Isoflurane-induced Facilitation of the Cardiac Sarcolemmal \( K_{ATP} \) Channel

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Background: Volatile anesthetics have cardioprotective effects that mimic ischemic preconditioning, including the involvement of adenosine triphosphate–sensitive potassium (\( K_{ATP} \)) channels. However, evidence for a direct effect of volatile anesthetic on the \( K_{ATP} \) channel is limited. In this study, the effects of isoflurane on the cardiac sarcolemmal \( K_{ATP} \) channel were investigated.

Methods: Single ventricular myocytes were enzymatically isolated from guinea pig hearts. Whole cell and single-channel configurations, specifically the cell-attached and inside-out patch mode, of the patch clamp technique were used to monitor sarcolemmal \( K_{ATP} \) channel current.

Results: In the cell-attached patch configuration, 2,4-dinitrophenol (150 \( \mu \)M) opened the sarcolemmal \( K_{ATP} \) channel. Isoflurane (0.5 mM) further increased channel open probability and the number of active channels in the patch. In contrast, in the inside-out patch experiments, isoflurane had no significant effect on the \( K_{ATP} \) channel activated by low ATP (0.2–0.5 mM). In addition, isoflurane had no effect on the \( K_{ATP} \) channel when activated by adenosine diphosphate, adenosine + guanosine triphosphate, bimakalim, and 2,4-dinitrophenol under inside-out patch configurations. When \( K_{ATP} \) current was monitored in the whole cell mode, isoflurane alone was unable to elicit channel opening. However, during sustained protein kinase C activation by 12,13-dibutryrate, isoflurane activated the \( K_{ATP} \) current that was sensitive to glibenclamide. In contrast, isoflurane had no effect on the \( K_{ATP} \) channel activated by 12,13-dibutryrate in a cell-free environment.

Conclusions: Isoflurane facilitated the opening of the sarcolemmal \( K_{ATP} \) channel in the intact cell, but not in an excised, inside-out patch. The isoflurane effect was not due to a direct interaction with the \( K_{ATP} \) channel protein, but required an intracellular component, likely including the translocation of specific protein kinase C isoforms. This suggests that the sarcolemmal \( K_{ATP} \) channel may have a significant role in anesthetic-induced preconditioning.

BRIEF periods of sublethal ischemia reduce the amount of myocyte necrosis produced by a subsequent sustained period of ischemia.\(^1\) This phenomenon, termed ischemic preconditioning (IPC), has been shown to be cardioprotective in several mammalian models, including dogs,\(^2,\) rabbits,\(^4\) rats,\(^5\) pigs,\(^6\) and humans.\(^7\) Although the underlying mechanism is still unclear, much has been documented of the involvement of cardiac sarcolemmal adenine triphosphate–sensitive potassium (\( K_{ATP} \)) channels in IPC. Several studies have shown that glibenclamide, a potent \( K_{ATP} \) channel blocker, abolishes the beneficial effects of IPC,\(^8\) while \( K_{ATP} \) channel openers, such as bimakalim, pinacidil, and cromakalim, mimicked the cardioprotective effects of IPC.\(^9,\)\(^10\) Recent studies have also implicated the contribution of the mitochondrial \( K_{ATP} \) channel in IPC.\(^11\) However, the relative contributions of the cardiac sarcolemmal and mitochondrial \( K_{ATP} \) channels in IPC are not clear. Recent evidence suggests that the mitochondrial \( K_{ATP} \) channel may play a more significant role in cardioprotection than the sarcolemmal channel.\(^12\) Yet the contribution by the sarcolemmal \( K_{ATP} \) channel cannot be irrefutably discounted. The various signaling pathways that underlie cardioprotection can potentially modulate the mitochondrial and sarcolemmal \( K_{ATP} \) channels. Both channel types may turn out to be important in cardioprotection by IPC.\(^13\)

Volatile anesthetics, particularly isoflurane, were recently found to mimic IPC of the heart.\(^14,\)\(^15\) The cellular mechanisms underlying anesthetic-induced preconditioning are not known but may parallel those of IPC. Since the isoflurane effects were abolished by glibenclamide, the \( K_{ATP} \) channels have been implicated. However, direct evidence at the cellular and molecular levels of the involvement of these channels in anesthetic-induced cardioprotection is limited.\(^16,\)\(^17\)

