Anesthesiology 2002; 97:50–6 © 2002 American Society of Anesthesiologists, Inc. Lippincott Williams & Wilkins, Inc.

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

Wai-Meng Kwok, Ph.D., Anne T. Martinelli, M.D.,† Kazuhiro Fujimoto, M.D., Ph.D.,‡ Akihiro Suzuki, M.D.,‡ Anna Stadnicka, Ph.D.,§ Zeljko J. Bosnjak, Ph.D.‖*

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 ± 11.1% and 58.4 ± 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 ± 5.8% and 72.1 ± 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[1–3] and may lead to an increased propensity to arrhythmias. However, recent studies have convincingly shown that volatile anesthetics can also be cardioprotective.[4–8] This cardioprotection, termed anesthetic-induced preconditioning, mimics ischemic preconditioning,[9] 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.[5,10] 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.[11,12] 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.[13–15] 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.[16] 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.[5] 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[17] and has previously been

* Assistant Professor, Departments of Anesthesiology and Pharmacology and Toxicology, ‡ Research Fellow, § Assistant Professor, Department of Anesthesiology, ‖ Professor, Departments of Anesthesiology and Physiology. † Medical Resident; current address: Department of Obstetrics and Gynecology, Metro-Health Medical Center, Cleveland, Ohio.

Received from the Department of Anesthesiology, Medical College of Wisconsin, Milwaukee, Wisconsin. Submitted for publication December 19, 2001. Accepted for publication March 19, 2002. Supported in part by grant Nos. GM-54568 (to Dr. Kwok) and NHLBI-34708 (to Dr. Bosnjak) from the National Institutes of Health, Bethesda, Maryland.

Address reprint requests to Dr. Kwok: Department of Anesthesiology, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, Wisconsin 53226. Address electronic mail to: wmkwok@mcw.edu. Individual article reprints may be purchased through the Journal Web site, www.anesthesiology.org.

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/97/1/50/407063/0000542-200207000-00008.pdf by guest on 19 October 2021

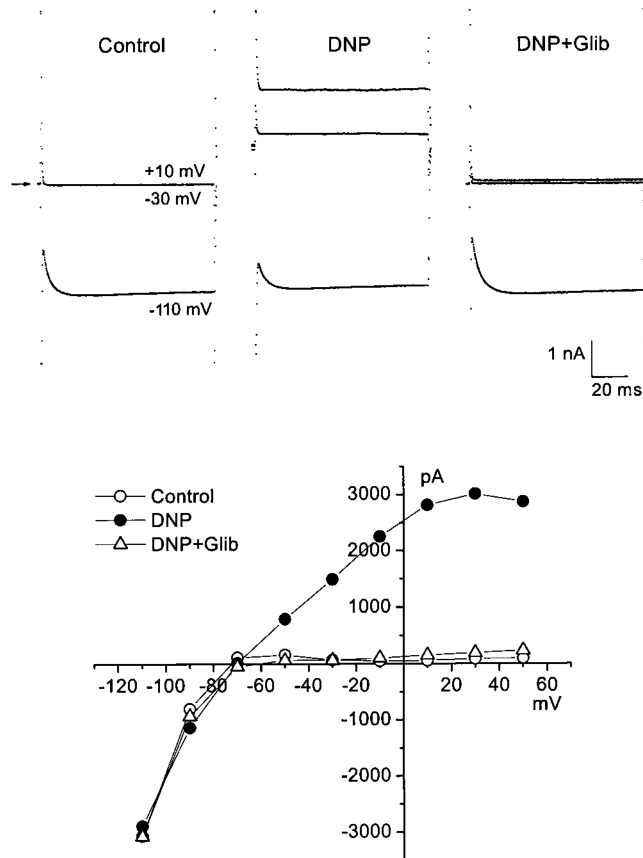

Fig. 1. Activation of I_{KATP} by 2,4-dinitrophenol (DNP). (*Top*) Sample whole cell current traces recorded in control, in the presence of 120 μM DNP, and in the presence of DNP + glibenclamide (Glib, 200 nM). Current was monitored at test potentials of −110, −30, and +10 mV from a holding potential 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.[3] 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_2-ATP, 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.

