Anesthesiology
1998; 88:233-44

Improved Contractility and Coronary Flow in Isolated Hearts after 1-Day Hypothermic Preservation with Isoflurane Is Not Dependent on K_{ATP} Channel Activation

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Background: **Isoflurane protects against reperfusion injury in isolated hearts when given before, during, and initially after hypoxia or ischemia and aids in preconditioning hearts if given before ischemia. The aims of the current study were to determine if isoflurane is cardioprotective during 1-day, severe hypothermic perfusion and if a mechanism of protection is K_{ATP} channel activation.**

Methods: **Guinea pig hearts (n = 60) were isolated, perfused with Kreb's solution initially at 37°C, and assigned to either a nontreated warm, time control group or one of five cold-treated groups: drug-free cold control, 1.3% isoflurane, 1.3% isoflurane plus glibenclamide (4 μM), 2.6% isoflurane, or 2.6% isoflurane plus glibenclamide. Isoflurane and glibenclamide were given 20 min before hypothermia, during low-flow hypothermia (3.8°C) for 22 h, and for 30 min after rewarming to 37°C. Heart rate, left ventricular pressure, $\%O_2$ extraction, and coronary flow were measured continuously, and responses to epinephrine, adenosine, 5-hydroxytryptamine, and nitroprusside were examined before and after hypothermia.**

Results: **Each group had similar initial left ventricular pressures, coronary flows, and responses to adenosine, 5-hydroxytryptamine, and nitroprusside. Before hypothermia, isoflurane with or without glibenclamide increased coronary flow while decreasing left ventricular pressure and $\%O_2$ extraction. After hypothermia, left ventricular pressure and coronary flow were reduced in all cold groups but least reduced in isoflurane-treated groups. During normothermic perfusion after isoflurane and glibenclamide, left ventricular pressure, coronary flow, $\%O_2$ extraction, and flow responses to adenosine, 5-hydroxytryptamine, and nitroprusside were similarly improved in isoflurane and isoflurane-plus-glibenclamide groups over the cold control group but not to levels observed in the warm-time control group.**

Conclusion: **Isoflurane, like halothane, given before, during, and initially after hypothermia markedly improved but did not restore cardiac perfusion and function. Protective effects of isoflurane were not concentration dependent and not inhibited by the K_{ATP} channel blocker glibenclamide. Volatile anesthetics have novel cardioprotective effects when given during long-term severe hypothermia. (Key words: Cardiac efficiency; guinea pig; glibenclamide; left ventricular pressure; oxygen extraction.)**

PRESERVATION of donor hearts for longer periods would allow a greater and more easily attainable supply of hearts for transplantation. Previous studies from our laboratory[1-5] indicate that long-term (1-day) *ex vivo* cardiac protection can be attained in guinea pig hearts by perfusing hearts with cold (3.8°C), normal ionic, oxygenated physiologic salt solution containing reversible metabolic inhibitors and vasodilators such as 2,3-butanedione monoxime, adenosine, nitrobenzylthioinosine, nitroprusside, or bimakalim. We reported recently that halothane also protected hearts when given during long-term hypothermic protection, that this protection was independent of the concentrations given, and that the protection was greater than that elicited by reducing extracellular calcium.[6]

Isoflurane and other volatile anesthetic agents may be cardioprotective because they depress contractile function by several interrelated mechanisms.[7-12] We have reported that isoflurane and halothane protect against cardiac damage after hypoxic perfusion[13] and that halothane improves function after reperfusion after ischemia.[14,15] Improved basal coronary flow (CF) and responsiveness to vasodilators after long-term hypothermia[1-3] also may play a role in cardioprotection by anes-

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Received from the Anesthesiology Research Laboratory, Departments of Anesthesiology and Physiology, Cardiovascular Research Center, and Veterans Affairs Medical Center, Medical College of Wisconsin, Milwaukee, Wisconsin. Submitted for publication February 5, 1997. Accepted for publication September 29, 1997. Supported in part by the Veterans Administration Merit grant 8204-04P to Dr. Stowe and the American Heart Association of Wisconsin grants 95-GS-85 to Dr. Stowe and 94-post-26 to Dr. Fujita. A preliminary report of the results presented here appears in abstract form in ANESTHESIOLOGY 1996;85:A551.

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thetics. Induced K_{ATP} opening affords cardioprotection during hypothermia[5]; thus, anesthetics may protect by inducing K_{ATP} channel opening during hypothermia. Coronary vasodilation by volatile anesthetics may be mediated in part by the K_{ATP} channel.[15,17]

The first aim was to examine if isoflurane, like halothane, is effective in improving cardiac function after 1-day, low-flow hypothermic perfusion. The second aim was to determine if isoflurane's protective effect is mediated through K_{ATP} channel activation, which results in K^+ efflux and reduced intracellular Ca^{2+}. Isolated hearts were treated before, during, and initially after hypothermia with either of two concentrations of isoflurane (1.3 or 2.6% by volume), with and without glibenclamide, a K_{ATP} channel antagonist. Cardioprotective effects of isoflurane were evidenced on normothermic reperfusion by improved cardiac rate and rhythm, left ventricular (LV) systolic and diastolic pressure, basal CF and vascular resistance, flow responses to endothelium-dependent (5-hydroxytryptamine [5-HT]) and -independent (nitroprusside) vasodilators, $\%O_2$ extraction, myocardial O_2 consumption, and relative cardiac efficiency.

