

# Differential Modulation of the Cardiac Adenosine Triphosphate-sensitive Potassium Channel by Isoflurane and Halothane

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**Background:** The cardiac adenosine triphosphate-sensitive potassium ( $K_{ATP}$ ) channel is activated during pathophysiological episodes such as ischemia and hypoxia and may lead to beneficial effects on cardiac function. Studies of volatile anesthetic interactions with the cardiac  $K_{ATP}$  channel have been limited. The goal of this study was to investigate the ability of volatile anesthetics halothane and isoflurane to modulate the cardiac sarcolemmal  $K_{ATP}$  channel.

**Methods:** The  $K_{ATP}$  channel current ( $I_{KATP}$ ) was monitored using the whole cell configuration of the patch clamp technique from single ventricular cardiac myocytes enzymatically isolated from guinea pig hearts.  $I_{KATP}$  was elicited by extracellular application of the potassium channel openers 2,4-dinitrophenol or pinacidil.

**Results:** Volatile anesthetics modulated  $I_{KATP}$  in an anesthetic-dependent manner. Isoflurane facilitated the opening of the  $K_{ATP}$  channel. Following initial activation of  $I_{KATP}$  by 2,4-dinitrophenol, isoflurane at 0.5 and 1.3 mM further increased current amplitude by  $40.4 \pm 11.1\%$  and  $58.4 \pm 20.6\%$ , respectively. Similar results of isoflurane were obtained when pinacidil was used to activate  $I_{KATP}$ . However, isoflurane alone was unable to elicit  $K_{ATP}$  channel opening. In contrast, halothane inhibited  $I_{KATP}$  elicited by 2,4-dinitrophenol by  $50.6 \pm 5.8\%$  and  $72.1 \pm 11.6\%$  at 0.4 and 1.0 mM, respectively. When  $I_{KATP}$  was activated by pinacidil, halothane had no significant effect on the current.

**Conclusions:** The cardiac sarcolemmal  $K_{ATP}$  channel is differentially modulated by volatile anesthetics. Isoflurane can facilitate the further opening of the  $K_{ATP}$  channel following initial channel activation by 2,4-dinitrophenol or pinacidil. The effect of halothane was dependent on the method of channel activation, inhibiting  $I_{KATP}$  activated by 2,4-dinitrophenol but not by pinacidil.

VOLATILE anesthetics have cardiac depressant effects and inhibit various ion channels in the heart. However, multiple effects of volatile anesthetics on the myocardium suggest the complexity of the underlying cellular and molecular mechanisms. Inhibition of cardiac voltage-gated calcium and sodium channels by volatile anesthetics is well documented<sup>1–3</sup> and may lead to an increased propensity to arrhythmias. However, recent studies have convincingly shown that volatile anesthetics

can also be cardioprotective.<sup>4–8</sup> This cardioprotection, termed anesthetic-induced preconditioning, mimics ischemic preconditioning,<sup>9</sup> whereby a small ischemic episode protects the myocardium from a subsequent, more devastating insult.

The underlying mechanisms involved in anesthetic-induced preconditioning have not been elucidated. Despite the potentially numerous targets of volatile anesthetics, including ion channels and intracellular second messenger systems, the adenosine triphosphate-sensitive potassium ( $K_{ATP}$ ) channel has been hypothesized to be one of the major target proteins involved in anesthetic-induced cardioprotection.<sup>5,10</sup> The sarcolemmal  $K_{ATP}$  channel is an attractive target since it acts as a metabolic sensor, and its activation leads to shortening of the cardiac action potential.<sup>11,12</sup> This, in turn, would lead to decreased calcium entry *via* the voltage-gated calcium channels and preservation of high-energy phosphates. Recent studies have also shown that the  $K_{ATP}$  channel on the inner membrane of mitochondria plays a more pivotal role in cardioprotection, particularly in ischemic preconditioning.<sup>13–15</sup> Volatile anesthetics, isoflurane and sevoflurane, were also recently reported to induce a redox-dependent increase in mitochondrial flavoprotein oxidation, an indicator of mitochondrial  $K_{ATP}$  channel opening.<sup>16</sup> Consequently, anesthetic-induced and ischemic preconditioning likely involve complex pathways that may include both the mitochondrial and sarcolemmal  $K_{ATP}$  channels.

Evidence for the involvement of the sarcolemmal  $K_{ATP}$  channel in anesthetic-induced preconditioning is derived from infarct-size studies using whole animal models.<sup>5</sup> On the other hand, direct studies of volatile anesthetic effects on the  $K_{ATP}$  channel have been limited. In the present study, the effects of two volatile anesthetics, isoflurane and halothane, on the cardiac sarcolemmal  $K_{ATP}$  channel were investigated using the whole cell configuration of the patch clamp technique.