Several endogenous factors also modulate the sarcolemmal \( K_{ATP} \) channel. Ischemic and hypoxic factors, such as an increase in adenosine diphosphate (ADP),\(^18\) production of adenosine,\(^19\) and changes in pH\(^20\) regulate \( K_{ATP} \) channel openings. Thus, despite the emergence of the role of the mitochondrial \( K_{ATP} \) channel in cardioprotection, effects on the sarcolemmal \( K_{ATP} \) channel by these endogenous factors make the channel an attractive end effector of cardioprotection. In anesthetic-induced cardioprotection, a direct action of isoflurane on the cardiac sarcolemmal \( K_{ATP} \) channel has been reported.\(^16\) Isoflurane decreased channel activity but also diminished the channel’s sensitivity to ATP. Our previous study showed that isoflurane and halothane differentially modulated the sarcolemmal \( K_{ATP} \) channel.\(^21\) Under whole cell conditions, isoflurane potentiated the opening of 2,4-dinitrophenol- and pinacidil-activated \( K_{ATP} \) channel current (\( I_{KATP} \)), while halothane inhibited the 2,4-dinitrophenol-activated \( I_{KATP} \) and had no effect on the pinacidil-activated \( I_{KATP} \).

The goal of the present study was to directly investigate the potentiating effects of isoflurane on the cardiac
sarcolemmal K\textsubscript{ATP} channel at the single-channel level and to determine whether modulators of the channel affected the anesthetic action.

Materials and Methods

**Preparation of Isolated Cardiac Ventricular Myocyte**

After approval was obtained from the Institutional Animal Care and Use Committee, single ventricular cells were isolated from enzymatically treated adult guinea pig (200–300 g) hearts. The procedure is a modification of that of Mitra and Morad,\textsuperscript{22} which has been previously described.\textsuperscript{25} In brief, immediately after thoracotomy, the heart was rapidly mounted on a Langendorff apparatus and perfused retrogradely through the aorta with warm (37°C) oxygenated buffer containing the Joklik medium and 0.25 mg/ml collagenase (Gibco Life Technologies, Grand Island, NY) and 0.13 mg/ml protease (Sigma Chemical Co., St. Louis, MO) for 8–12 min. The isolated myocytes were washed and stored in a standard Tyrode solution. Only cells with clear borders and well-defined striations were selected and used for experiments within 12 h after isolation.

**Solutions**

The isolated myocytes were initially placed in a standard Tyrode solution that contained the following ingredients: 132 mM NaCl, 4.8 mM KCl, 3 mM MgCl\textsubscript{2}, 1 mM CaCl\textsubscript{2}, 5 mM dextrose, and 10 mM HEPES, with pH adjusted to 7.3 with NaOH. After establishing a gigaohm seal, the external solution was changed to one that was identical to the external solution. The volatile anesthetic iso-urane (Ohmeda Caribe Inc., Liberty Corner, NJ) was mixed by adding known aliquots of concentrated anesthetics to graduated syringes with the appropriate bath solutions. Isoflurane superfusion was achieved using a syringe pump with a constant flow of 1 ml/min. Clinically relevant concentrations of iso-urane (0.5 and 1.0 mM, equivalent to 1.048 and 2.095 vol\%, respectively) 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.

**Recording Procedure and Data Analysis**

K\textsubscript{ATP} channel activity was monitored using the whole cell, cell-attached, and excised, inside-out patch configurations of the patch clamp technique. Patch pipettes with resistances ranging from 2 to 10 MΩ were pulled by a microforge (MF-83; Narishige, Tokyo, Japan) using a programmable micropipette puller (Sachs-Flaming PC-84; Sutter Instruments, Novato, CA). Pipette tips were heat polished using a microforge (MF-83; Narishige, Tokyo, Japan). Current was measured using a patch clamp amplifier (EPC-7; List, Darmstadt, Germany) interfaced to a computer via an Axon Instrument 1200A Digidata board (Axon Instruments, Foster City, CA). Data acquisition and analysis were performed using the pClamp software package versions 6.0.3 and 8.0 (Axon Instruments). Additional data and statistical analyses were performed on Origin (OriginLab, Northampton, MA).

