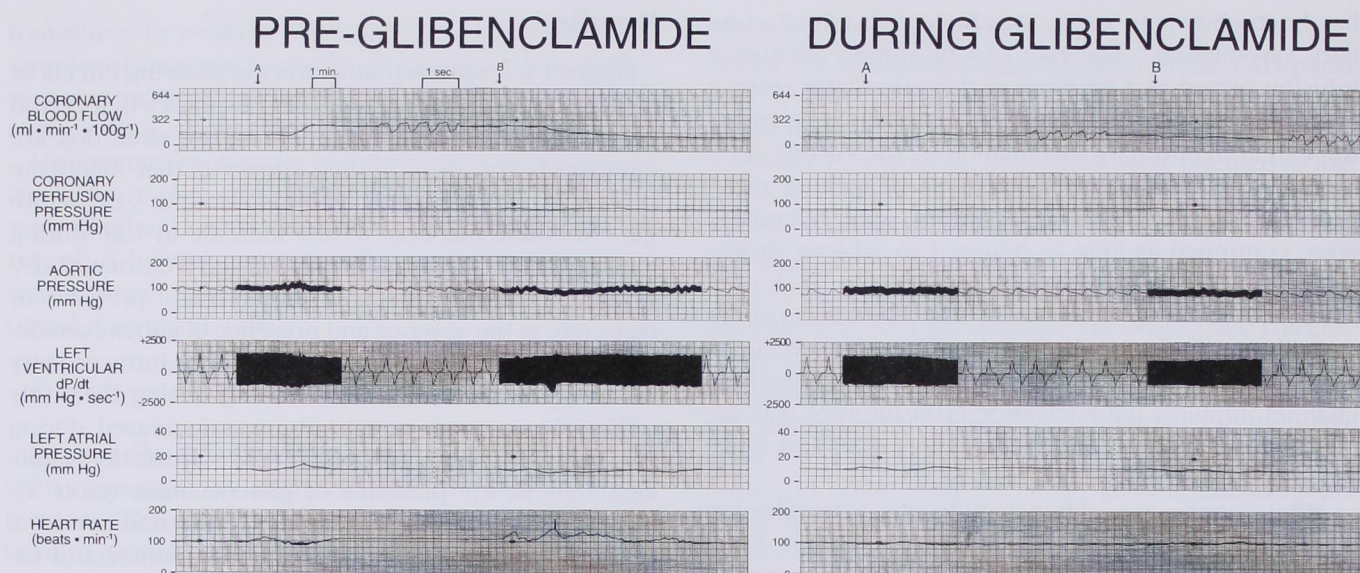


Fig. 1. Original tracing showing effects of selective exposure of the left anterior descending coronary artery (LAD) to blood equilibrated with 1 minimum alveolar concentration halothane before and during glibenclamide. (A) Perfusion of the LAD was switched to the halothane-equilibrated blood reservoir. (B) Perfusion was returned to the halothane-free reservoir. Note that glibenclamide attenuated the halothane-induced increase in coronary blood flow.

Cromakalim caused modest, dose-dependent decreases in SS, although  $\text{MVO}_2$  did not change consistently (table 5). Glibenclamide abolished the cromakalim-induced decreases in SS.

Table 6 presents the baseline effects of intracoronary glibenclamide on SS,  $\text{MVO}_2$ , and CBF. Glibenclamide reduced CBF by 30%; however, a proportional increase in oxygen extraction resulted in no change in  $\text{MVO}_2$ . Glibenclamide had no effect on SS.

## Discussion

### Critique of Methods

We have used the model of regional coronary perfusion in canine hearts in previous studies to evaluate the direct coronary vascular effects of the volatile anesthetics<sup>1-3</sup> and to clarify mechanisms responsible for these effects, such as nitric oxide.<sup>28</sup> In the present study, control values for myocardial oxygen extraction in the extracorporeally per-

Table 1. Effect of Intracoronary Halothane on Systemic Hemodynamic Parameters and Coronary Arterial Blood Gases before, during, and after Glibenclamide

	Before Glibenclamide		During Glibenclamide		After Glibenclamide	
	Control	Halothane	Control	Halothane	Control	Halothane
MAP (mmHg)	83 ± 6	82 ± 7	85 ± 7	82 ± 7	81 ± 9	74 ± 10
MLAP (mmHg)	8.0 ± 1.0	8.6 ± 0.9	8.2 ± 1	8.4 ± 1.1	8.5 ± 1.4	9.4 ± 1.7
dP/dt <sub>max</sub> (mmHg/sec)	1,430 ± 76	1,360 ± 110*	1,460 ± 93	1,300 ± 94*	1,488 ± 144	1,288 ± 146*
HR (beats/min)	139 ± 6	138 ± 6	140 ± 7	141 ± 8	133 ± 7	134 ± 9
CPP (mmHg)	81 ± 1	82 ± 1	81 ± 1	81 ± 1	80 ± 2	83 ± 1
Coronary arterial blood values						
P <sub>O<sub>2</sub></sub> (mmHg)	317 ± 50	407 ± 55	236 ± 44	356 ± 50*	279 ± 61	399 ± 38
P <sub>CO<sub>2</sub></sub> (mmHg)	36 ± 2	32 ± 1*	35 ± 1	31 ± 1	33 ± 3	32 ± 1
pH	7.39 ± 0.01	7.44 ± 0.01*	7.39 ± 0.02	7.43 ± 0.01	7.42 ± 0.04	7.43 ± 0.02
Hct (%)	35 ± 1	34 ± 1	31 ± 1	32 ± 1	31 ± 2	32 ± 2

Values are mean ± SE for 10 observations. Findings during isoflurane and enflurane were comparable. MAP = mean aortic pressure; MLAP = mean left arterial pressure; HR = heart rate; CPP = coronary perfusion pressure; Hct = hematocrit.