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/97/1/50/407063/0000542-200207000-00008.pdf by guest on 19 October 2021

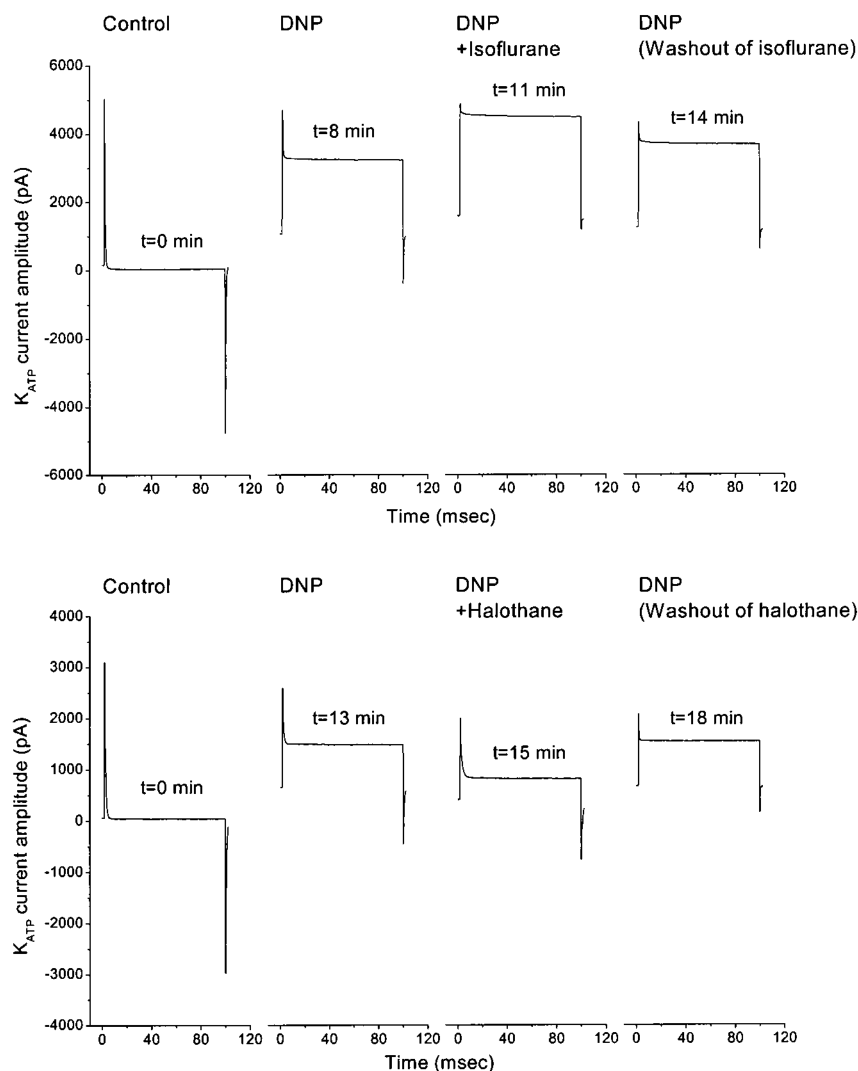

Fig. 2. Effect of isoflurane and halothane on 2,4-dinitrophenol (DNP)-activated I_{KATP}. Current was monitored every 15 s during a 100-ms test pulse to 0 mV from a holding potential of −40 mV. Traces are sequentially shown at the time depicted. The time t = 0 min denotes current recordings in control 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.