## Materials and Methods

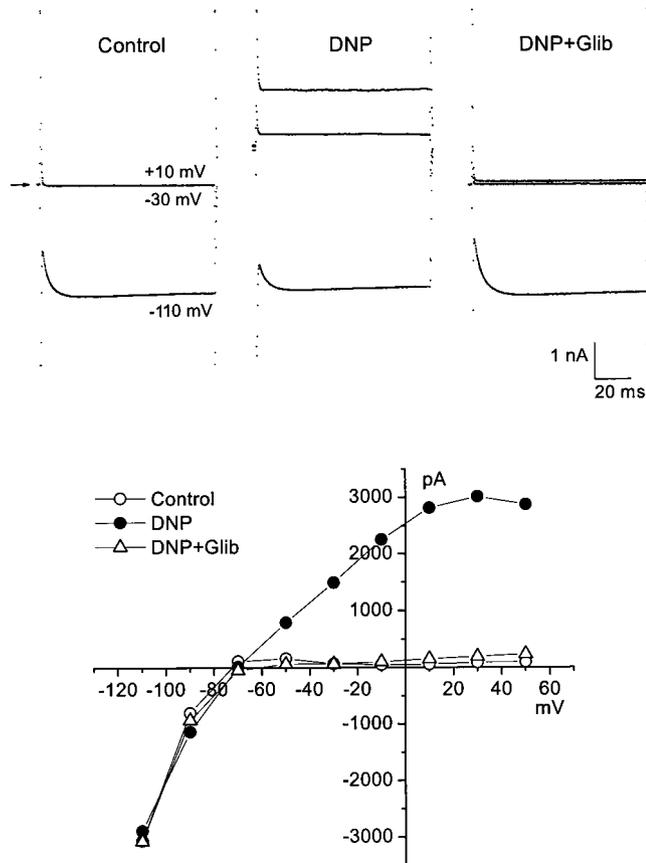
### Preparation of Isolated Cardiac Ventricular Myocyte

After approval was obtained from the Institutional Animal Care and Use Committee, cardiac myocytes were enzymatically isolated from guinea pigs weighing 200–300 g. The procedure of the cell isolation is a modification of that of Mitra and Morad<sup>17</sup> and has previously been

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**Fig. 1.** Activation of  $I_{KATP}$  by 2,4-dinitrophenol (DNP). (Top) Sample whole cell current traces recorded in control, in the presence of 120  $\mu$ M DNP, and in the presence of DNP + glibenclamide (Glib, 200 nM). Current was monitored at test potentials of -110, -30, and +10 mV from a holding potential of -40 mV. Current at -110 mV is the inward-rectifier K current. The outward current sensitive to glibenclamide is  $I_{KATP}$ . (Bottom) The corresponding current-voltage relation. Current amplitude was measured at the end of the 100-ms test pulses.

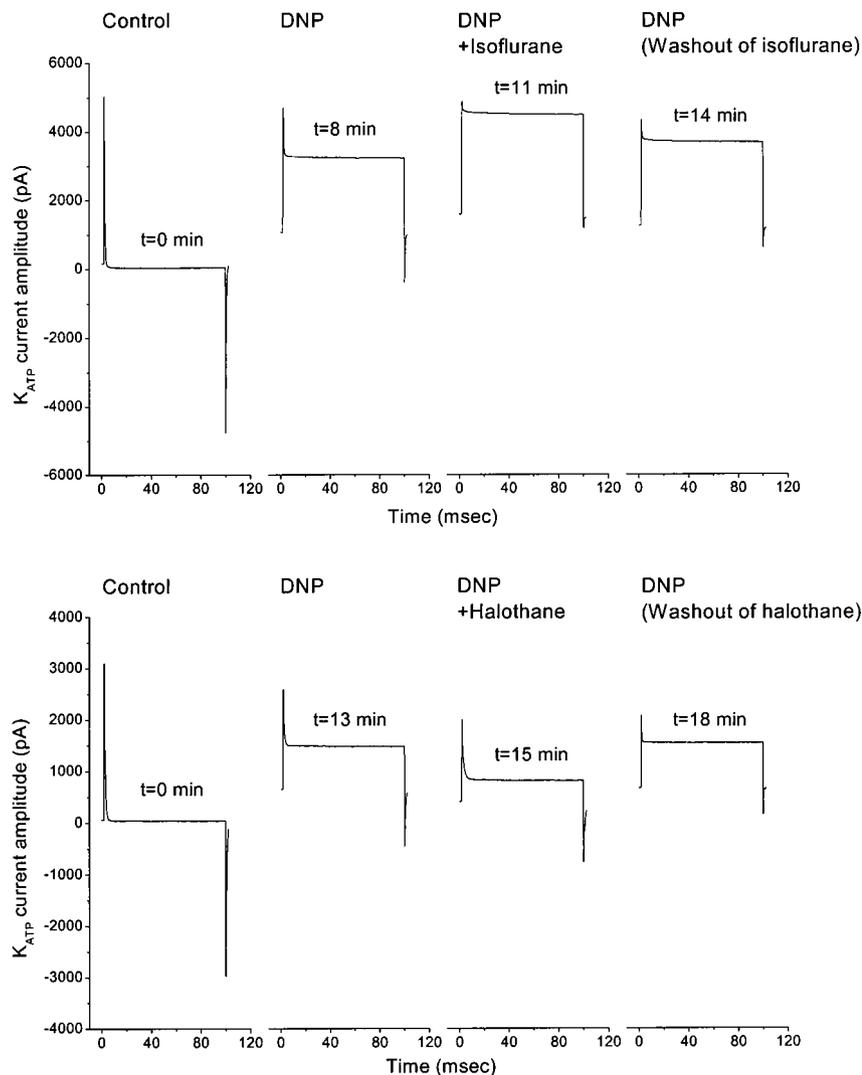
reported.<sup>3</sup> In brief, the guinea pigs were anesthetized by intraperitoneal injection of 180 mg/kg pentobarbital sodium and injected with 1,000 U heparin to hinder coagulation. During deep anesthesia, the hearts were quickly excised and mounted *via* the ascending aorta on a Langendorff-type apparatus. Each heart was perfused retrogradely at a rate of 6–8 ml/min with Joklik's medium containing 2.5 U/ml heparin at pH 7.23. After 3–4 min to allow for clearing of blood, the perfusing solution was replaced with an enzyme solution containing Joklik's medium with 0.25 mg/ml collagenase (Gibco Life Technologies, Grand Island, NY), 0.13 mg/ml protease (Type XIV, Sigma, St. Louis, MO), and 1 mg/ml bovine serum albumin (Serologicals Proteins, Kankakee, IL) at pH 7.23. The perfusion solutions were oxygenated (95%  $O_2$ -5%  $CO_2$ ) and maintained at 37°C. After 14 min of recirculating the enzyme solution, the ventricles were removed, cut into small fragments, and incubated for approximately 3–8 min in a shaker bath in the enzyme solution.

