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Mechanism of Cardiac Sarcolemmal Adenosine Triphosphate–sensitive Potassium Channel Activation by Isoflurane in a Heterologous Expression System

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Background: **Activation of the cardiac sarcolemmal adenosine triphosphate–sensitive potassium (K_{ATP}) channel during metabolic stress initiates cellular events that preserve cardiac performance. Previous studies showed that halogenated anesthetics prime K_{ATP} channels under whole cell voltage clamp and act in intracellular pH (pHi)–dependent manner on K_{ATP} channels in excised membrane patches. However, it is not known how halogenated anesthetics interact with these channels.**

Methods: **The authors evaluated the effect of pHi and isoflurane on the K_{ATP} channel subunits, the pore-forming inward rectifier Kir6.2, and the regulatory sulfonylurea receptor SUR2A, using HEK293 cells as a heterologous expression system. Single channel activity was recorded in the inside-out patch configuration.**

Results: **At pHi 7.4, isoflurane had negligible effect on activity of wild-type Kir6.2/SUR2A, but at pHi 6.8, the channel open probability was increased by isoflurane (0.177 ± 0.077 to 0.364 ± 0.164). By contrast, the open probability of truncated Kir6.2ΔC26, which forms a functional channel without SUR2A, was attenuated by isoflurane at both pHi 7.4 and pHi 6.8. Coexpression of Kir6.2ΔC26 with SUR2A restored pHi sensitivity of channel activation by isoflurane. Site-directed mutagenesis within the Walker motifs of SUR2A abolished isoflurane activation of K_{ATP} channel at pHi 6.8. In addition, the pancreatic-type channels expressing sulfonylurea receptor SUR1 could not be activated by isoflurane.**

Conclusions: **The nucleotide binding domains of SUR2A play a crucial role in isoflurane facilitation of the K_{ATP} channel activity at moderately acidic pHi as would occur during early ischemia. These findings support direct and differential interaction of isoflurane with the subunits of the cardiac sarcolemmal K_{ATP} channel.**

ACTIVATION of the cardiac sarcolemmal adenosine triphosphate–sensitive potassium (K_{ATP}) channel during metabolic stress such as ischemia initiates cellular mechanisms that preserve cardiac performance.[1–4] Under normal physiologic conditions, the cardiac K_{ATP} channels are predominantly closed. When they open during severe metabolic inhibition, the action potential is shortened,[2] and Ca^{2+} entry *via* voltage-dependent calcium channels is reduced, leading to preservation of the energy stores.[5] The protective function of the sarcolemmal K_{ATP} channel has been most characterized in studies on preconditioning where brief periods of ischemia or exposure to halogenated anesthetics or other drugs improved recovery and reduced infarct size resulting from a subsequent prolonged ischemic injury.[6,7] Participation of the sarcolemmal K_{ATP} channel in preconditioning by halogenated anesthetics has been demonstrated in animal and isolated cardiomyocyte models.[8–10] Under whole cell or cell-attached patch clamp conditions, isoflurane increases the open probability (Po) of K_{ATP} channels previously activated by uncouplers of oxidative phosphorylation or the channel openers,[11,12] and this effect is mediated by protein kinase C.[13,14] However, how halogenated anesthetics enhance activity of the K_{ATP} channels has not been clarified.

Intracellular protons modulate activity of the K_{ATP} channels in a variety of tissues,[15,16] and the molecular structures responsible for this effect have been identified.[17,18] Recently, we have demonstrated that in addition to modulating basal activity of the K_{ATP} channel, intracellular pH (pHi) also modifies the interaction of isoflurane with this channel whereby channel opening is enhanced by isoflurane at moderately acidic pHi similar to that occurring in early ischemia.[19] This effect was observed in the excised membrane patches, suggesting a possibility of direct anesthetic interaction with the K_{ATP} channel. To further investigate the mechanism of this interaction, the current study evaluated the effects of pHi and isoflurane on subunits of the sarcolemmal K_{ATP} channel, the pore-forming inward rectifier Kir6.2 and the regulatory sulfonylurea receptor SUR2A (fig. 1) in the heterologous expression system.

