## LABORATORY INVESTIGATIONS

Anesthesiology
1996; 85:794–807


# *Mechanism of Myocardial Protection by Isoflurane*

## *Role of Adenosine Triphosphate-regulated Potassium ($K_{ATP}$) Channels*

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***Background:*** **The mechanism of the protective actions of volatile anesthetics in ischemic myocardium has not been clearly elucidated. The role of myocardial adenosine triphosphate-regulated potassium ($K_{ATP}$) channels in isoflurane-induced enhancement of recovery of regional contractile function after multiple brief occlusions and reperfusion of the left anterior descending coronary artery (LAD) was studied in dogs anesthetized with barbiturates.**

***Methods:*** **Dogs (n = 32) were instrumented to measure left ventricular and aortic blood pressure, cardiac output, LAD coronary blood flow velocity, and subendocardial segment length. Regional myocardial perfusion was measured using radioactive microspheres. Hemodynamics and percentage segment shortening (%SS) in the LAD perfusion territory were evaluated after instrumentation was complete; after pretreatment with the $K_{ATP}$ channel antagonist, glyburide (0.05 mg/kg$^{-1}$) or drug vehicle (polyethylene glycol in ethyl alcohol; control experiments); and in the presence or absence of 1 MAC isoflurane administered for 30 min before and during five 5-min occlusions and reperfusion of the LAD in four experimental groups. Isoflurane was discontinued at the onset of the final reperfusion period. Measurements of hemodynamics, %SS, and myocardial perfusion were repeated at several intervals during 180 min after reperfusion of the LAD.**

***Results:*** **Left anterior descending coronary artery occlusion caused regional dyskinesia during each 5-min occlusion in each dog. Control and glyburide-pretreated dogs demonstrated poor recovery of %SS by 180 min after reperfusion (2 ± 10 and 7 ± 6% of baseline, respectively). In contrast, dogs anesthetized with isoflurane exhibited complete recovery of function (%SS) by 180 min after reperfusion (82 ± 8% of baseline). Enhanced recovery of regional contractile function by isoflurane was abolished by pretreatment with glyburide 180 min after reperfusion (16 ± 10% of baseline). Improvement of functional recovery of stunned myocardium by isoflurane, and the blockade of this action by glyburide, was not associated with changes in hemodynamics or regional myocardial perfusion.**

***Conclusions:*** **The results indicate that isoflurane prevents decreased systolic shortening caused by multiple episodes of ischemia and reperfusion. These actions result in improved recovery of contractile function of postischemic, reperfused myocardium and are mediated by isoflurane-induced activation of $K_{ATP}$ channels. (Key words: Adenosine 5′ triphosphate-dependent potassium channels, antagonists: glyburide. Anesthetics, volatile: isoflurane. Heart, myocardial performance: left ventricular function; myocardial contractility. Heart, myocardial stunning).**

ACTIVATION of adenosine triphosphate-regulated potassium ($K_{ATP}$) channels in cardiac muscle has been shown to produce important antiischemic effects. These channel agonists enhance the functional recovery of postischemic-reperfused (stunned) myocardium,[1] decrease myocardial infarct size,[2] and mimic the effects of myocardial ischemic preconditioning[3] *in vivo*, actions that are blocked by glyburide (glibenclamide), a selective $K_{ATP}$ channel antagonist.[1,3,4] $K_{ATP}$ channel activation may improve recovery of regional contractile function of stunned myocardium by shortening action potential duration and attenuation of membrane depolarization. These effects in turn reduce the duration of calcium influx through voltage-gated calcium channels and increase the time when the sodium-calcium exchanger can extrude calcium from the cell, both of which will reduce intracellular calcium over-

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Received from the Departments of Anesthesiology, Pharmacology, and Medicine (Division of Cardiology), Medical College of Wisconsin, and Zablocki VA Medical Center, Milwaukee, Wisconsin. Supported in part by a FAER Young Investigator Award (Judy R. Kersten), U.S. Public Health Service grant HL 54280 (David C. Warltier), and Anesthesiology Research Training Grant GM 08377 (David C. Warltier). Submitted for publication February 9, 1996. Accepted for publication April 2, 1996.

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load and myocardial tissue injury.[5] Thus opening of $K_{ATP}$ channels may help maintain normal intracellular calcium concentrations and prevent injury to cellular organelles damaged by calcium overload. Multiple, brief episodes of coronary artery occlusion and reperfusion cause reversible contractile dysfunction (stunning)[6-9] and may occur in patients with unstable angina or coronary artery vasospasm. $K_{ATP}$ channels have also been implicated in the mechanism of myocardial stunning associated with repetitive ischemia and reperfusion.[6]

Volatile anesthetics, including isoflurane, exert protective effects during myocardial ischemia.[10-19] Reductions in myocardial oxygen consumption and beneficial alterations in intracellular calcium homeostasis have been proposed as potential mechanisms for this phenomenon. Recently, volatile anesthetic-induced coronary vasodilation was shown to be mediated by activation of $K_{ATP}$ channels.[20,21] These findings raise the intriguing possibility that myocardial protection afforded by these agents during ischemia and reperfusion injury may also involve $K_{ATP}$ activation. The present investigation tested the hypothesis that activation of $K_{ATP}$ channels contributes to isoflurane-induced enhanced recovery of contractile function after repetitive episodes of myocardial ischemia and reperfusion.

## Materials and Methods

All experimental procedures and protocols used in this investigation were reviewed and approved by the Animal Care and Use Committee of the Medical College of Wisconsin. Furthermore, all conformed to the *Guiding Principles in the Care and Use of Animals* of the American Physiologic Society and were in accordance with the *Guide for the Care and Use of Laboratory Animals* (DHEW [DHHS] publication no. [NIH] 85-23, revised 1985).

### *General Preparation*

Mongrel dogs of either sex weighing 24 to 31 kg were fasted overnight. Anesthesia was induced and maintained with an intravenous bolus of sodium barbital (200 mg/kg$^{-1}$) and sodium pentobarbital (15 mg/kg$^{-1}$). The dogs' lungs were ventilated using positive pressure with an air and oxygen mixture after tracheal intubation. Arterial blood gas tensions were monitored at selected intervals and maintained within a physiologic range by adjusting respiratory rate, tidal volume, and inspired oxygen concentration when necessary during each experiment. Arterial blood glucose concentrations were measured at intervals using a glucometer (Tracer II; Boehringer Mannheim, Indianapolis, IN) and maintained at baseline values with an intravenous infusion of 10% dextrose as needed during the experiment. Body temperature was maintained with a heating pad.

A double pressure transducer-tipped catheter (PC 771; Millar Instruments, Houston, TX) was inserted into the aorta and left ventricle *via* the carotid artery to measure aortic and left ventricular pressures. The maximum rate of increase of left ventricular pressure ($dP/dt_{max}$) was determined by electronic differentiation of the left ventricular pressure waveform. A thoracotomy was performed at the left fifth intercostal space, the left lung was gently retracted, and the pericardium was incised. An ultrasonic flow probe (Transonics, Ithaca, NY) was positioned around the ascending thoracic aorta to measure aortic blood flow. Heparin-filled catheters were inserted into the left atrial appendage and the right femoral artery to administer radioactive microspheres and withdraw reference blood flow samples (used to calculate myocardial blood flow), respectively. A 1.5- to 2-cm segment of the proximal left anterior descending coronary artery (LAD) distal to the first diagonal branch was isolated, and a precalibrated Doppler ultrasonic flow transducer was placed around the vessel to measure phasic coronary blood flow velocity. A silk ligature was placed around the LAD to produce coronary artery occlusion and reperfusion. A pair of ultrasonic segment-length transducers (5 MHz) used to measure changes in regional contractile function (percentage segment shortening [%SS]) was implanted in the subendocardium of the LAD perfusion territory. Segment-length and coronary blood flow velocity signals were monitored using ultrasonic amplifiers (Crystal Biotech, Hopkinton, MA). Relative diastolic coronary vascular resistance was calculated as the ratio of end-diastolic arterial pressure to peak diastolic coronary blood flow velocity. The pressure work index, an estimate of myocardial oxygen consumption, was determined using a previously validated formula.[22] All hemodynamic data were monitored continuously on a polygraph (model 7758A; Hewlett-Packard, San Francisco, CA) and digitized using a computer interfaced with an analog-to-digital converter.

