Delayed cardioprotection by isoflurane: role of \( K_{ATP} \) channels

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The protective effects of volatile anesthetics on ischemic myocardium have been termed anesthetic-induced preconditioning because of its remarkable similarity to ischemic preconditioning. Isoflurane improves functional recovery of stunned myocardium in chronically or acutely instrumented dogs after brief periods of coronary artery occlusion and reperfusion (25, 26, 44). Low doses of the ATP-sensitive K (\( K_{ATP} \)) channel blocker glibenclamide completely abolished the cardioprotective effects of isoflurane (25). These results indicate that \( K_{ATP} \) channels are activated by isoflurane and mediate the beneficial effects of isoflurane in stunned myocardium in animal models that respond to acute ischemic preconditioning in a similar fashion to the human heart (5, 45).

\( K_{ATP} \) channel openers mimic early ischemic preconditioning, whereas \( K_{ATP} \) channel blockers prevent ischemic preconditioning. These effects were initially attributed to opening and closing of sarcolemmal \( K_{ATP} \) channels, respectively. New evidence suggests, however, that mitochondrial \( K_{ATP} \) channels might very well have even a greater influence on preconditioning (35, 37). For instance, diazoxide, an opener of mitochondrial \( K_{ATP} \) channels, was demonstrated to exert cardioprotection at concentrations that open only mitochondrial \( K_{ATP} \) channels. Furthermore, the mitochondrial \( K_{ATP} \) channel blocker sodium 5-hydroxydecanoate (5-HD) was shown to prevent cardioprotection produced by diazoxide or ischemic preconditioning (1). Therefore, the role of mitochondrial \( K_{ATP} \) channels needs to be further explored by examining the effects of volatile anesthetics such as isoflurane and/or various channel modulators on the sarcolemmal \( K_{ATP} \) and mitochondrial \( K_{ATP} \) channels and the acute reduction of infarct size following ischemia and reperfusion in vivo.

In addition, it is unknown whether isoflurane exerts a delayed cardioprotective effect and whether such an effect is mediated by sarcolemmal or mitochondrial \( K_{ATP} \) channels. We hypothesized that isoflurane pro-

BRIEF PERIODS OF ISCHEMIA lead to a reduced severity of cardiac injury following a second sustained period of ischemia. This cardioprotective effect has been termed ischemic preconditioning and has been demonstrated in all species studied. Certain pharmacological agents can mimic the cardioprotective effect of ischemic preconditioning. Recent clinical and experimental data indicate that volatile anesthetics also exert acute protective effects on the myocardium.
Isoflurane can induce delayed cardioprotection via enhanced opening of K\textsubscript{ATP} channels. The objectives of our study were to determine whether isoflurane can induce delayed cardioprotection, the involvement of K\textsubscript{ATP} channels, the cellular location of the channels, and the dose dependency of this effect.

**MATERIALS AND METHODS**

**Animals**

Animals used in this study received humane care in compliance with the Guide for the Care and Use of Laboratory Animals formulated by the National Research Council, 1986. Pregnant New Zealand White rabbits were obtained from a commercial breeder. The kits were born in the Animal Resource Center at the Medical College of Wisconsin, raised from birth by their biological mothers, and were studied at 7–10 days of age. Rabbits were anesthetized with pentobarbital sodium (30 mg/kg ip). Pentobarbital anesthesia does not confer cardioprotection in the rabbit (11).

**Isoflurane Treatment**

Neonatal New Zealand White rabbits (7–10 days of age) were exposed to 1% isoflurane (minimal alveolar concentration, MAC)-100% oxygen for 2 h. New Zealand White rabbits exposed to 2 h of 100% oxygen served as untreated controls. Twenty-four hours later, resistance to myocardial ischemia was determined using an isolated perfused heart model.

**Perfusion System**

Isolated rabbit hearts were instrumented as previously described (3). A three-way tap, located immediately above the site of cannulation, allowed the entire perfusate to be diverted away from the heart to produce global, no-flow ischemia. Reperfusion was achieved by repositioning the tap to allow perfusate to be delivered to the heart.