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/97/1/50/407063/0000542-200207000-00008.pdf by guest on 19 October 2021

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 MΩ. 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 ± 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

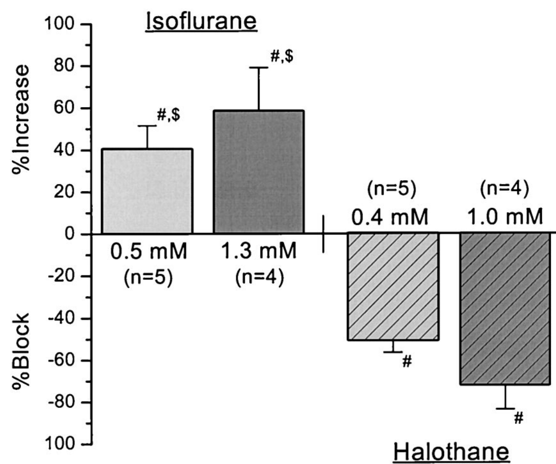

Fig. 3. Summary of the effects of isoflurane and halothane on 2,4-dinitrophenol (DNP)-activated I_{KATP}. Percent increase or block of K_{ATP} current amplitude was measured from the steady state DNP concentration prior to application of the anesthetics. Current amplitude was measured at the end of the 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 μM), 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 μM 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_{KAPT}

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.[18] 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

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/97/1/50/407063/0000542-200207000-00008.pdf by guest on 19 October 2021

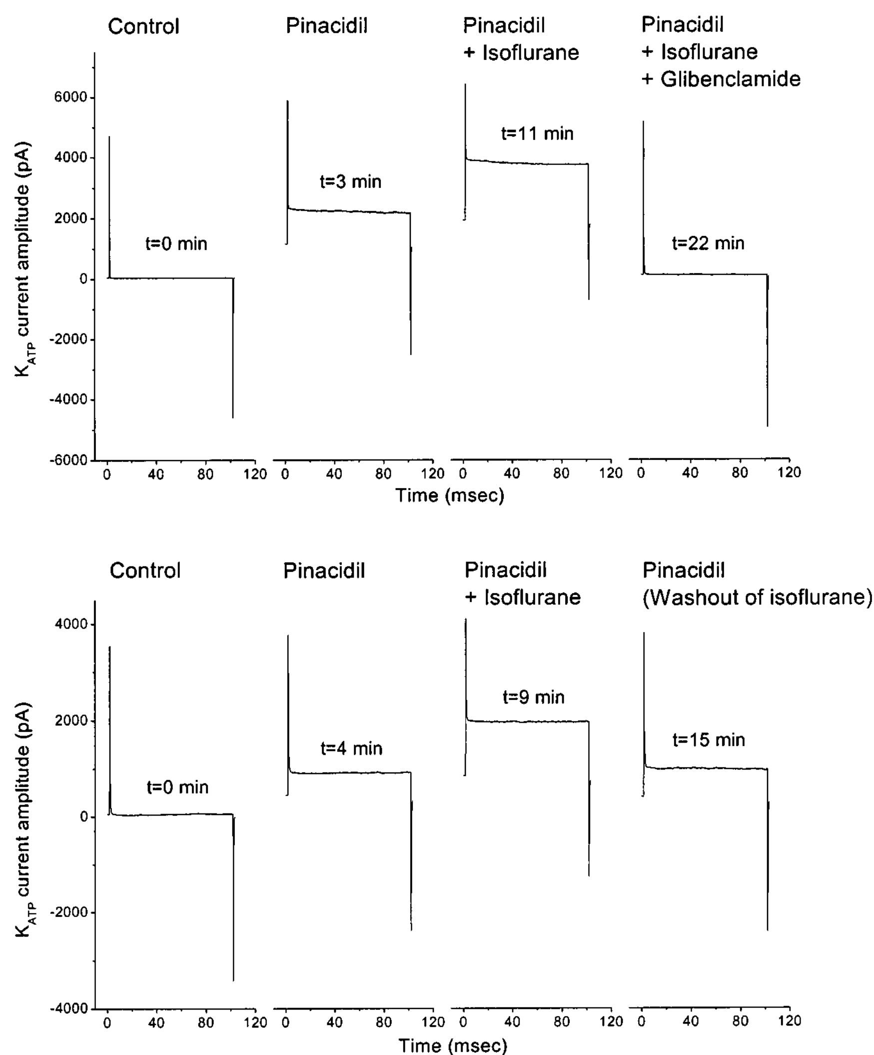

Fig. 4. Effects of isoflurane on pinacidil-activated I_{KATP}. Whole cell current monitored during a 100-ms test pulse to 0 mV from a −40-mV holding potential was recorded in control, in 5 μM pinacidil, and in pinacidil + 0.6 mM isoflurane. (*Top*) The inhibition of the isoflurane-potentiated current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