\* $P < 0.05$  versus control.



## VOLATILE ANESTHETICS AND POTASSIUM CHANNELS

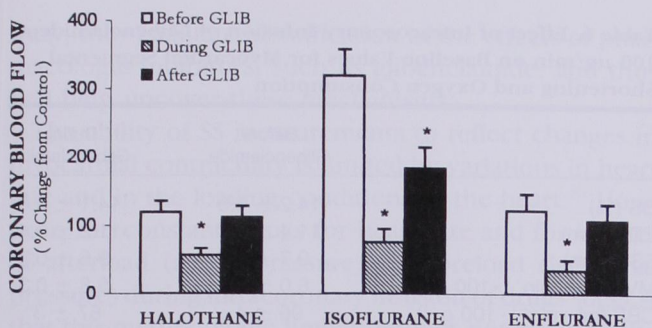


Fig. 2. Changes in coronary blood flow during intracoronary administration of 1 minimum alveolar concentration halothane ( $n = 10$ ), isoflurane ( $n = 8$ ), and enflurane ( $n = 7$ ) before, during, and after glibenclamide (GLIB) infusion.  $*P > 0.05$  compared with values before GLIB infusion. Values are means  $\pm$  SE.

fused LAD bed were moderately lower than those usually found in anesthetized dogs with naturally perfused left coronary circulations.<sup>29</sup> This reflects modest vasodilation in the control preparation, probably because of vasodilators released from blood cells in the pumps, bottles, and tubing contained in the extracorporeal circuit.<sup>30</sup> Nevertheless, vascular responsiveness to an endothelium-dependent vasodilator (acetylcholine),<sup>31</sup> an endothelium-independent vasodilator (sodium nitroprusside),<sup>31</sup> and a  $K_{ATP}$  channel opener (cromakalim) remained pronounced. Furthermore, vasodilator reserve was appreciable, as demonstrated by the fivefold increases in CBF during adenosine infusion. We have shown that the oxygenator used to add the volatile anesthetics to the coronary arterial blood does not itself release vasoactive or inotropic substances.<sup>1-3,32</sup>

The experimental approaches used in the current study were designed to clarify, under well-controlled

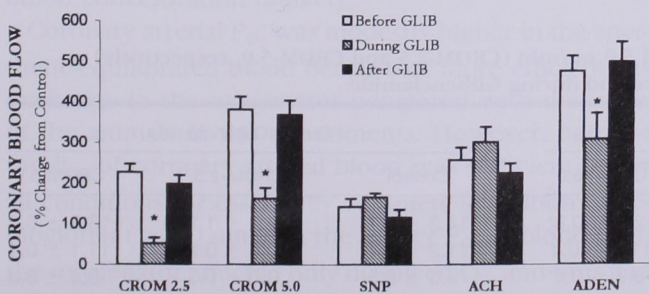


Fig. 3. Changes in coronary blood flow during cromakalim (CROM; 2.5 and 5  $\mu\text{g}/\text{min}$ ), sodium nitroprusside (SNP; 80  $\mu\text{g}/\text{min}$ ), acetylcholine (ACH; 20  $\mu\text{g}/\text{min}$ ), and adenosine (ADEN; 8  $\text{mg}/\text{min}$ ) before, during, and after glibenclamide (GLIB) infusion.  $*P < 0.05$  compared with control values. Values are means  $\pm$  SE for 25 observations.

Table 2. Effect of Intracoronary Halothane on Myocardial Segmental Shortening and Oxygen Consumption before and during Glibenclamide

	Before Glibenclamide		During Glibenclamide	
	Control	Halothane	Control	Halothane
SS (%)	14.8 $\pm$ 0.9	9.0 $\pm$ 2.1*	14.1 $\pm$ 0.7	8.7 $\pm$ 1.4*
EDL (mm)	11.1 $\pm$ 0.5	11.6 $\pm$ 0.5	11.2 $\pm$ 0.5	11.4 $\pm$ 0.4
ESL (mm)	9.4 $\pm$ 0.4	10.6 $\pm$ 0.6*	9.6 $\pm$ 0.4	10.4 $\pm$ 0.5*
MV <sub>O<sub>2</sub></sub> (ml $\cdot$ min <sup>-1</sup> $\cdot$ 100 g <sup>-1</sup> )	7.2 $\pm$ 0.5	5.2 $\pm$ 0.7*	6.2 $\pm$ 0.4	4.8 $\pm$ 0.5*
CBF (ml $\cdot$ min <sup>-1</sup> $\cdot$ 100 g <sup>-1</sup> )	100 $\pm$ 6	221 $\pm$ 21*	74 $\pm$ 7	119 $\pm$ 14*
E <sub>O<sub>2</sub></sub> (%)	44 $\pm$ 2	15 $\pm$ 2*	60 $\pm$ 2	31 $\pm$ 5*

Values are mean  $\pm$  SE for 10 observations, except for SS, EDL, and ESL, which were for seven observations.