**Perfusion Media**

The standard perfusate was modified Krebs-Henseleit bicarbonate buffer (3) in the following composition (mmol/l): 118.5 NaCl, 25.0 NaHCO\textsubscript{3}, 4.8 KCl, 1.2 MgSO\textsubscript{4}-6H\textsubscript{2}O, 1.2 KH\textsubscript{2}PO\textsubscript{4}, and 1.8 CaCl\textsubscript{2}-2H\textsubscript{2}O (pH 7.4 when gassed with 95% O\textsubscript{2}-5% CO\textsubscript{2}). Glucose (11.1 mmol/l) was added to the perfusate. Before use, all perfusion fluids were filtered through cellulose acetate membranes with a pore size of 5.0 μm to remove particulate matter. K\textsubscript{ATP} channel blockers were added to the perfusate as needed.

**Assessment of Ventricular Function**

Left ventricular function was monitored continuously throughout each experiment using a compliant saline-filled latex balloon placed in the cavity of the ventricle as previously described (3). End-diastolic pressure was initially set to 3 mmHg for 2 min. The balloon was then progressively inflated with a microsyringe to set end-diastolic pressures to 8 mmHg, and developed pressure was recorded during steady-state conditions. Coronary flow rate was measured throughout the experiment by timed collections of the coronary effluent from the right side of the heart into a graduated cylinder. Coronary flow rate was expressed as milliliters per minute.

**Infarct Size Determination**

After 3 h of reperfusion, hearts were subsequently perfused with 1% triphenyltetrazolium chloride (Sigma Chemical; St. Louis, MO) in phosphate buffer (pH 7.4) at 38°C for 10 min via a sidearm immediately above the point of aortic cannulation. The hearts were then removed from the perfusion apparatus and sliced across the long axis of the left ventricle, from apex to base, into 2-mm-thick transverse sections. Infarct areas were visually enhanced by storage in 10% formaldehyde solution for 24 h before final measurement. In the globally ischemic heart, the entire ventricle is at risk of infarction and therefore measurement of collateral flow and the area at risk is not required. Global ischemia resulted in multiple small areas of triphenyltetrazolium chloride staining. We were careful to separate the areas of viable and necrotic tissue using a surgical blade. The tissues were then weighed by an independent observer (M. Tonkovic-Capin) who did not know the origin of the hearts. Infarct weight was expressed as a percentage of left ventricular weight for each heart (4).

**Enzyme Leakage**

The entire coronary effluent was collected during the initial 40-min reperfusion period. The volume was recorded and an aliquot taken for determination of lactate dehydrogenase activity. Enzyme leakage was expressed as international units per 40 min per gram wet weight of heart (4).

**Delayed Cardioprotection with Isoflurane**

We performed the following experiments in a random order using eight hearts from two groups to determine whether isoflurane induces delayed cardioprotection. The two experimental groups were as follows: *group 1*, exposure of rabbits to 100% O\textsubscript{2} for 2 h; and *group 2*, exposure of rabbits to isoflurane (1% MAC)-100% O\textsubscript{2} for 2 h. Isoflurane-oxygen was then discontinued. Rabbits were allowed to recover 24 h, and resistance to myocardial ischemia was determined. Immediately after aortic cannulation, hearts were perfused in the Langendorff mode at constant perfusion pressure with a balloon placed in the left ventricle (4). Ventricular function and coronary flow rate were recorded under steady-state conditions. During the initial 40-min reperfusion period, indexes of cardiac function were measured under steady-state conditions, and the entire coronary effluent was collected for the determination of lactate dehydrogenase activity (4). At the end of the 3-h reperfusion period, hearts were processed and stained with triphenyltetrazolium dye for infarct size determination.

**K\textsubscript{ATP} Channel Studies**

We performed the following experiments in a random order using 5–16 hearts from six groups to determine whether the mitochondrial and sarcolemmal K\textsubscript{ATP} channels contribute to delayed cardioprotection in isoflurane-exposed hearts. The six experimental groups were as follows: *group 3*, control hearts treated with 5-HD; *group 4*, control hearts treated with HMR-1098; *group 5*, control hearts treated with 5-HD plus HMR-1098; *group 6*, isoflurane-exposed hearts treated with 5-HD; *group 7*, isoflurane-exposed hearts treated with HMR-1098; and *group 8*, isoflurane-exposed hearts treated with 5-HD and HMR-1098. 5-HD (100 μmol/l) or HMR 1098 (5 μmol/l) were added alone or in combination for 20 min before a global ischemic period of 30 min, followed by 3 h of reperfusion.