intervals is depicted in figure 6. After allowing for diffusional exchange of 1 mM ATP between the pipette solution and the cell's interior, isoflurane (0.6 mM) was applied extracellularly. However, during a 10-min application, isoflurane failed to elicit any outward current characteristic of I_{KATP}. Upon washout of isoflurane, an application of a K_{ATP} channel opener, bimakalim, resulted in the activation of I_{KATP}, 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 mM, near the threshold for channel opening, isoflurane failed to activate I_{KATP} (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_{KATP} 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_{KATP}, respectively. In addition, although isoflurane facilitated opening of the K_{ATP} channel, the anesthetic by itself was unable to directly activate I_{KATP}. 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.[5,6] However, direct evidence of volatile anesthetic modulation of the sarcolemmal K_{ATP} channels has been limited.[19,20] In the rabbit ventricular myocytes, isoflurane shifted the K_{ATP} channel's sensitivity to ATP and increased the mean closed time.[19] 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-

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/97/1/50/407063/0000542-200207000-00008.pdf by guest on 19 October 2021

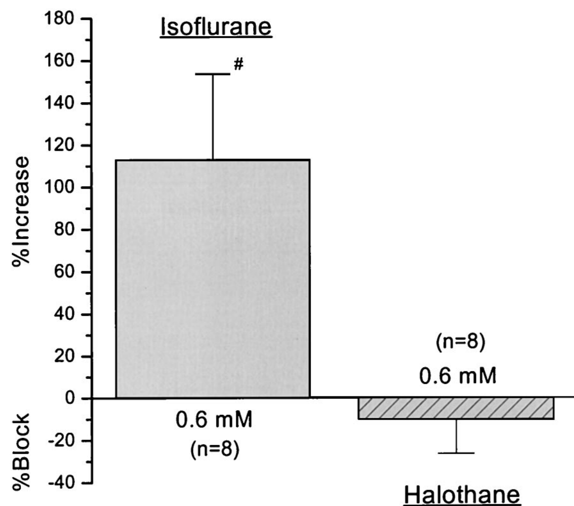

Fig. 5. Summary of the effects of isoflurane and halothane on pinacidil-activated I_{KATP}. Percent changes in K_{ATP} current amplitude were measured from the steady state pinacidil concentration prior to application of the anesthetics. #Significantly different from control.

dence of volatile anesthetic action on the sarcolemmal K_{ATP} channel was reported in studies on human atrial trabecular muscles.[20] In these cells, halothane decreased and isoflurane had no effect on I_{KATP}. Thus, the inhibitory effect of halothane on I_{KATP} 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.[21] 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,[12,22–24] 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_{KATP}. 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_{KATP} 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_{KATP} 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_{KATP}. Studies on a rabbit model have shown that halothane has cardioprotective effects mimicking ischemic preconditioning.[25] However, results from the human atrial studies suggest that halothane diminishes the protective effects of ischemic preconditioning, while isoflurane induces protection.[20] 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.[26] Consequently, it is conceivable that the halothane effect may be differentially dependent on the intracellular changes resulting from alterations in

