Anesthesiology 2003; 98:114–20

Isoflurane Sensitizes the Cardiac Sarcolemmal Adenosine Triphosphate–Sensitive Potassium Channel to Pinacidil

Susanne Gassmayr, M.D., Anna Stadnicka, Ph.D.,† Akihiro Suzuki, M.D.,* Wai-Meng Kwok, Ph.D.,‡ Zeljko J. Bosnjak, Ph.D.§*

Background: **Cardioprotective effects of isoflurane are partially mediated by the sarcolemmal adenosine triphosphate–sensitive potassium ($sarcK_{ATP}$) channel. The authors tested the hypothesis that isoflurane sensitizes $sarcK_{ATP}$ channels to a potassium channel opener, pinacidil, *via* adenosine- and phospholipid-mediated pathways.**

Methods: **Activation by pinacidil of the K_{ATP} current (I_{KATP}) was monitored in guinea pig ventricular myocytes at 0.5 and 5 mM intracellular ATP in the whole cell configuration of the patch clamp technique. The sensitization effect was evaluated by pretreating each myocyte with isoflurane (0.57 ± 0.04 mM) before application of pinacidil (5 μM) in the continued presence of the anesthetic. To investigate whether intracellular signaling pathways may be involved in isoflurane sensitization, the authors used the adenosine receptor antagonist theophylline (100 μM) and the phosphatidylinositol kinase inhibitor wortmannin (100 μM).**

Results: **The density of pinacidil-activated I_{KATP} was higher at 0.5 mM ATP (20.7 ± 3.2 pA/pF) than at 5 mM ATP (2.0 ± 0.3 pA/pF). At 0.5 mM ATP, pretreatment with isoflurane caused an increase in density of pinacidil-activated I_{KATP} (42.4 ± 6.2 pA/pF) and accelerated the rate of current activation (from 5.4 ± 1.2 to 39.0 ± 7.9 $pA \cdot pF^{-1} \cdot min^{-1}$). Theophylline attenuated current activation by pinacidil (9.4 ± 3.9 pA/pF) and abolished the sensitization effect of isoflurane on I_{KATP} (10.0 ± 2.5 pA/pF). Wortmannin did not alter pinacidil activation of I_{KATP} (13.2 ± 1.7 pA/pF) but prevented sensitization by isoflurane (15.8 ± 4.5 pA/pF).**

Conclusions: **These results suggest that isoflurane increases sensitivity of cardiac $sarcK_{ATP}$ channels to the potassium channel opener pinacidil. Blockade of adenosine receptors or phosphatidylinositol kinases abolishes the sensitization effect, suggesting that the adenosine and phospholipid signaling pathways may be involved in the actions by isoflurane.**

VOLATILE anesthetic–induced preconditioning appears as effective as ischemic preconditioning (IPC) in protecting the heart against ischemia–reperfusion injury by decreasing myocardial infarct size and improving postischemic functional recovery.[1–4] This important finding may in the future have an impact in clinical settings where an increasing number of surgical patients with coronary artery disease are at a high risk for perioperative myocardial ischemia. While the mechanisms underlying IPC has been a major field of investigation since the first report by Murry *et al.*,[5] the cellular and molecular mechanisms of anesthetic-induced preconditioning are not yet defined, although the pathways involved are thought to mimic IPC.

Two distinct populations of myocardial adenosine triphosphate–sensitive potassium (K_{ATP}) channel, the sarcolemmal K_{ATP} ($sarcK_{ATP}$) and the mitochondrial K_{ATP} ($mitoK_{ATP}$) channels, contribute to IPC, but their exact roles are not elucidated. Recent evidence supports a predominant role of $mitoK_{ATP}$ channels in the initiation of IPC.[6–9] The $sarcK_{ATP}$ channels are thought to mediate cardioprotection during the reoxygenation phase.[10] Similarly, in anesthetic-induced preconditioning, both the $sarcK_{ATP}$ and $mitoK_{ATP}$ channels have been indicated to contribute to cardioprotection,[11] although their specific roles are not yet determined.

There is limited direct evidence on the interaction of volatile anesthetic with the cardiac $sarcK_{ATP}$ channel. Isoflurane was shown to have no significant effect on $sarcK_{ATP}$ channel in human atrial cells.[12] Other investigators reported that isoflurane inhibited single K_{ATP} channel current in the cell-free membrane patches from rabbit ventricular myocytes and attenuated channel sensitivity to ATP.[13] The *in vivo* studies have suggested that the effects of volatile anesthetics on K_{ATP} channels may involve intracellular signaling.[14] It has been shown that blockade of adenosine receptors and G_i proteins abolishes the cardioprotective effects of volatile anesthetics.[14,15] The contribution of intracellular signaling to modulation of volatile anesthetic effects on the K_{ATP} channel has also been demonstrated at the single cell level.[16–18]

The *in vitro* effects of volatile anesthetics on the $sarcK_{ATP}$ channel have been investigated during conditions where channel activity was monitored during application of the anesthetic. It is uncertain, however, whether pretreatment with volatile anesthetic can facilitate the opening of K_{ATP} channel, a condition relevant to anesthetic-induced preconditioning. We have previously reported that isoflurane alone is unable to elicit $sarcK_{ATP}$ channel opening under whole cell conditions.[19] In the current study, we tested the hypothesis that pretreatment with isoflurane increases sensitivity of $sarcK_{ATP}$ channels to the potassium channel opener pinacidil, facilitating K_{ATP} current (I_{KATP}) activated by pinacidil. In addition, an involvement of the adenosine-

* Postdoctoral Fellow, † Assistant Professor, Department of Anesthesiology. ‡ Assistant Professor, Departments of Anesthesiology and Pharmacology & Toxicology. § Professor, Departments of Anesthesiology and Physiology.