**Dose-Response Studies**

We performed the following experiments in random order using eight hearts from three groups to determine dose...
dependency of delayed cardioprotection with isoflurane. The three experimental groups were as follows: group 9, exposure of rabbits to isoflurane (2% MAC - 100% O₂) for 2 h; group 10, exposure of rabbits to isoflurane (1% MAC - 100% O₂) for 2 h; and group 11, exposure of rabbits to 100% O₂ for 2 h. Isoflurane-oxygen was then discontinued. Rabbits were allowed to recover 24 h, and resistance to myocardial ischemia was determined as described above.

Isoflurane Washout Studies

We performed the following studies in random order using six hearts from three groups of animals to determine the circulating levels of anesthetic in the blood following 2 h of isoflurane (1% MAC) treatment. The three experimental groups were as follows: group 12, 0 h following isoflurane treatment; group 13, 3 h following isoflurane treatment; and group 14, 24 h following isoflurane treatment. Rabbits were anesthetized with pentobarbital sodium (30 mg/kg ip). The abdomen was opened and the abdominal artery cannulated. One milliliter of blood was withdrawn, and isoflurane levels were determined by gas chromatography in the analytical core laboratory in the Department of Anesthesiology. Data are expressed as blood isoflurane concentration (in mmol/l).

Statistical Evaluation

All hearts that were entered into the study were included in the analysis. Recovery of developed pressure was expressed as a percentage of its predrug, preischemic value. Results are expressed as means ± SD. Statistical analysis was performed by use of repeated measures ANOVA with the Greenhouse-Geisser adjustment used to correct for the inflated risk of a Type I error (3) and, where this proved significant, the Mann-Whitney test was used as a second step to identify which groups were significantly different. After ANOVA the data were reanalyzed for differences related to multiple comparisons (3). Significance was accepted at a level of P < 0.05.

RESULTS

Delayed Cardioprotection With Isoflurane

The hemodynamic stability of the model was assessed by perfusing hearts in the Langendorff mode for 120 min with nonrecirculating perfusate. No significant changes in heart rate or developed pressure occurs until after 110 min. In the studies described that we used to test our hypothesis, the hearts were perfused for a maximum of 80 min to measure hemodynamic function, well within the stability limits of the preparation. Aerobic cardiac function before ischemia was determined in untreated and isoflurane-treated hearts (Table 1). Heart rate was unaffected by isoflurane treatment. Coronary flow rate in hearts treated with isoflurane was unchanged compared with untreated control hearts. Left ventricular developed pressure was unchanged by isoflurane treatment.

To determine the effect of isoflurane treatment on resistance to myocardial ischemia, recovery of postischemic function was examined in control and isoflurane-treated hearts. Isoflurane (1% MAC) significantly reduced infarct size/area at risk (means ± SD) by 50% (10 ± 5%) versus untreated controls (20 ± 6%) (Fig. 1). Isoflurane also increased recovery of postischemic left ventricular developed pressure by 28% (69 ± 4%) versus untreated controls (54 ± 6%) (Fig. 2). Postischemic leakage of lactate dehydrogenase into the coronary effluent during the first 40 min of reperfusion was unaffected by isoflurane treatment (Fig. 1). Exposure to 100% oxygen for 2 h did not exert any effect on delayed cardioprotection as measured by infarct size (19 ± 5%) or recovery of developed pressure (55 ± 2%).

To determine whether inspired oxygen levels exerted

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<td>1. 2 h Oxygen exposure, no intervention</td>
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<td>2. 2 h 1% Isoflurane exposure + no intervention</td>
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<td>3. 2 h Oxygen exposure + 5-HD (100 μmol/l)</td>
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<td>4. 2 h Oxygen exposure + HMR-1098 (5 μmol/l)</td>
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<td>8. 2 h 1% Isoflurane exposure + 5-HD (100 μmol/l) + HMR-1098 (5 μmol/l)</td>
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Values are means ± SD from 5 to 16 hearts per group. 5-HD, 5-hydroxydecanoate. †P < 0.05, predrug vs. reperfusion.
any affect upon resistance to ischemia, an additional control group of rabbits breathing room air (21% O₂) alone was studied. There was no difference in infarct size, postischemic enzyme leakage, or recovery of developed pressure. Thus the fraction of inspired oxygen did not affect resistance to myocardial ischemia 24 h later. The ambient oxygen levels during isoflurane exposure were supplemented with 100% O₂ to counter any possible anesthetic-induced respiratory and cardiac depression. The control group was also supplemented with 100% O₂. Thus both isoflurane and control groups were treated with oxygen in an identical manner.