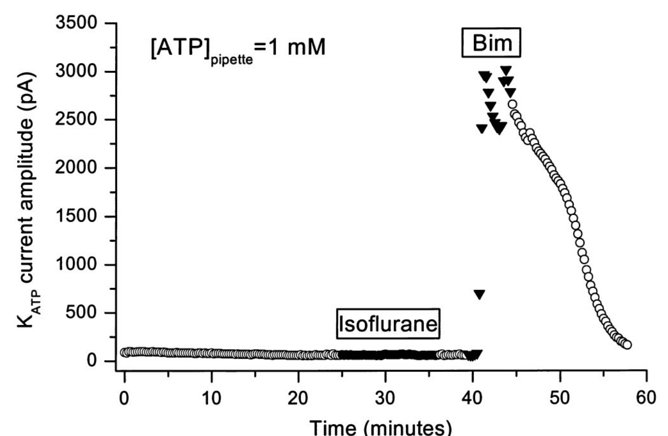

Fig. 6. Isoflurane alone does not activate I_{KATP}. K_{ATP} current was monitored every 15 s during a test pulse to 0 mV from a holding potential of −40 mV. Current amplitude was measured at the end of the 100-ms test pulse. Control recordings were obtained for 25 min to allow for the diffusional exchange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

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/97/1/50/407063/0000542-200207000-00008.pdf by guest on 19 October 2021

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.[27] 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.[13,14,28] 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.[28]

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.[29] In addition, the cardioprotective effects of desflurane were found to involve both the sarcolemmal and mitochondrial K_{ATP} channels.[30] 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.[16]

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}.