Currents recorded in the cell-attached and inside-out patch configurations were low-pass-filtered at 500 Hz and sampled at 1 kHz. An opening was interpreted as a crossing of a 50% threshold level from the baseline to the first open channel amplitude. Channel activity was recorded in 2-min durations. For the cell-attached patch experiments, K\textsubscript{ATP} channel activity was monitored at a pipette (command) potential (V\textsubscript{pip}) of −40 mV. Since both the pipette and bath solutions contained 5 mM

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potassium, the resting membrane potential ($V_{\text{resting}}$) was calculated to be $-86$ mV. Taking into account the polarity of the pipette potential relative to the membrane potential, the resultant membrane potential was $-46$ mV according to the standard relation for a cell-attached patch: $V_m = V_{\text{resting}} - V_{\text{pip}}$. For the excised, inside-out patch experiments, KATP channel activity was monitored at a membrane potential of 0 mV in external 5-mM K and internal 140-mM K concentrations. The KATP channels were identified by channel conductance and by their sensitivity to ATP and glibenclamide. All recordings were made at room temperature (20–25°C).

Because of multiple channels in a patch, open probability ($P_o$) was calculated as a cumulative $P_o$, which is defined as a fraction of the total length of time the channels were in an open state during the total recording duration. $P_o$ was determined from the ratios of the area under the peaks in the all-points amplitude histograms of KATP channel activity recorded in the presence of 2,4-dinitrophenol, 2,4-dinitrophenol/isoflurane, and 2,4-dinitrophenol/isoflurane/glibenclamide. The times shown in the brackets denote the period at which channel activity was recorded. The arrow indicates zero current level.

![Fig. 1. Effect of isoflurane on the sarcolemmal KATP channel activated by 2,4-dinitrophenol (DNP). Channel activity was monitored in the cell-attached patch mode at a membrane potential of $-46$ mV. The pipette and bath solution contained 5 mM K. Single channel traces and corresponding amplitude histograms are shown in DNP, DNP + isoflurane, and DNP + isoflurane + glibenclamide. The times shown in the brackets denote the period at which channel activity was recorded. The arrow indicates zero current level.](http://pubs.asahq.org/anesthesiology/article-pdf/97/1/57/407157/0000542-200207000-00009.pdf)

**Results**

Effects of Isoflurane on KATP Channel Activated by 2,4-Dinitrophenol

The effects of isoflurane on the sarcolemmal KATP channel were first investigated in the presence of 2,4-dinitrophenol, an uncoupler of oxidative phosphorylation. The cell-attached patch configuration was used. During control conditions, due to millimolar concentrations of ATP, the KATP channel was inactive. Figure 1 shows representative traces and corresponding all-points amplitude histograms of KATP channel activity recorded in the presence of 2,4-dinitrophenol, 2,4-dinitrophenol + isoflurane, and 2,4-dinitrophenol + isoflurane + glibenclamide. The effect of 150 μM 2,4-dinitrophenol was evident after approximately 5–10 min as KATP channels were activated. Recording of the KATP channel activity was then commenced (denoted by $t = 0$ min in fig. 1) and continued for 2–4 min before application of the anesthetic. The subsequent application of isoflurane (0.5 mM) in the continued presence of 2,4-dinitrophenol appeared to increase the cumulative Po of the KATP channel. Glibenclamide (500 nM), a potent inhibitor of the KATP channel, completely blocked the channel activity. In 8 patches, isoflurane significantly increased the cumulative channel Po from 0.036 ± 0.022 to 0.410 ± 0.086 ($P < 0.05$). The increase in Po of the KATP channel in the presence of both 2,4-dinitrophenol and isoflurane is also reflected in the opening of more channels present in the membrane patch.
to 3.9 ± 0.5 (n = 8). However, the single-channel conductance was not affected. The chord conductances in 2,4-dinitrophenol alone and in the presence of 2,4-dinitrophenol + isoflurane were 12.1 ± 0.6 and 12.3 ± 0.3 pS, respectively, in agreement with that previously reported under physiologic conditions.24