Methods

Preparation and Measurements

Approval from the institutional Animal Care Committee was obtained before initiation of this study. The investigation conformed with the *Guide for the Care and Use of Laboratory Animals* published by the United States National Institutes of Health. Albino English short-haired guinea pigs weighing 400–600 g were injected intraperitoneally with 10 mg of ketamine and 1,000 U of heparin and decapitated when unresponsive to noxious stimulation. Methods of isolation and preparation of hearts in this study have been detailed in recent reports.[1–6,14,15] The inferior and superior venae cavae were cut after thoracotomy, and the aorta was cannulated distal to the aortic valve. Each heart was immediately perfused and then excised. Except during hypothermia, all hearts were perfused *via* the aortic root at a constant pressure of 55 mmHg. The perfusate, a modified Krebs-Ringer solution, was filtered (5-μm pore size) in-line (Cole Palmer, Vernon Hills, IL) and had the following control composition in mM: Na^+, 137.0; K^+, 5.0; Mg^{2+}, 1.2; Ca^{2+}, 2.5; Cl^-, 134.0; HCO_3^-, 15.5; $H_2PO_4^-$, 1.2; pyruvate, 2.0; glucose, 11.5; mannitol, 16.0; glutamate, 0.05; ethylenediaminetetraacetic acid, 0.05; and insulin, 5 U/l. Isovolumetric LV pressure (LVP) was measured by a transducer connected to a thin, saline-filled latex balloon inserted into the left ventricle through the mitral valve from a cut in the left atrium. Balloon volume was adjusted during the initial control period to obtain a diastolic LVP of 0 mmHg. Two pairs of bipolar electrodes were placed in the right atrial and ventricular walls to monitor intracardiac electrograms, sinoatrial rate, and atrioventricular conduction time, as noted previously.[1–6] Coronary sinus effluent was collected by placing a cannula into the right ventricle through the pulmonic valve after ligating the venae cavae. Coronary inflow (aortic) was measured at constant temperature by an ultrasonic flowmeter.

Coronary inflow and outflow (coronary sinus) O_2 tensions were measured continuously on-line (Instech® 203B O_2 electrode, Plymouth Meeting, PA) and verified *via* a blood gas analyzer as described previously.[18] O_2 consumption and $\%O_2$ extraction were measured in all studies to assess direct vasodilatory responses of drugs apart from responses due to metabolic factors (*e.g.,* a decrease in CF and O_2 delivery secondary to decreased contractility and O_2 consumption). Percent O_2 extraction, O_2 consumption rate, and relative cardiac efficiency (heart rate · LVP per O_2 consumption rate) were calculated as reported previously.[1–6] During normothermia, coronary perfusion pressure was held constant at 55 mmHg while CF was measured. During hypothermia, CF was held constant at 2 ml/min while perfusion pressure was measured. Coronary vascular resistance was calculated as perfusion pressure divided by coronary flow per gram of heart weight. Electrograms, heart rate, atrioventricular conduction time, outflow O_2 tension, perfusion pressure, CF, and LV systolic and diastolic pressure were printed graphically on an eight-channel recorder (Astro-Med® MT9500, West Warwick, RI) and digitally recorded (MacLab, AD Instruments, Castle Hills, Australia) for later detailed analysis. Description and identification of dysrhythmias in this model have been described in detail previously.[1–6]

Protocol

Hearts were assigned randomly to one of six groups (n 11 for each): (1) time (warm) control (6 h of normothermic perfusion); (2) 1.3% isoflurane; (3) 2.6% isoflurane; (4) 1.3% isoflurane plus glibenclamide; (5) 2.6% isoflurane plus glibenclamide; and (6) cold treatment control. Isoflurane and glibenclamide were administered 0.5 h before, during, and 0.5 h after 22 h of hypothermic perfusion. The five hypothermia (cold)

groups were perfused normothermically at constant perfusion pressure for 3 h, hypothermically at reduced constant CF for 22 h, and again normothermically at constant pressure for 3 h after hypothermia for a total of 28 h. The period of normothermia in the warm, time control group was 6 h, which is the same duration of normothermia as in the hypothermia groups.

Perfusate and bath temperature were maintained at 37.4 ± 0.1°C (mean ± SEM) before and after hypothermia using a thermostatically controlled water circulator. Perfusate and bath temperature were maintained at 3.75 ± 0.1°C during the 22-h period of cold, low-flow perfusion in all groups. A switch to low constant flow at 3.75°C was accomplished by use of a separate refrigerated jacket and perfusion circuit placed in parallel with the warm perfusion circuit.[1] After hypothermic perfusion for 3 h, normothermic perfusion was reinstated by switching back to the warm circuit. Warm and cold perfusion circulation circuits were temperature equilibrated in advance. Time to reach half the temperature decrease from 37.4 to 3.75°C was 5 min.

On lowering temperature to 15°C, cardiac perfusion was switched from constant pressure to low constant flow (equivalent to 1.7 $ml \cdot g^{-1} \cdot min^{-1}$), which was approximately one fourth the baseline normothermic flow during constant pressure perfusion. With depressed cardiac metabolism during hypothermia, low constant flow perfusion maintained adequate and equal global perfusion of all hearts. When temperature reached 25°C during normothermic reperfusion after hypothermia, cardiac perfusion was returned to the constant pressure (55 mmHg) mode. Time to reach half the temperature increase from 3.75°C to 37.4°C was 3 min.

Warm and cold perfusate solutions were equilibrated with 96% O_2 + 4% CO_2. For hearts in all groups during the initial normothermic period, mean ± SEM coronary arterial (inflow) pH averaged 7.45 ± 0.02; pCO_2 averaged 24 ± 1 mmHg; and pO_2 averaged 608 ± 7 mmHg. Inflow samples, collected at 3.75°C during the hypothermic period but measured at 37°C, had values of 7.12 ± 0.02, 48 ± 2, and 778 ± 15 mmHg, respectively. The acidity and hyperoxia of the cold perfusate reflect the greater solubility of O_2 and CO_2 in the hypothermic solution. There were no significant differences for these pH and gas values among the groups at each of the two temperatures.

Figure 1 shows the time course of the experimental groups. In all six groups, peak coronary responsiveness was tested with adenosine to arrest hearts temporarily. A bolus of adenosine (0.2 ml of a 200 μM solution) was injected directly into the aortic (coronary perfusion) cannula to assess this response. In all six groups, endothelium-dependent responses were tested with a 3-min infusion of 1 μM of 5-HT; endothelium-independent responses were tested with a 3-min infusion of 100 μM of nitroprusside; and inotropic and chronotropic responses were tested with a 1-min infusion of 0.5 μM of epinephrine. All variables were measured during the last minute of (1) a 0.5-h initial control (C1) period (h 0.5); (2) during initial test infusions of adenosine, 5-HT, nitroprusside, and epinephrine (D1); (3) during the prehypothermia period before giving isoflurane with and without 4 μM of glibenclamide; (4) during isoflurane with and without glibenclamide before the hypothermic period; (5) during isoflurane with and without glibenclamide after 22 h of hypothermic perfusion or during continued normothermia; (6) every subsequent 0.5-h period beginning after treatment (C2) in cold groups (h 26–28) or in the warm group (h 4–6); and (7) during repeat infusions of adenosine, 5-HT, nitroprusside, and epinephrine (D2).