As a member of the ABC transporter family, SUR2A possesses two nucleotide binding domains (NBD1 and NBD2) that are important for the metabolic sensing properties of the K_{ATP} channel. Specific interactions of intracellular adenine nucleotides with NBD1 and NBD2 have been indicated to be involved in K_{ATP} channel regulation.[20] We mutated four highly conserved amino acids within the Walker motifs of NBD1 and NBD2 to test the importance of these domains for isoflurane-induced channel activation. The residues K708 and K1349 are involved in γ-phosphate coordination, and D833 and D1470 are essential for Mg binding within NBD1 and NBD2, respectively.[21] The truncated Kir6.2 (Kir6.2ΔC26) that forms a functional channel without SUR2A subunit was also used.[22] This construct allows us to test the effects of pHi and isoflurane on the K_{ATP}

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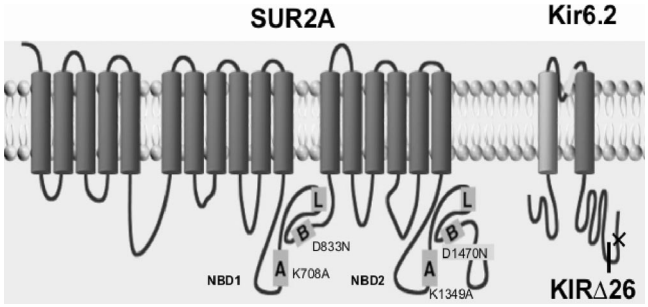

Fig. 1. A structural model of the cardiac sarcolemmal adenosine triphosphate–sensitive potassium channel comprising two subunits, the pore-forming inward rectifier Kir6.2 and the regulatory sulfonylurea receptor SUR2A. Locations of the nucleotide binding domains NBD1 and NBD2, each with Walker motifs A and B and L-linker regions, and the amino acids changed by site-directed mutagenesis (K708A, D833N, K1349A, D1470N) are indicated in SUR2A. A deletion of 26 amino acids in the C-terminus (KIRΔ26), which allows expression of a functional Kir6.2ΔC26 channel without SUR2A, is indicated in Kir6.2.

channel pore without SUR subunit, thus enabling us to determine which subunit is targeted by isoflurane.

Materials and Methods

Site-directed Mutagenesis

Point mutations in the core consensus sequence of the Walker motifs of NBD1 (K708A and D833N) and NBD2 (K1349A and D1470N) in the mouse cardiac SUR2A isoform, and an early stop codon in Kir6.2ΔC26 (fig. 1) were introduced in the pCDNA3.1 plasmid by polymerase chain reaction amplification of both DNA strands with complementary primers containing desired amino acid changes (QuickChange, Stratagene, La Jolla, CA) as described previously.[21] The following forward primers were used (mutations are marked bold):

K708A: GGCCAAGTGGGTTGTGGA**GC**ATCATCTCTTC TGG,
D833N: CCAACATCGTCTTTTTG**AAC**GACCCATTCTC TGC,
K1349A: GTCGAACTGGCAGTGGG**GC**GTCCTCCTTAT CCC,
D1470N: GCAGTATACTCATCATG**A**ATGAAGCCACTGC TTC,
Kir6.2Δ26: GACCCTCGCCTCGTCG**T**G**A**GGGCCCCTGC GCAAG.

The reverse primers were complementary. Mutated constructs were sequenced to confirm point mutations and rule out additional changes in the sequence.

Cell Culture and Transfection of K_{ATP} Channel Subunits

HEK293 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and seeded at 1×10^6 cells per 35-mm dish 24 h before transfection. The Kir6.2ΔC26, Kir6.2, and wild-type and mutated SUR2A and SUR1 subcloned into the expression vector pCDNA3.1 were transiently transfected into HEK293 cells using Fugene 6 (Roche, Indianapolis, IN). For each 35-mm dish (2 ml medium), pCDNA3.1-SURx (5 μg) and pCDNA3.1-Kir6.2 (1 μg) were cotransfected together with the expression vector for green fluorescent protein (0.5 μg pGREEN-lantern; Invitrogen, Carlsberg, CA) serving as a reporter gene. Cells were used 48–72 h after transfection. Transfection started to decline after 72 h. Transfection efficiency was between 70 and 90% as indicated by green fluorescence. Under our experimental conditions, HEK293 cells transfected with cDNA for green fluorescent protein alone exhibited green fluorescence, but no ionic current of any type could be detected in these cells (data not shown).