The cell suspension was then filtered, centrifuged, and washed twice in Tyrode solution before the cells were ready for experiments. The cells were stored in Tyrode solution at room temperature (20–25°C) and used within 12 h after isolation. For the patch clamp experiments, cells were transferred to a recording chamber mounted on the stage of an inverted microscope.

### Solutions

The isolated myocytes were initially washed in a standard Tyrode solution that contained the following ingredients: 132.0 mM NaCl, 4.8 mM KCl, 1.2 mM  $MgCl_2$ , 1.0 mM  $CaCl_2$ , 5.0 mM dextrose, and 10.0 mM HEPES, with pH adjusted to 7.4 with NaOH. After establishing a gigaohm seal, the external Tyrode solution was changed to one appropriate for measurement of potassium channel currents and contained 132.0 mM *N*-methyl-D-glucamine (substitute for sodium), 1.0 mM  $CaCl_2$ , 2.0 mM  $MgCl_2$ , 10.0 mM HEPES, and 5.0 mM KCl, with pH adjusted to 7.4 with HCl. Nisoldipine (200 nM), supplied by Miles Pentex (West Haven, CT), was also added to block the L-type Ca channel current. To elicit activation of the  $K_{ATP}$  current, 2,4-dinitrophenol or pinacidil, a  $K_{ATP}$  channel opener, was used. 2,4-Dinitrophenol (Sigma Chemical) was added directly to the external buffer solution to obtain a desired concentration. Pinacidil (Sigma/RBI) was prepared as a 10-mM stock in dimethyl sulfoxide and diluted to the desired concentration in the external solution. In a specified set of experiments, bimakalim was used as a potassium channel opener. Bimakalim was supplied by Garrett Gross, Ph.D. (Professor, Department of Pharmacology and Toxicology, Medical College of Wisconsin) and was prepared in dimethyl sulfoxide. The final concentration of dimethyl sulfoxide (0.025%) had no effect on the whole cell K currents. The standard pipette solution contained 60.0 mM K-glutamate, 50.0 mM KCl, 1.0 mM  $CaCl_2$ , 1.0 mM  $MgCl_2$ , 11.0 mM EGTA, and 0.1–1.0 mM  $K_2ATP$ , with pH adjusted to 7.4 with KOH.

The volatile anesthetics, isoflurane (Ohmeda Caribe Inc., Liberty Corner, NJ) and halothane (Halocarbon Laboratories, River Edge, NJ), were mixed by adding known aliquots of concentrated anesthetics to graduated syringes with the appropriate bath solutions. Isoflurane and halothane superfusions were achieved using a syringe pump with a constant flow of 1 ml/min. Clinically relevant concentrations of isoflurane (0.5–1.3 mM, equivalent to 1.048–2.723 vol%) and halothane (0.4–1.0 mM, equivalent to 0.643–1.610 vol%) were used. To determine anesthetic concentrations, 1 ml of the superfusate was collected in a metal-capped 2-ml glass vial at the end of each experiment. The superfusate concentration of the anesthetic was then determined by gas chromatography (head-space analysis) utilizing flame ionization detection Perkin-Elmer Sigma 3B gas chromatograph.