SS = segmental shortening; EDL = end-diastolic length; ESL = end-systolic length; MV<sub>O<sub>2</sub></sub> = myocardial oxygen consumption; CBF = coronary blood flow; E<sub>O<sub>2</sub></sub> = myocardial oxygen extraction.

\* $P < 0.05$  versus control.

hemodynamic conditions, the role of the  $K_{ATP}$  channels in the coronary vasodilation (and the cardiac depression) caused by the volatile anesthetics halothane, isoflurane, and enflurane. This was accomplished by comparing the changes in CBF and SS by a volatile anesthetic in the absence and presence of glibenclamide in the same preparation. The validity of our findings depends on the duplicate responses to the individual volatile anesthetic being independent of one another. In previous studies using the same model, we found that

Table 3. Effect of Intracoronary Isoflurane on Myocardial Segmental Shortening and Oxygen Consumption before and during Glibenclamide

	Before Glibenclamide		During Glibenclamide	
	Control	Isoflurane	Control	Isoflurane
SS (%)	16.1 $\pm$ 1.7	9.2 $\pm$ 3.4*	14.8 $\pm$ 2.2	10.6 $\pm$ 2.3*
EDL (mm)	12.5 $\pm$ 1.3	12.7 $\pm$ 1.3	12.6 $\pm$ 1.3	12.7 $\pm$ 1.2
ESL (mm)	10.4 $\pm$ 0.9	11.5 $\pm$ 1.1	10.7 $\pm$ 0.9	11.4 $\pm$ 1.1
MV <sub>O<sub>2</sub></sub> (ml $\cdot$ min <sup>-1</sup> $\cdot$ 100 g <sup>-1</sup> )	6.4 $\pm$ 0.7	4.1 $\pm$ 0.5*	6.5 $\pm$ 0.6	6.7 $\pm$ 1.2
CBF (ml $\cdot$ min <sup>-1</sup> $\cdot$ 100 g <sup>-1</sup> )	103 $\pm$ 9	422 $\pm$ 37*	78 $\pm$ 5	137 $\pm$ 16*
E <sub>O<sub>2</sub></sub> (%)	41 $\pm$ 5	6 $\pm$ 1*	54 $\pm$ 5	37 $\pm$ 7*

Values are mean  $\pm$  SE for eight observations, except for SS, EDL, and ESL, which were for four observations. Abbreviations are the same as in table 2.

\* $P < 0.05$  versus control.



**Table 4. Effect of Intracoronary Enflurane on Myocardial Segmental Shortening and Oxygen Consumption before and during Glibenclamide**

	Before Glibenclamide		During Glibenclamide	
	Control	Enflurane	Control	Enflurane
SS (%)	12.4 ± 0.9	0.1 ± 1.8*	12.8 ± 0.7	2.8 ± 2.6*
EDL (mm)	10.8 ± 0.5	11.1 ± 0.6	10.2 ± 0.5	10.2 ± 0.7
ESL (mm)	9.5 ± 0.5	11.1 ± 0.6*	8.9 ± 0.5	9.9 ± 0.4*
MV <sub>O<sub>2</sub></sub> (ml · min <sup>-1</sup> · 100 g <sup>-1</sup> )	5.1 ± 0.3	3.6 ± 0.9*	5.2 ± 0.6	3.8 ± 0.6*
CBF (ml · min <sup>-1</sup> · 100 g <sup>-1</sup> )	98 ± 10	223 ± 45*	66 ± 10	90 ± 14*
E <sub>O<sub>2</sub></sub> (%)	41 ± 6	12 ± 3*	57 ± 6	36 ± 10*

Values are mean ± SE for seven observations. Abbreviations are the same as in table 2.

\**P* < 0.05 versus control.

the coronary vasodilating effects of volatile anesthetics were blunted when arterial blood concentration was increased gradually,<sup>33</sup> or when exposure to the anesthetics was prolonged.<sup>28</sup> These findings implied a tendency for coronary vascular smooth muscle to adapt to the relaxing effects of these drugs. Such vascular adaptation could have attenuated responses to the second exposure to the volatile anesthetic, regardless of an effect of glibenclamide. To reduce this possibility, we used abrupt, relatively brief exposures of the LAD to blood previously equilibrated with a volatile anesthetic. The recovery of volatile anesthetic-induced coronary vasodilation during a third exposure in the absence of glibenclamide provided evidence that this protocol was successful in avoiding vascular adaptation, and that the diminution in the response to the volatile anesthetics

**Table 6. Effect of Intracoronary Infusion of Glibenclamide at 100 μg/min on Baseline Values for Myocardial Segmental Shortening and Oxygen Consumption**

	Before Glibenclamide	During Glibenclamide
SS (%)	14.2 ± 0.9	14.1 ± 0.8
EDL (mm)	11.4 ± 0.4	11.2 ± 0.4
ESL (mm)	9.7 ± 0.3	9.6 ± 0.3
MV <sub>O<sub>2</sub></sub> (ml · min <sup>-1</sup> · 100 g <sup>-1</sup> )	6.0 ± 0.4	5.8 ± 0.3
CBF (ml · min <sup>-1</sup> · 100 g <sup>-1</sup> )	96 ± 4	67 ± 3*
E <sub>O<sub>2</sub></sub> (%)	42 ± 2	58 ± 2*

Values are mean ± SE for 25 observations, except for SS, EDL, and ESL, which were for 18 observations. Abbreviations are the same as in table 2.