Isolation of Cardiac Myocytes

Single ventricular cardiomyocytes were isolated from adult guinea pig hearts by enzymatic treatment with collagenase (Type II; Invitrogen, Carlsbad, CA) and protease (Type XIV; Sigma, St. Louis, MO) as reported previously.[23] Myocytes were kept at room temperature in a Tyrode solution (132 mM NaCl, 5.0 mM KCl, 1.0 mM $MgCl_2$, 1.0 mM $CaCl_2$, 10 mM HEPES, and 5 mM glucose, at pH 7.3 with NaOH) and were used for patch clamping within 6 h after isolation.

Electrophysiology and Data Analysis

The inside-out configuration of the patch clamp technique was used to monitor channel activity. The cytosolic side of membrane patches was superfused with the intracellular/bath solution containing 136 mM KCl, 0.5 mM $MgCl_2$, 10 mM HEPES, 2 mM EGTA, and 0.1 mM K_2ATP, at pH 7.4 adjusted with KOH, or at pH 6.8 adjusted with HCl. The extracellular/pipette solution contained 144 mM KCl, 0.5 mM $MgCl_2$, 0.5 mM $CaCl_2$, and 10 mM HEPES, at pH 7.4 adjusted with KOH. The final concentration of K^+ on both sides of the membrane patches was 145 mM. Isoflurane (FORANE; Baxter Healthcare, Deerfield, IL) was applied in the intracellular solution at a clinical concentration of 0.5 mM equivalent to 1 vol% or 1 minimum alveolar concentration in humans at room temperature (20°–22°C). The concentrations of isoflurane in the bath solution sampled from the recording chamber were measured by the headspace gas chromatography method, using a Shimadzu GC8A chromatograph (Shimadzu, Kyoto, Japan). The patch pipettes were pulled from standard borosilicate glass tubing (Garner, Claremont, CA) using a horizontal PC-84 micropipette puller (Sutter Instruments, Novato, CA). Pipette tips were fire-polished with a microforge (Narishige, Tokyo, Japan). The resistance of pipettes filled with the extracellular solution was 6–10 MΩ. Experiments were conducted in the RC-13 perfusion chamber (Warner, Hamden, CT) mounted on the stage of an inverted IMT-2