### *Myocardial Segment Shortening*

End-systolic segment length (ESL) was determined 10 ms before maximum negative left ventricular dP/dt,

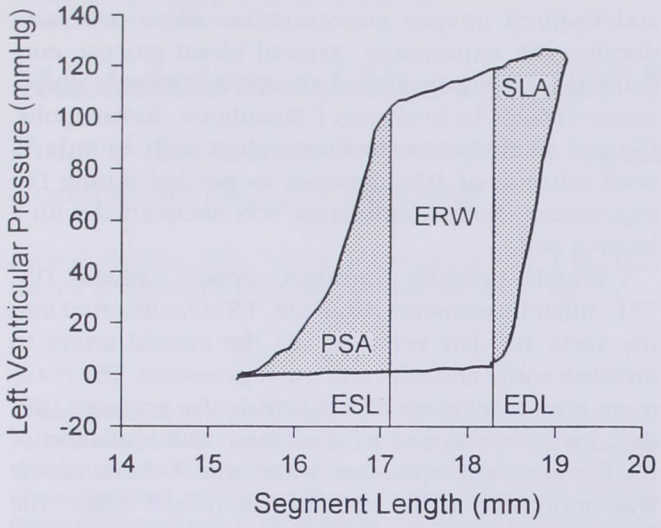


**Fig. 1. Left ventricular pressure-segment length diagram from a representative dog demonstrating effective regional work (ERW), postsystolic shortening area (PSA), and systolic lengthening area (SLA).**

and end-diastolic segment length (EDL) was determined 10 ms before dP/dt first exceeded 140 mmHg/sec$^{-1}$ (immediately before the onset of left ventricular isovolumic contraction). Percentage segment shortening was calculated using the formula %SS = (EDL − ESL) · 100 · EDL$^{-1}$. Left ventricular pressure-segment length diagrams were analyzed to determine effective regional work at selected intervals during each experiment.[23] Effective regional work (fig. 1) was determined by planimetry of the rectangular area delineated horizontally by the EDLs and ESLs and represents the total loop area minus postsystolic shortening area (defined as the loop area to the left of a perpendicular line drawn vertically through the end-systolic point) and systolic lengthening area (defined as the area of the loop to the right of a perpendicular line drawn vertically through the end-diastolic point).

*Regional Myocardial Blood Flow*

Carbonized plastic microspheres (15 ± 2 μm [SD] in diameter; New England Nuclear, Boston, MA) labeled with $^{141}$Ce, $^{103}$Ru, $^{51}$Cr, or $^{95}$Nb were used to measure regional myocardial perfusion. Immediately before injection, the microsphere suspension was ultrasonicated (model 450, E/MC) for 15 min. The injection consisted of 2 to 3 × 10$^6$ microspheres administered into the left atrium as a bolus during a 10-s period and flushed in with 10 ml warm (37°C) saline. A few seconds before the microsphere injection, a timed collection of reference arterial flow was started (precalibrated infusion-withdrawal pump, model 1941; Harvard, Natick, MA) from the femoral arterial catheter and withdrawn at a constant rate of 7 ml/min$^{-1}$ for 3 min.

Transmural tissue samples were selected for mapping tissue flow in the myocardium at the end of each experiment. The samples were obtained from two regions of the left ventricle: (1) normal zone (myocardium supplied by the left circumflex coronary artery) and (2) ischemic zone (distal to the LAD occlusion). At the conclusion of each experiment, two colored dyes (India ink and Patent Blue Dye) were injected into the coronary circulation immediately distal to the LAD occlusion and into the proximal left circumflex coronary artery at a pressure of 100 mmHg to identify accurately the ischemic and normal zones, respectively. The heart was immediately fibrillated, removed, and fixed in formalin overnight. On the following day, myocardial tissue samples were subdivided into subepicardial, mid-myocardial, and subendocardial layers of approximately equal thickness. Samples were weighed and placed in scintillation vials, and the activity of each isotope was determined. Similarly, the activity of each isotope in the reference blood sample was assessed. Tissue blood flow (ml · min$^{-1}$ · g$^{-1}$) was calculated as $Q_r \cdot C_m \cdot C_r,^{-1}$ where $Q_r$ = rate of withdrawal of the reference blood flow sample (ml/min$^{-1}$); $C_m$ = activity (cpm/g$^{-1}$) of the myocardial tissue sample; and $C_r$ = activity (cpm) of the reference blood flow sample. Transmural blood flow was considered as the average of the subepicardial, mid-myocardial, and subendocardial blood flows.

*Experimental Protocol*

Figure 2 shows the experimental design. Dogs were randomly assigned to receive 2 ml drug vehicle (50% polyethylene glycol in ethyl alcohol; control experiments) or glyburide (0.05 mg/kg$^{-1}$ administered intravenously) in the presence or absence of 1 MAC (end-tidal) isoflurane in four experimental groups. End-tidal concentrations of isoflurane were measured at the tip of the endotracheal tube by an infrared anesthetic analyzer (Datex Capnomac, Helsinki, Finland). The infrared analyzer was calibrated with known standards before and during the experiments. Thirty minutes after instrumentation was completed, baseline systemic and coronary hemodynamics were recorded. All dogs were subjected to five 5-min periods of LAD occlusion sepa-

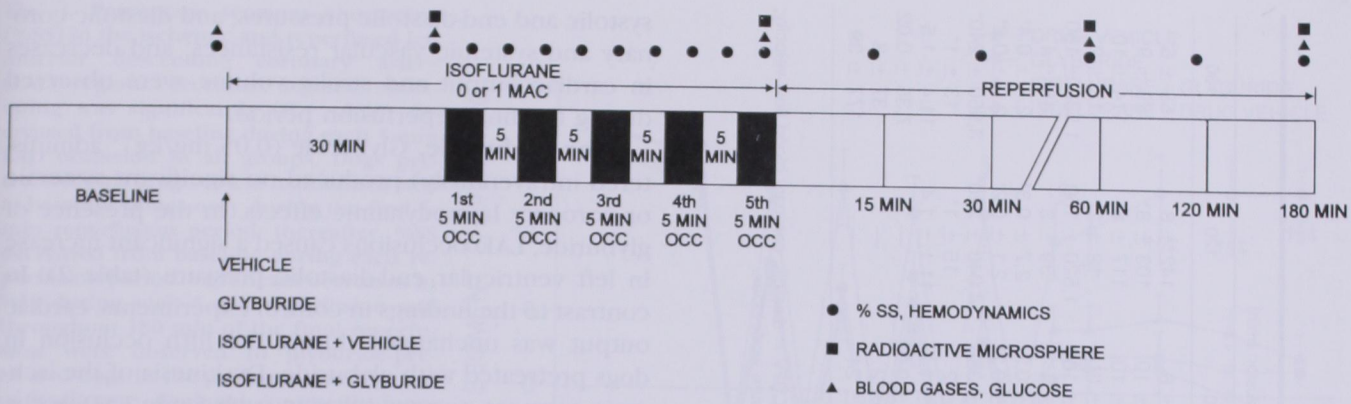


**Fig. 2. Schematic diagram of the experimental protocol used to study the role of isoflurane and $K_{ATP}$ channels in stunned myocardium produced by multiple left anterior descending (LAD) coronary artery occlusions and reperfusion. All dogs were subjected to five 5-min periods of LAD occlusion (OCC), interspersed with 5-min periods of reperfusion and followed by a final 180 min of reperfusion. Percentage segment shortening (%SS), systemic and coronary hemodynamics, arterial blood gases, blood glucose, and regional myocardial blood flow were measured at the indicated time intervals.**

rated by 5-min periods of reperfusion and followed by a final 180 min of reperfusion during which hemodynamics and contractile function were monitored continuously. Regional myocardial blood flow was measured at baseline, during the fifth coronary artery occlusion, and after 60 and 180 min of reperfusion. In the control group, dogs received drug vehicle during a period of 10 min and then 20 min later they underwent repetitive occlusion and reperfusion. In a second group of experiments conducted in an identical manner, dogs were pretreated with glyburide (0.05 mg/kg$^{-1}$) to examine the effect of $K_{ATP}$ channel blockade on recovery of function after multiple episodes of ischemia and reperfusion. In two other groups of experiments, the effects of isoflurane on recovery of stunned myocardium were assessed in dogs pretreated with drug vehicle or glyburide. Immediately after treatment with drug vehicle or glyburide, dogs received isoflurane (1 MAC) for a 30-min period before and continued throughout brief periods of LAD occlusion. Isoflurane was discontinued at the onset of the final reperfusion.