Kₐₜₚ Channel Studies

To determine the optimal concentration for 5-HD and HMR-1098 for use in the Kₐₜₚ channel studies, we performed concentration-response studies for each drug (5-HD: 0–300 μmol/l, HMR-1098: 0–30 μmol/l) in isoflurane-treated hearts. In isoflurane-treated hearts both 5-HD and HMR-1098 exhibited a "U"-shaped response profile for recovery of left ventricular developed pressure and drug concentration. The optimal concentrations for reducing the cardioprotective effect of isoflurane treatment with 5-HD and HMR-1098 was 100 and 5 μmol/l, respectively. In untreated hearts, 100 μmol/l 5-HD and 5 μmol/l HMR-1098 did not affect recovery of left ventricular developed pressure compared with drug-free controls.

The effect of Kₐₜₚ channel blockers on aerobic function before ischemia was determined in untreated and isoflurane-treated hearts. The mitochondrial-selective Kₐₜₚ channel blocker 5-HD (100 μmol/l) did not affect heart rate or developed pressure in isoflurane-treated and untreated hearts. The sarcolemmal-selective Kₐₜₚ channel blocker HMR-1098 (5 μmol/l) did not affect heart rate or developed pressure in isoflurane-treated and untreated hearts. The combination of 5-HD (100 μmol/l) plus HMR-1098 (5 μmol/l) had no effect on heart rate or ventricular developed pressures in either untreated or isoflurane-treated hearts (Table 1).

To determine the contribution of the mitochondrial and sarcolemmal Kₐₜₚ channels upon resistance to myocardial ischemia, recovery of postischemic function was measured in untreated and isoflurane-treated hearts receiving 5-HD and HMR-1098 either alone or in combination before ischemia. The mitochondrial Kₐₜₚ channel blocker 5-HD completely (55 ± 3%) and the sarcolemmal Kₐₜₚ channel blocker HMR-1098 partially (62 ± 3%) abolished the cardioprotective effects of isoflurane (Fig. 2). The combination of 5-HD and HMR-1098 completely abolished the cardioprotective effect of isoflurane (56 ± 5%) (Fig. 2). 5-HD alone, HMR-109 alone, and the combination of 5-HD plus HMR-1098 did not affect recovery in control groups (55 ± 3%) (Fig. 2). These data indicate the mitochondrial and sarcolemmal Kₐₜₚ channels were not active in control hearts. The direction of change for infarct size for all groups studied was inversely related to the changes observed for recovery of developed pressure.

Dose-Response Studies

To determine whether delayed cardioprotection with isoflurane is dose dependent, rabbits were treated with 1% MAC or 2% MAC isoflurane. 1% MAC isoflurane treatment increased recovery of postischemic left ventricular developed pressure (69 ± 4%) compared with untreated controls (54 ± 6%). 1% MAC isoflurane also decreased infarct size (10 ± 5%) compared with un-
treated controls (20 ± 6%). However, 2% MAC isoflurane treatment did not increase recovery of left ventricular developed pressure (54 ± 6%) versus untreated controls (54 ± 6%). 2% MAC isoflurane also had no effect on infarct size (21 ± 5%) compared with untreated controls (20 ± 6%) (Fig. 3). These data suggest isoflurane exerts a delayed cardioprotective role over a narrow dose range. Postischemic leakage of lactate dehydrogenase into the coronary effluent during the first 40 min of reperfusion in untreated control hearts was 34 ± 4 IU/g wet wt. 1% MAC or 2% MAC isoflurane treatment did not affect enzyme leakage with values for enzyme leakage 33 ± 3 and 34 ± 3 IU/g wet wt, respectively.

Isoflurane Washout Studies

To determine the time course of anesthetic elimination following isoflurane treatment, circulating anesthetic levels were determined at 0, 3, and 24 h following discontinuation of treatment. The isoflurane concentration in blood declined from 0.3 ± 0.06 mmol/l at 0 h to 0.1 ± 0.05 mmol/l at 3 h. Isoflurane was not detected in blood 24 h following discontinuation of treatment (Fig. 4), suggesting the mechanism of cardioprotection is indirect.