**Fig. 2.** Effect of isoflurane and halothane on 2,4-dinitrophenol (DNP)-activated  $I_{KATP}$ . Current was monitored every 15 s during a 100-ms test pulse to 0 mV from a holding potential of -40 mV. Traces are sequentially shown at the time depicted. The time  $t = 0$  min denotes current recordings in control obtained immediately prior to application of DNP. The effects of 1.3 mM isoflurane (*top*) and 0.4 mM halothane (*bottom*) are shown. Isoflurane and halothane were applied after steady state effects of DNP were reached. Note that the holding current at -40 mV was increased due to activation of  $I_{KATP}$  by DNP and by DNP + isoflurane. In contrast, the holding current was depressed by DNP + halothane.

### Electrophysiology

Adenosine triphosphate-sensitive potassium current ( $I_{KATP}$ ) was recorded in the whole cell configuration of the patch clamp technique. Pipettes were pulled from borosilicate glass capillary tubes (Garner Glass, Claremont, CA) using a horizontal two-stage puller (Sachs-Flaming PC-84; Sutter Instruments, Novato, CA) and heat polished (Narishige microforge; MF-83, Tokyo, Japan). In standard solutions, pipette resistance ranged from 2.5 to 3.5 M $\Omega$ . Current was monitored during 100-ms test pulses from -110 to +50 mV in 10-mV increments from a holding potential of -40 mV. During this recording condition, contributions from the cardiac delayed-rectifier potassium current was minimal due to its activation kinetics of several hundred milliseconds at room temperature. To monitor changes in current amplitude over time,  $I_{KATP}$  was recorded every 15 s during a 100-ms test pulse to 0 mV from a -40-mV holding potential.  $I_{KATP}$  amplitude was measured at the end of the 100-ms test pulse. Series resistance compensation was adjusted to give the fastest possible cell capacity transients

without producing ringing. Current was measured with a List EPC-7 patch clamp amplifier (Adams & List Assoc., Great Neck, NY), and the output was lowpass filtered at 3 kHz to reduce high-frequency noise. Experiments were performed at room temperature (20–25°C). Data were acquired and analyzed with the pClamp software package (versions 6.02 and 8.0; Axon Instruments, Inc., Foster City, CA) and ORIGIN (OriginLab, Northampton, MA).

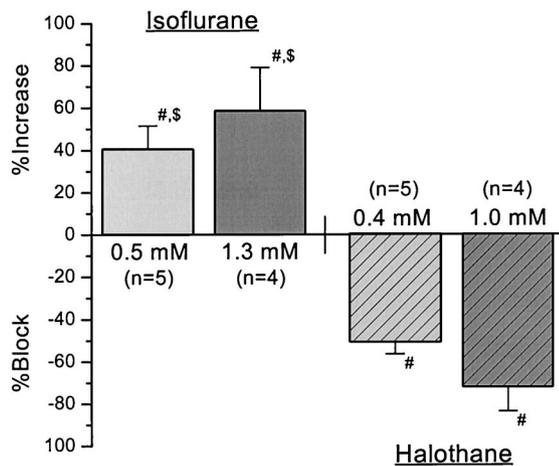
### Statistics

Data are expressed as means  $\pm$  SEM. Statistical differences were determined using paired or unpaired Student  $t$  test. Differences were considered statistically significant at  $P < 0.05$ .

### Results

#### Effect of 2,4-Dinitrophenol on Whole Cell $K^+$ Current

The effect of 2,4-dinitrophenol on whole cell  $K^+$  current recorded from a cardiac myocyte is demonstrated in



**Fig. 3.** Summary of the effects of isoflurane and halothane on 2,4-dinitrophenol (DNP)-activated  $I_{KATP}$ . Percent increase or block of  $K_{ATP}$  current amplitude was measured from the steady state DNP concentration prior to application of the anesthetics. Current amplitude was measured at the end of the 100-ms test pulse to 0 mV from a  $-40$ -mV holding potential. #Significantly different from control; \$Significantly different from 0.4 and 1.0 mM halothane,  $P < 0.05$ . Isoflurane did not show significantly different effects on  $I_{KATP}$  at 0.5 and 1.3 mM. Similarly, halothane did not show significantly different effects at 0.4 and 1.0 mM.

figure 1. During control conditions, the only prominent K current was the inward-rectifier  $K^+$  current recorded at potentials negative to the potassium equilibrium potential,  $E_K$ . In the presence of 2,4-dinitrophenol ( $120 \mu M$ ), an outward current was elicited at potentials positive to  $E_K$ . This current was identified as the sarcolemmal  $K_{ATP}$  current ( $I_{KATP}$ ) by its sensitivity to glibenclamide ( $200 nM$ ). At potentials negative to  $E_K$ , glibenclamide had no effect on the inward-rectifier  $K^+$  current.