\**P* < 0.05 versus Before Glibenclamide.

during the second exposure was an effect of glibenclamide.

Our model typically demonstrates increases in CBF that are more pronounced than those observed when the volatile anesthetics are administered in the inspired gas.<sup>34,35</sup> One factor contributing to this difference is that our model obviates decreases in coronary perfusion pressure and global cardiac work demand (secondary to reduced ventricular wall tension) that accompany inhalation of the anesthetics. Furthermore, when a volatile anesthetic is administered *via* the lungs, its blood concentration increases gradually in accordance with the pharmacokinetics of the anesthetic in the alveoli and pulmonary capillary bed,<sup>20</sup> thus providing an opportunity for vascular adaptation. As previously noted, our model excluded this factor. The more pronounced CBF responses in our model do not detract from its value as a tool to clarify the mechanisms underlying volatile anesthetic-induced coronary vasodilation. On the con-

**Table 5. Effect of Intracoronary Infusion of Cromakalim at 2.5 and 5.0 μg/min (CROM-2.5 and CROM-5.0, respectively) on Myocardial Segmental Shortening and Oxygen Consumption before and during Glibenclamide**

	Before Glibenclamide			During Glibenclamide		
	Control	CROM-2.5	CROM-5.0	Control	CROM-2.5	CROM-5.0
SS (%)	15.6 ± 0.8	13.1 ± 1.2*	12.6 ± 0.8*	14.3 ± 0.7	13.3 ± 1.1	13.8 ± 0.8
EDL (mm)	11.3 ± 0.4	10.2 ± 0.3*	11.4 ± 0.4	11.2 ± 0.4	10.4 ± 0.2	11.1 ± 0.4
ESL (mm)	9.5 ± 0.3	8.9 ± 0.3*	9.9 ± 0.4*	9.6 ± 0.3	9.0 ± 0.3	9.6 ± 0.3
MV <sub>O<sub>2</sub></sub> (ml · min <sup>-1</sup> · 100 g <sup>-1</sup> )	6.1 ± 0.4	5.7 ± 0.7	5.4 ± 0.4	5.8 ± 0.3	5.5 ± 0.3	5.4 ± 0.3
CBF (ml · min <sup>-1</sup> · 100 g <sup>-1</sup> )	93 ± 4	295 ± 17*	433 ± 26*	68 ± 3	105 ± 8*	175 ± 16*
E <sub>O<sub>2</sub></sub> (%)	44 ± 2	13 ± 2*	12 ± 2*	59 ± 2	47 ± 5*	30 ± 3*

Values are mean ± SE for 25 observations, except for SS, EDL, and ESL, which were for 18 observations. Abbreviations are the same as in table 2.

\**P* < 0.05 versus control.



## VOLATILE ANESTHETICS AND POTASSIUM CHANNELS

trary, they facilitate identification of the effects of pharmacologic inhibitors, such as glibenclamide, and thus will help uncover these mechanisms.

The ability of SS measurements to reflect changes in myocardial contractility is limited by variations in heart rate and in the loading conditions of the heart.<sup>36</sup> However, the constant values for heart rate and for indices of afterload (aortic pressure) and preload (left atrial pressure) during intracoronary infusion of drugs suggest that this methodologic limitation does not apply to the present study.

Interpretation of our results depends on glibenclamide being an effective and specific blocker of  $K_{ATP}$  channels. Such blockade was demonstrated by the ability of glibenclamide to attenuate significantly, in a readily reversible manner, the coronary vasodilating effects of the  $K_{ATP}$  channel-opening drug cromakalim while having no effect on the coronary vasodilating effects of the  $K_{ATP}$  channel-independent drugs, sodium nitroprusside and acetylcholine. Our intracoronary dose of glibenclamide (100  $\mu$ g/min) was based on preliminary studies, which indicated that it was the highest dose that could be used without causing nonspecific inhibition of coronary vascular smooth muscle reactivity, and it was in the range of the dose used by previous investigators.<sup>11,13</sup> The failure of glibenclamide to abolish the coronary vasodilating effects of cromakalim is consistent with the known competitive antagonism between the two compounds.<sup>37</sup>

Glibenclamide acts on  $K_{ATP}$  channels in the beta cells of the pancreas to increase insulin release, which, in turn, decreases glucose concentration in the blood.<sup>38</sup> The use of intracoronary infusions of glibenclamide in our study kept the systemic blood concentrations low, thus making significant changes in insulin or glucose blood concentration unlikely.