References

1. Pancrazio JJ: Halothane and isoflurane preferentially depress a slowly inactivating component of Ca2+ channel current in guinea-pig myocytes. J Physiol 1996; 494:91–103
2. Stadnicka A, Bosnjak ZJ, Kampine JP, Kwok WM: Effects of sevoflurane on inward rectifier K+ current in guinea pig ventricular cardiomyocytes. Am J Physiol 1997; 273:H324–32
3. Weigt HU, Kwok WM, Rehmert GC, Turner LA, Bosnjak ZJ: Voltage-dependent effects of volatile anesthetics on cardiac sodium current. Anesth Analg 1997; 84:285–93
4. Kersten JR, Schmeling TJ, Hettrick DA, Pagel PS, Gross GJ, Waritier DC: Mechanism of myocardial protection by isoflurane: Role of adenosine triphosphate-regulated potassium (KATP) channels. Anesthesiology 1996; 85:794–807; discussion 27A
5. Kersten JR, Schmeling TJ, Pagel PS, Gross GJ, Warltier DC: Isoflurane mimics ischemic preconditioning via activation of K(ATP) channels: Reduction of myocardial infarct size with an acute memory phase. Anesthesiology 1997; 87:361–70
6. Cason BA, Gamperl AK, Slocum RE, Hickey RF: Anesthetic-induced preconditioning: Previous administration of isoflurane decreases myocardial infarct size in rabbits. Anesthesiology 1997; 87:1182–90
7. Novalija E, Fujita S, Kampine JP, Stowe DF: Sevoflurane mimics ischemic preconditioning effects on coronary flow and nitric oxide release in isolated hearts. Anesthesiology 1999; 91:701–12
8. Toller WG, Kersten JR, Gross ER, Pagel PS, Warltier DC: Isoflurane preconditions myocardium against infarction via activation of inhibitory guanine nucleotide binding proteins. Anesthesiology 2000; 92:1400–7
9. Murry CE, Jennings RB, Reimer KA: Preconditioning with ischemia: A delay of lethal cell injury in ischemic myocardium. Circulation 1986; 74:1124–36
10. Kersten JR, Gross GJ, Pagel PS, Warltier DC: Activation of adenosine triphosphate-regulated potassium channels: Mediation of cellular and organ protection. Anesthesiology 1998; 88:495–513
11. Noma A: ATP-regulated K+ channels in cardiac muscle. Nature 1983; 305:147–8
12. Nichols CG, Lederer WJ: The regulation of ATP-sensitive K+ channel activity in intact and permeabilized rat ventricular myocytes. J Physiol 1990; 423:91–110
13. Garlid KD, Paucek P, Yarov-Yarovoy V, Murray HN, Darbenzio RB, D'Alonzo AJ, Lodge NJ, Smith MA, Grover GJ: Cardioprotective effect of diazoxide and its interaction with mitochondrial ATP-sensitive K+ channels: Possible mechanism of cardioprotection. Circ Res 1997; 81:1072–82
14. Liu Y, Sato T, O'Rourke B, Marban E: Mitochondrial ATP-dependent potassium channels: Novel effectors of cardioprotection? Circulation 1998; 97: 2463–9
15. Fryer RM, Hsu AK, Gross GJ: Mitochondrial KATP channel opening is important during index ischemia and following myocardial reperfusion in ischemic preconditioned rat hearts. J Mol Cell Cardiol 2001; 33:831–4
16. Kohro S, Hogan QH, Nakae, Y, Yamakage, M, Bosnjak, ZJ: Anesthetic effects on mitochondrial ATP-sensitive K channel. Anesthesiology 2001; 95:1435–40
17. Mitra R, Morad M: A uniform enzymatic method for dissociation of myocytes from hearts and stomachs of vertebrates. Am J Physiol 1985; 249:H1056–60
18. Alekseev AE, Gomez LA, Aleksandrova LA, Brady PA, Terzic A: Opening of cardiac sarcolemmal KATP channels by dinitrophenol separate from metabolic inhibition. J Membr Biol 1997; 157:203–14
19. Han J, Kim E, Ho WK, Earm YE: Effects of volatile anesthetic isoflurane on ATP-sensitive K+ channels in rabbit ventricular myocytes. Biochem Biophys Res Commun 1996; 229:852–6
20. Roscoe AK, Christensen JD, Lynch C 3rd: Isoflurane, but not halothane, induces protection of human myocardium via adenosine A1 receptors and adenosine triphosphate-sensitive potassium channels. Anesthesiology 2000; 92:1692–701
21. Camara AK, Begic Z, Kwok WM, Bosnjak ZJ: Differential modulation of the cardiac L- and T-type calcium channel currents by isoflurane. Anesthesiology 2001; 95:515–24
22. Nichols CG, Lederer WJ: Adenosine triphosphate-sensitive potassium channels in the cardiovascular system. Am J Physiol 1991; 261:H1675–86
23. Fan Z, Makielski JC: Anionic phospholipids activate ATP-sensitive potassium channels. J Biol Chem 1997; 272:5388–95
24. Fan Z, Makielski JC: Phosphoinositides decrease ATP sensitivity of the cardiac ATP-sensitive K(+) channel: A molecular probe for the mechanism of ATP-sensitive inhibition. J Gen Physiol 1999; 114:251–69
25. Cope DK, Impastato WK, Cohen MV, Downey JM: Volatile anesthetics protect the ischemic rabbit myocardium from infarction. Anesthesiology 1997; 86:699–709
26. Liu Y, Ren G, O'Rourke B, Marban E, Seharaseyon J: Pharmacological comparison of native mitochondrial KATP channels with molecularly defined surface KATP channels. Mol Pharmacol 2001; 59:225–30
27. Belhomme D, Peynet J, Louzy M, Launay JM, Kitakaze M, Menasche P: Evidence for preconditioning by isoflurane in coronary artery bypass graft surgery. Circulation 1999; 100:II340–4
28. Light PE, Kanji HD, Fox JE, French RJ: Distinct myoprotective roles of cardiac sarcolemmal and mitochondrial KATP channels during metabolic inhibition and recovery. Faseb J 2001; 15:2586–94
29. Jovanovic A, Jovanovic S, Lorenz E, Terzic A: Recombinant cardiac ATP-sensitive K+ channel subunits confer resistance to chemical hypoxia-reoxygenation injury. Circulation 1998; 98:1548–55
30. Toller WG, Gross ER, Kersten JR, Pagel PS, Gross GJ, Warltier DC: Sarcolemmal and mitochondrial adenosine triphosphate-dependent potassium channels: Mechanism of desflurane-induced cardioprotection. Anesthesiology 2000; 92:1731–9

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/97/1/50/407063/0000542-200207000-00008.pdf by guest on 19 October 2021