Since 2,4-dinitrophenol gradually decreases the intracellular ATP concentration, the effect of isoflurane on the KATP channel may, in part, be due to a time-dependent 2,4-dinitrophenol effect. To test this hypothesis, the effects of 2,4-dinitrophenol alone on the KATP channel were monitored over time and compared with those in the presence of isoflurane. Figure 2 demonstrates KATP channel activity monitored during a 10-min period in the presence of 2,4-dinitrophenol alone. Between the t = 0- and 6-min periods, there was no marked change in channel Po. However, Po was increased at the t = 10-min mark. This is in contrast to the result of 2,4-dinitrophenol + isoflurane demonstrated in figure 1. At the t = 6-min mark, channel Po was higher with the addition of isoflurane compared with 2,4-dinitrophenol alone at the t = 0-min mark. The results summarized in figure 3 show the data for 2,4-dinitrophenol alone superimposed on the 2,4-dinitrophenol + isoflurane data. The time at which initial KATP channel activity was observed in the presence of 2,4-dinitrophenol is denoted by t = 0 min. In the 2,4-dinitrophenol-alone group, a time-dependent increase in channel Po was observed only during the t = 10-min period. However, in the 2,4-dinitrophenol + isoflurane group, the application of isoflurane at the t = 6-min mark significantly increased channel Po. There was a significant difference in channel Po between the 2,4-dinitrophenol-alone group and 2,4-dinitrophenol + isoflurane group at t = 6 min. At time t = 10 min, upon washout of isoflurane, channel activity remained high. Consequently, at the t = 10-min mark, the time-dependent effect of 2,4-dinitrophenol was evident. These results suggest a kinetic difference in KATP channel opening between the 2,4-dinitrophenol-alone and 2,4-dinitrophenol + isoflurane groups. In the presence of isoflurane, the opening of the KATP channel by 2,4-dinitrophenol was accelerated.

![Fig. 2. Time course of the effect of 2,4-dinitrophenol (DNP) on the KATP channel. The time-dependent effect of DNP on KATP channel activity was monitored in a cell-attached patch as described in figure 1. The times shown in the brackets denote the period at which channel activity was recorded. The sample recording was obtained from one patch exposed to 150 μM DNP and monitored over the time period depicted.](image-url)

![Fig. 3. The kinetic effect of isoflurane on 2,4-dinitrophenol (DNP)-activated KATP channel. Cumulative channel open probability is plotted against time for the DNP group (●, n = 6) and the DNP + isoflurane group (○, n = 8). For the DNP + isoflurane group, isoflurane was added during the 6-min mark and washed out at the 10-min mark, as denoted on the plot. *P < 0.01 versus DNP group; #P < 0.01 versus 0 min of isoflurane + DNP group.](image-url)
Effect of Isoflurane on KATP Channel Activated by Low Adenosine Triphosphate

To better control the recording environment, experiments using the excised, inside-out patch configuration were carried out. Furthermore, due to the kinetic effect of isoflurane in the presence of 2,4-dinitrophenol on KATP channel Po, all subsequent excised patch experiments were conducted in a similar time course. The activation of the KATP channel by various agents and the effect of isoflurane in the continued presence of these agents were monitored within a 6-min period.

In the initial set of inside-out patch experiments, low internal ATP concentrations were used to elicit KATP channel activity. This circumvented the use of 2,4-dinitrophenol to activate the channel. Figure 4 shows the effect of isoflurane on the KATP channel activated by low ATP on the internal side of the membrane. Control conditions were obtained in 0.2–0.5 mM ATP. Isoflurane (0.5 mM) was added to the internal (bath) solution. The arrows indicate zero current level. (Bottom) Summarized data from 7 patches. Control ATP concentrations were 0.2–0.5 mM.

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Effect of Isoflurane on KATP Channel Activated by Elevated Adenosine Diphosphate

Since the initial cell-attached patch experiments showed an acceleration of the opening of KATP channels by isoflurane in the presence of 2,4-dinitrophenol, the result from the excised patch suggested that this effect required intracellular agents. Therefore, the isoflurane effect on modulators of the KATP channel was tested. ADP is one of the endogenous modulators known to shift the channel’s sensitivity to ATP.18 Figure 5 shows the effect of isoflurane on the KATP channel activated by ADP. Control condition was obtained in 0.5 mM ATP on the internal side of the membrane. The addition of 0.1 mM ADP to the internal side increased the channel’s cumulative Po. However, a subsequent addition of 0.5 mM isoflurane did not significantly affect Po. The cumulative Po in 0.1 mM ADP was 0.32 ± 0.09 and in isoflurane was 0.35 ± 0.10.