Received from the Department of Anesthesiology, Medical College of Wisconsin, Milwaukee, Wisconsin. Submitted for publication April 10, 2002. Accepted for publication August 20, 2002. Supported in part by grants No. HL-34708 (to Dr. Bosnjak) and GM-54568 (to Dr. Kwok) from the National Institutes of Health, Bethesda, Maryland, and a Max Kade grant (to Dr. Gassmayr) from the Max Kade Foundation, IMC, New York, New York, and Graz, Austria. Presented at the annual meeting of the American Society of Anesthesiologists, New Orleans, Louisiana, October 13–17, 2001, and the 76th International Anesthesia Research Society Meeting, San Diego, California, March 16–20, 2002.

Address reprint requests to Dr. Kwok: Medical College of Wisconsin, Department of Anesthesiology, 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.

and phospholipid-mediated signaling pathways to the actions by isoflurane were tested.

Materials and Methods

The experimental procedures of this study were approved by the Animal Use and Care Committee of the Medical College of Wisconsin.

Cell Isolation

Single ventricular myocytes were enzymatically isolated from adult Hartley guinea pigs (either sex) weighing 150–300 g using a modified isolation method by Mitra and Morad.[20] The guinea pigs were anesthetized by pentobarbital sodium (325 mg/kg, administered intraperitoneally) and injected with heparin (1,000 U/ml, administered intraperitoneally). After thoracotomy, the hearts were quickly excised, mounted on a Langendorff apparatus, and perfused retrogradely *via* the aorta at a flow of 7–8 ml/min with oxygenated Joklik medium (Gibco BRL, Invitrogen, Grand Island, NY) containing 2.5 U/ml heparin. After blood has been washed out from the heart, this medium was replaced by an enzyme solution containing Joklik medium, 0.4 mg/ml collagenase type II (Gibco BRL, Invitrogen), 0.1 mg/ml protease XIV (Sigma-Aldrich, St. Louis, MO), and 1 mg/ml bovine serum albumin (Serologicals, Kankakee, IL), at pH 7.23. The temperature was maintained at 37°C, and oxygen and carbon dioxide concentrations were kept at 95% and 5%, respectively, by continuously bubbling the solution at a constant gas flow. After 14 min of enzyme treatment, the ventricular tissue was minced and incubated in the enzyme solution for additional 3–10 min in a shaker bath at 37°C. The cell suspension was filtered through a 200-μm mesh and centrifuged. The pellet was washed twice in modified Tyrode solution. Myocytes were stored in Tyrode solution at room temperature (22°C) up to 12 h.

Solutions

The modified Tyrode solution had the following composition: 132 mM NaCl, 4.8 mM KCl, 1.2 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM HEPES, and 5 mM glucose, at pH 7.4 adjusted with NaOH. The intracellular–pipette solution contained the following: 60 mM L-glutamic acid, 50 mM KCl, 10 mM HEPES, 1 mM $MgCl_2$, 11 mM EGTA, 1 mM $CaCl_2$, and either 0.5 or 5°K_2ATP, at pH 7.4 adjusted with KOH. The external–bath solution contained: 132 mM *N*-methyl-D-glucamine, 2 mM $MgCl_2$, 1 mM $CaCl_2$, and 10 mM HEPES, at pH 7.4 adjusted with HCl. Nisoldipine (Miles-Pentex, West Haven, CT) was added to the external solution at a concentration of 200 nM to block the L-type calcium channels. The 1-mM stock solution of nisoldipine was made in polyethylene glycol. The 10-mM stock solution of pinacidil, an opener of K_{ATP} channels, was prepared in 0.1 N HCl. The 1-mM stock solution of glibenclamide, a blocker of K_{ATP} channels, was prepared in DMSO. Wortmannin (Calbiochem-Novabiochem, San Diego, CA), an inhibitor of phosphatidylinositol kinases, was dissolved in DMSO and added to the pipette solution. After final dilution in the pipette solution, DMSO at a concentration of 0.005% had no effect on the whole cell I_{KATP}. Theophylline was applied in the external solution at 100 μM. At this concentration, theophylline shows high affinity for adenosine receptors and has only minimal if any effects on phosphodiesterase activity, cyclic adenosine monophosphate production, or intracellular Ca^{2+} translocation. These cellular events may be affected by theophylline concentrations higher than 200 μM. Unless stated otherwise, all chemicals were purchased from Sigma (Sigma-Aldrich). Isoflurane (Abbott Laboratories, North Chicago, IL) was delivered after sonicating into the external solution. The concentrations of isoflurane in the recording chamber were measured using the flame ionization detection method and Shimadzu GC8A gas chromatograph (Shimadzu, Kyoto, Japan). The concentration of isoflurane used in this study was 0.55 mM, which is equivalent to 1.0 vol% at 22°C. The external solutions were delivered *via* a set of syringe infusion pumps at a rate of 2 ml/min and were removed by vacuum suction.

Electrophysiologic Recordings and Data Analysis

The I_{KATP} was measured in the whole cell configuration of the patch clamp technique,[21] using the EPC-7 patch clamp amplifier (List, Darmstadt-Eberstadt, Germany) and Digidata 1322A interface (Axon Instruments, Foster City, CA). The pClamp8 software (Axon Instruments) was used for data acquisition and analysis. Pipettes were pulled from borosilicate glass (Garner Glass, Claremont, CA) with a multistage PC-84 puller (Sutter, Novato, CA) and heat polished using a microforge MF-83 (Narishige, Japan). The pipette resistances ranged from 2 to 3 MΩ. The cells suspended in Tyrode solution were placed in the recording chamber on the stage of an inverted IMT2 microscope (Olympus, Tokyo, Japan). Only quiescent, rod-shaped cells with distinct striations were selected for experiments.