### *Statistical Analysis*

Statistical analysis of data within and between groups under baseline conditions, during drug and anesthetic interventions, LAD occlusions, and reperfusion was performed with multiple analysis of variance for repeated measures followed by application of the Student's *t* test with Bonferroni's correction for multiplicity. Changes within and between groups were considered statistically significant when the *P* value was less than 0.05. All data are expressed as mean ± SEM.

## Results

Thirty-two dogs (eight in each group) were instrumented to obtain 29 successful experiments. Three dogs died of ventricular fibrillation during the first 5 min of reperfusion. These dogs were excluded from data analysis.

### *Hemodynamic Effects of Ischemia and Reperfusion*

**In Control Experiments.** There were no differences in systemic and coronary hemodynamics, arterial blood gas tensions, or blood glucose concentrations across groups after completion of instrumentation and before experimental intervention. Left anterior descending coronary artery occlusions caused significant ($P < 0.05$) increases in left ventricular end-diastolic pressure and decreases in cardiac output (table 1). No changes in heart rate, mean arterial and left ventricular systolic pressures, $dP/dt_{max}$, systemic vascular resistance, stroke volume, and pressure-work index were observed. Systolic aneurysmal bulging of ischemic myocardium occurred during each 5-min LAD occlusion (fig. 3). Percentage segment shortening was unchanged from baseline during the first 5-min reperfusion in dogs (control) pretreated with drug vehicle. However, %SS was significantly decreased from baseline during each subsequent 5-min reperfusion and during the entire 180 min of final reperfusion. Impaired contractile function during reperfusion was also indicated by sustained decreases in effective regional work (fig. 4). Increases in heart rate, mean arterial pressure, left ventricular

**Table 1. Hemodynamic Effects of Myocardial Stunning in Control Dogs**

| | N | Baseline | Vehicle | 5th Occlusion | Final Reperfusion (min) | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | 15 | 30 | 60 | 120 | 180 |
| HR (beats/min) | 7 | 137 ± 6 | 137 ± 4 | 131 ± 7 | 142 ± 5 | 137 ± 6 | 142 ± 6 | 142 ± 8 | 158 ± 6* |
| MBP (mmHg) | 7 | 86 ± 5 | 82 ± 3 | 86 ± 5 | 96 ± 7 | 94 ± 8 | 98 ± 10 | 103 ± 8* | 99 ± 9 |
| LVSP (mmHg) | 7 | 98 ± 6 | 93 ± 4 | 95 ± 4 | 108 ± 7 | 108 ± 9 | 113 ± 10 | 117 ± 8* | 114 ± 10 |
| LVEDP (mmHg) | 7 | 7 ± 1 | 8 ± 1 | 13 ± 2* | 9 ± 2 | 10 ± 2 | 11 ± 2* | 12 ± 2* | 12 ± 2* |
| $+dP/dt_{max}$ (mmHg · s$^{-1}$) | 7 | 1,660 ± 170 | 1,600 ± 190 | 1,380 ± 140 | 1,450 ± 150 | 1,450 ± 170 | 1,500 ± 180 | 1,520 ± 160 | 1,530 ± 160 |
| DCBFV (Hz · $10^2$) | 7 | 45 ± 5 | 41 ± 4 | 0 | 40 ± 5 | 39 ± 4 | 39 ± 4 | 39 ± 3 | 38 ± 4 |
| DCVR (mmHg · Hz$^{-1}$ · $10^{-2}$) | 7 | 2.0 ± 0.3 | 2.1 ± 0.3 | — | 2.5 ± 0.4 | 2.5 ± 0.4 | 2.5 ± 0.3 | 2.7 ± 0.3* | 2.7 ± 0.4* |
| CO (L · min$^{-1}$) | 6 | 2.8 ± 0.3 | 2.6 ± 0.3 | 2.2 ± 0.2* | 2.3 ± 0.3 | 2.3 ± 0.2 | 2.2 ± 0.2* | 2.1 ± 0.2* | 2.0 ± 0.2* |
| SVR (dyne · s · cm$^{-5}$) | 6 | 2,630 ± 280 | 2,740 ± 280 | 3,350 ± 230 | 3,540 ± 320* | 3,410 ± 290 | 3,630 ± 380* | 4,040 ± 220* | 3,950 ± 240* |
| SV (ml) | 6 | 21 ± 3 | 19 ± 3 | 18 ± 3 | 16 ± 2 | 17 ± 2 | 16 ± 2 | 15 ± 1* | 13 ± 1* |
| PWI (ml · min$^{-1}$ · 100 g$^{-1}$) | 6 | 10.2 ± 0.5 | 9.4 ± 0.3 | 9.1 ± 0.6 | 10.5 ± 0.8 | 10.2 ± 1.0 | 10.7 ± 1.2 | 11.1 ± 1.4 | 11.4 ± 1.5 |
| *p*H | 7 | 7.40 ± 0.03 | 7.40 ± 0.03 | 7.35 ± 0.03 | — | — | 7.35 ± 0.02 | — | 7.35 ± 0.02 |
| $p_{CO_2}$ (mmHg) | 7 | 31 ± 3 | 30 ± 2 | 32 ± 2 | — | — | 34 ± 2 | — | 37 ± 3 |
| $p_{O_2}$ (mmHg) | 7 | 141 ± 26 | 124 ± 18 | 134 ± 17 | — | — | 164 ± 24 | — | 177 ± 39 |

Data are mean ± SEM.

HR = heart rate; MBP = mean aortic blood pressure; LVSP and LVEDP = left ventricular systolic and end-diastolic pressure, respectively; DCBFV = diastolic coronary blood flow velocity; DCVR = diastolic coronary vascular resistance; CO = cardiac output; SV = stroke volume; SVR = systemic vascular resistance; PWI = pressure work index.

*Significantly ($P < 0.05$) different from baseline.

systolic and end-diastolic pressures, and diastolic coronary and systemic vascular resistances; and decreases in cardiac output and stroke volume were observed during the final reperfusion period.

**After Glyburide.** Glyburide (0.05 mg/kg$^{-1}$ administered intravenously) produced no significant systemic or coronary hemodynamic effects. In the presence of glyburide, LAD occlusions caused a significant increase in left ventricular end-diastolic pressure (table 2). In contrast to the findings in control experiments, cardiac output was unchanged during the fifth occlusion in dogs pretreated with glyburide. Dyskinesia of the ischemic segment occurred during each 5-min LAD occlusion. Significant decreases in contractile function (%SS and effective regional work) were observed during the first 5-min reperfusion and continued throughout 180 min of the final reperfusion (figs. 3 and 4). Left ventricular end-diastolic pressure and systemic vascular resistance increased, and $dP/dt_{max}$ and cardiac output decreased during the final reperfusion in dogs pretreated with glyburide. No differences in hemodynamics, %SS, or effective regional work were observed between control and glyburide-pretreated dogs.