Effect of Isoflurane on KATP Channel Activated by Adenosine

The next series of experiments tested whether isoflurane interacted with the membrane delimited coupling of the adenosine receptor and the sarcolemmal KATP channel. Adenosine modulates the KATP channel via Gi protein activation under existing GTP19 as demonstrated in figures 6A and B. In the absence of adenosine, GTP applied from internal side of the membrane did not affect KATP activity. In the presence of adenosine...
Effect of Isoflurane on K<sub>ATP</sub> Channel Activated by Bimakalim

Under excised patch conditions, isoflurane had no significant effects on the K<sub>ATP</sub> channel activated by endogenous agents. This is in contrast to the enhancing effect isoflurane had on the K<sub>ATP</sub> channel recorded under cell-attached patch conditions in the presence of 2,4-dinitrophenol. To test whether this difference was due to the method of channel activation, the effect of isoflurane on the K<sub>ATP</sub> channel opened by bimakalim, a potassium channel opener, was investigated using the excised, inside-out patch configuration. The results are summarized in figure 7A. Control condition was obtained with 3.0 mM ATP on the internal side of the membrane. During this condition, channel activity was mostly inhibited, with Po = 0.006 ± 0.003. Bimakalim (5.0 µM) significantly increased activity, with Po = 0.15 ± 0.04. Again, the addition of isoflurane did not change K<sub>ATP</sub> channel activity.

Effect of 2,4-Dinitrophenol during Excised Patch Conditions

In the cell-attached patch configuration, activation of the K<sub>ATP</sub> channel by 2,4-dinitrophenol occurs via uncoupling of oxidative phosphorylation and also by directly interacting with the K<sub>ATP</sub> channel. Consequently, additional experiments were conducted to test whether the isoflurane effect observed under cell-attached patch mode was dependent on a direct interaction of 2,4-dinitrophenol with the channel. Experiments were conducted in the inside-out patch configuration, and the effect of isoflurane on the 2,4-dinitrophenol-activated K<sub>ATP</sub> channel was monitored. Figure 7B summarizes the results from these experiments. During control conditions obtained in 0.8–1.0 mM ATP on the internal side of the membrane, K<sub>ATP</sub> channel activity was limited, with Po = 0.023 ± 0.007. When 150 µM 2,4-dinitrophenol (100 µM) on the external side of the membrane (in the pipette solution), GTP increased K<sub>ATP</sub> channel activity. Figure 6C summarizes the effect of isoflurane on the K<sub>ATP</sub> channel activated by adenosine and GTP. Control condition was obtained with 0.2–0.5 mM ATP on the internal side of the membrane. Adenosine (100 µM) was included in the pipette solution throughout the experiment. The addition of 0.2 mM GTP significantly increased Po from 0.15 ± 0.06 to 0.55 ± 0.15. However, addition of isoflurane (0.5 mM) did not result in any significant changes in Po.

Fig. 6. Effect of adenosine and guanosine triphosphate (GTP) on the K<sub>ATP</sub> channel. Single-channel current traces were monitored in the inside-out patch mode. The effect of GTP (200 µM) in the absence (A) and presence (B) of adenosine in the pipette solution is shown. The arrows indicate zero current level. (C) Summarized data for the isoflurane effect on K<sub>ATP</sub> channel open probability in the presence of adenosine and GTP. *P < 0.05 versus control group, n = 5.

Fig. 7. Effect of isoflurane on the K<sub>ATP</sub> channel activated by bimakalim and 2,4-dinitrophenol (DNP). Channel activity was monitored in the inside-out patch mode. (A) Summary of the effect of isoflurane (0.5 mM) on the K<sub>ATP</sub> channel activated by 5 µM bimakalim (n = 7). Control condition was obtained in 3.0 mM adenosine triphosphate (ATP). (B) Summary of the effect of isoflurane (0.5 mM) on the K<sub>ATP</sub> channel activated by DNP (150–200 µM) (n = 8). Control condition was obtained with 0.8–1.0 mM ATP in the internal solution. DNP was added in the internal side of the membrane. *P < 0.05 versus control.
was applied to the internal side, Po increased significantly to 0.116 ± 0.034. Yet the addition of isoflurane had no further effect on $K_{ATP}$ channel Po.