After a gigaohm seal was formed and the whole cell configuration was established by membrane rupture, the series resistance was adjusted to give the fastest possible capacitance transient without causing ringing. Whole cell currents were elicited by a 100-ms depolarizing voltage step to 0 mV from a holding potential of −40 mV applied every 15 s. Current amplitude was measured at the end of each voltage step. To allow for comparisons among cells, currents were normalized to cell capacitance and reported as current density (pA/pF).

Statistical Analysis

Data were analyzed using the pClamp8 software (Axon Instruments) and Origin 6 software (OriginLab, Northampton, MA). Results are reported as mean ± SEM. Statistical analysis was performed using analysis of variance and Student *t* test. Differences were considered significant at $P < 0.05$.

Results

Effects of Pinacidil on Whole Cell Adenosine Triphosphate–sensitive Potassium Current

In all experiments of the current study, the sarcK_{ATP} channel current, I_{KATP}, was elicited using a specific potassium channel opener, pinacidil. Pinacidil, a vasodilator and antihypertensive drug, activates sarcK_{ATP} channels during normal physiologic conditions.[22–25] During control conditions of our study, spontaneous activation of I_{KATP} was not observed in the absence of pinacidil.

Because the degree of activation of the K_{ATP} channel by pinacidil is dependent on the concentration of intracellular ATP, we first evaluated the effects of pinacidil on I_{KATP} in guinea pig ventricular myocytes at two different concentrations of intracellular ATP, 0.5 mM and 5.0 mM. Figure 1A shows representative traces of the whole cell I_{KATP} activated by pinacidil (5 μM) at 0.5 mM intracellular ATP. Pinacidil was applied in the bath solution after 30-min dialysis of each cell with the internal solution to allow for equilibration of intracellular ATP. Pinacidil elicited an outward, time-independent current that was inhibited by glibenclamide (0.5 μM). The time course of I_{KATP} activation by pinacidil is shown in figure 1B. Current amplitude was measured at the end of a 100-ms test pulse to 0 mV from a holding potential of −40 mV, applied every 15 s. At 0.5 mM intracellular ATP, the density of pinacidil activated I_{KATP} was 20.7 ± 3.2 pA/pF (n = 6), and the rate of current activation as determined from a linear regression fit was 5.4 ± 1.2 pA · pF^{-1} · min^{-1} (n = 5). At 5.0 mM intracellular ATP, the density of pinacidil-activated I_{KATP} was 2.0 ± 0.3 pA/pF (n = 6). These results are in agreement with reports by other investigators showing that the magnitude of pinacidil activated I_{KATP} depends on intracellular ATP.[23–25]

Sensitization of Sarcolemmal Adenosine Triphosphate–sensitive Potassium Channel to Pinacidil by Isoflurane

Recent studies from our laboratory have suggested that isoflurane alone does not activate sarcK_{ATP} channel in guinea pig ventricular myocytes at 0.5 mM intracellular ATP.[19] However, we had also reported that volatile anesthetics may have sensitization effects on other cardiac sarcolemmal ion channels, for example, the sodium channel.[26] Therefore, we tested whether isoflurane can sensitize the sarcK_{ATP} channel to pinacidil. The experimental protocol included a 20-min dialysis of each cell

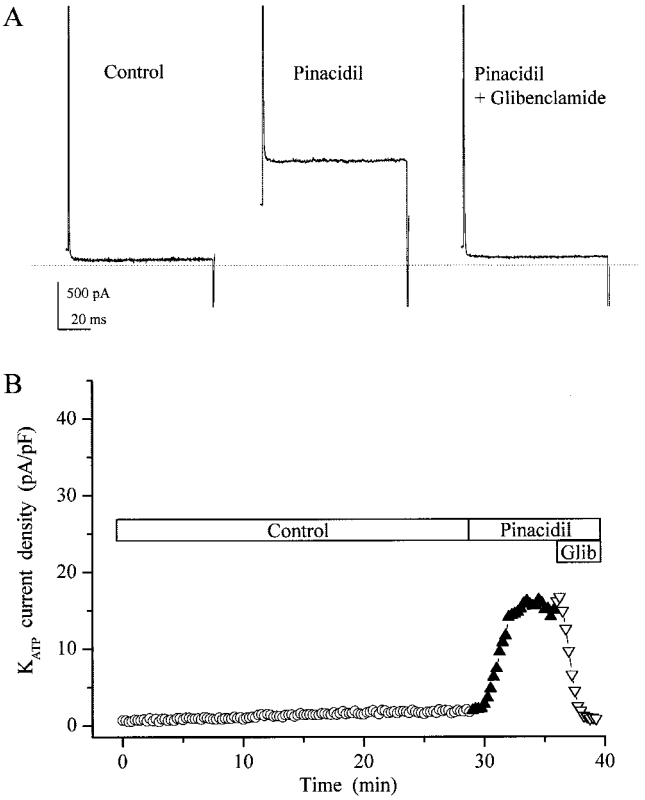

Fig. 1. Activation of sarcolemmal adenosine triphosphate–sensitive potassium current (I_{KATP}) by pinacidil at 0.5 mM intracellular ATP. (*A*) Traces of I_{KATP} elicited by a 100-ms voltage pulse to 0 mV from a holding potential of −40 mV in control, in the presence of 5 μM pinacidil, and in the presence of pinacidil and 0.5 μM glibenclamide. Pinacidil elicited current sensitive to glibenclamide. (*B*) Corresponding time course of I_{KATP} activation by pinacidil. Current was monitored every 15 s using voltage protocol described in (*A*). Current amplitude was normalized to cell capacitance, and the resulting current density was plotted against time. No I_{KATP} was elicited during 30-min control dialysis of the cell with 0.5 mM ATP. Subsequent application of 5 μM pinacidil activated I_{KATP} that was blocked by 0.5 μM glibenclamide (Glib).