**After Isoflurane.** Isoflurane significantly decreased heart rate, mean arterial and left ventricular systolic pressures, $dP/dt_{max}$, cardiac output, diastolic coronary vascular resistance, pressure-work index (table 3), and effective regional work (66 ± 10% of baseline values). An increase in left ventricular end-diastolic pressure occurred during LAD occlusions. However, all other hemodynamic variables remained unchanged during repetitive ischemia and reperfusion. As compared with dogs that did not receive isoflurane, heart rate, mean arterial and left ventricular systolic pressures, myocardial contractility, and pressure-work index were significantly decreased by isoflurane during LAD occlusions. Equivalent degrees of systolic dyskinesia were produced by LAD occlusions in isoflurane-anesthetized dogs when compared with dogs not receiving this agent (figs. 3). In contrast to control and glyburide-pretreated dogs, %SS recovered to baseline values during the first and second 5-min reperfusion periods in dogs anesthetized with isoflurane. Regional contractile function also recovered to baseline values by 60 min during the final reperfusion (figs. 3 and 4). Percentage segment shortening and effective regional work were greater in dogs receiving isoflurane 30, 60, 120, and 180 min after reperfusion when compared with those findings in dogs not receiving isoflurane. Left ventricular $dP/dt_{max}$, cardiac output, and stroke volume were

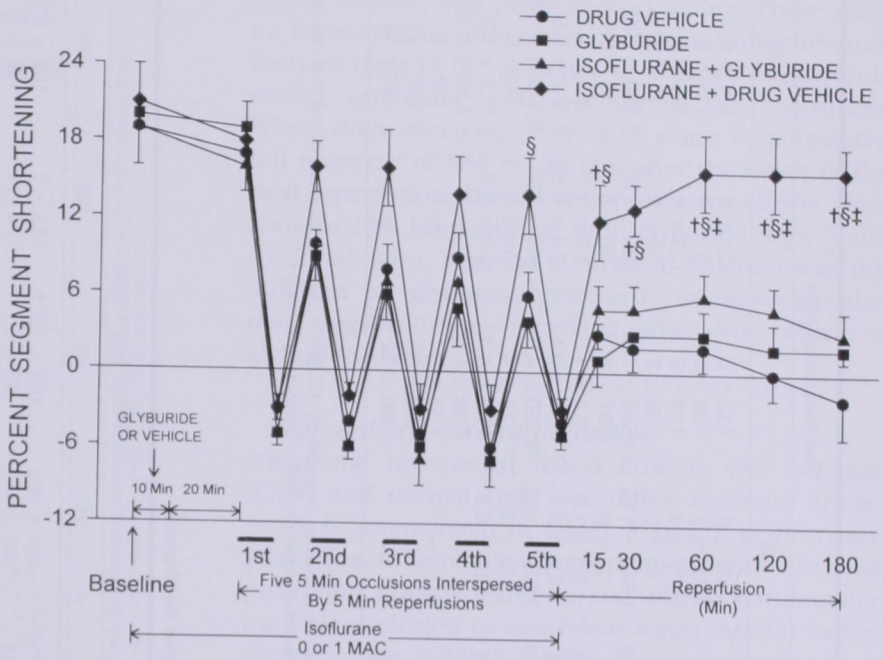


Fig. 3. Percentage segment shortening (%SS) in the ischemic and reperfused left anterior descending coronary artery (LAD) region. Percentage segment shortening was significantly ($P < 0.05$) decreased from baseline during each 5-min LAD occlusion in all groups. Dogs pretreated with drug vehicle maintained %SS at baseline values only during the first 5-min reperfusion period; thereafter, %SS decreased from baseline during each reperfusion period. Significant decreases in %SS during each 5-min reperfusion and throughout 180 min of the final reperfusion were observed in glyburide-pretreated dogs in the presence and absence of isoflurane. Regional contractile function recovered to baseline values during the first and second 5-min reperfusions and 60, 120, and 180 min after the final reperfusion in dogs receiving isoflurane alone.

†Significantly ($P < 0.05$) different from drug vehicle-pretreated dogs; §significantly ($P < 0.05$) different from glyburide-pretreated dogs; and ‡significantly ($P < 0.05$) different from dogs receiving glyburide and isoflurane.

decreased, and systemic vascular resistance was increased during reperfusion compared with baseline values. Changes in these variables in dogs receiving isoflurane were similar to those findings in control or glyburide-pretreated dogs.

**After Glyburide and Isoflurane.** Isoflurane caused hemodynamic effects in glyburide-pretreated dogs (table 4) that were very similar to those observed in dogs receiving isoflurane and drug vehicle. Effective regional work was similarly decreased by isoflurane (60 ± 9% of baseline values) in glyburide-pretreated dogs. Left anterior descending coronary artery occlusions increased left ventricular end-diastolic pressure and systemic vascular resistance and decreased cardiac output,

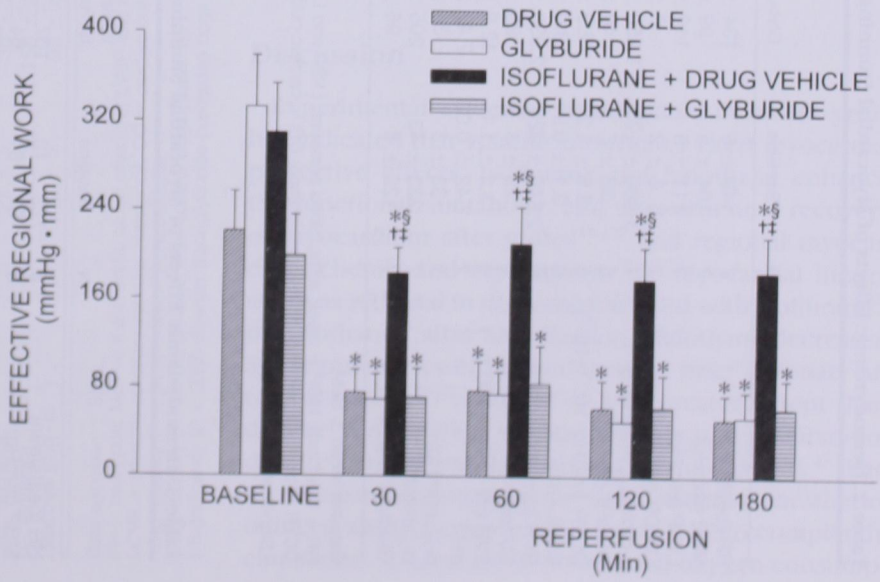


Fig. 4. Effective regional work at baseline and 30, 60, 120, and 180 min after reperfusion.

*Significantly ($P < 0.05$) different from baseline; †significantly ($P < 0.05$) different from drug vehicle-pretreated dogs; §significantly ($P < 0.05$) different from dogs receiving glyburide alone; and ‡significantly ($P < 0.05$) different from dogs receiving glyburide and isoflurane.

**Table 2. Hemodynamic Effects of Myocardial Stunning in Dogs Receiving Glyburide**

| | N | Baseline | Glyburide | 5th Occlusion | Final Reperfusion (min) 15 | 30 | 60 | 120 | 180 |
|---|---|---|---|---|---|---|---|---|---|
| HR (beats/min) | 7 | 140 ± 6 | 134 ± 3 | 125 ± 5 | 125 ± 5 | 127 ± 5 | 132 ± 8 | 133 ± 8 | 126 ± 9 |
| MBP (mmHg) | 7 | 104 ± 5 | 99 ± 3 | 96 ± 4 | 112 ± 6 | 109 ± 3 | 108 ± 6 | 117 ± 4 | 115 ± 6 |
| LVSP (mmHg) | 7 | 111 ± 5 | 110 ± 4 | 105 ± 5 | 121 ± 7 | 117 ± 3 | 118 ± 6 | 123 ± 4 | 122 ± 6 |
| LVEDP (mmHg) | 7 | 6 ± 2 | 7 ± 1 | 12 ± 2* | 9 ± 3 | 11 ± 3 | 9 ± 2 | 10 ± 2 | 11 ± 2* |
| $+dP/dt_{max}$ ($mmHg \cdot s^{-1}$) | 7 | 1,980 ± 120 | 1,850 ± 150 | 1,590 ± 200 | 1,620 ± 120 | 1,540 ± 110 | 1,550 ± 100 | 1,580 ± 120 | 1,530 ± 120* |
| DCBFV ($Hz \cdot 10^2$) | 5 | 39 ± 7 | 40 ± 9 | 0 | 38 ± 8 | 37 ± 8 | 37 ± 8 | 36 ± 8 | 35 ± 9 |
| DCVR ($mmHg \cdot Hz^{-1} \cdot 10^{-2}$) | 5 | 3.5 ± 1.2 | 2.9 ± 0.6 | — | 3.3 ± 0.9 | 3.5 ± 1.0 | 3.7 ± 1.0 | 4.0 ± 1.1 | 4.3 ± 1.7 |
| CO ($L \cdot min^{-1}$) | 6 | 3.1 ± 0.3 | 3.0 ± 0.4 | 2.8 ± 0.4 | 2.8 ± 0.4 | 2.7 ± 0.4 | 2.7 ± 0.4 | 2.4 ± 0.5* | 2.2 ± 0.5* |
| SVR ($dyne \cdot s \cdot cm^{-5}$) | 6 | 2,840 ± 220 | 2,930 ± 410 | 3,020 ± 320 | 3,640 ± 460 | 3,610 ± 500 | 3,520 ± 410 | 4,590 ± 700 | 5,170 ± 1100* |
| SV (ml) | 6 | 23 ± 3 | 23 ± 3 | 23 ± 4 | 23 ± 3 | 21 ± 3 | 21 ± 4 | 19 ± 5 | 20 ± 6 |
| PWI ($ml \cdot min^{-1} \cdot 100\ g^{-1}$) | 6 | 13.2 ± 1.1 | 12.0 ± 0.9 | 11.4 ± 0.9 | 12.3 ± 0.8 | 12.0 ± 0.8 | 11.8 ± 1.1 | 12.3 ± 0.9 | 11.4 ± 1.3 |
| *p*H | 7 | 7.42 ± 0.02 | 7.42 ± 0.03 | 7.39 ± 0.03 | — | — | 7.38 ± 0.03 | — | 7.38 ± 0.02 |
| $p_{CO_2}$ (mmHg) | 7 | 33 ± 3 | 34 ± 3 | 34 ± 2 | — | — | 33 ± 3 | — | 32 ± 2 |
| $p_{O_2}$ (mmHg) | 7 | 233 ± 43 | 205 ± 37 | 197 ± 43 | — | — | 156 ± 28 | — | 182 ± 47 |
| Glucose ($mg \cdot dl^{-1}$) | 7 | 120 ± 8 | 96 ± 10 | 77 ± 14 | — | — | 86 ± 12 | — | 96 ± 12 |