#### Volatile Anesthetic Effects on 2,4-Dinitrophenol-induced $I_{KATP}$

The effects of isoflurane and halothane on the sarcolemmal  $K_{ATP}$  channel activated by 2,4-dinitrophenol were investigated in the next series of experiments, where we monitored  $K_{ATP}$  current amplitude every 15 s.  $I_{KATP}$  was activated by  $120 \mu M$  2,4-dinitrophenol. The effects of the volatile anesthetics are demonstrated in figure 2. The current monitored at time  $t = 0$  min was recorded immediately prior to the application of 2,4-dinitrophenol. The effects of isoflurane and halothane were recorded in the continued presence of 2,4-dinitrophenol but after the effect of 2,4-dinitrophenol has reached steady state. The example shows that isoflurane ( $1.3$  mM) potentiated  $I_{KATP}$  that was activated by 2,4-dinitrophenol. The increase in current amplitude was approximately 39%. In contrast, halothane ( $0.5$  mM) had an inhibitory effect, decreasing current amplitude by approximately 45%. In both cases, the effects of the anesthetics were reversible. A summary of the effects of the volatile anesthetics on  $I_{KATP}$  is shown in figure 3. At the concentrations tested, isoflurane further increased

$I_{KATP}$  amplitude initially activated by 2,4-dinitrophenol, while halothane decreased 2,4-dinitrophenol-activated  $I_{KATP}$ . For both the isoflurane and halothane groups, the anesthetic effects on  $I_{KATP}$  had a tendency to be greater at the higher concentrations. However, within each anesthetic group, there were no significant concentration-dependent differences.