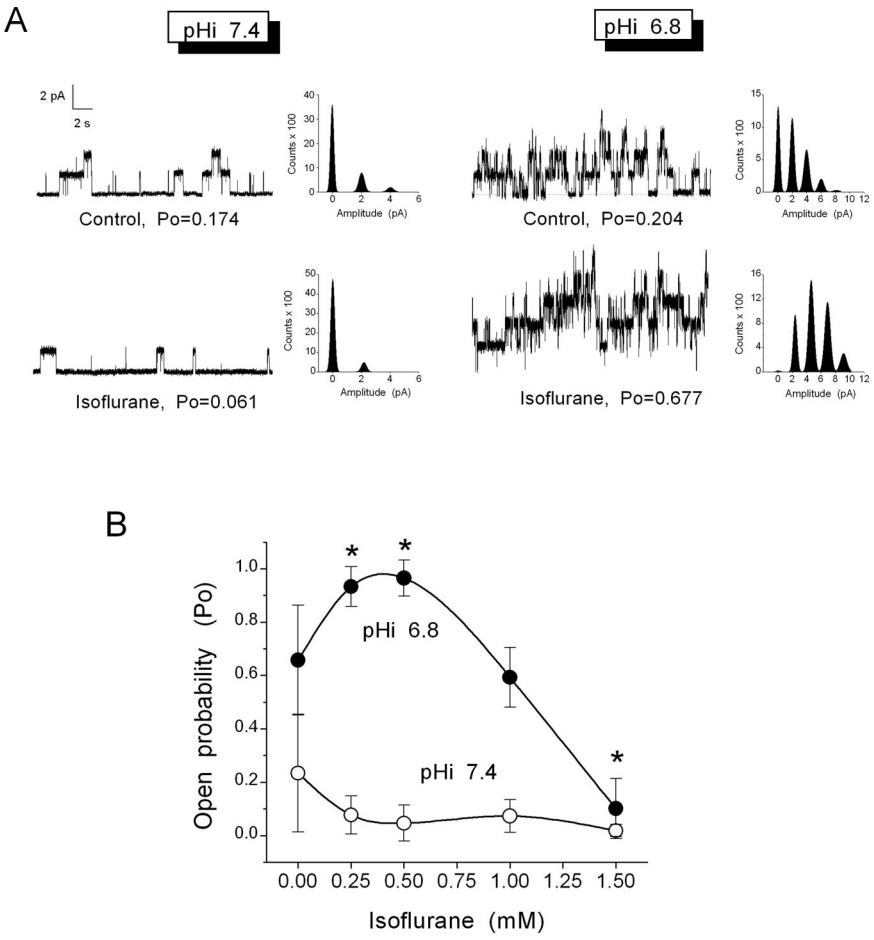

Fig. 2. Isoflurane modulates activity of native sarcolemmal adenosine triphosphate–sensitive potassium channels from guinea pig ventricular cardiomyocytes in a pH-dependent manner. (*A*) Representative records of single channel activity at intracellular pH (pHi) 7.4 and 6.8 in the absence (control) and the presence of 0.5 mM isoflurane. Compared with pHi 7.4, basal channel activity was greater at pHi 6.8, and isoflurane further increased the probability of channel opening (Po) at pHi 6.8, but not at pHi 7.4. (*B*) Summary of results from experiments shown in *A*. Isoflurane effects on Po were pHi sensitive and concentration related. At pHi 7.4, Po was not significantly affected by isoflurane. At pHi 6.8, Po was increased in the presence of 0.25 and 0.50 mM isoflurane, but remained unchanged at 1.0 mM isoflurane, and was decreased at 1.50 mM isoflurane. Data are mean ± SD; n = 5 per data point. * $P <$ 0.05 *versus* anesthetic-free control (0.00 mM isoflurane).

microscope (Olympus, Tokyo, Japan). Single channel currents were recorded at the membrane potential of +40 mV using a List EPC-7 patch clamp amplifier (ALA Scientific Instruments, Westbury, NY), digitized (Digidata 1322A; Axon Instruments, Foster City, CA), and stored on the hard disk of a computer for subsequent analysis. Data were sampled at 1 kHz and low-pass filtered at 0.5 kHz through the eight-pole Bessel filter. pClamp9 software (Axon Instruments) and Origin6 software (OriginLab, Northampton, MA) were used for data acquisition and analysis. Criteria to confirm identity of the K_{ATP} channels were the unitary current amplitude, single channel conductance, sensitivity to inhibition by intracellular adenosine triphosphate (ATP; 1–2 mM), and blockade by glibenclamide (1–5 μM). The half-amplitude threshold method was used when detecting single channel openings. Amplitude of unitary current was determined from the all-point amplitude histograms constructed from 60-s-long data recordings. The channel Po was calculated from the ratios of the area under the peaks in the all-points amplitude histograms fitted by a multigaussian distribution. Reported here is cumulative Po, defined as a fraction of the total length of time the channels were in the open state during 60-s-long recordings.

Statistical Analysis

Data are presented as means ± SD; n indicates the number of experiments. Statistical significance was determined using the Student paired *t* test and one-way analysis of variance with *post hoc* Scheffé test. All comparisons were two tailed. Differences with $P < 0.05$ were accepted as significant.

Results

We first investigated how pHi affects the relation between isoflurane concentration and the Po of native cardiac K_{ATP} channels in guinea pig cardiomyocytes. Figure 2 shows the results that were obtained for pHi 7.4 and 6.8. At pHi 7.4, isoflurane tended to decrease Po irrespective of tested anesthetic concentration, but the change was not significant. In contrast, at moderately acidic pHi 6.8, 0.25 and 0.5 mM isoflurane significantly increased Po to 0.933 ± 0.074 (42%, n = 5) and 0.965 ± 0.068 (47%, n = 5), respectively, from control Po of 0.657 ± 0.206, but 1.0 mM isoflurane had a negligible effect on Po, and 1.5 mM isoflurane decreased Po to 0.101 ± 0.112 (n = 5). These results confirmed that isoflurane modulates activity of the cardiac sarcolemmal K_{ATP} channel in a pHi-dependent manner.

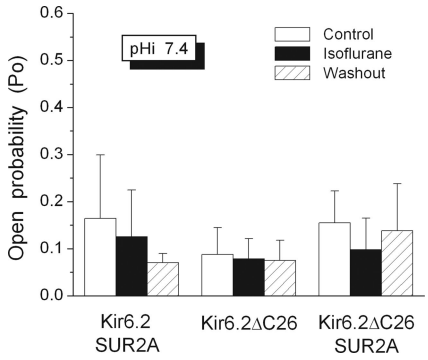

Fig. 3. Effects of isoflurane on the open probability (Po) of expressed wild-type Kir6.2/SUR2A WT (n = 6), truncated Kir6.2ΔC26 (n = 7), and Kir6.2ΔC26/SUR2A (n = 6) channels at intracellular pH (pHi) 7.4. Data are mean ± SD. At pHi 7.4, isoflurane had a negligible, nonsignificant effect on Po. Note that the basal/control Po of truncated Kir6.2ΔC26 channel is much lower than Po of the channels expressing SUR2A.