Data are mean ± SEM.

HR = heart rate; MBP = mean aortic blood pressure; LVSP and LVEDP = left ventricular systolic and end-diastolic pressure, respectively; DCBFV = diastolic coronary blood flow velocity; DCVR = diastolic coronary vascular resistance; CO = cardiac output; SV = stroke volume; SVR = systolic vascular resistance; PWI = pressure work index.

* Significantly ($P < 0.05$) different from baseline.

**Table 3. Hemodynamic Effects of Myocardial Stunning in Dogs Receiving Drug Vehicle and Isoflurane**

| | N | Baseline | Vehicle + Isoflurane | 5th Occlusion | Final Reperfusion (min) 15 | 30 | 60 | 120 | 180 |
|---|---|---|---|---|---|---|---|---|---|
| HR (beats/min) | 8 | 122 ± 10 | 107 ± 8*†‡ | 104 ± 6*† | 106 ± 7*† | 104 ± 9*† | 104 ± 10*† | 113 ± 7 | 109 ± 8*† |
| MBP (mmHg) | 8 | 97 ± 8 | 64 ± 8*‡ | 67 ± 4*†‡ | 82 ± 4‡ | 87 ± 4 | 96 ± 5 | 104 ± 6 | 106 ± 3 |
| LVSP (mmHg) | 8 | 108 ± 8 | 77 ± 8*‡ | 77 ± 4*‡ | 93 ± 6‡ | 97 ± 5 | 106 ± 5 | 114 ± 7 | 115 ± 4 |
| LVEDP (mmHg) | 8 | 7 ± 2 | 9 ± 2 | 11 ± 2* | 9 ± 2 | 8 ± 2 | 8 ± 2 | 8 ± 2 | 9 ± 2 |
| $+dP/dt_{max}$ ($mmHg \cdot s^{-1}$) | 8 | 1,620 ± 170 | 1,090 ± 150*‡ | 860 ± 70*‡ | 1,150 ± 100* | 1,240 ± 120* | 1,320 ± 100* | 1,410 ± 120* | 1,570 ± 130 |
| DCBFV ($Hz \cdot 10^2$) | 8 | 35 ± 4 | 36 ± 5 | 0 | 36 ± 5 | 31 ± 3 | 32 ± 5 | 31 ± 4 | 33 ± 5 |
| DCVR ($mmHg \cdot Hz^{-1} \cdot 10^{-2}$) | 8 | 2.7 ± 0.3 | 1.7 ± 0.3* | — | 2.3 ± 0.3 | 2.7 ± 0.3 | 3.1 ± 0.4 | 3.4 ± 0.3 | 3.3 ± 0.4 |
| CO ($L \cdot min^{-1}$) | 8 | 2.8 ± 0.3 | 2.0 ± 0.3* | 1.8 ± 0.2* | 2.2 ± 0.2 | 2.1 ± 0.2* | 1.9 ± 0.2* | 1.8 ± 0.1* | 1.8 ± 0.2* |
| SVR ($dyne \cdot s \cdot cm^{-5}$) | 8 | 3,270 ± 730 | 2,700 ± 310 | 3,070 ± 370 | 3,150 ± 310 | 3,750 ± 500 | 4,480 ± 630 | 4,970 ± 670* | 5,020 ± 610* |
| SV (ml) | 8 | 23 ± 2 | 19 ± 2 | 17 ± 2 | 21 ± 2 | 20 ± 2 | 18 ± 2 | 16 ± 1* | 17 ± 1* |
| PWI ($ml \cdot min^{-1} \cdot 100\ g^{-1}$) | 8 | 10.2 ± 1.0 | 6.6 ± 1.0*‡ | 6.4 ± 0.5*†‡ | 7.8 ± 0.6*‡ | 7.9 ± 0.7*‡ | 8.1 ± 0.6* | 9.0 ± 0.6 | 9.0 ± 0.5 |
| *p*H | 8 | 7.38 ± 0.04 | 7.40 ± 0.05 | 7.42 ± 0.05 | — | — | 7.43 ± 0.04 | — | 7.41 ± 0.04 |
| $p_{CO_2}$ (mmHg) | 8 | 32 ± 2 | 31 ± 2 | 29 ± 2 | — | — | 29 ± 1 | — | 28 ± 2 |
| $p_{O_2}$ (mmHg) | 8 | 157 ± 35 | 127 ± 8 | 172 ± 20 | — | — | 207 ± 30 | — | 248 ± 45 |

Data are mean ± SEM.

HR = heart rate; MBP = mean aortic blood pressure; LVSP and LVEDP = left ventricular systolic and end-diastolic pressure, respectively; DCBFV = diastolic coronary blood flow velocity; DCVR = diastolic coronary vascular resistance; CO = cardiac output; SV = stroke volume; SVR = systemic vascular resistance; PWI = pressure work index.

* Significantly ($P < 0.05$) different from baseline.

† Significantly ($P < 0.05$) different from control dogs (Table 1).

‡ Significantly ($P < 0.05$) different from glyburide-pretreated dogs (Table 2).

Table 4. Hemodynamic Effects of Myocardial Stunning in Dogs Receiving Glyburide and Isoflurane