#### Volatile Anesthetic Effects on Pinacidil-induced

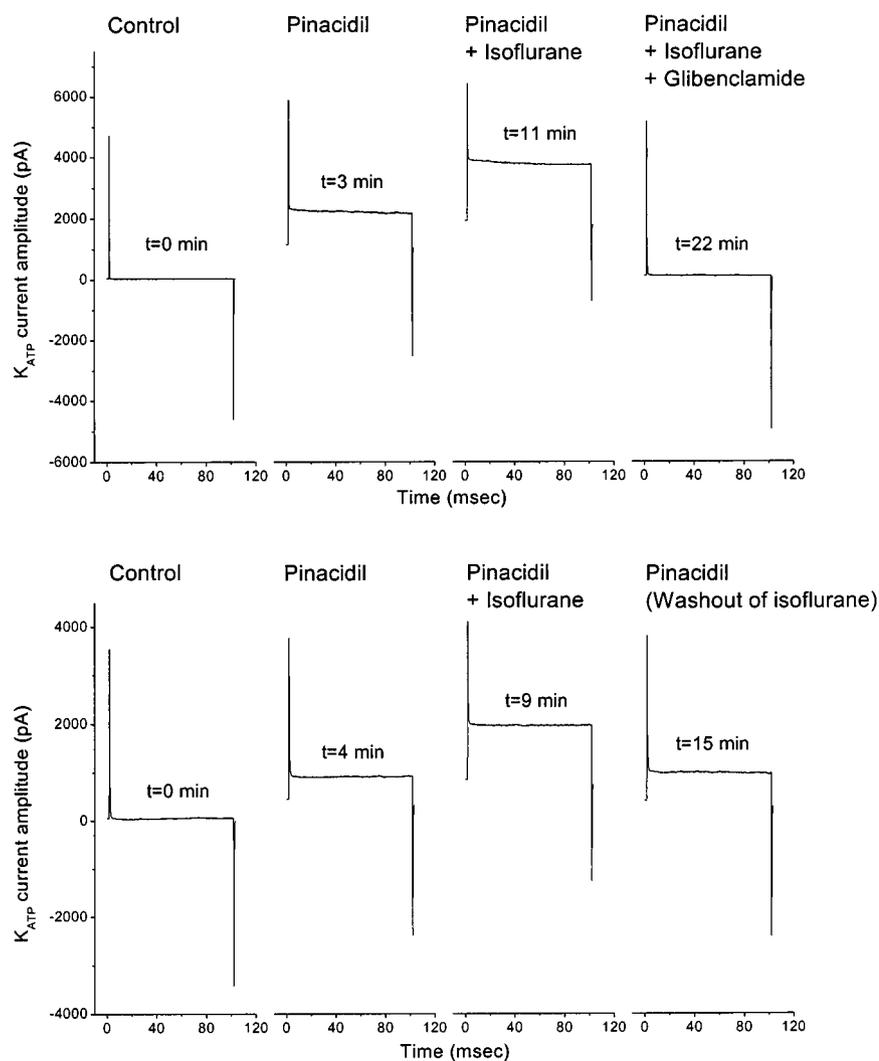
##### $I_{KATP}$

The results from the 2,4-dinitrophenol studies showed differential and contrasting effects of isoflurane and halothane on  $I_{KATP}$ . The effects of these anesthetics can possibly be due to the method of  $K_{ATP}$  channel activation. The activation of the  $K_{ATP}$  channel by 2,4-dinitrophenol is due to the uncoupling of oxidative phosphorylation, leading to decreased intracellular ATP. 2,4-Dinitrophenol has also been shown to directly interact with the  $K_{ATP}$  channel protein, leading to opening of the channel.<sup>18</sup> Consequently, to test whether the anesthetic effects were unique to 2,4-dinitrophenol-activated  $I_{KATP}$ , experiments using pinacidil, a  $K_{ATP}$  channel opener, were carried out. Figure 4 demonstrates the effects of isoflurane on a pinacidil-activated  $I_{KATP}$ . Whole cell current trace was recorded during a test-pulse potential of 0 mV from a  $-40$ -mV holding potential. During control, 25 min was allowed to elapse before the extracellular application of pinacidil to allow for diffusional exchange of  $0.5$  mM ATP between the recording pipette and the cell's interior. At this concentration of ATP, the  $K_{ATP}$  channel remained inhibited. In figure 4, the time  $t = 0$  min denotes current recordings in control immediately prior to application of pinacidil. In the presence of pinacidil,  $K_{ATP}$  current was activated. After the effect of pinacidil reached steady state,  $0.6$  mM isoflurane was applied in the continued presence of pinacidil, resulting in a further increase in  $I_{KATP}$ . The isoflurane-potentiated current was inhibited by glibenclamide. The effect of isoflurane on pinacidil-activated  $I_{KATP}$  was also reversible (fig. 4).

In 8 cells, isoflurane at  $0.6$  mM further increased  $K_{ATP}$  current amplitude in the presence of pinacidil, as summarized in figure 5. This result was similar to those observed with 2,4-dinitrophenol-activated  $I_{KATP}$ . On the other hand, halothane had no significant effect on the pinacidil-activated current (fig. 5). This is in contrast to the inhibitory effects of halothane on the 2,4-dinitrophenol-activated current.

#### The Effect of Isoflurane on Whole Cell $I_{KATP}$

The previous sets of experiments demonstrated that isoflurane can facilitate the further opening of the  $K_{ATP}$  channel after initial activation by either 2,4-dinitrophenol or pinacidil. Therefore, whether isoflurane alone can open the  $K_{ATP}$  channel was tested. A time-course experiment where  $I_{KATP}$  amplitude was monitored at 15-s



**Fig. 4.** Effects of isoflurane on pinacidil-activated  $I_{K_{ATP}}$ . Whole cell current monitored during a 100-ms test pulse to 0 mV from a  $-40$ -mV holding potential was recorded in control, in  $5 \mu\text{M}$  pinacidil, and in pinacidil +  $0.6 \text{ mM}$  isoflurane. (*Top*) The inhibition of the isoflurane-potentiated current by  $500 \text{ nM}$  glibenclamide. (*Bottom*) The reversibility of the isoflurane effect on pinacidil-activated  $I_{K_{ATP}}$ . Traces are sequentially shown at the time depicted. The time  $t = 0 \text{ min}$  denotes the recording of current during control conditions immediately prior to application of pinacidil. Note that the holding current at  $-40 \text{ mV}$  also increased due to activation of  $I_{K_{ATP}}$  by pinacidil and by pinacidil + isoflurane.

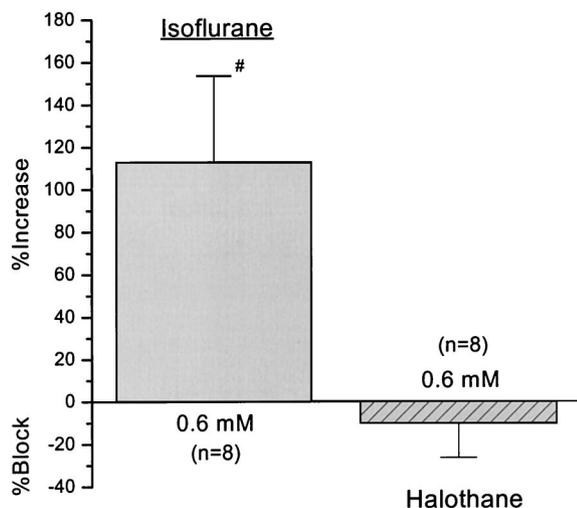
intervals is depicted in figure 6. After allowing for diffusional exchange of  $1 \text{ mM}$  ATP between the pipette solution and the cell's interior, isoflurane ( $0.6 \text{ mM}$ ) was applied extracellularly. However, during a 10-min application, isoflurane failed to elicit any outward current characteristic of  $I_{K_{ATP}}$ . Upon washout of isoflurane, an application of a  $K_{ATP}$  channel opener, bimakalim, resulted in the activation of  $I_{K_{ATP}}$ , confirming the functional existence of the  $K_{ATP}$  channel in this myocyte. In six cells tested, isoflurane failed to elicit  $K_{ATP}$  channel opening. Even after lowering the intracellular ATP to  $0.5 \text{ mM}$ , near the threshold for channel opening, isoflurane failed to activate  $I_{K_{ATP}}$  (data not shown).