with the pipette solution containing 0.5 or 5 mM ATP and 10-min exposure to isoflurane (0.57 ± 0.04 mM, n = 27), followed by a 10-min exposure to pinacidil (5 μM) in the continued presence of isoflurane. Glibenclamide (0.5 μM) was applied at the end of each protocol to confirm the identity of I_{KATP}. Figure 2A shows sample traces of I_{KATP} in control, in the presence of isoflurane alone, and in the presence of isoflurane and pinacidil. A corresponding time course of current activation is depicted in figure 2B. After pretreatment and in the continued presence of isoflurane at 0.5 mM intracellular ATP, the pinacidil-activated current was markedly increased compared with anesthetic-free controls (compare with fig. 1). With isoflurane, the density of pinacidil-activated current was increased to 42.4 ± 6.2 pA/pF (n = 8; $P < 0.05$, isoflurane + pinacidil *vs.* pinacidil alone). Furthermore, in the presence of isoflurane, the rate of current activation by pinacidil increased to 39.0 ± 7.9 pA · pF^{-1} · min^{-1} (n = 5), indicating that activation of I_{KATP} by pinacidil

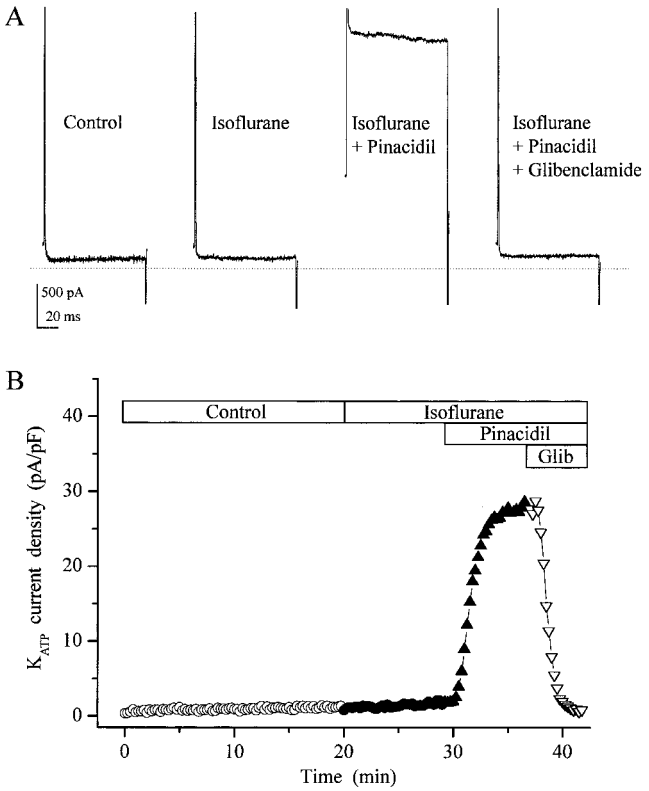

Fig. 2. Effect of isoflurane on activation of adenosine triphosphate–sensitive potassium current (I_{KATP}) by pinacidil. (*A*) Traces of I_{KATP} activated by pinacidil after pretreatment with isoflurane. The voltage protocol was as described in figure 1. Isoflurane alone did not activate I_{KATP}. After a 10-min pretreatment with isoflurane, activation of I_{KATP} by 5 μM pinacidil was monitored in the continued presence of 0.5 mM isoflurane. Current activated by pinacidil was blocked by 0.5 μM glibenclamide. (*B*) Corresponding time course of I_{KATP} activation by pinacidil after pretreatment and in the presence of isoflurane. The 20-min control dialysis of the cell with the pipette solution containing 0.5 mM ATP was allowed before isoflurane, and subsequently isoflurane and pinacidil were added to the bath solution. Current activated by pinacidil was blocked by glibenclamide (Glib).

was accelerated in the presence of isoflurane. At 5 mM intracellular ATP, pretreatment with isoflurane caused an increase in pinacidil-activated I_{KATP} to 5.9 ± 2.3 pA/pF (n = 6), but compared with the corresponding control this change was not significant ($P = 0.292$). Figure 3 summarizes the effects of 0.5 and 5 mM ATP on I_{KATP} activation by pinacidil alone and by isoflurane + pinacidil following 10-min pretreatment of cells with anesthetic. These results suggest that at lower intracellular ATP, isoflurane may sensitize the sarcK_{ATP} channel to pinacidil, resulting in a greater current density and accelerated current activation.

Involvement of Adenosine Signaling in the Sensitization by Isoflurane

Experimental evidence supports an important role of adenosine signaling in myocardial protection.[27] The cardioprotective effects of isoflurane may also be mediated

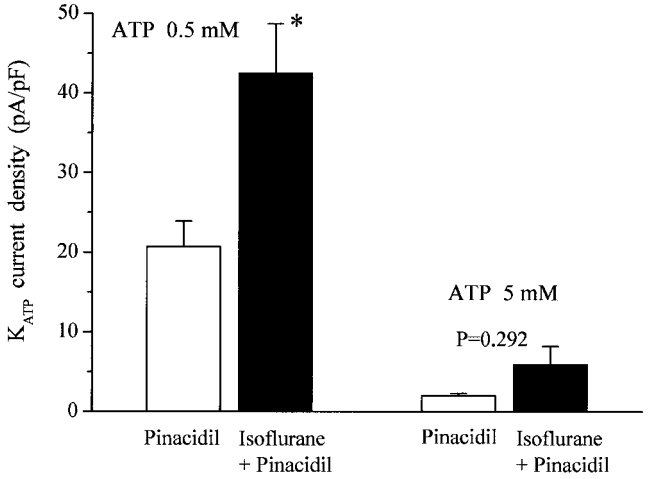

Fig. 3. Summary of adenosine triphosphate–sensitive potassium current (I_{KATP}) activation by pinacidil in the absence and presence of isoflurane at 0.5 and 5.0 mM intracellular ATP. At 0.5 mM ATP, the density of pinacidil activated I_{KATP} was significantly increased after 10-min pretreatment with isoflurane (*$P < 0.05$, isoflurane + pinacidil *vs.* pinacidil alone; n = 8). At 5.0 mM ATP, the density of pinacidil-activated I_{KATP} was not significantly changed in the presence of isoflurane ($P = 0.292$; n = 6).