In preliminary experiments, perfusion of normothermic hearts with isoflurane for 1 h had no lasting effect on any variable measured. Isolated guinea pig hearts perfused normothermically for 25 h, with or without drug protection, exhibited only very slow erratic atrial beating and no ventricular rhythm or contractile function; hearts stored hypothermically for 22 h without perfusion were contractured and exhibited no myocardial activity (Stowe DF, unpublished data, 1995). Rewarming provoked ventricular dysrhythmias in a few hearts at ≈25°C, so each heart in the cold groups received, as prophylactic, one bolus injection of 0.1 ml of 10-mg lidocaine HCl during rewarming at 25°C to reduce the occurrence of such dysrhythmias. Only dysrhythmias occurring at 37°C were tabulated.

Isoflurane was administered by an agent-specific vaporizer by switching to perfusate solution preequilibrated with isoflurane. Samples were collected at an aortic inflow port during delivery of the anesthetic before, during, and after hypothermic perfusion for determination of isoflurane concentration by gas chromatography as described elsewhere.[12,18] Isoflurane's effective vapor fraction (vol%) was calculated before and after hypothermia, that is, at 37°C, using 0.61 as its partition coefficient in Kreb's solution at 37°C. Perfusate concentrations (mean ± SEM) of isoflurane for each group before, during, and after hypothermia, respectively, were 0.28 ± 0.03, 1.16 ± 0.12, and 0.34 ± 0.06 mM for 1.3% isoflurane; 0.33 ± 0.02, 1.17 ± 0.06, and 0.33

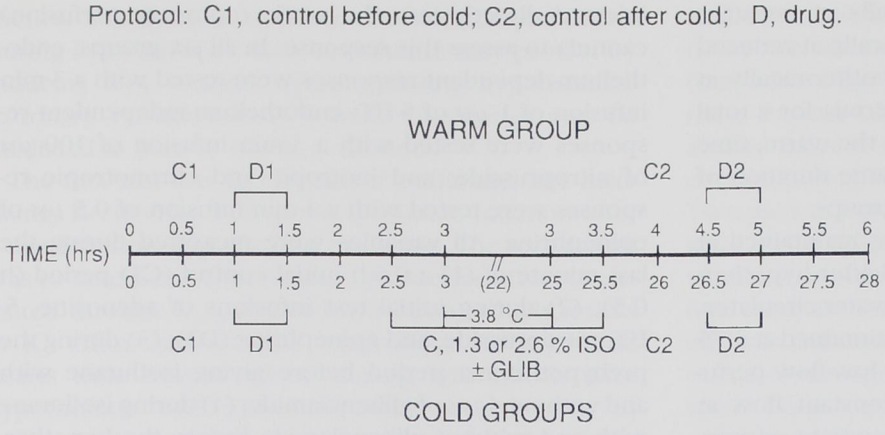

Fig. 1. Time schema for protocol. The warm group was treated similarly to the cold groups except that the warm group did not undergo 22 h of hypothermia or exposure to isoflurane or glibenclamide (GLIB). C1 and C2 = initial and final controls; D1 and D2 = initial and final responses to adenosine (ADE), 5 hydroxytryptamine (5-HT), nitroprusside (NP), and epinephrine (EPI). The five cold groups are: C = cold control; 1 (1.3%) and 2 (2.6%), isoflurane alone or with GLIB.

± 0.03 mM for 1.3% isoflurane plus glibenclamide; 0.68 ± 0.05, 2.47 ± 0.17, and 0.72 ± 0.04 mM for 2.6% isoflurane; and 0.81 ± 0.02, 2.12 ± 0.14, and 0.72 ± 0.03 mM for 2.6% isoflurane plus glibenclamide. The markedly higher isoflurane concentrations during hypothermia are due to increased anesthetic solubility, but the effective vapor fraction during hypothermia is similar to that obtained during normothermia.[19] The average effective vapor fractions were calculated as 1.3 and 2.6% by volume during normothermia. Hearts were weighed immediately after each experiment (28 h for hypothermia groups and 6 h for the warm, time control group), and dehydrated hearts were weighed to calculate water heart weight expressed as a percentage of total heart weight.

Statistical Analysis

All data are expressed graphically as means ± SEM for clarity of presentation. Mean values were considered significant at $P \leq 0.05$. Calculated variables were computed using Microsoft Excel™ (Microsoft Corporation, Redmond, WA). For data expressed over time (figs. 2–5), the six groups were compared for variability at each time interval by two-way analysis of variance (CLR ANOVA™, Clear Lake Research, Houston, TX) and comparison among means (least significant difference tests). (The symbols mentioned in this and the subsequent section refer to those in figures 2–7.) For each variable, each cold group was compared with the 6-h warm, time control group (†); each cold group was compared with the cold control group (*); the 1.3% isoflurane group was compared with the 2.6% isoflurane group (§); and each isoflurane group was compared with its corresponding isoflurane plus glibenclamide group (¶). Statistical notations are not marked in the time portions of figures 2–5 if there was no change from the previous time period. Group comparisons are always noted during initial or final administration of isoflurane, during adenosine or epinephrine, and at the final time control.

Coronary flow (fig. 6) and $\%O_2$ extraction (fig. 7) responses to the vasodilators adenosine, 5-HT, and nitroprusside were tested using one-way analysis of variance with repeated measures. The following comparisons were made: vasodilator responses to adenosine, 5-HT, and nitroprusside (•) *versus* group C1 and *versus* group C2; each cold group *versus* the warm, time control group (†); each group *versus* the cold control group (*); the 1.3% isoflurane group *versus* the 2.6% isoflurane group (§); and isoflurane groups *versus* isoflurane-plus-glibenclamide groups (¶). Fisher's least significant difference test was used to compare means.