**Effect of Protein Kinase C on $K_{ATP}$ Current Activation by Isoflurane**

Results obtained from the excised patch experiments strongly suggest an intracellular component in the regulation of the $K_{ATP}$ channel by isoflurane. Since activation of protein kinase C (PKC) is seen as a critical step in IPC, the role of PKC activation on the isoflurane effect was investigated during whole cell conditions. For PKC activation, 12,13-dibutyrate (1 mM) was applied extracellularly throughout the course of the experiment. A time of 30 min was allowed to elapse before application of isoflurane, 12,13-dibutyrate alone did not activate $I_{K_{ATP}}$. The average isoflurane induced $K_{ATP}$ current density in the presence of 12,13-dibutyrate was 14.1 ± 4.2 pA/pF ($n = 6$).

**Effect of Protein Kinase C Activation during Excised Patch Conditions**

The whole cell experiments with 12,13-dibutyrate showed that PKC activation facilitated the opening of the sarcolemmal $K_{ATP}$ channel by isoflurane. To confirm whether PKC activation is sufficient to modulate the effect of isoflurane on the $K_{ATP}$ channel, inside-out, excised patch experiments were carried out. Based on the whole cell experiment described above, isoflurane would be expected to increase $K_{ATP}$ channel activity in a cell-free environment during activation of PKC. In an excised membrane patch, stimulation of endogenous PKC activity results in increased $K_{ATP}$ channel activity. As summarized in figure 9, application of 0.5 mM 12,13-dibutyrate to the intracellular side of the membrane patch resulted in an increase in Po from the control condition obtained in 500 mM ATP. Surprisingly, the subsequent application of isoflurane (0.5 mM) did not significantly increase channel Po. This result was obtained from 6 patches.

**Discussion**

The present study provides direct evidence at the single-channel level that isoflurane facilitates opening of the sarcolemmal $K_{ATP}$ channel. Under cell-attached patch conditions, isoflurane, in combination with 2,4-dinitrophenol, increased the cumulative Po of the $K_{ATP}$ channel. This increase appeared to be the result of an
acceleration of channel opening in the presence of 2,4-dinitrophenol. The result confirms our previous observation that isoflurane potentiated the $K_{ATP}$ current at the whole cell level.\textsuperscript{21} 2,4-Dinitrophenol, an uncoupler of oxidative phosphorylation, inhibits ATP synthesis at the mitochondrial membrane and mimics cellular hypoxia. A drawback in using 2,4-dinitrophenol is that the depletion of ATP occurs gradually. Despite the time-dependent effect of 2,4-dinitrophenol, the time-control experiments showed that the enhancing effect of isoflurane hastened the opening of the $K_{ATP}$ channel. Thus, these results show that isoflurane can enhance $K_{ATP}$ channel opening during conditions where intracellular ATP concentration is decreased.

The results from the excised, inside-out patch studies suggest that the effect of isoflurane on the $K_{ATP}$ channel requires an intracellular component. Isoflurane had no effect on the $K_{ATP}$ channel that was previously activated by low intracellular ATP. This suggested that isoflurane does not interact directly with the channel protein but requires an intracellular component missing in a cell-free environment. Endogenous agents that are produced during ischemic conditions, ADP\textsuperscript{18} and adenosine,\textsuperscript{19} are also modulators of the $K_{ATP}$ channel. Isoflurane, however, had no additional effect on the $K_{ATP}$ channel activated by ADP or adenosine in an excised patch environment. Moreover, in an excised patch configuration, a 2,4-dinitrophenol-activated $K_{ATP}$ channel was also not affected by isoflurane. This indicated that the isoflurane effect observed during cell-attached conditions were not due to the use of 2,4-dinitrophenol as an opener of the channel. $K_{ATP}$ channels opened by a potassium channel opener, bimakalin, also was not affected by isoflurane in a cell-free environment. This further confirms the involvement of intracellular agents since in our previous study, isoflurane potentiated pinacidil-activated $I_{K_{ATP}}$ recorded during a whole cell condition.\textsuperscript{21}