by adenosine-triggered signaling pathway.[14,15] To test whether this pathway is involved in the sensitization effect by isoflurane, we used a broad-spectrum antagonist of adenosine receptors, theophylline. The experiments were conducted at 0.5 mM intracellular ATP. Figure 4 shows that extracellularly applied theophylline (100 μM) reduced I_{KATP} elicited by pinacidil (5 μM), and the current density was 9.4 ± 3.9 pA/pF (n = 6; $P < 0.05$, pinacidil + theophylline *vs.* pinacidil alone).

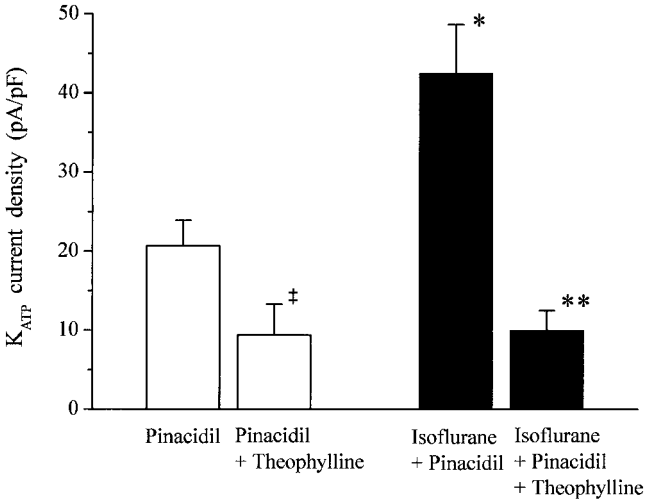

Fig. 4. Effect of theophylline on activation of adenosine triphosphate–sensitive potassium current (I_{KATP}) by pinacidil at 0.5 mM internal ATP. The voltage protocol was as described in figures 1 and 2. Theophylline (100 μM) was present in the extracellular solution throughout the course of experiment. Theophylline decreased the density of 5 μM pinacidil-activated I_{KATP} (‡$P < 0.05$, pinacidil + theophylline *vs.* pinacidil alone; n = 6) and abolished the sensitization by isoflurane (*$P < 0.05$, isoflurane + pinacidil *vs.* pinacidil alone, n = 8; **$P < 0.05$, isoflurane + pinacidil + theophylline *vs.* isoflurane + pinacidil, n = 6).

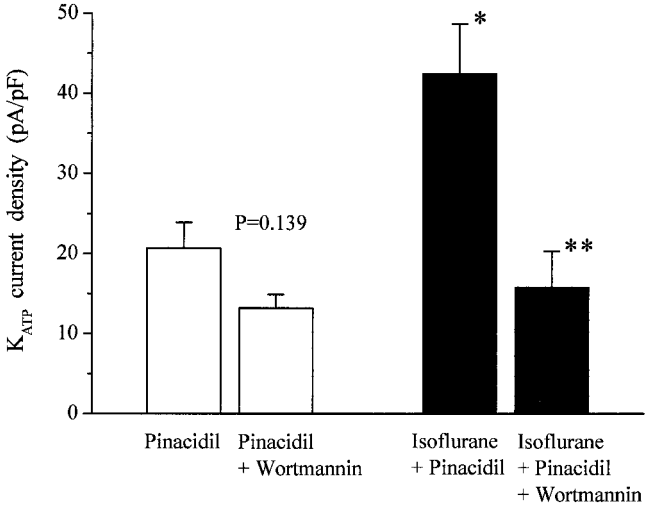

Fig. 5. Effects of wortmannin on pinacidil activated adenosine triphosphate–sensitive potassium current (I_{KATP}) at 0.5 mM internal ATP. The voltage protocol was as described in figures 1 and 2. Wortmannin (100 μM) was applied intracellularly *via* the recording pipette. Wortmannin did not significantly affect I_{KATP} activated by pinacidil alone ($P = 0.139$; n = 6) but prevented the sensitization effect of isoflurane (*$P < 0.05$, isoflurane + pinacidil *vs.* pinacidil alone, n = 8; **$P < 0.05$, isoflurane + pinacidil + wortmannin *vs.* isoflurane + pinacidil, n = 7).

Furthermore, when present throughout the course of the experiment, theophylline prevented sensitization by isoflurane. In the presence of theophylline, isoflurane did not increase the pinacidil-activated I_{KATP}, and current density was 10.0 ± 2.5 pA/pF (n = 6; $P < 0.05$, isoflurane + pinacidil + theophylline *vs.* isoflurane + pinacidil). Thus, blockade of adenosine receptors caused a decrease in magnitude of pinacidil-activated current through $sarcK_{ATP}$ channel and prevented sensitization by isoflurane.