| | N | Baseline | Glyburide + Isoflurane | 5th Occlusion | Final Reperfusion (min) 15 | 30 | 60 | 120 | 180 |
|---|---|---|---|---|---|---|---|---|---|
| HR (beats/min) | 7 | 125 ± 7 | 102 ± 6*† | 103 ± 5*† | 104 ± 6*† | 104 ± 6*† | 106 ± 8*† | 109 ± 9*† | 111 ± 10† |
| MBP (mmHg) | 7 | 89 ± 4 | 66 ± 6‡ | 71 ± 6‡ | 83 ± 6‡ | 86 ± 7 | 92 ± 8 | 98 ± 10 | 96 ± 9 |
| LVSP (mmHg) | 7 | 100 ± 4 | 74 ± 4‡ | 83 ± 6 | 94 ± 6‡ | 97 ± 7 | 104 ± 7 | 107 ± 9 | 109 ± 7 |
| LVEDP (mmHg) | 7 | 9 ± 2 | 10 ± 2 | 14 ± 2* | 10 ± 2 | 9 ± 1 | 9 ± 1 | 9 ± 2 | 10 ± 1 |
| $+dP/dt_{max}$ (mmHg · $s^{-1}$) | 7 | 1,580 ± 90 | 990 ± 60*†‡ | 905 ± 80*‡ | 1,110 ± 100*‡ | 1,130 ± 90* | 1,260 ± 130 | 1,280 ± 100 | 1,300 ± 110 |
| DCBFV (Hz · $10^2$) | 6 | 37 ± 7 | 35 ± 4 | 0 | 33 ± 5 | 32 ± 5 | 33 ± 5 | 33 ± 5 | 34 ± 5 |
| DCVR (mmHg · $Hz^{-1}$ · $10^{-2}$) | 6 | 2.4 ± 0.3 | 1.7 ± 0.2 | — | 2.3 ± 0.3 | 2.5 ± 0.3 | 2.5 ± 0.2 | 2.8 ± 0.3 | 2.8 ± 0.3 |
| CO (L · $min^{-1}$) | 6 | 3.4 ± 0.3 | 2.3 ± 0.3* | 1.5 ± 2*‡ | 2.3 ± 0.3* | 2.1 ± 0.3* | 2.1 ± 0.2* | 1.7 ± 0.3* | 1.7 ± 0.3* |
| SVR (dyne · s · $cm^{-5}$) | 6 | 2,150 ± 230 | 2,460 ± 360 | 4,240 ± 430* | 3,190 ± 340 | 3,630 ± 270 | 3,710 ± 340 | 4,980 ± 600* | 5,180 ± 770* |
| SV (ml) | 6 | 27 ± 4 | 22 ± 3 | 14 ± 2* | 21 ± 2 | 19 ± 2 | 19 ± 1 | 14 ± 1* | 14 ± 2* |
| PWI (ml · $min^{-1}$ · 100 $g^{-1}$) | 6 | 10.7 ± 0.5 | 6.7 ± 0.7*‡ | 6.4 ± 0.7*‡ | 8.2 ± 0.8‡ | 8.4 ± 1.0‡ | 9.1 ± 1.0 | 8.5 ± 1.3 | 8.3 ± 1.2 |
| *pH* | 7 | 7.44 ± 0.03 | 7.42 ± 0.03 | 7.43 ± 0.02 | — | — | 7.40 ± 0.02 | — | 7.39 ± 0.02 |
| $P_{CO_2}$ (mmHg) | 7 | 27 ± 3 | 30 ± 3 | 27 ± 2 | — | — | 30 ± 3 | — | 28 ± 2 |
| $P_{O_2}$ (mmHg) | 7 | 182 ± 32 | 158 ± 25 | 173 ± 35 | — | — | 173 ± 9 | — | 165 ± 13 |
| Glucose (mg · $dl^{-1}$) | 7 | 110 ± 5 | 92 ± 8 | 74 ± 12 | — | — | 87 ± 12 | — | 83 ± 13 |

Data are mean ± SEM.
HR = heart rate; MBP = mean aortic blood pressure; LVSP and LVEDP = left ventricular systolic and end-diastolic pressure, respectively; DCBFV = diastolic coronary blood flow velocity; DCVR = diastolic coronary vascular resistance; CO = cardiac output; SV = stroke volume; SVR = systemic vascular resistance; PWI = pressure work index.
* Significantly ($P < 0.05$) different from baseline.
† Significantly ($P < 0.05$) different from control dogs (Table 1).
‡ Significantly ($P < 0.05$) different from glyburide-pretreated dogs (Table 2).

stroke volume, and pressure-work index. There were no hemodynamic differences between isoflurane-anesthetized dogs in the presence or absence of glyburide during isoflurane, LAD occlusions, or reperfusion. While dogs receiving isoflurane alone demonstrated full recovery of %SS by 60 min after the onset of the final reperfusion, these cardioprotective effects were abolished by pretreatment with glyburide (figs. 3 and 4). In addition, regional contractile function was not different in glyburide-pretreated, isoflurane-anesthetized dogs from dogs receiving either drug vehicle or glyburide alone.

*Regional Myocardial Perfusion*

Regional myocardial blood flow in the ischemic (LAD) and normal (left circumflex coronary artery) zones is summarized in tables 5 and 6, respectively. Blood flow to ischemic myocardium decreased in subepicardium, mid-myocardium, and subendocardium during LAD occlusion to equivalent levels in each experimental group (table 5), indicating a similar degree of coronary collateral blood flow is present in dogs receiving drug vehicle or glyburide with or without isoflurane. During baseline conditions, blood flow to the subendocardium in the LAD region (table 5) and flow to the mid-myocardium and subendocardium of the normal left circumflex coronary artery region (table 6) was lower in dogs receiving glyburide and isoflurane compared with glyburide alone. There were no differences between experimental groups in blood flow to ischemic or normal myocardium during reperfusion.

## Discussion

Experimental evidence accumulated in recent years has indicated that volatile anesthetics exert myocardial protective effects. Isoflurane and halothane enhance the functional, metabolic, and ultrastructural recovery of myocardium after global[10–15] and regional myocardial ischemia and reperfusion.[16–19] Myocardial infarct size was reduced in dogs anesthetized with isoflurane[24] or halothane[25] after LAD ligation. Halothane decreased S-T segment elevation produced by brief coronary artery occlusion[26,27] and did so to a greater extent than did the combination of nitroprusside and propranolol despite causing similar hemodynamic changes.[27] The mechanism responsible for these volatile anesthetic-induced antiischemic actions has not been completely elucidated. A decrease in myocardial oxygen consump-

**Table 5. Effects of Myocardial Stunning on Myocardial Perfusion ($ml \cdot min^{-1} \cdot g^{-1}$) in the Ischemic (LAD) Region**

| | | | Final Reperfusion (min) | |
|---|---|---|---|---|
| | Baseline | 5th Occlusion | 60 | 180 |
| Control | | | | |
| Subepicardium | 0.89 ± 0.10 | 0.12 ± 0.04* | 1.12 ± 0.15 | 1.19 ± 0.08 |
| Midmyocardium | 0.85 ± 0.04 | 0.09 ± 0.04* | 0.86 ± 0.08 | 0.94 ± 0.06 |
| Subendocardium | 0.91 ± 0.05 | 0.05 ± 0.03* | 0.82 ± 0.06 | 0.89 ± 0.07 |
| Transmural | 0.88 ± 0.02 | 0.09 ± 0.02* | 0.93 ± 0.09 | 1.01 ± 0.06 |
| Glyburide | | | | |
| Subepicardium | 0.88 ± 0.11 | 0.15 ± 0.07* | 1.20 ± 0.31 | 1.14 ± 0.14 |
| Midmyocardium | 0.90 ± 0.12 | 0.07 ± 0.03* | 0.84 ± 0.15 | 0.74 ± 0.08 |
| Subendocardium | 0.95 ± 0.11 | 0.05 ± 0.02* | 0.94 ± 0.17 | 0.73 ± 0.11 |
| Transmural | 0.91 ± 0.10 | 0.09 ± 0.04* | 0.99 ± 0.20 | 0.87 ± 0.10 |
| Vehicle + isoflurane | | | | |
| Subepicardium | 0.88 ± 0.14 | 0.14 ± 0.04* | 1.01 ± 0.23 | 1.11 ± 0.19 |
| Midmyocardium | 0.76 ± 0.13 | 0.06 ± 0.03* | 0.75 ± 0.07 | 0.77 ± 0.06 |
| Subendocardium | 0.78 ± 0.09 | 0.07 ± 0.02* | 0.74 ± 0.06 | 0.78 ± 0.08 |
| Transmural | 0.81 ± 0.10 | 0.09 ± 0.02* | 0.83 ± 0.10 | 0.89 ± 0.08 |
| Glyburide + isoflurane | | | | |
| Subepicardium | 0.74 ± 0.09 | 0.09 ± 0.01* | 1.26 ± 0.23 | 1.23 ± 0.23 |
| Midmyocardium | 0.51 ± 0.04 | 0.05 ± 0.02* | 0.82 ± 0.14 | 0.73 ± 0.14 |
| Subendocardium | 0.58 ± 0.03† | 0.09 ± 0.04* | 0.87 ± 0.11* | 0.76 ± 0.13 |
| Transmural | 0.61 ± 0.05 | 0.07 ± 0.02* | 0.98 ± 0.16* | 0.90 ± 0.15 |

Data are mean ± SEM.

LAD = left anterior descending coronary artery.

* Significantly ($P < 0.05$) different from baesline.