Coronary arterial  $P_{O_2}$  was modestly higher in the anesthetic-equilibrated blood because of more efficient gas exchange in the oxygenator compared with the lungs of the animals in the experiments. However, because the  $P_{O_2}$  of coronary arterial blood was sufficient under all conditions for essentially complete saturation of hemoglobin (>200 mmHg), the higher  $P_{O_2}$  in blood from the oxygenator affected only dissolved  $O_2$ , and thus had only a negligible influence on arterial  $O_2$  content.

#### Coronary Effects of Volatile Anesthetics

The main finding from this study was that glibenclamide (a specific  $K_{ATP}$  channel blocker) attenuated the in-

creases in CBF but had no effect on the decreases in SS, caused by the volatile anesthetics, halothane, isoflurane, and enflurane in canine hearts *in situ*.

The increases in CBF caused by the volatile anesthetics were accompanied by decreases in SS and  $MVO_2$  (reflecting a direct negative inotropic effect), and thus the values for  $O_2$  extraction decreased markedly. These decreases in  $O_2$  extraction indicated an uncoupling of coronary oxygen supply from the myocardial oxygen demands, which is the hallmark of a coronary vasodilating drug.<sup>4</sup> The coronary vasodilating potency of the volatile anesthetics varied by agent; that is, isoflurane > halothane = enflurane.

We observed that glibenclamide significantly attenuated the coronary vasodilation caused by the volatile anesthetics, suggesting that an opening of  $K_{ATP}$  channels plays an important role in this response. These findings correspond with the findings obtained by Larach and Schuler<sup>16</sup> during administration of halothane in crystalloid-perfused rat hearts arrested with tetrodotoxin. However, in contrast to our findings, Cason *et al.*<sup>17</sup> found that glibenclamide completely inhibited the direct coronary vasodilating effect of isoflurane, thus unmasking a vasoconstrictor effect. This vasoconstrictor effect was attributed to the metabolic mechanisms matching CBF to the reduced  $MVO_2$  due to the negative inotropic effect of isoflurane. An explanation for the greater effectiveness of glibenclamide in the study by Cason *et al.* compared with the present study is not clear. Several methodologic differences may have contributed, including a difference in species, a higher dose for glibenclamide in one half the studies by Cason *et al.*, and a difference in the protocol used for intracoronary administration of isoflurane. Because Cason *et al.* used graded and relatively prolonged intracoronary administrations of isoflurane, the coronary vasodilating responses during their second exposure to isoflurane (in the presence of glibenclamide) may have been limited by vascular adaptation. This factor played no apparent role in the present study.

The mechanism(s) by which halothane, isoflurane, and enflurane open the  $K_{ATP}$  channels in coronary vascular smooth muscle remains to be clarified. Several potential mechanisms may be proposed. (1) The volatile anesthetics may have interacted directly with the  $K_{ATP}$  channels. (2) A reduction in ATP concentration within the vascular smooth muscle cell may have caused an opening of the  $K_{ATP}$  channels. (3) Prostacyclin may have been released (perhaps from the vascular endothelium), which in turn opens the  $K_{ATP}$  channels, perhaps by



a G-protein-mediated pathway. This mechanism was implied by the study of Jackson *et al.*,<sup>39</sup> who reported that glibenclamide inhibited coronary vasodilation caused by exogenous prostacyclin or iloprost (the stable analog of prostacyclin) in saline-perfused rabbit hearts. (4) An adenosine vascular receptor may have been activated, leading to an opening of  $K_{ATP}$  channels *via* a G protein.<sup>7,40-43</sup> (5) An interaction may have occurred with phosphorylation-regulating enzymes, such as protein kinase C, which in turn may regulate the activity of the  $K_{ATP}$  channel. Further investigations are required to determine which, if any, of the above mechanisms are involved in the opening of  $K_{ATP}$  channels in coronary vascular smooth muscle by the volatile anesthetics.

The decreases in SS and  $MVO_2$  during the intracoronary administration of the volatile anesthetics are consistent with the well-documented negative inotropic effect of these agents.<sup>44-48</sup> The relative potency of this negative inotropic effect in our study was as follows: enflurane > halothane = isoflurane. A comparison of our results to most previous *in vivo* findings is complicated by the reductions in cardiac afterload and baroreceptor-arousal of the sympathoadrenal system that accompany administration of the volatile anesthetics in inspired gas.<sup>48</sup> However, some studies used experimental approaches that minimized these complicating factors. Coetzee *et al.*,<sup>47</sup> using a load-independent index of myocardial contractility (the end-systolic pressure-length relation) in dogs anesthetized with fentanyl, found relative negative inotropic effects for 1 minimum alveolar concentration of halothane, isoflurane, and enflurane that were similar to those that we found in this study. However, Pagel *et al.*,<sup>48</sup> using another load-independent index of myocardial contractility (preload recruitable stroke work) in chronically instrumented autonomically blocked dogs, found that 1.5 and 2 minimum alveolar concentrations of halothane had a greater negative inotropic effect than did equianesthetic concentrations of isoflurane. These latter results supported findings obtained in cardiac samples *in vitro*.<sup>44-46</sup> The findings to date suggest that the relative negative inotropic effects of halothane, isoflurane, and enflurane may depend on the experimental preparation and the anesthetic concentration.