Results

Figures 2–5 compare time-dependent changes in four measured or calculated variables among the six groups. Bar figures (figs. 6 and 7) detail the differences in basal and stimulated flow and oxygen extraction responses to endothelium-dependent and -independent vasodilators before and after hypothermic preservation. These figures show that all groups had similar control baseline values and responsiveness to epinephrine, adenosine, 5-HT, and nitroprusside *before* administration of isoflurane and before hypothermia. Administration of 1.3% and 2.6% isoflurane before hypothermia similarly reduced heart rate from 227 ± 5 and 224 ± 4 beats/min to 218 ± 6 (not significant) and 209 ± 7 ($P \leq 0.05$) beats/min, respectively. Isoflurane, with or without gli-

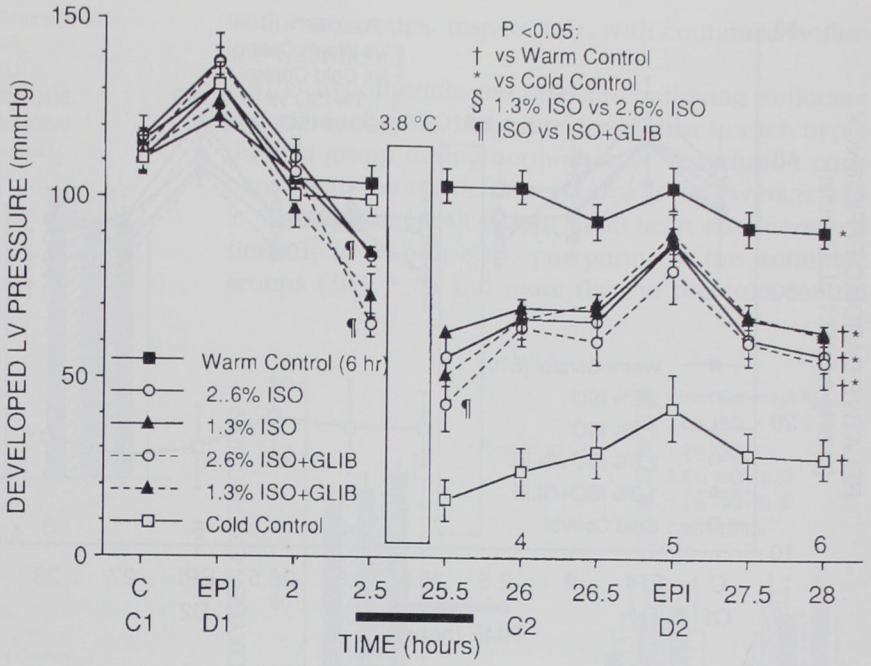

Fig. 2. Changes in left ventricular (LV) systolic minus diastolic pressure over 28 h in five groups of isolated hearts perfused at low flow for 22 h during hypothermia at 3.8°C and in one time control group perfused at normal flow for 6 h during normothermia. Each group consists of 9–11 hearts. The interval 2.5–25.5 h represents the period before, during (rectangle), and initially after hypothermia during treatment with isoflurane (ISO) either alone or with glibenclamide (GLIB). Above the time line is the time course in the time control group only. EPI = the response to a 1-min infusion of 0.5 μM of epinephrine. Not all statistical notations are shown for each time point. See figure 1 for other details.

benclamide, decreased LVP before hypothermia (fig. 2). Percent O_2 extraction was reduced by 2.6% isoflurane (fig. 3), and cardiac efficiency remained unaltered in the presence of isoflurane with or without glibenclamide (fig. 4) before hypothermia at 2.5 h. Compared with the cold control group, CF was higher in the 2.6% isoflurane groups (fig. 5). With the onset of hypothermia, the 15 ± 4% increase in LVP at ≈15°C in the cold control group was not evident in any isoflurane group.

Peak coronary vascular resistance (mmHg ÷ ml·

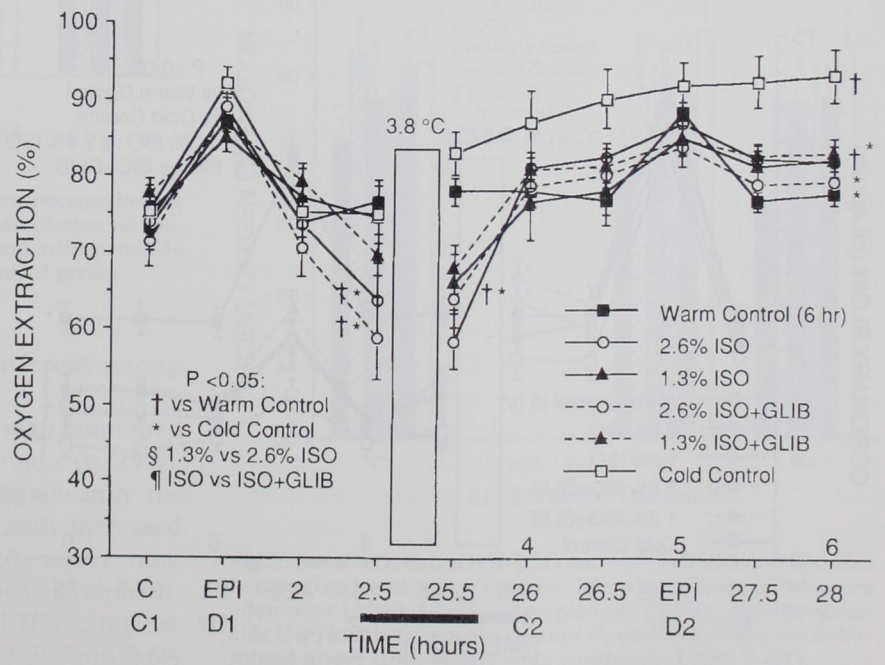

Fig. 3. Changes in $\%O_2$ extraction over 28 h in five groups of isolated hearts perfused at low flow for 23 h during hypothermia at 3.8°C and in one time control group perfused at normal flow for 6 h during normothermia. See figures 1 and 2 for other details.