† Significantly ($P < 0.05$) different from glyburide-pretreated dogs.

tion produced by volatile anesthetics has been proposed as a potential mechanism of protection by these agents in ischemic myocardium, although this is controversial. Halothane, but not isoflurane, decreased myocardial oxygen consumption as estimated by the rate-pressure product, although both anesthetics were equally protective against myocardial stunning in a previous investigation from this laboratory.[19] Recent evidence indicates that halothane may even produce beneficial effects during complete functional cardiac arrest.[15] Isoflurane has been shown to exert antiischemic actions during ischemia and reperfusion despite maintenance of heart rate and arterial pressure at control values.[18] These findings suggest that favorable changes in myocardial oxygen supply-and-demand relations are not solely responsible for the beneficial actions of isoflurane and halothane in this setting, and they support the hypothesis that volatile anesthetics may also cause myocardial protection *via* other mechanisms, such as a reduction in excessive intracellular calcium accumulation. To date, the mechanism of action of volatile anesthetics to reduce ischemia and reperfusion injury remains highly speculative.

Acute coronary artery occlusion results in a contractile deficit within the ischemic myocardium characterized by initial dyskinesia that progresses rapidly to holosystolic aneurysmal bulging.[28] Brief periods of coronary artery occlusion (less than 20 min) followed by reperfusion, although they do not cause tissue necrosis, produce prolonged contractile dysfunction, declines in tissue adenine nucleotide content, and abnormalities in myocyte ultrastructure. This condition is called myocardial stunning.[29] Brief, repetitive periods of myocardial ischemia have also been shown to sensitively elicit contractile dysfunction after ischemia and reperfusion.[6-9] Multiple mechanisms probably contribute to the phenomenon of myocardial stunning, and altered intracellular calcium regulation is likely to assume a key role in its pathogenesis.[30] Activation of $K_{ATP}$ channels was recently shown to enhance the functional recovery of stunned myocardium,[1] reduce myocardial infarct size,[2] and modulate ischemic preconditioning.[3] $K_{ATP}$ channel activation may exert these antiischemic effects *via* a reduction in abnormal intracellular calcium concentration associated with myocardial injury. The current investigation examined the actions of

**Table 6. Effects of Myocardial Stunning on Myocardial Perfusion ($ml \cdot min^{-1} \cdot g^{-1}$) in the Normal (LCCA) Region**

| | Baseline | 5th Occlusion | Final Reperfusion (min) | |
|---|---|---|---|---|
| | | | 60 | 180 |
| Control | | | | |
| Subepicardium | 0.88 ± 0.12 | 0.98 ± 0.18 | 0.88 ± 0.13 | 1.13 ± 0.12 |
| Midmyocardium | 0.92 ± 0.04 | 1.10 ± 0.07 | 1.03 ± 0.10 | 1.19 ± 0.08 |
| Subendocardium | 0.88 ± 0.07 | 1.07 ± 0.04 | 1.03 ± 0.10 | 1.08 ± 0.10 |
| Transmural | 0.89 ± 0.02 | 1.05 ± 0.09 | 0.98 ± 0.10 | 1.14 ± 0.10 |
| Glyburide | | | | |
| Subepicardium | 0.81 ± 0.14 | 0.84 ± 0.15 | 0.87 ± 0.21 | 0.99 ± 0.12 |
| Midmyocardium | 1.02 ± 0.14 | 1.13 ± 0.13 | 1.00 ± 0.18 | 1.05 ± 0.10 |
| Subendocardium | 1.16 ± 0.16 | 1.30 ± 0.16 | 1.17 ± 0.19 | 1.13 ± 0.17 |
| Transmural | 0.99 ± 0.14 | 1.09 ± 0.13 | 1.01 ± 0.19 | 1.06 ± 0.11 |
| Vehicle + isoflurane | | | | |
| Subepicardium | 0.79 ± 0.09 | 0.89 ± 0.16 | 0.82 ± 0.06 | 0.87 ± 0.13 |
| Midmyocardium | 0.82 ± 0.11 | 0.87 ± 0.12 | 0.82 ± 0.06 | 0.91 ± 0.06 |
| Subendocardium | 0.90 ± 0.09 | 0.95 ± 0.16 | 0.89 ± 0.06 | 0.95 ± 0.03 |
| Transmural | 0.84 ± 0.09 | 0.91 ± 0.13 | 0.84 ± 0.06 | 0.91 ± 0.06 |
| Glyburide + isoflurane | | | | |
| Subepicardium | 0.55 ± 0.06 | 0.93 ± 0.16 | 1.01 ± 0.16 | 0.96 ± 0.16 |
| Midmyocardium | 0.54 ± 0.04* | 0.84 ± 0.12 | 0.90 ± 0.12 | 0.84 ± 0.11 |
| Subendocardium | 0.62 ± 0.04* | 0.96 ± 0.13 | 1.00 ± 0.12 | 0.92 ± 0.10 |
| Transmural | 0.57 ± 0.05* | 0.91 ± 0.13 | 0.97 ± 0.13 | 0.91 ± 0.11 |

Data are mean ± SEM.

LCCA = left circumflex coronary artery.

* Significantly ($P < 0.05$) different from glyburide-pretreated.

isoflurane to enhance functional recovery of stunned myocardium and tested the hypothesis that the beneficial actions of isoflurane are mediated by $K_{ATP}$ channel activation.

The present results confirm and extend previous findings from this laboratory[19] and demonstrate that isoflurane promotes recovery of ischemic zone contractile function to baseline values within 60 min after reperfusion. Isoflurane caused reversal of diastolic creep (increases in unstressed segment length) associated with ischemia and reperfusion, also indicating enhancement of recovery of contractile function.[31] Isoflurane favorably altered ischemia-induced abnormalities in the configuration of the left ventricular pressure-segment length diagram (fig. 5).[23,32] The effective regional myocardial shortening that contributes to ejection (effective regional work) was shown to be a sensitive index of contractile function during and after myocardial ischemia.[23] Dogs receiving isoflurane demonstrated significantly greater effective regional work (fig. 4) after repetitive ischemia and reperfusion than did dogs receiving drug vehicle or glyburide alone. The improvement in recovery of function produced by isoflurane was eliminated, however, in those dogs pretreated with glyburide.

The protective effects of isoflurane on postischemic reperfused myocardium in the present investigation were probably not mediated by isoflurane-induced reductions in myocardial oxygen consumption. The pressure-work index, a calculated estimate of myocardial oxygen consumption previously validated in dogs,[22] decreased to equivalent degrees in dogs receiving isoflurane in the presence or absence of glyburide. However, recovery of contractile function during reperfusion occurred only in dogs receiving isoflurane and drug vehicle. In addition, no differences in systemic hemodynamics were observed between drug vehicle- and glyburide-pretreated dogs receiving isoflurane. Although the pressure-work index has been shown to accurately reflect measured myocardial oxygen consumption under a wide variety of contractile states and loading conditions *in vivo*,[33] coronary sinus oxygen tension was not determined and direct measurements of myocardial oxygen consumption were not made in the present investigation. The present results suggest that isoflur-