## Discussion

The results from this study show that volatile anesthetics, isoflurane and halothane, have differential effects on the sarcolemmal  $K_{ATP}$  channel in guinea pig ventricular myocytes. During conditions where  $I_{K_{ATP}}$  was initially activated by 2,4-dinitrophenol or pinacidil, isoflurane

further increased  $K_{ATP}$  current amplitude. In contrast, halothane either inhibited or had no significant effects on 2,4-dinitrophenol- or pinacidil-activated  $I_{K_{ATP}}$ , respectively. In addition, although isoflurane facilitated opening of the  $K_{ATP}$  channel, the anesthetic by itself was unable to directly activate  $I_{K_{ATP}}$ . Thus, isoflurane alone is not an effective  $K_{ATP}$  channel opener.

Since the studies by Kersten *et al.* and Cason *et al.* reporting on the cardioprotective effects of volatile anesthetics that mimic ischemic preconditioning, anesthetic effects on the sarcolemmal  $K_{ATP}$  channels have been implicated.<sup>5,6</sup> However, direct evidence of volatile anesthetic modulation of the sarcolemmal  $K_{ATP}$  channels has been limited.<sup>19,20</sup> In the rabbit ventricular myocytes, isoflurane shifted the  $K_{ATP}$  channel's sensitivity to ATP and increased the mean closed time.<sup>19</sup> The net result of a decreased ATP sensitivity coupled with an increase in mean closed time is ambiguous. The results from the present study show that the net outcome is an increase in whole cell  $K_{ATP}$  current amplitude, specifically in guinea pig ventricular myocytes. Another recent evi-



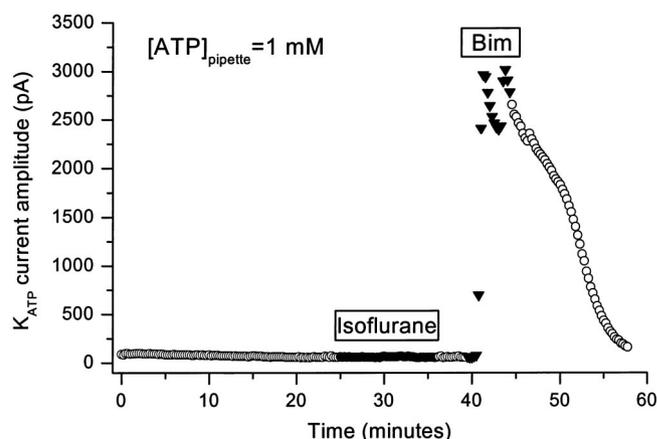
**Fig. 5.** Summary of the effects of isoflurane and halothane on pinacidil-activated  $I_{K_{ATP}}$ . Percent changes in  $K_{ATP}$  current amplitude were measured from the steady state pinacidil concentration prior to application of the anesthetics. #Significantly different from control.

dence of volatile anesthetic action on the sarcolemmal  $K_{ATP}$  channel was reported in studies on human atrial trabecular muscles.<sup>20</sup> In these cells, halothane decreased and isoflurane had no effect on  $I_{K_{ATP}}$ . Thus, the inhibitory effect of halothane on  $I_{K_{ATP}}$  is similar for the human atrial and guinea pig ventricular myocytes. On the other hand, the effects of isoflurane were different. This cannot be attributed to differences in the method of  $K_{ATP}$  channel activation, where in both cases 2,4-dinitrophenol was used. It is conceivable that the difference is due to species or tissue differences. Whether the  $K_{ATP}$  channels in atrial myocytes are less sensitive to isoflurane than those in the ventricles remain to be determined. Our previous studies have shown that the L-type Ca channels in the guinea pig atria were more sensitive to isoflurane than those in the ventricles.<sup>21</sup> Thus, differential sensitivity to isoflurane may also be characteristic of  $K_{ATP}$  channels in the atria and ventricle.

The effects of isoflurane and halothane on the  $K_{ATP}$  channel showed no significant differences between the two concentrations tested. This suggests that the concentrations of volatile anesthetic used were at saturating levels of  $K_{ATP}$  channel modulation. On the other hand, the observed variability suggests the disadvantage and difficulty in controlling the effects of 2,4-dinitrophenol. The consequence of uncoupling oxidative phosphorylation by 2,4-dinitrophenol leads to a decrease in intracellular ATP concentrations. However, the rate of ATP decrease and the accompanying intracellular changes will differ from cell to cell. Given the complexity of  $K_{ATP}$  channel modulation, which is sensitive to ATP, ADP,  $Mg^{2+}$ , and phospholipids, to name a few intracellular modulators,<sup>12,22-24</sup> the microenvironment surrounding the channel protein will likely differ from cell to cell. The isoflurane effect on the  $K_{ATP}$  channel may also be