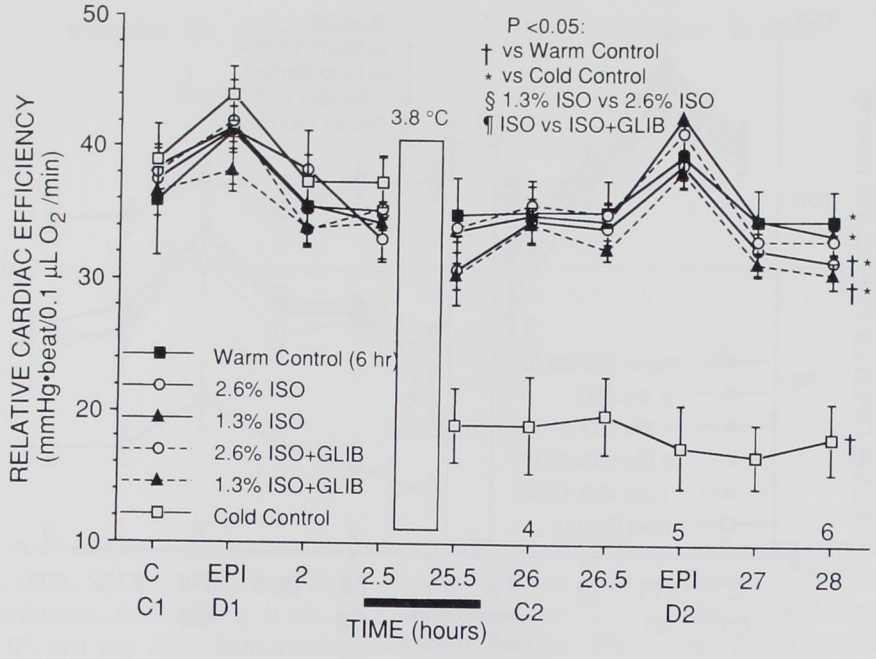

Fig. 4. Changes in relative cardiac efficiency (left ventricular pressure [LVP] · heart rate/O_2 consumption) over 28 h in five groups of isolated hearts perfused at low flow for 22 h during hypothermia at 3.8°C and in one time control group perfused at normal flow for 6 h during normothermia. See figures 1 and 2 for other details.

$g^{-1} \cdot min^{-1}$) *during* hypothermia was 12 ± 2 for 1.3% isoflurane alone and 10 ± 2 for 1.3% isoflurane plus glibenclamide; 12 ± 1 for 2.6% isoflurane alone and 12 ± 2 for 2.6% isoflurane plus glibenclamide; 30 ± 5 for cold controls; and 8 ± 2 for warm, time controls. Resistance to flow was higher in the cold control group ($P \leq 0.05$) than in all other groups and was statistically similar in the isoflurane groups and the warm, time control group. Water weight as a fraction of total heart weight averaged 88.2 ± 0.3% for all

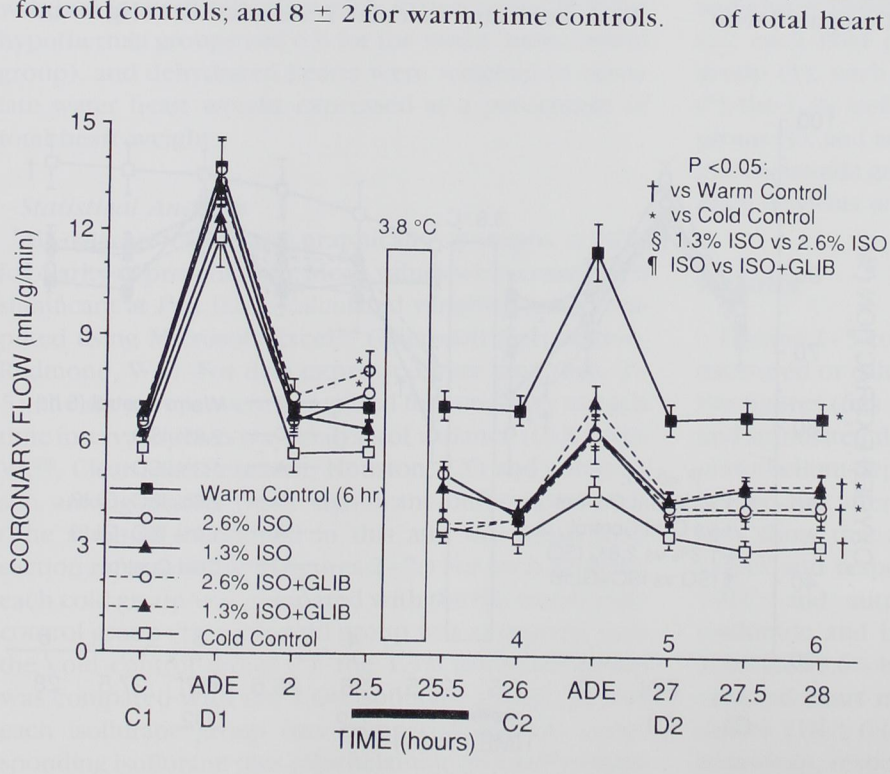

Fig. 5. Changes in coronary flow over 26 h in five groups of isolated hearts perfused at low flow for 22 h during hypothermia at 3.8°C and in one time control group perfused at normal flow for 8 h during normothermia. See figures 1 and 2 for other details.

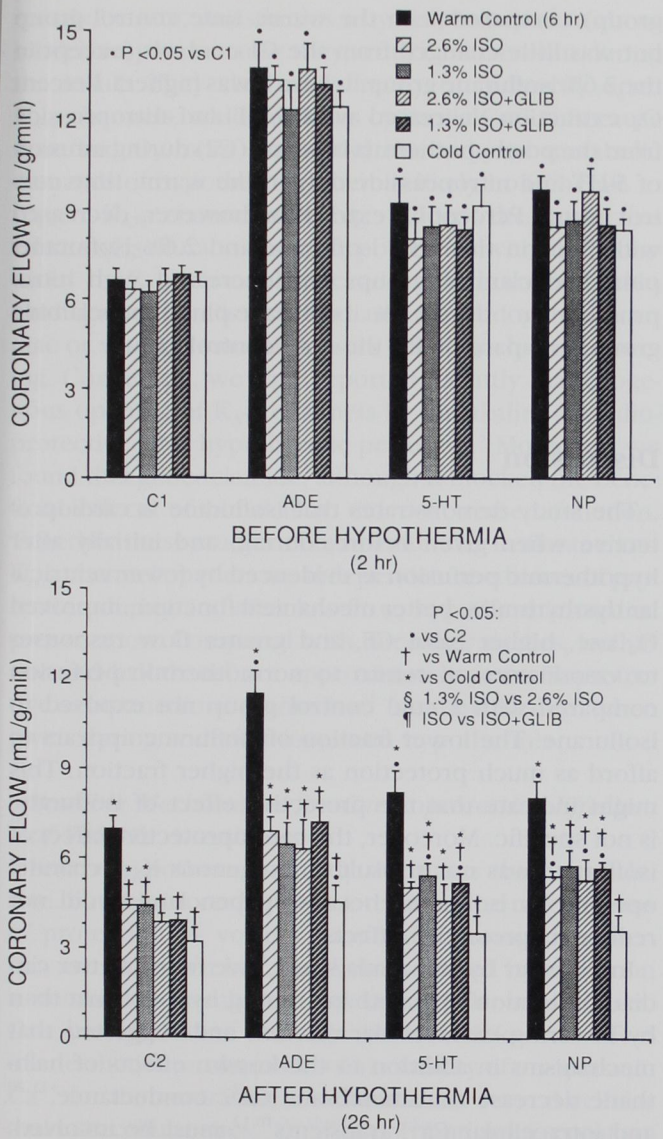

Fig. 6. Basal flow (C1) and coronary flow responses before *(top)* and after *(bottom)* hypothermia to vasodilators adenosine (ADE), 5-hydroxytryptamine (5-HT), and nitroprusside (NP) in all five cold groups and the time control group.

six groups; this was not significantly different among groups.