Although systolic lengthening (indicative of total arrest of mechanical function) is usually associated with localized myocardial ischemia, it has been observed when a potent negative inotrope, such as lidocaine, is selectively administered into a branch of the left main

coronary artery.<sup>49</sup> Several lines of evidence suggest this latter mechanism was responsible for the systolic lengthening observed during intracoronary enflurane in our study. First, enflurane increased, rather than decreased, CBF. Second, in a previous study, we found that regional myocardial lactate uptake was maintained during intracoronary enflurane infusion, suggesting a lack of anaerobic metabolism and myocardial ischemia.<sup>3</sup> Finally, we also found that systolic lengthening persisted when CBF was increased maximally with adenosine during intracoronary enflurane administration.<sup>3</sup>

In this study, the intracoronary infusions of cromakalim caused marked increases in CBF (300–400%), accompanied by modest decreases in SS (15–20%). This small effect of  $K_{ATP}$  channel activation on cardiac function is consistent with previous findings obtained in isolated perfused rat hearts<sup>50</sup> and in swine hearts *in vivo*.<sup>51</sup> The relative changes in CBF and SS caused by cromakalim in the present study are consistent with observations obtained *in vitro*, indicating that vascular smooth muscle may be as much as 2,000 times more sensitive to  $K_{ATP}$  channel openers than is cardiac muscle.<sup>8,9</sup>

The failure of glibenclamide to blunt the decreases in SS by the volatile anesthetics suggests that the cardiac  $K_{ATP}$  channels were not activated by these agents. Another possibility is that the volatile anesthetics caused activation of the cardiac  $K_{ATP}$  channels, but because this mechanism made only a modest contribution to the overall negative inotropic effect, its influence was overshadowed by other more dominant mechanisms, such as the alterations in  $Ca^{++}$  flux.<sup>44-46</sup> An opening of cardiac  $K_{ATP}$  channels by volatile anesthetics has been suggested by recent work in chronically instrumented dogs, indicating that isoflurane can provide protection from myocardial stunning, which can be attenuated with glibenclamide.<sup>52</sup>

The ability of glibenclamide to prevent the reduction in  $MVO_2$  during isoflurane administration was an interesting and new observation. Because the decreases in SS were preserved, this effect could not be explained by an obtunded negative inotropic response. Rather, it apparently reflected an ability of glibenclamide in the presence of isoflurane to uncouple myocardial oxygen use from the cardiac workload, *via* a direct effect on mitochondrial function. This effect of glibenclamide was not observed in the absence of the volatile anesthetics; that is, glibenclamide itself had no effect on SS or  $MVO_2$  (table 6) or in the presence of halothane or enflurane. Because little is known about the indepen-



dent influences of the  $K_{ATP}$  channels and isoflurane on mitochondrial function,<sup>12</sup> it is difficult to speculate how these factors might interact to produce the present findings. Further studies using sophisticated *in vitro* techniques are needed to clarify this mechanism.

We found that glibenclamide inhibited adenosine-induced coronary vasodilation, implying that adenosine activates the  $K_{ATP}$  channels in coronary vascular smooth muscle. This observation confirms findings obtained both *in vivo*<sup>40-42</sup> and in isolated heart preparations.<sup>42,43</sup> Although adenosine causes coronary vasodilation *via*  $A_1$  and  $A_2$  receptors, only the  $A_1$  receptor-mediated component could be inhibited with glibenclamide.<sup>43</sup> This suggests that the  $A_1$  receptors are coupled to the  $K_{ATP}$  channels. Findings in isolated ventricular myocytes suggest that this coupling may occur through G proteins.<sup>55</sup>

Other investigators using intracoronary infusions of glibenclamide also reported decreases in baseline CBF, implying that the  $K_{ATP}$  channel is active under basal conditions in coronary vascular smooth muscle and that it contributes to the maintenance of basal coronary tone.<sup>11,12</sup> Despite the decrease in CBF during glibenclamide, neither  $MVO_2$  nor SS was affected. Myocardial oxygen consumption was maintained because oxygen extraction increased sufficiently to offset the reduction in CBF.

Our findings suggest that the  $K_{ATP}$  channels play an important role in coronary vasodilation but are not apparently involved in the cardiac depression caused by halothane, isoflurane, and enflurane in canine hearts *in situ*.

The authors thank Derrick L. Harris for technical assistance.