DISCUSSION

Our study indicates that the volatile anesthetic isoflurane induces delayed cardioprotection at 24 h following exposure, which is manifest as an increase in recovery of postischemic developed pressure and a decrease in infarct size. This delayed cardioprotective effect of isoflurane is dose dependent and mediated by the sarcolemmal and mitochondrial K<sub>ATP</sub> channels. The cardioprotective effects of isoflurane appear indirect, because circulating levels of this volatile anesthetic are undetectable 24 h following exposure. Our study is the first to demonstrate delayed cardioprotection induced by a volatile anesthetic.

K<sub>ATP</sub> channels are not active under normal physiological conditions but are known to be activated following ischemia or hypoxia. However, our results indicate that isoflurane activates both the mitochondrial and sarcolemmal K<sub>ATP</sub> channels to confer delayed cardioprotection in the absence of ischemic or hypoxic stimuli. We recently showed cardioprotection induced by adaptation to chronic hypoxia is associated with activation of the mitochondria and sarcolemmal K<sub>ATP</sub> channel (27). In contrast, cardioprotection induced by ischemic preconditioning involves activation of the mitochondrial but not the sarcolemmal K<sub>ATP</sub> channel (23). We combined 5-HD with HMR-1098 to address the possibility of cross-talk between the mitochondrial and the sarcolemmal K<sub>ATP</sub> channels. Our data did not indicate cross talk was present in the current study.

Beneficial effects of acute treatment with volatile anesthetics on myocardial ischemia-reperfusion injury have been described. Halothane has been shown to reduce S-T segment alterations during coronary artery occlusion (22), and both halothane (15) and isoflurane (16) decrease infarct size in dogs. Enflurane was observed to reduce lactate production in myocardium distal to a coronary artery stenosis (42). In isolated hearts, halothane (9), enflurane (20), isoflurane (29), and sevoflurane (29) have been shown to improve function following global ischemia. Halothane and isoflu-
Isoflurane markedly enhance functional recovery of stunned myocardium (44) and preserve myocardial ATP and creatine phosphate (24). These studies, in combination with evidence documenting the importance of the K<sub>ATP</sub> channel in delayed ischemic preconditioning (7, 8, 30), prompted us to test the hypothesis that the delayed cardioprotective effects of the volatile anesthetic isoflurane are mediated by K<sub>ATP</sub> channels.

The mechanisms by which volatile anesthetics exert their acute protective actions on ischemic myocardium have previously been attributed to an improvement in the relationship between myocardial oxygen supply and demand. Volatile anesthetics are negative inotropes that decrease left ventricular contractility and depress sinoatrial nodal function, actions that decrease myocardial oxygen demand (9, 39). Although a portion of the acute cardioprotective effects of volatile anesthetics following ischemia and reperfusion may be attributed to a reduction in energy requirements and ultimate preservation of energy-dependent vital cellular processes, other studies have demonstrated this not to be necessary for cardioprotection. For example, halothane exerts cardioprotective effects even when administered during cardioplegic arrest and at reperfusion (31, 38). Thus a decrease in oxygen demand is unlikely to be the only mechanism responsible for the acute anti-ischemic actions of volatile anesthetics. This mechanism may be excluded in delayed cardioprotection, because circulating isoflurane levels are undetectable 24 h following discontinuation of treatment.

The identity of the mediator through which isoflurane exerts its delayed cardioprotective effect and the signal transduction pathway leading to activation of K<sub>ATP</sub> channels are unknown. Reactive oxygen species can mediate cardioprotection by ischemic preconditioning (13, 14, 32, 41, 43) and pharmacological preconditioning induced by diazoxide, an opener of the mitochondrial K<sub>ATP</sub> channel (19). The present study indicates the mitochondrial K<sub>ATP</sub> channel mediates isoflurane-induced delayed cardioprotection. Preconditioning can result in an increase of reactive oxygen species (41, 43) with cardioprotection abolished by free radical scavengers (32, 43). Isoflurane-induced constriction of rabbit coronary arteries is mediated by reactive oxygen species (33). We speculate that reactive oxygen species may mediate isoflurane-induced cardioprotection.