Initially *after* long-term hypothermia, with continued isoflurane or isoflurane plus glibenclamide (h 25.5), LVP, cardiac efficiency, and CF were significantly decreased and $\%O_2$ extraction was significantly increased compared with values in the untreated, warm, time control group (not all statistical notations are shown). Heart rate (beats/min) was 193 ± 7 and 186 ± 8 ($P \leq 0.05$ compared with group C1) in the 1.3% and 2.6% isoflurane groups, respectively, with continued isoflurane treatment.

After hypothermia and *after* discontinuing isoflurane and glibenclamide, heart rate was similar in each hypothermia group during normothermic reperfusion compared with the warm, time control group (average 215 ± 5 beats/min for all groups), and heart rate increased similarly in response to epinephrine in the isoflurane groups (303 ± 7) and more than in the cold control

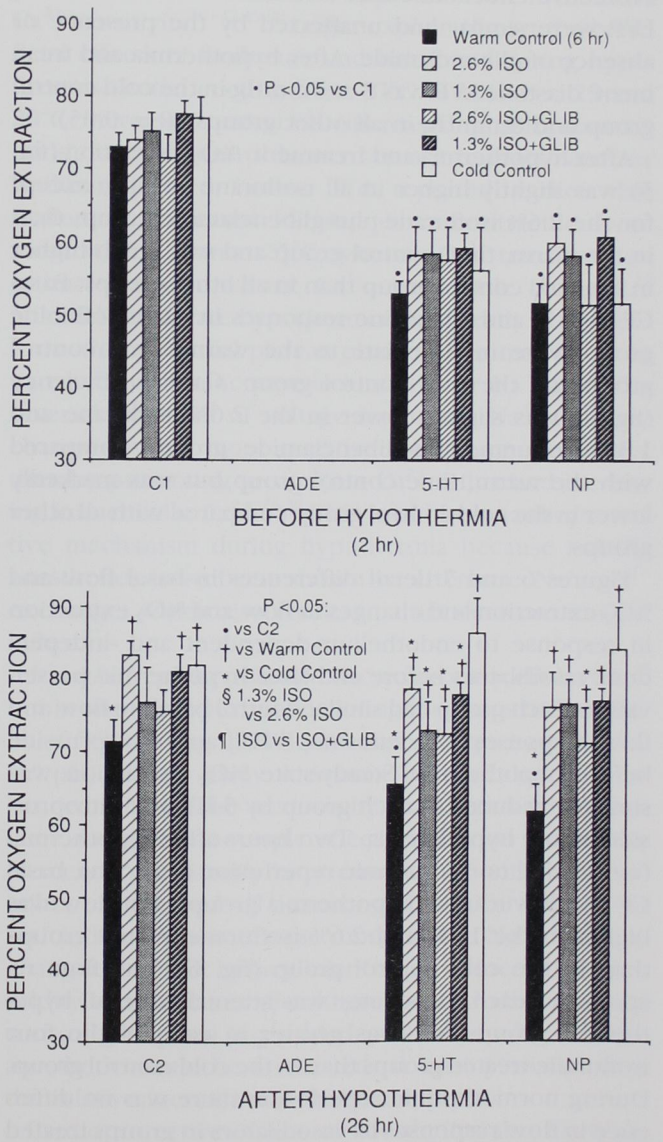

Fig. 7. Basal $\%O_2$ extraction (C1) and $\%O_2$ extraction responses before *(top)* and after *(bottom)* hypothermia to vasodilators adenosine (ADE), 5-hydroxytryptamine (5-HT), and nitroprusside (NP) in all six groups. Percent O_2 extraction was not determined under non–steady state conditions (ADE, 5-HT).

group (271 ± 7). The incidence of ventricular fibrillation (36%) *versus* normal sinus rhythm was significant ($P \leq 0.05$) only in the cold control group. All hearts remained in or had reverted to sinus rhythm with boluses of lidocaine within 2 h of warm reperfusion. There were no significant ventricular dysrhythmias in any isoflurane-treated group.

After hypothermia and treatment, developed LVP (fig. 2) in each isoflurane group was intermediate to the warm, time control group and the cold control group. Protective effects of either isoflurane concentration on LVP were similar and unaffected by the presence or absence of glibenclamide. After hypothermia and treatment, diastolic LVP was 8 ± 3 mmHg in the cold control group and 0 mmHg in all other groups ($P \leq 0.05$).

After hypothermia and treatment, $\%O_2$ extraction (fig. 3) was slightly higher in all isoflurane groups, except for the 2.6% isoflurane-plus-glibenclamide group, than in the warm, time control group and was much higher in the cold control group than in all other groups. Basal CF (fig. 5) and adenosine responses in each isoflurane group were intermediate to the warm, time control group and the cold control group. Cardiac efficiency (fig. 4) was slightly lower in the 2.6% isoflurane and 1.3% isoflurane-plus-glibenclamide groups compared with the warm, time control group but was markedly lower in the cold control group compared with all other groups.