## References

- Crystal GJ, Khoury E, Gurevicius J, Salem MR: The direct effects of halothane on coronary blood flow, myocardial oxygen consumption, and myocardial segmental shortening in *in situ* canine hearts. *Anesth Analg* 1995; 80:256-62
- Crystal GJ, Kim S-J, Czinn EA, Salem MR, Mason WR, Abdel-Latif M: Intracoronary isoflurane causes marked vasodilation in canine hearts. *ANESTHESIOLOGY* 1991; 74:757-65
- Gurevicius J, Holmes CB, Salem MR, Abdel-Halim A, Crystal GJ: The direct effects of enflurane on coronary blood flow, myocardial oxygen consumption, and myocardial segmental shortening in *in situ* canine hearts. *Anesth Analg* 1996; 83:68-74
- Feigl EO: Coronary physiology. *Physiol Rev* 1983; 63:1-205
- Noma A: ATP-regulated  $K^+$  channels in cardiac muscle. *Nature* (London) 1983; 305:147-8
- Standen NB, Quayle JM, Davies NW, Brayden JE, Huang Y, Nelson MT: Hyperpolarizing vasodilators activate ATP-sensitive potassium channels in arterial smooth muscle. *Science* 1989; 245:177-80
- Nelson MT, Quayle JM: Physiological roles and properties of potassium channels in arterial smooth muscle. *Am J Physiol* 1995; 268:C799-822
- Longman SD, Clapham JC, Wilson C, Hamilton TC: Cromakalim, a potassium channel activator: A comparison of its cardiovascular haemodynamic profile and tissue specificity with those of pinacidil and nicorandil. *J Cardiovasc Pharmacol* 1988; 12:535-42
- Sanguinetti MC, Scott AL, Zingaro GJ, Siegl PKS: BRL 34915 (cromakalim) activates ATP-sensitive  $K^+$  current in cardiac muscle. *Proc Natl Acad Sci USA* 1988; 85:8360-4
- Nelson MT, Patlak JB, Worley JF, Standen NB: Calcium channels, potassium channels, and voltage dependence of arterial smooth muscle tone. *Am J Physiol* 1990; 259:C3-18
- Imamura Y, Tomoike H, Narishige T, Takahashi T, Kasuya T, Takeshita T: Glibenclamide decreases basal coronary blood flow in anesthetized dogs. *Am J Physiol* 1992; 263:H399-404
- Samaha FF, Heineman FW, Ince C, Fleming J, Balaban RS: ATP-sensitive potassium channel is essential to maintain basal coronary vascular tone *in vivo*. *Am J Physiol* 1992; 262:C1220-7
- Narishige T, Egashira K, Akatsuka Y, Imamura Y, Takashashi T, Kasuya H, Takeshita A: Glibenclamide prevents coronary vasodilation induced by  $\beta_1$ -adrenoceptor stimulation in dogs. *Am J Physiol* 1994; 266:H84-92
- Komaru T, Lamping KG, Eastham CL, Dellsperger KC: Role of ATP-sensitive potassium channels in coronary microvascular autoregulatory responses. *Circ Res* 1991; 69:1146-51
- Narishige T, Egashira K, Akatsuka Y, Katsuda Y, Numaguchi K, Sakata M, Takeshita A: Glibenclamide, a putative ATP-sensitive  $K^+$  channel blocker, inhibits coronary autoregulation in anesthetized dogs. *Circ Res* 1993; 73:771-6
- Larach DR, Schuler HG: Potassium channel blockade and halothane vasodilation in conduction and resistance coronary arteries. *J Pharmacol Exp Ther* 1993; 267:72-81
- Cason BA, Shubayev I, Hickey RF: Blockade of adenosine triphosphate-sensitive potassium channels eliminates isoflurane-induced coronary arterial vasodilation. *ANESTHESIOLOGY* 1994; 81:1245-55
- Nichols CG, Ripoll C, Lederer WJ: ATP-sensitive potassium channel modulation of the guinea pig ventricular action potential and contraction. *Circ Res* 1991; 68:280-7
- Nichols CG, Lederer WJ: Adenosine triphosphate-sensitive potassium channels in the cardiovascular system. *Am J Physiol* 1991; 261:H1675-86
- Eger EI II: Uptake of inhaled anesthetics: The alveolar to inspired anesthesia difference. *Anesthetic Uptake and Action*, 4th edition. Baltimore, Williams and Wilkins, 1976, pp 77-96
- Roberts DL, Nakazawa HK, Klocke FJ: Origin of great cardiac vein and coronary sinus drainage within the left ventricle. *Am J Physiol* 1976; 230:486-92
- Vinten-Johannsen J, Johnston WE, Crystal GJ, Mills SA, Santamore WP, Cordell AR: Validation of local venous sampling within the at risk left anterior vascular bed in the canine left ventricle. *Cardiovasc Res* 1987; 21:646-51
- Crystal GJ, Gurevicius J: Nitric oxide does not modulate myocardial contractility acutely in *in situ* canine hearts. *Am J Physiol* 1996; 270:H1568-76
- Crystal GJ, Rock MH, Kim S-J, Salem MR: Effect of intracoronary infusions of amrinone and dobutamine on segment shortening, blood flow, and oxygen consumption in *in situ* canine hearts. *Anesth Analg* 1994; 79:1066-74