Involvement of the Membrane Phospholipids in Isoflurane Sensitization

Membrane phospholipids, and particularly phosphatidylinositol 4,5-biphosphate (PIP_2), modulate the activity of $sarcK_{ATP}$ channel by increasing channel open probability and reducing sensitivity to ATP.[28,29] However, recent work has also demonstrated that phospholipids may reduce sensitivity of K_{ATP} channel to potassium channel openers and glibenclamide.[30] The intracellular concentrations of phospholipids are up-regulated by the action of phosphatidylinositol kinases and down-regulated by phospholipid lipases and phosphatases. To test the hypothesis that sensitization by isoflurane may involve the phosphatidylinositol kinase-dependent pathway, we investigated the effects of wortmannin, an inhibitor of phosphatidylinositol kinases, on pinacidil-activated I_{KATP} at 0.5 mM internal ATP. Wortmannin was applied at 100 μM intracellularly in the pipette solution. Figure 5 shows that wortmannin alone did not significantly alter activation of I_{KATP} by pinacidil (5 μM), and current density was 13.2 ± 1.7 pA/pF (n = 6; $P = 0.139$, pinacidil + wortmannin *vs.* pinacidil alone). However, in the continued presence of wortmannin, isoflurane failed to sensitize I_{KATP} to pinacidil. In the presence of wortmannin and isoflurane, the density of pinacidil-activated I_{KATP} was 15.8 ± 4.5 pA/pF (n = 7). Thus, current density was not different from that determined in the absence of isoflurane ($P = 0.085$, isoflurane + pinacidil + wortmannin *vs.* pinacidil + wortmannin) but was significantly less than in the presence of isoflurane and pinacidil ($P < 0.05$, isoflurane + pinacidil + wortmannin *vs.* isoflurane + pinacidil). These results suggest that wortmannin, an inhibitor of phosphatidylinositol kinases, may prevent isoflurane-mediated sensitization of $sarcK_{ATP}$ channel to pinacidil.

Discussion

This study investigated the effects of isoflurane on the sensitivity of cardiac $sarcK_{ATP}$ channels to a potassium channel opener, pinacidil, as measured by changes in the whole cell I_{KATP}. The results show that, although isoflurane alone may not activate whole cell I_{KATP} in guinea pig ventricular cells, pretreatment with isoflurane increases sensitivity of these channels to pinacidil, as reflected in the increased current density and accelerated rate of I_{KATP} activation. Furthermore, the sensitization effect is abolished by theophylline and wortmannin. This is a novel finding showing that a volatile anesthetic can enhance sensitivity of the cardiac $sarcK_{ATP}$ channel to its opener *via* adenosine-triggered signaling cascade and membrane phospholipids.

Sensitization effect of isoflurane was greater at 0.5 mM than at 5 mM intracellular ATP. This suggests that impaired metabolic conditions that may cause a local depletion of ATP could facilitate isoflurane actions, an important finding regarding cardioprotection under compromised cellular function. A possibility of isoflurane modulation of ATP sensitivity of K_{ATP} channel has been suggested by the study showing that isoflurane may decrease ATP sensitivity despite increasing channel closed time.[13] However, it has also been reported that trifluoroacetic acid, a metabolite of isoflurane, may modulate ATP sensitivity of K_{ATP} channels.[31] Taking under account a very low metabolism of isoflurane *in vivo*, and our *in vitro* experimental conditions where single myocytes are directly exposed to isoflurane, the results of the current study suggest that isoflurane itself rather than its metabolite sensitizes the channel. Since pinacidil effects are ATP-dependent, whereby a decrease in ATP leads to a greater activation of I_{KATP},[24,25] the sensitization of $sarcK_{ATP}$ channel to pinacidil by isoflurane may, in part, result from its ability to alter ATP sensitivity.

Released under metabolic stress, adenosine may exert cardioprotective effects by activating A_1 and A_3 receptors coupled *via* G_o/G_i proteins to multiple effectors.

These include phospholipases C and D, phosphoinositides, protein kinases such as protein kinase C (PKC), and the K_{ATP} channel.[27] Recent studies demonstrated that in cardioprotection, the triggering action of adenosine is not dependent on K_{ATP} channel activation or free radical production, but rather results from a direct activation of the kinases.[32] Although adenosine was not used in our study, an antagonist of adenosine receptors, theophylline, prevented sensitization by isoflurane. This suggests that isoflurane may alter activity of adenosine receptors, since it has been demonstrated that isoflurane does not increase the production or release of adenosine.[33] Isoflurane may act on other components of the adenosine signaling pathway, including not only PKC, but also the phospholipases or phosphatidylinositol kinase activity upstream of PKC. Attenuation of I_{KATP} activation by pinacidil in the presence of theophylline could be explained by a decreased potency of pinacidil resulting from adenosine receptor blockade, since it is known that activation of adenosine receptors can enhance the potency of potassium channel openers nicorandil and levcromakalim in the arterial smooth muscle.[34]

The membrane phospholipids regulate the $sarcK_{ATP}$ channels by modifying its sensitivity to ATP, sulfonylureas, and potassium channel openers.[30] Phosphatidylinositol phosphates, and particularly PIP_2, greatly reduce sensitivity to inhibition by ATP that is mediated *via* the pore-forming Kir6.2 subunit of K_{ATP} channel.[28,30] In addition, sensitivities to the openers and glibenclamide that are mediated by the SUR2A subunit[35,36] are also decreased by PIP_2. The levels of PIP_2 are up-regulated *via* a rapid activation of phosphatidylinositol kinases and phosphorylation of phosphatidylinositol and PI-4-P, and down-regulated by phospholipid lipase–mediated hydrolysis or the action of phospholipid phosphatases.[29] Stimulation of phosphatidylinositol kinases would therefore be expected to increase PIP_2 concentrations and thus alter channel sensitivity to its openers. Consequently, either inhibition of phosphatidylinositol kinases to prevent PIP_2 synthesis, or activation of phospholipases to enhance PIP_2 breakdown, would be expected to have an enhancing effect on sensitivity of the $sarcK_{ATP}$ channel to specific potassium channel openers. It is possible that isoflurane sensitization results from altered activity of some of these enzymes. Previous studies demonstrated that volatile anesthetics may increase activity of phospholipase C in erythrocyte membranes[37] or skeletal muscle.[38] Isoflurane may also interact with the PKC signaling pathway where adenosine and PIP_2 pathways merge, since it has been suggested that elements distal to PIP_2 may be involved in the PIP_2-induced modification of ATP sensitivity.[39]