Figures 6 and 7 detail differences in basal flow and $\%O_2$ extraction and changes in flow and $\%O_2$ extraction in response to endothelium-dependent and -independent vasodilators before and after hypothermic preservation. Each group had similar control baseline flow and flow responses to adenosine, 5-HT, and nitroprusside before hypothermia. Steady-state $\%O_2$ extraction was similarly reduced in each group by 5-HT and nitroprusside before hypothermia. Two hours after hypothermia (*i.e.,* during normothermic reperfusion at 26.5 h), basal CF was lower in all hypothermia groups, but flow was higher in the 1.3% and 2.6% isoflurane-treated groups than in the cold control group (fig. 6). The flow response to each vasodilator was attenuated in all hypothermia groups but was greater in each of the four isoflurane-treated groups than in the cold control group. During normothermic reperfusion there was no difference in flow responses to vasodilators in groups treated with either of the two concentrations of isoflurane with or without glibenclamide. Basal $\%O_2$ extraction (fig. 7) after hypothermia (C2) was elevated in most cold groups (except the 2.6% isoflurane-plus-glibenclamide group) compared with the warm, time control group but was little changed from the C1 controls (except in the 2.6% isoflurane group, where it was higher). Percent O_2 extraction decreased with 5-HT and nitroprusside from the posthypothermia baseline (C2) during infusion of 5-HT and nitroprusside only in the warm, time control group. Percent O_2 extraction, however, decreased with 5-HT in the 1.3% isoflurane and 2.6% isoflurane-plus-glibenclamide groups and decreased with nitroprusside in the 2.6% isoflurane-plus-glibenclamide group, compared with the cold control group.

Discussion

The study demonstrates that isoflurane is cardioprotective when given before, during, and initially after hypothermic perfusion as evidenced by fewer ventricular dysrhythmias, better mechanical function, improved O_2 use, higher basal CF, and greater flow responses to vasodilators on return to normothermic perfusion compared with a cold control group not exposed to isoflurane. The lower fraction of isoflurane appears to afford as much protection as the higher fraction. This might indicate that the protective effect of isoflurane is not specific. Moreover, the cardioprotective effect of isoflurane was not a result of exogenous K_{ATP} channel opening by isoflurane because glibenclamide did not reduce its protective effects.

In a similar hypothermia study,[6] we found better cardiac protection by halothane during hypothermia than by lowering extracellular calcium and suggested that mechanisms in addition to the known effects of halothane decrease transmembrane Ca^{2+} conductance,[11,20] and intracellular Ca^{2+} transients[10,12] must be involved. This companion study shows that isoflurane is similarly protective compared with halothane, and, like halothane, that protection is not dependent on the concentrations tested. Qualitatively, isoflurane gave results similar to those of halothane except for cardiac efficiency, which appeared to be more improved by isoflurane than by halothane.

We have shown before that volatile anesthetics preserve function in isolated hearts during hypoxia and normoxic reperfusion[13] and during ischemia and reperfusion.[14,15] It seems likely that protective mechanisms afforded by anesthetics with hypothermia are different than those with hypoxic perfusion or global coronary occlusion. The latter two insults produce ischemia because O_2 delivery or flow is insufficient to maintain the

metabolic rate. Anesthetics may be protective in those models in part because they reduce metabolism during the insult. They also may directly or indirectly open K_{ATP} channels, which reduces cytosolic Ca^{2+}.[21] Because flow and O_2 more than meet the reduced metabolic demands of hypothermia, there is likely no ischemia to trigger K_{ATP} channel opening. Indeed, the protective effects of isoflurane during hypothermia were not attenuated by K_{ATP} channel blockade, which suggests that cardioprotection is not mediated by endogenous cardiac or vascular smooth muscle cell K_{ATP} channel opening. Conversely, we have reported recently that exogenous opening of K_{ATP} channels by bimakalim is cardioprotective after hypothermic perfusion.[5] Moreover, we found that glibenclamide, although it blocked the beneficial effects of bimakalim, did not worsen function. Together these studies demonstrate that isoflurane and K_{ATP} channel openers are independent mediators of protection during and after low-flow, hypothermic cardiac preservation. Compared with our other studies, isoflurane and halothane[6] are approximately as cardioprotective as butanedione monoxime[1-4] and bimakalim[5] when given during and after long-term hypothermia.

Possible Mechanisms for Cardioprotective Effects of Volatile Anesthetics

Severe hypothermia greatly decreases the metabolic rate, but it is incompletely protective. The mechanisms of protection by volatile anesthetics during hypothermia are unknown but are likely mediated by action on a number of membrane and intracellular messengers. One mechanism to consider is the well-known effect of anesthetics on alteration of availability of myoplasmic Ca^{2+} for the contractile mechanism *via* several cellular and intracellular sites.[7-12,19,20] Contributing effects of anesthetics might include (1) voltage-dependent depression of transsarcolemmal Ca^{2+} current, (2) decreased net sarcoplasmic reticular Ca^{2+} uptake, (3) increased rate of Ca^{2+} leak from the sarcoplasmic reticulum during diastole, (4) altered affinity of troponin C for Ca^{2+}, and (5) decreased response of the myofilaments to a given level of occupancy of the Ca^{2+} binding sites on troponin C. Volatile anesthetics have been reported to inhibit rapid cooling (5°C)-induced contractions[21,22] activated by Ca^{2+} release through sarcoplasmic reticular channels.[23] In skinned myocardium at 23°C, only halothane was found to inhibit caffeine-induced contractures due to opening of sarcoplasmic reticular Ca^{2+} release channels, whereas isoflurane and halothane similarly inhibited rapid cooling-induced contractures.[22] Differential effects of anesthetics on attenuating induced sarcoplasmic reticular Ca^{2+} release during normothermia, however, may be no longer evident during hypothermia. Differences among anesthetics have been found for effects on myocardial cell Ca^{2+},[7-13,20,22] but we have observed that cardioprotection by isoflurane was comparable to that of halothane.[6]

Mild hypothermia, *per se,* causes a positive inotropic effect. At 25°C, contractile force as a function of extracellular calcium is steeper and shifted to the left compared with that at 35°C.[19] Depression of myofibrillar Ca^{2+} sensitivity[10-12,19,20] might explain in part the beneficial effects of isoflurane and halothane during hypothermia. Initial cooling from 37°C to between 25 and 20°C increases peak LVP (increased phasic contractions) in cold control hearts but not in halothane-[6] or isoflurane-treated hearts. We reported earlier that isoflurane decreased Purkinje fiber contractile force more at 35°C than at 25°C, whereas Ca^{2+} transients, an estimate of myoplasmic Ca^{2+}, were little changed.[19] Mild and moderate hypothermia also greatly prolonged the action potential plateau phase.[24] This may occur in part because an increase in intracellular Na^+ shifts the reversal potential of voltage-dependent Na^+-Ca^{2+} exchange toward more negative membrane potentials, thereby promoting greater net Ca^{2+} influx and increasing intracellular Ca^{2+}.[25,26] This could be a cardioprotective mechanism during hypothermia because volatile anesthetics have been shown to attenuate Na^+-Ca^{2+} exchange, which also results in a net decrease in Ca^{2+} influx.[27]