25. Streeter DD, Spotnitz HM, Patel DP, Ross J, Sonnenblick EH: Fiber orientation in the canine left ventricle during diastole and systole. *Circ Res* 1969; 40:158-65
26. Lange R, Ware J, Kloner RA: Absence of cumulative deterioration of regional function during three repeated 5 or 15 minute coronary occlusions. *Circulation* 1984; 69:400-8
27. Zar JH: *Biostatistical Analysis*. Englewood Cliffs, NJ, Prentice-Hall, 1974, pp 121-4, 151-62
28. Crystal GJ, Kim S-J, Salem MR, Khoury E, Gurevicius J: Nitric oxide does not mediate coronary vasodilation by isoflurane. *ANESTHESIOLOGY* 1994; 81:209-20
29. Crystal GJ, Kim S-J, Salem MR: Right and left ventricular O<sub>2</sub> uptake during hemodilution and adrenergic stimulation. *Am J Physiol* 1993; 265:H1769-77
30. Borgdorff P, Sipkema P, Westerhof N: Pump perfusion abolishes autoregulation possibly via prostaglandin release. *Am J Physiol* 1988; 225:H280-7
31. Moncada S, Palmer RMJ, Higgs EA: Nitric oxide: Physiology, pathophysiology, and pharmacology. *Pharmacol Rev* 1991; 43:109-41
32. Gurevicius J, Salem MR, Metwally AA, Silver JM, Crystal GJ: Contribution of nitric oxide to coronary vasodilation during hypercapnic acidosis. *Am J Physiol* 1995; 263:H39-47
33. Crystal GJ, Czinn EA, Silver JM, Salem MR: Coronary vasodilation by isoflurane: Abrupt versus gradual administration. *ANESTHESIOLOGY* 1995; 82:542-9
34. Abdel-Latif M, Kim S-J, Salem MR, Crystal GJ: Phenylephrine does not limit myocardial blood flow or oxygen delivery during isoflurane-induced hypotension in dogs. *Anesth Analg* 1992; 74:870-6
35. Hickey RF, Cason BA, Shubayev I: Regional vasodilating properties of isoflurane in normal swine myocardium. *ANESTHESIOLOGY* 1994; 80:574-81
36. Aversano T, Maughan WL, Hunter WC, Kass D, Becken LC: End-systolic measures of regional ventricular performance. *Circulation* 1986; 73:938-50
37. Cyrus S, Daut J: The sensitivity of coronary vascular tone to glibenclamide: A study on the isolated perfused guinea pig heart. *Cardiovasc Res* 1994; 28:888-93
38. Cook NS: The pharmacology of potassium channels and their therapeutic potential. *Trends Pharmacol Sci* 1988; 9:21-8
39. Jackson WF, König A, Dambacher T, Busse R: Prostacyclin-induced vasodilation in rabbit heart is mediated by ATP-sensitive potassium channels. *Am J Physiol* 1993; 264:H238-43
40. Aversano T, Ouyang P, Silverman H: Blockade of ATP-sensitive potassium channel modulates reactive hyperemia in the canine circulation. *Circulation* 1991; 69:618-23
41. Belloni FL, Hintze TH: Glibenclamide attenuates adenosine-induced bradycardia and coronary vasodilation. *Am J Physiol* 1991; 261:H720-7
42. Clayton FC, Hess TA, Smith MA, Grover GJ: Coronary reactive hyperemia and adenosine-induced vasodilation are mediated partially by a glyburide-sensitive mechanism. *Pharmacology* 1992; 44:92-100
43. Nakhostine N, Lamontagne D: Adenosine contributes to hypoxia-induced vasodilation through ATP-sensitive K<sup>+</sup> channel activation. *Am J Physiol* 1993; 265:H1289-93
44. Lynch C III: Differential depression of myocardial contractility by halothane and isoflurane in vitro. *ANESTHESIOLOGY* 1986; 64:620-31
45. Housmans PR: Negative inotropy of halogenated anesthetics in ferret ventricular myocardium. *Am J Physiol* 1990; 259:H827-34
46. Housmans PR, Murat I: Comparative effects of halothane, enflurane, and isoflurane at equipotent anesthetic concentrations on isolated ventricular myocardium of the ferret. I. Contractility. *ANESTHESIOLOGY* 1988; 69:451-63
47. Coetzee A, Fourie P, Badenhorst E: Effect of halothane, enflurane, and isoflurane on the end-systolic pressure-length relationship. *Can J Anaesth* 1987; 34:351-7
48. Pagel PS, Kampine JP, Schmeling WT, Wartier DC: Comparison of end-systolic pressure-length relations and preload recruitable stroke work as indices of myocardial contractility in the conscious and anesthetized, chronically instrumented dog. *ANESTHESIOLOGY* 1990; 73:278-90
49. Gayheart PA, Vinten-Johansen J, Johnston WE, Hester TO, Cordell AR: Oxygen requirements of the dyskinetic myocardial segment. *Am J Physiol* 1989; 257:H1184-91
50. Grover GJ, Sleph PG, Dzwonczyk S: Pharmacologic profile of cromakalim in the treatment of myocardial ischemia in isolated rat hearts and anesthetized dogs. *J Cardiovasc Pharmacol* 1990; 16:853-64
51. Rohman S, Weygandt GH, Schelling P, Soei LK, Becker K-H, Verdouw PD, Lues I, Häusler: Effect of bimakalim (EMD 52692), an opener of ATP sensitive potassium channels, on infarct size, coronary blood flow, regional wall function, and oxygen consumption in swine. *Cardiovasc Res* 1994; 28:858-63
52. Kersten JR, Lowe D, Hettrick DA, Pagel PS, Gross GJ, Wartier DC: Glyburide, a K<sub>ATP</sub> channel antagonist, attenuates the cardioprotective effects of isoflurane in stunned myocardium. *Anesth Analg* 1996; 83:27-33
53. Kirsch GE, Codina J, Birnbaumer L, Brown AM: Coupling of ATP-sensitive K<sup>+</sup> channels to A<sub>1</sub> receptors by G proteins in rat ventricular myocytes. *Am J Physiol* 1990; 259:H820-6