modulated by these agents. For example, at 1 mM ATP, isoflurane failed to open the  $K_{ATP}$  channel when applied alone. This showed that isoflurane alone was unable to overcome the inhibitory effect of ATP. However, during conditions where the  $K_{ATP}$  channel was initially activated, isoflurane facilitated its opening, leading to an increase in  $I_{K_{ATP}}$ . It appears that prior channel opening is a "precursor" to the isoflurane effect. One possible underlying mechanism is that isoflurane may partially desensitize the channel to ATP, resulting in a greater current flow. However, since isoflurane alone was unable to elicit  $I_{K_{ATP}}$  even during conditions of 0.5 mM ATP, which is close to the threshold for channel opening, other intracellular mechanisms are likely to be involved.

The pinacidil experiments showed that the effect of isoflurane on  $I_{K_{ATP}}$  is independent of the method of channel activation. In contrast, the effect of halothane was dependent on the method of channel activation, suggesting that different mechanisms may underlie the actions of isoflurane and halothane on  $I_{K_{ATP}}$ . Studies on a rabbit model have shown that halothane has cardioprotective effects mimicking ischemic preconditioning.<sup>25</sup> However, results from the human atrial studies suggest that halothane diminishes the protective effects of ischemic preconditioning, while isoflurane induces protection.<sup>20</sup> This discrepancy may be attributed to the different models used and may imply potential species-dependent differences in the mechanism underlying cardioprotection. For example, the action of 2,4-dinitrophenol on the mitochondria results in uncoupling of oxidative phosphorylation. Pinacidil acts directly on the sarcolemmal  $K_{ATP}$  channel but also opens the mitochondrial  $K_{ATP}$  channel.<sup>26</sup> Consequently, it is conceivable that the halothane effect may be differentially dependent on the intracellular changes resulting from alterations in



**Fig. 6.** Isoflurane alone does not activate  $I_{K_{ATP}}$ .  $K_{ATP}$  current was monitored every 15 s during a test pulse to 0 mV from a holding potential of -40 mV. Current amplitude was measured at the end of the 100-ms test pulse. Control recordings were obtained for 25 min to allow for the diffusional exchange of 1 mM adenosine triphosphate from the pipette solution to the cell's interior prior to application of 1.0 mM isoflurane. Bimakalim (20  $\mu M$ , Bim) was applied in the absence of isoflurane.

mitochondrial function initiated by 2,4-dinitrophenol or pinacidil.

Cardioprotection by isoflurane mimicking ischemic preconditioning is well documented in laboratory and, more recently, clinical studies.<sup>27</sup> However, the underlying mechanism for this protection has not been elucidated. Earlier studies have hypothesized that the sarcolemmal  $K_{ATP}$  channel was the end effector in both ischemic and anesthetic preconditioning. Recent studies have demonstrated that the mitochondrial  $K_{ATP}$  channel may play a more significant role, particularly in ischemic preconditioning.<sup>13,14,28</sup> Diazoxide, a potassium channel opener more specific for the cardiac mitochondrial rather than the sarcolemmal  $K_{ATP}$  channel, can mimic ischemic preconditioning. Opening of the mitochondrial  $K_{ATP}$  channel may subsequently trigger intracellular changes, leading to cardioprotection. On the other hand, activation of the cardiac sarcolemmal  $K_{ATP}$  channel may play a larger role during reperfusion and reoxygenation.<sup>28</sup>

Although recent evidence supports the greater role of the mitochondrial  $K_{ATP}$  channel, possible contributions by the sarcolemmal  $K_{ATP}$  cannot be entirely excluded. It has been demonstrated that transfecting a cell with the sarcolemmal  $K_{ATP}$  channel can lead to the protection against hypoxia.<sup>29</sup> In addition, the cardioprotective effects of desflurane were found to involve both the sarcolemmal and mitochondrial  $K_{ATP}$  channels.<sup>30</sup> Furthermore, although the pathways involved in ischemic preconditioning are better characterized than those for anesthetic preconditioning, that identical mechanisms are involved in the two types of cardioprotection has not been established. It is possible that divergent pathways are involved since the initial trigger mechanism, ischemic *versus* volatile anesthetic, is different. In addition, the result that isoflurane can facilitate the opening of the sarcolemmal  $K_{ATP}$  channel suggests that it may be involved in anesthetic preconditioning in conjunction with activation of the mitochondrial  $K_{ATP}$  channel.<sup>16</sup>

In summary, the results from this study show differential effects of isoflurane and halothane on the cardiac sarcolemmal  $K_{ATP}$  channel. Isoflurane facilitated the opening of the  $K_{ATP}$  channel after prior activation by either 2,4-dinitrophenol or pinacidil. In contrast, halothane inhibited the 2,4-dinitrophenol-activated  $I_{KATP}$  but had no significant effect on the pinacidil-activated  $I_{KATP}$ .

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