The absence of a relationship between functional recovery and posts ischemic lactate dehydrogenase leakage calls into question the utility of enzyme leakage as a meaningful index of myocardial protection in the immature heart. Our observation is consistent with previous studies where we observed a bell-shaped dose-response profile for pH of St. Thomas’ II cardioplegic solution during ischemia and recovery of posts ischemic aortic flow but not for posts ischemic creatine kinase leakage in the immature heart (2). This contrasts with the mature heart, where a bell-shaped dose-response curve existed for recovery of aortic flow and a U-shaped curve existed for creatine kinase leakage (2).

We considered whether altered hemodynamics are responsible for the delayed cardioprotective effect of isoflurane. However, there was no effect of isoflurane treatment on preischemic heart rate, coronary flow rate, and left ventricular developed pressure (Table 1). In studies on the effect of isoflurane on hemodynamic function in the intact rabbit, Bell (6) showed a decrease in mean arterial pressure without a change in heart rate upon increasing the concentration of isoflurane from 1.0% MAC to 2.0% MAC. Our data indicates that increasing the concentration of isoflurane from 1.0% MAC to 2.0% MAC did not result in a corresponding increase in delayed cardioprotection. Treatment of rabbits with 2% MAC isoflurane did not confer any delayed cardioprotection. Thus there appears to be no role for hemodynamic changes in the induction of delayed cardioprotection by isoflurane. In addition, this observation also suggested the cardioprotective effects of isoflurane were indirect as isoflurane treatment was discontinued 24 h before determining resistance to ischemia. To address this observation, we measured circulating levels of isoflurane in blood at 0, 3, and 24 h following discontinuation of treatment. Isoflurane was not detected in the blood 24 h following treatment. In addition, the isolated perfused heart was anesthetic free, further suggesting an indirect protective effect of the anesthetic. Thus isoflurane appears to act as a trigger to initiate a signal transduction pathway that culminates in the activation of the mitochondrial and sarcoslemmal K<sub>ATP</sub> Channels. Delayed cardioprotection in the isoflurane-treated group was abolished with concentrations of 5-HD and HMR-1098 that had no effect on aerobic function before ischemia and where the concentrations for 5-HD and HMR-1098 are lower than those described previously to block cardioprotection in adult hearts. This observation may indicate a different mechanism of K<sub>ATP</sub> channel activation compared with cardioprotection conferred by adaptation to chronic hypoxia and ischemic preconditioning.

**Clinical Relevance**

K<sub>ATP</sub> channels appear to be involved in the process of ischemic preconditioning in humans (12, 17, 18, 36). Ischemic preconditioning of isolated human myocytes can be abolished by the K<sub>ATP</sub> channel blocker glibenclamide and by the PKC inhibitor chelerythrine. One recent clinical outcome study of acute myocardial infarction suggests that sulfonylurea use is associated with increased risks of mortality in diabetic patients undergoing coronary angioplasty (21). Preconditioning is induced in humans undergoing percutaneous transluminal coronary angioplasty with a coronary occlusion of 2 min duration (17). Thus information regarding ischemic preconditioning in humans is rapidly expanding. In comparison, information regarding anesthetic-induced preconditioning in humans is limited. Despite this, anesthetic-induced preconditioning has been recently demonstrated in humans undergoing coronary artery bypass graft surgery (5, 34, 40).
Our results raise the possibility that delayed cardioprotection with isoflurane may have important clinical applications in two areas. The first is protection of the myocardium in the postoperative period. Patients with coronary artery disease undergoing general anesthesia for noncardiac surgery have the greatest severity of perioperative myocardial ischemia at times over 24 h postoperatively (28). Isoflurane may be able to increase resistance to myocardial ischemia in these patients. The second area is pretreatment to protect the heart during subsequent elective cardiac ischemia. Protection of immature myocardium during surgical correction of congenital birth defects remains incomplete resulting in postoperative cardiac dysfunction (10). In addition, sulfonylurea drug use is associated with increased risk of mortality in diabetic patients undergoing coronary angioplasty for acute myocardial infarction (21). Thus isoflurane may also be able to increase resistance of the heart to ischemia in the setting of cardiac surgery and coronary angioplasty.

We conclude both mitochondrial and sarcolemmal K$_{ATP}$ channels contribute to isoflurane-induced delayed cardioprotection in the neonatal rabbit. The signal transduction pathways are currently unknown, and the duration of cardioprotection requires further characterization. Further studies are warranted to determine whether delayed cardioprotection with isoflurane is present in the adult heart.

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