In vivo, hypothermia also decreases intracellular H^+,[28] but in our study perfusate cooling increases the solubility of CO_2 gassing the solution and decreases extracellular pH, which likely increases intracellular H^+. Enhanced intracellular acidosis by anesthetics might produce a negative inotropic effect directly on contractile elements or indirectly by attenuating H^+ efflux for Na^+ influx *via* the Na^+-H^+ exchanger, and Ca^{2+} efflux for Na^+ influx *via* the reverse mode of the Na^+-Ca^{2+} exchanger,[29] so that intracellular Ca^{2+} is reduced. Solution acidity was similar in all cold groups, including the cold control group, so any added protective effect of the anesthetic could be associated, at least partially, with the relative increase in solution acidity.

Cardioprotective effects of anesthetics also likely result from additional interrelated actions on diverse cellular and subcellular structures because anesthetics nonspecifically and reversibly alter function in multiple organelles.[30] Volatile agents might protect during

hypothermia by depressing the temperature for transition of the lipid membrane from fluid to gel.[31] Volatile anesthetics might alter lipid and protein interactions within membrane structures, modulate free radical production, alter pump-regulated ion gradients, uncouple contraction from excitation, and produce other unknown effects. During hypothermia, anesthetics might alter the function of receptor- and second messenger-associated proteins of the sarcolemma and sarcoplasmic reticulum, and other intracellular structures, in a way that preserves cardiac tissue.

K_{ATP} Channel Function during Hypothermia versus Ischemia

It is important to distinguish the protective effects of volatile agents with ischemia and with hypothermia and the role of endogenous K_{ATP} channel opening. Brief ischemic preconditioning maneuvers applied before a longer period of ischemia can reduce the degree of dysfunction, or "myocardial stunning," by reducing the extent or severity of ischemic damage. K_{ATP}-sensitive channel openers[32–34] when given before a long period of ischemia reduce the extent of infarction and the degree of reversible myocardial dysfunction on reperfusion. These drugs may mimic endogenous K_{ATP} channel opening during a very brief transient occlusion, which gives protection against stunning and infarction before a much longer occlusion.[35]

Isoflurane has been shown to protect the myocardium against ischemia in intact dogs.[35–37] Isoflurane (2%) given 45 min before a left anterior descending artery occlusion for 15 min nearly restored cardiac function in the ischemic zone after 5 h of reperfusion; moreover, the protective effect of isoflurane was greatly attenuated by glibenclamide.[37] In another study, the marked functional benefit of isoflurane given 30 min before and during five left anterior descending artery occlusions lasting 5 min with 5-min reperfusion periods was completely blocked by glibenclamide.[38] These studies suggest that isoflurane exerts cardioprotective effects in stunned myocardium, at least in part, *via* activation of K_{ATP} channels.

It is not wholly unexpected that cardioprotective effects of volatile anesthetics during hypothermic preservation are not mediated *via* activation of K_{ATP} channels. Although we found that the K_{ATP} channel opener bimakalim is cardioprotective during long-term hypothermia,[5] we suggest that the K_{ATP} channel is not open during low-flow hypothermia as during normothermic ischemia. It is unlikely that ischemia occurs in our long-term hypothermia model because hearts are continuously perfused. Because hypothermia itself abolishes rhythm and contractile activity, it is not possible from this study to ascertain how isoflurane better protects the myocardial function during hypothermia.

Perfusion Protection by Volatile Anesthetics

Ischemia also damages the endothelial lining so that production of intrinsic endothelium-dependent vasodilators, like nitric oxide or prostaglandins, is diminished, and underlying vascular smooth muscle contracts so that perfusion is restricted because of vasoconstriction, obstruction, or both (microvascular stunning).[39,40] Inadequate production of vasodilators, poor vascular smooth muscle responses to vasodilators, and production of vasoconstrictor factors would lead to a level of O_2 and nutrient supply inadequate to match metabolic demands. It is not known if nonischemic hypothermia injures the endothelium or vasculature. The mechanism for the improved CF by halothane and isoflurane after hypothermia is unclear. Clearly, improved perfusion after hypothermia contributes to improved contractile function. In isolated hearts, isoflurane, like halothane, is a myocardial depressant that produces a relative over perfusion of the myocardium.[18]

It is interesting that coronary conductance was higher in isoflurane-treated groups *during* hypothermia. If, conversely, there were a relative decrease in coronary conductance on normothermic reperfusion, ischemia could occur to activate endogenous K_{ATP} channel opening. Thus improved perfusion appears to play a role in the cardioprotective effects of isoflurane, halothane, and other drugs examined during hypothermia. Responses to a maximal vasodilator agent (adenosine) and to endothelium-dependent (5-HT) and endothelium-independent (nitroprusside) agents were tested to assess microvascular injury. Although responses to these agents were attenuated after hypothermia in each cold group, each isoflurane-treated group exhibited better flow responses to these agents than did the cold control group. It is likely that the nitric oxide synthase–guanylyl cyclase vasodilator system and ion gradient stabilization systems are not fully operational during hypothermia, but they may be differentially protected before hypothermia or differentially activated during rewarming in the presence of volatile anesthetics. K_{ATP} channel blockade has been reported to reduce or eliminate anesthetic-induced vasodilation of isolated coronary arteries, so it has been suggested that volatile anesthetics produce vasorelaxation in part by activating K_{ATP} chan-

nels.[16,17] Although isoflurane moderately increased coronary conductance and decreased $\%O_2$ extraction before and after hypothermia, we could not demonstrate significant differences in vascular responses before or after hypothermia in isoflurane groups treated with glibenclamide to block K_{ATP} channel activation.

In conclusion, volatile anesthetics such as isoflurane enhance the cardioprotective effects of severe long-term hypothermic perfusion on coronary vascular compliance, O_2 use, and cardiac mechanical function, but the mechanisms of protection require further study.

The authors are especially grateful to James S. Heisner for his excellent technical assistance and to research medical students Richard A. Paulsen and Wynda W. Chung for assistance with this study.

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