To test whether isoflurane sensitization involves modulation of phosphatidylinositol kinases, we used wortmannin, an inhibitor of phosphatidylinositol-3 and -4 kinases.[40,41] In the presence of wortmannin, isoflurane failed to sensitize the K_{ATP} channel to pinacidil. This suggests that modulation of pinacidil effects by isoflurane may occur *via* phosphatidylinositol kinases. The phosphatidylinositol kinases, particularly phosphatidylinositol-3 kinase, are key signaling enzymes mediating activation of other kinases such as protein kinase B, p70 kinase, numerous PKC isoforms, and endothelial nitric oxide synthase.[42] Thus, we cannot exclude a possibility that isoflurane effects involves one of these pathways. Nevertheless, the wortmannin experiments suggest that isoflurane sensitizes the cardiac $sarcK_{ATP}$ channel to pinacidil by a mechanism that is upstream of PKC and may involve phospholipid-mediated control of the channel.

Compared with other K_{ATP} channel subtypes, the cardiac $sarcK_{ATP}$ channel composed of Kir6.2 and SUR2A subunits shows high sensitivity to potassium channel openers.[43] Binding of pinacidil to the identified binding sites on the SUR2A subunit[35] is modulated by nucleotides and requires ATP hydrolysis to induce a conformational change that stabilizes the pinacidil-activated state.[44] Thus, we cannot disregard a possibility of allosteric modulation by isoflurane of channel protein, which could cause an increase in sensitivity to pinacidil possibly by enhancing the accessibility of channel opener to its binding sites on the SUR2A subunit.

The current study and the study by Kohro *et al.*[45] suggest that isoflurane may have differential effects on the $sarcK_{ATP}$ and $mitoK_{ATP}$ channels in ventricular myocytes. Isoflurane alone does not open $sarcK_{ATP}$ channel, but enhances activity of the channels activated by specific channel openers or metabolic inhibitors.[16,19] In contrast, isoflurane appears to directly activate the $mitoK_{ATP}$ channel, as measured by an increase in flavoprotein oxidation.[45] Isoflurane may also enhance the $mitoK_{ATP}$ channel activated by diazoxide. These findings suggest differential sensitivity of sarcolemmal and mitochondrial channels to isoflurane. However, isoflurane may enhance activity of both types of K_{ATP} channels previously opened by specific channel activators.

In summary, this study is the first to show that pretreatment with a volatile anesthetic, isoflurane, enhances sensitivity of the cardiac $sarcK_{ATP}$ channel to pinacidil. Isoflurane sensitization is modulated by intracellular ATP and may involve components of adenosine and phospholipid signaling pathways. Sensitization of cardiac K_{ATP} channel to specific openers may be one of the cellular mechanisms by which isoflurane and other volatile anesthetics produce myocardial protection.

References

1. Warltier DC, al-Wathiqui MH, Kampine JP, Schmeling WT: Recovery of contractile function of stunned myocardium in chronically instrumented dogs is enhanced by halothane or isoflurane. ANESTHESIOLOGY 1988; 69:552–65

2. Kersten JR, Schmeling TJ, Pagel PS, Gross GJ: Isoflurane mimics ischemic preconditioning via activation of K_{ATP} channels. ANESTHESIOLOGY 1997; 87:361–70

3. Freedman BM, Hamm DP, Everson CT, Wechsler AS, Christian CMI: Enflurane enhances postischemic functional recovery in the isolated rat heart. ANESTHESIOLOGY 1985; 62:29–33

4. Coetzee JF, le Roux PJ, Genade S, Lochner A: Reduction of postischemic contractile dysfunction of the isolated rat heart by sevoflurane: Comparison with halothane. Anesth Analg 2000; 90:1089–97

5. Murry CE, Jennings RB, Reimer KA: Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. Circulation 1986; 74:1124–36

6. O'Rourke B: Myocardial K_{ATP} channels in preconditioning. Circ Res 2000; 87:845–55

7. Liu Y, Sato T, O'Rourke B, Marban E: Mitochondrial ATP-dependent potassium channels: Novel effectors of cardioprotection. Circulation 1998; 97:2463–9

8. Ghosh S, Standen NB, Galinanes M: Evidence for mitochondrial K_{ATP} channels as effectors of human myocardial preconditioning. Cardiovasc Res 2000; 45:934–40

9. Gross GJ, Fryer RM: Sarcolemmal versus mitochondrial ATP-sensitive K^+ channels and myocardial preconditioning. Circ Res 1999; 84:973–9

10. Toyoda Y, Friehs I, Parker RA, Levitsky S, McCully JD: Differential role of sarcolemmal and mitochondrial K(ATP) channels in adenosine-enhanced ischemic preconditioning. Am J Physiol Heart Circ Physiol 2000; 279:H2694–703

11. 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

12. 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

13. 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

14. 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

15. Ismaeil MS, Tkachenko I, Gamperl AK, Hickey RF, Cason BA: Mechanisms of isoflurane-induced myocardial preconditioning in rabbits. ANESTHESIOLOGY 1999; 90:812–21

16. Fujimoto K, Bosnjak ZJ, Kwok WM: Isoflurane-induced facilitation of the cardiac sarcolemmal K_{ATP} channel. ANESTHESIOLOGY 2002; 97:57–65

17. Stadnicka A, Kwok WM, Gassmayr S, Bosnjak ZJ: Activation of cardiac sarcolemmal K_{ATP} channel by isoflurane and protein tyrosine kinase inhibitor genistein (abstract). ANESTHESIOLOGY 2001; 95:A607

18. Turner LA, Stadnicka A, Fujimoto K, Kwok WM, Bosnjak ZJ: Isoflurane activates cardiac K_{ATP} channels after stimulation of PKC by diacylglycerol (abstract). ANESTHESIOLOGY 2001; 95:A613