The results obtained in this study appear to be in disagreement with those obtained by Han \textit{et al.}\textsuperscript{16} In their study, in an excised patch configuration, isoflurane increased the mean closed time of the $K_{ATP}$ channel. However, isoflurane also shifted the $K_{ATP}$ channel's sensitivity to ATP, making it less sensitive. The latter effect would result in an enhanced current flow at a particular intracellular ATP concentration. Due to multiple channels in a patch, no kinetic analysis of the channel activity was attempted in our study. Thus, in an excised patch condition, isoflurane may have subtle kinetic effects not evident by measuring Po alone. However, our study showed that isoflurane had no net effect on the $K_{ATP}$ channel in a cell-free environment. In addition, species differences may account for the discrepancy in the mechanism of anesthetic action on the $K_{ATP}$ channel, where rabbit ventricular myocytes were used in the study by Han \textit{et al.}\textsuperscript{16} Nevertheless, results from both studies support the hypothesis of $K_{ATP}$ channel activation during anesthetic-induced cardioprotection. In rabbit ventricular myocytes, the mechanism may involve decreased sensitivity to ATP, and in guinea pig ventricular myocytes, it may involve an increase in channel Po via an intracellular signaling cascade.

A strong candidate that may be the missing link between isoflurane and the $K_{ATP}$ channel is PKC. Recently, PKC has been implicated as a crucial component in IPC.\textsuperscript{27-29} PKC also modulates the sarcolemmal $K_{ATP}$ channel by shifting its sensitivity to intracellular ATP.\textsuperscript{30} The whole cell experiments in this study demonstrated that PKC activation can "prime" the sarcolemmal $K_{ATP}$ channel to opening by isoflurane. This may provide the appropriate environment for isoflurane to facilitate the opening of the $K_{ATP}$ channel. However, surprisingly, in a cell-free environment, activation of PKC alone was not sufficient to enhance the opening of the $K_{ATP}$ channel by isoflurane. Under this condition, the effect of PKC on the $K_{ATP}$ channel is due to membrane-bound PKC. Hence, it is likely that the isoflurane effect on the sarcolemmal $K_{ATP}$ channel involves the translocation of specific PKC isoforms. Studies have shown that, in IPC, translocation of the PKC-ε isoform has a pivotal role in cardioprotection.\textsuperscript{31,32}

Another possible mechanism underlying the effect of isoflurane on the $K_{ATP}$ channel is that isoflurane itself may decrease intracellular ATP concentration. The cell-attached patch experiments showed that isoflurane can facilitate opening of the $K_{ATP}$ channel after applying 2,4-dinitrophenol to decrease intracellular ATP. Isoflurane alone, in the absence of 2,4-dinitrophenol, was unable to elicit channel activity. This may suggest that isoflurane enhances $K_{ATP}$ channel opening by facilitating the decrease in intracellular ATP by 2,4-dinitrophenol. This is supported by the study that isoflurane may affect mitochondrial $K_{ATP}$ channel,\textsuperscript{35} and opening of this channel may decrease ATP synthesis.\textsuperscript{34} However, other studies have reported that volatile anesthetics do not affect ATP concentrations in the heart or myocyte. For instance, halothane did not change ATP concentrations during normal conditions.\textsuperscript{35} Another study showed that isoflurane and halothane did not change ATP concentrations during control, ischemia, and reperfusion compared to a nonanesthetic condition.\textsuperscript{36} Other volatile anesthetics, enflurane and sevoflurane, also did not affect ATP concentrations after the reperfusion period.\textsuperscript{37}

The effect of isoflurane from the 2,4-dinitrophenol and PKC experiments may be linked, although it is rather speculative at this point. It is possible that 2,4-dinitrophenol, via its effect on the mitochondria that results in the uncoupling of oxidative phosphorylation, can subsequently trigger an intracellular signaling cascade leading to translocation of a PKC isoform. This, in turn, can facilitate the opening of the $K_{ATP}$ channel. The excised patch experiments with 2,4-dinitrophenol would support this hypothesis since there was no effect of isoflu-
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