The pore-forming subunit Kir6.2 is known to be involved in the pHi dependency of K_{ATP} channel opening.[16,17] To investigate whether this also holds true for pHi dependency of isoflurane–channel interaction, we expressed the K_{ATP} channel subunits heterologously in HEK293 cells. In addition, we used a deletion mutant of Kir6.2 (Kir6.2ΔC26) lacking the last 26 amino acids in the C-terminus. This deletion allows trafficking of Kir6.2 to the plasma membrane, resulting in expression of a functional K_{ATP} channel without SUR2A.[22] As shown in figure 3, at pHi 7.4, isoflurane produced a slight, but not significant decrease in Po of wild-type Kir6.2/SUR2A WT (n = 6), Kir6.2ΔC26 (n = 7), and Kir6.2ΔC26/SUR2A (n = 6) channels. By contrast, at pHi 6.8, a twofold increase in Po from 0.177 ± 0.077 to 0.364 ± 0.164 (n = 8) was observed for Kir6.2/SUR2A WT channels (figs. 4 and 5). Summary data demonstrate that this increase was abolished and reversed in the SUR2A-lacking Kir6.2ΔC26, where isoflurane decreased Po from 0.204 ± 0.062 to 0.153 ± 0.081 (n = 7; fig. 5). Coexpression of SUR2A with Kir6.2ΔC26 restored the isoflurane-induced increase in Po (0.221 ± 0.062 from control 0.135 ± 0.053, n = 8), confirming that SUR2A is responsible for this effect. The effects of isoflurane on the constructs were reversed on anesthetic washout (fig. 5).

We mutated four highly conserved amino acids within the Walker motifs of NBD1 and NBD2 to test the impor-