19. Kwok WM, Martinelli AT, Fujimoto K, Suzuki A, Stadnicka A, Bosnjak ZJ: Differential modulation of the cardiac ATP-sensitive potassium channel by isoflurane and halothane. ANESTHESIOLOGY 2002; 97:50–6

20. Mitra R, Morad M: A uniform enzymatic method for dissociation of myocytes from hearts and stomachs of vertebrates. Am J Physiol Heart Circ Physiol 1985; 249:H1056–60

21. Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ: Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflügers Arch Eur J Physiol 1981; 391:85–100

22. Edwards G, Weston AH: The pharmacology of ATP-sensitive potassium channels. Annu Rev Pharmacol Toxicol 1993; 33:597–637

23. Arena JP, Kass RS: Enhancement of potassium-sensitive current in heart cells by pinacidil: Evidence for modulation of the ATP-sensitive potassium channel. Circ Res 1989; 65:436–45

24. Tseng GN, Hoffman BF: Actions of pinacidil on membrane currents in canine ventricular myocytes and their modulation by intracellular ATP and cAMP. Pflügers Arch Eur J Physiol 1990; 415:414–24

25. Arena JP, Kass RS: Activation of ATP-sensitive K channels in heart cells by pinacidil: Dependence on ATP. Am J Physiol Heart Circ Physiol 1989; 257: H2092–6

26. Weigt HU, Kwok WM, Rehmert GC, Bosnjak ZJ: Modulation of the cardiac sodium current by inhalational anesthetics in the absence and presence of beta-stimulation. ANESTHESIOLOGY 1998; 88:114–24

27. Mubagwa K, Flameng W: Adenosine, adenosine receptors and myocardial protection: An updated overview. Cardiovasc Res 2001; 52:25–39

28. Baukrowitz T, Schulte U, Oliver D, Herlitze S, Krauter T, Tucker SJ, Ruppersberg JP, Fakler B: PIP2 and PIP as determinants for ATP inhibition of KATP channels. Science 1998; 282:1141–4

29. Baukrowitz T, Fakler B: K(ATP) channels: Linker between phospholipid metabolism and excitability. Biochem Pharmacol 2000; 60:735–40

30. Krauter T, Ruppersberg JP, Baukrowitz T: Phospholipids as modulators of K(ATP) channels: Distinct mechanisms for control of sensitivity to sulphonylureas, K(+) channel openers, and ATP. Mol Pharmacol 2001; 59:1086–93

31. Han J, Kim N, Kim E: Trifluoroacetic acid activates ATP-sensitive K+ channels in rabbit ventricular myocytes. Biochem Biophys Res Commun 2001; 285:1136–42

32. Cohen MV, Yang XM, Liu GS, Heusch G, Downey JM: Acetylcholine, bradykinin, opioids, and phenylephrine, but not adenosine, trigger preconditioning by generating free radicals and opening mitochondrial K(ATP) channels. Circ Res 2001; 89:273–8

33. Kersten JR, Orth KG, Pagel PS, Mei DA, Gross GJ, Warltier DC: Role of adenosine in isoflurane-induced cardioprotection. ANESTHESIOLOGY 1997; 86:1128–39

34. Davie CS, Everitt DE, Standen NB: Increase in the vasorelaxant potency of K(ATP) channel opening drugs by adenosine A(1) and A(2) receptors in the pig coronary artery. Eur J Pharmacol 1999; 383:155–62

35. Babenko AP, Gonzalez G, Bryan J: Pharmaco-topology of sulfonylurea receptors: Separate domains of the regulatory subunits of K(ATP) channel isoforms are required for selective interaction with K(+) channel openers. J Biol Chem 2000; 275:717–20

36. Uhde I, Toman A, Gross I, Schwanstecher C, Schwanstecher M: Identification of the potassium channel opener site on sulfonylurea receptors. J Biol Chem 1999; 274:28079–82

37. Rooney TA, Hager R, Stubbs CD, Thomas AP: Halothane regulates G-protein-dependent phospholipase C activity in turkey erythrocyte membranes. J Biol Chem 1993; 268:15550–6

38. Kudoh A, Matsuki A: Sevoflurane stimulates inositol 1,4,5-trisphosphate in skeletal muscle. Anesth Analg 2000; 91:440–5

39. Okamura M, Kakei M, Ichinari K, Miyamura A, Oketani N, Koriyama N, Tei C: State-dependent modification of ATP-sensitive K+ channels by phosphatidylinositol 4,5-bisphosphate. Am J Physiol Cell Physiol 2001; 280:C303–8

40. Fruman DA, Meyers RE, Cantley LC: Phosphoinositide kinases. Annu Rev Biochem 1998; 67:481–507

41. Xie LH, Takano M, Kakei M, Okamura M, Noma A: Wortmannin, an inhibitor of phosphatidylinositol kinases, blocks the MgATP-dependent recovery of Kir6.2/SUR2A channels. J Physiol (Lond) 1999; 514:655–65

42. Tong H, Chen W, Steenbergen C, Murphy E: Ischemic preconditioning activates phosphatidylinositol-3-kinase upstream of protein kinase C. Circ Res 2000; 87:309–15

43. Gribble FM, Reimann F, Ashfield R, Ashcroft FM: Nucleotide modulation of pinacidil stimulation of the cloned K(ATP) channel Kir6.2/SUR2A. Mol Pharmacol 2000; 57:1256–61

44. Schwanstecher M, Sieverding C, Dorschner H, Gross I, Aguilar-Bryan L, Schwanstecher C, Bryan J: Potassium channel openers require ATP to bind to and act through sulfonylurea receptors. EMBO J 1998; 17:5529–35

45. Kohro S, Hogan QH, Nakae Y, Yamakage M, Bosnjak ZJ: Anesthetic effects on mitochondrial ATP-sensitive K channel. ANESTHESIOLOGY 2001; 95:1435–40