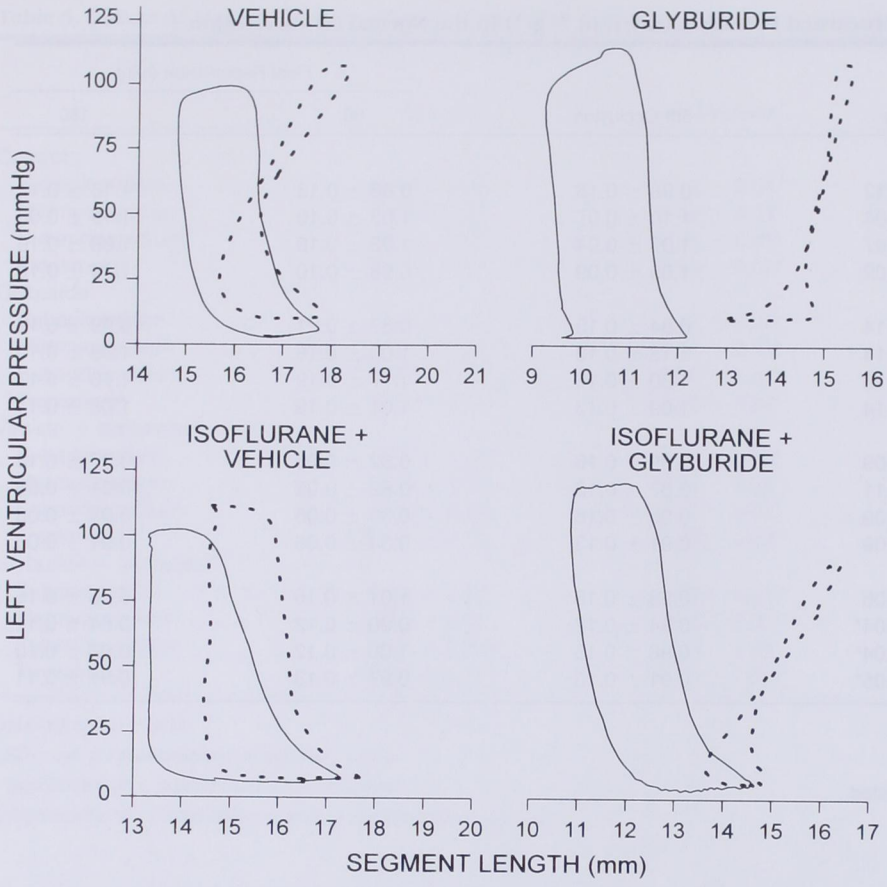


**Fig. 5. Left ventricular pressure-segment length diagrams from the ischemic region in a representative dog from each experimental group. The solid lines indicate diagrams obtained at baseline; the dashed lines represent diagrams obtained 180 min after coronary blood flow was reestablished. Myocardial contractile dysfunction is evident in dogs pretreated with drug vehicle, glyburide, and isoflurane and glyburide after 180 min of final reperfusion, in contrast to findings in dogs receiving isoflurane alone.**

ane-induced alterations in primary determinants of myocardial oxygen consumption and ventricular loading conditions do not account for the antiischemic effects of this volatile anesthetic. Alternatively, isoflurane-induced myocardial protection might require both $K_{ATP}$ channel activation and favorable alterations in myocardial oxygen supply-and-demand relations.

Dogs receiving isoflurane and drug vehicle showed recovery of segment shortening to 76 ± 6% of baseline 120 min after reperfusion. The present results are similar to those observed with the $K_{ATP}$ channel agonist, aprikalim, in the same multiple occlusion model of myocardial stunning, and in the absence of any hemodynamic effects. Dogs receiving aprikalim demonstrated recovery of segment shortening to 67 ± 6% of baseline 120 min after reperfusion.[6] Pretreatment with glyburide abolished the myocardial protective effects of isoflurane and aprikalim in the present and in a previous investigation,[1] respectively. These findings indicate that the actions of isoflurane to enhance functional recovery of myocardium are mediated by $K_{ATP}$ channel activation. The selectivity of glyburide for antagonism of $K_{ATP}$ channels in ischemic myocardium has been shown by findings that the antiischemic effects of calcium channel blockers, sodium channel antagonists, and calmodulin antagonists were not inhibited by glyburide.[34] Although glyburide lacks complete specificity, the low dose used in this investigation was unlikely to cause nonspecific effects on functional recovery of myocardium. In dogs pretreated with glyburide or drug vehicle that did not receive isoflurane, equivalent degrees of contractile dysfunction occurred during reperfusion. Thus the dose of glyburide used in this investigation did not exacerbate the impairment of contractile function during reperfusion in the absence of isoflurane. Importantly, dyskinesia (negative %SS) that occurred during each 5-min occlusion was identical in all four experimental groups, indicating that the severity of ischemia was similar among groups. Extracellular acidification, as occurs during ischemia, markedly enhances the effectiveness of low doses of glyburide to

block myocardial $K_{ATP}$ channels.[35] Therefore, nonspecific actions of glyburide are not likely to account for the findings of this investigation.

Multiple brief periods of ischemia limit myocardial injury after a subsequent, more sustained ischemic period, a phenomenon known as preconditioning.[36] A similar mechanism may be evoked during myocardial stunning induced with multiple 5-min coronary occlusions.[7] Ischemic preconditioning is inhibited by glyburide and mimicked by $K_{ATP}$ channel agonists administered before myocardial ischemia.[3] In the present investigation, dogs receiving isoflurane alone demonstrated full recovery of %SS during the first 5-min reperfusion period. Thus it is possible that isoflurane-induced activation of $K_{ATP}$ channels before and during the first ischemic period may have delayed or reduced myocardial injury through preconditioning. $K_{ATP}$ channels open during ischemia after a reduction in intracellular adenosine triphosphate concentration or, more likely, after a decrease in the ratio of adenosine triphosphate to adenosine diphosphate.[37] These changes decrease action potential duration and attenuation of membrane depolarization.[38] These effects may reduce intracellular calcium overload and delay ischemic cellular injury. In contrast, dogs receiving isoflurane and glyburide showed a significant decrement in %SS during the first reperfusion period, consistent with a previous finding indicating that glyburide blocks preconditioning.[3] Whether isoflurane administered before but not during ischemia produces protective effects similar to that of myocardial preconditioning has not yet been determined. $K_{ATP}$ channels were also shown to be coupled to adenosine receptors by G proteins in ventricular myocytes.[39] Whether isoflurane interacts with adenosine or adenosine receptors to alter mechanical recovery of postischemic reperfused myocardium is unknown.

$K_{ATP}$ channels have been identified in both ventricular myocardium[38] and in coronary vascular smooth muscle cells.[40] Activation of these channels in vascular smooth muscle by ischemia, hypoxia, or $K_{ATP}$ channel agonists causes vasodilation.[40] Recently isoflurane *in vivo*[20] and halothane *in vitro*[21] have been shown to produce coronary vasodilation through activation of $K_{ATP}$ channels, an effect that was also inhibited by glyburide. In the present investigation, isoflurane caused decreases in diastolic coronary vascular resistance in drug vehicle- but not glyburide-pretreated dogs, suggesting that isoflurane may cause coronary artery vasodilation mediated by $K_{ATP}$ channel activation. Myocardial perfusion was not different during control conditions in dogs receiving isoflurane with and without glyburide. In addition, coronary collateral blood flow was low (less than 0.10 $ml \cdot min^{-1} \cdot g^{-1}$) and no differences in collateral perfusion were observed between these groups. These results support the conclusion of a previous study that glyburide blocks functional recovery of stunned myocardium independent of changes in coronary collateral blood flow.[1]

The ability of glyburide to alter blood glucose concentration by blocking $K_{ATP}$ channels in $\beta$ cells of the pancreas may have influenced myocardial recovery after ischemia. However, the dose of glyburide used to inhibit $K_{ATP}$ channel function in this investigation was lower than that previously reported,[2,3] and blood glucose levels remained unchanged in dogs receiving glyburide. Recovery of function after multiple brief coronary artery occlusions was shown previously to occur independent of changes in blood glucose, but instead is linked to $K_{ATP}$ channel activation with subsequent shortening of monophasic action potential duration.[6] Glyburide prevented these reductions in monophasic action potential duration and blocked the associated recovery of mechanical function. Thus it is unlikely that a metabolic effect of glyburide was responsible for abolishing the effects of isoflurane to enhance recovery of postischemic reperfused myocardium. High doses of barbiturates inhibit $K_{ATP}$ channels *in vitro*.[41] However, previous work showed that functional recovery of stunned myocardium after coronary artery occlusion and reperfusion is not different in barbiturate-anesthetized compared with conscious dogs.[42] Therefore, it is unlikely that basal barbiturate anesthesia substantially altered the findings of this investigation.

In summary, the present results show that isoflurane is associated with enhanced recovery of ischemic zone contractile function during reperfusion after repetitive coronary artery occlusions. This beneficial effect is abolished by pretreatment with the selective $K_{ATP}$ channel antagonist glyburide. The results indicate that the beneficial actions of isoflurane in postischemic, reperfused myocardium are mediated by $K_{ATP}$ channel activation in barbiturate-anesthetized dogs during open-chest procedures.

The authors thank David Schwabe for technical assistance and Angela Barnes and Anita Tredeau for assistance in preparing the manuscript.

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