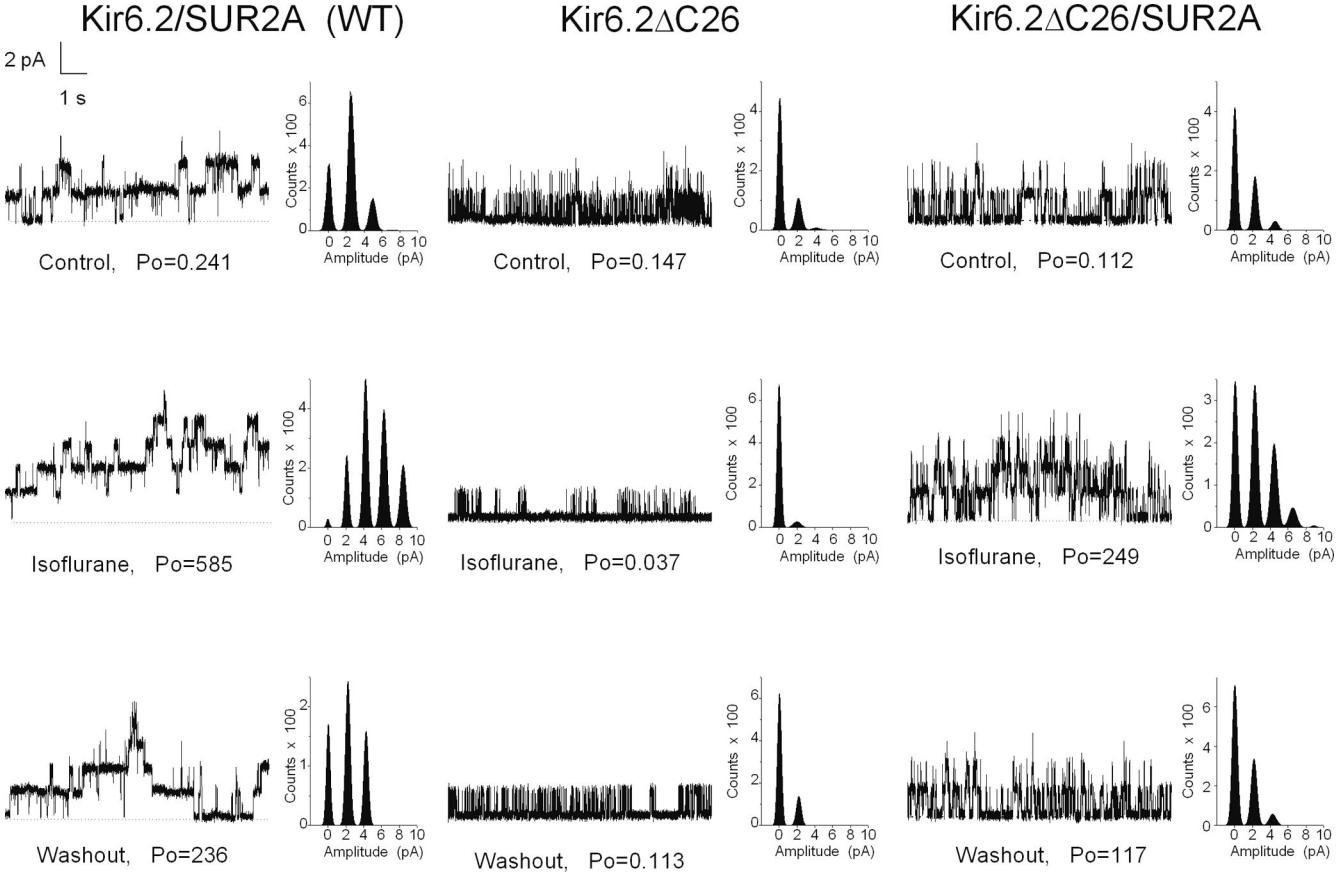

Fig. 4. Effects of isoflurane on expressed adenosine triphosphate–sensitive potassium channels at intracellular pH 6.8. Representative raw traces of single channel currents through Kir6.2/SUR2A WT, Kir6.2ΔC26, and Kir6.2ΔC26/SUR2A recorded from inside-out patches at the membrane potential of +40 mV in control, during application of 0.5 mM isoflurane, and after anesthetic washout. The corresponding amplitude histograms show that isoflurane increased the number of open channels and open probability (Po) of Kir6.2/SUR2A and Kir6.2ΔC26/SUR2A. By contrast, isoflurane tended to decrease Po of truncated Kir6.2ΔC26 channels without SUR2A.

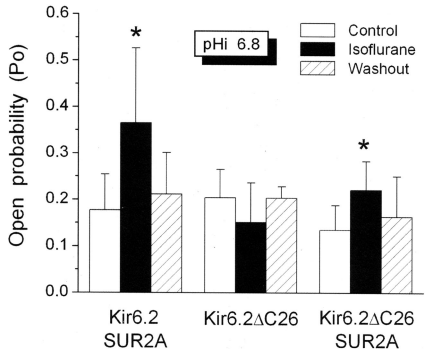

Fig. 5. Summary of isoflurane effects on the open probability (Po) of adenosine triphosphate–sensitive potassium channels determined from experiments shown in figure 4. At intracellular pH (pHi) 6.8, isoflurane (0.5 mM) significantly increased Po of Kir6.2/SUR2A WT and Kir6.2ΔC26/SUR2A channels (n = 9 and n = 8, respectively; * $P < 0.05$ *vs.* control), but not Kir6.2ΔC26 channels (n = 7). Data are mean ± SD. Note that basal/control Po of truncated Kir6.2ΔC26 channels is higher at pHi 6.8 than at pHi 7.4. This is because some of the protein domains crucial for pHi sensitivity are located in the Kir6.2.

tance of these domains for isoflurane-induced increase in Po at pHi 6.8. After neutralizing all four amino acids (SUR2A-4X, n = 6), isoflurane did not increase channel Po at pHi 6.8 (fig. 6). To identify the protein region responsible for isoflurane actions, we created a single point mutation in each nucleotide binding domain, K708A in NBD1 and D1470N in NBD2. Disrupting the function of NBD2 (SUR2A-D1470N, n = 7) had no effect on isoflurane-induced increase in Po compared with the wild-type SUR2A-expressing channels. However, altering NBD1 function (SUR2A-K708A, n = 5) abolished the isoflurane effect similar to that which was observed after mutating all four Walker motif residues in NBD1 and NBD2 (SUR2A-4X). This strongly suggested that the intact NBD1 of SUR2A is critical for isoflurane enhancing effect on open probability of the K_{ATP} channel. Interestingly, in contrast to Kir6.2/SUR2A, the channels composed of Kir6.2 and the wild-type SUR1 (n = 7) were not sensitive to activation by isoflurane.

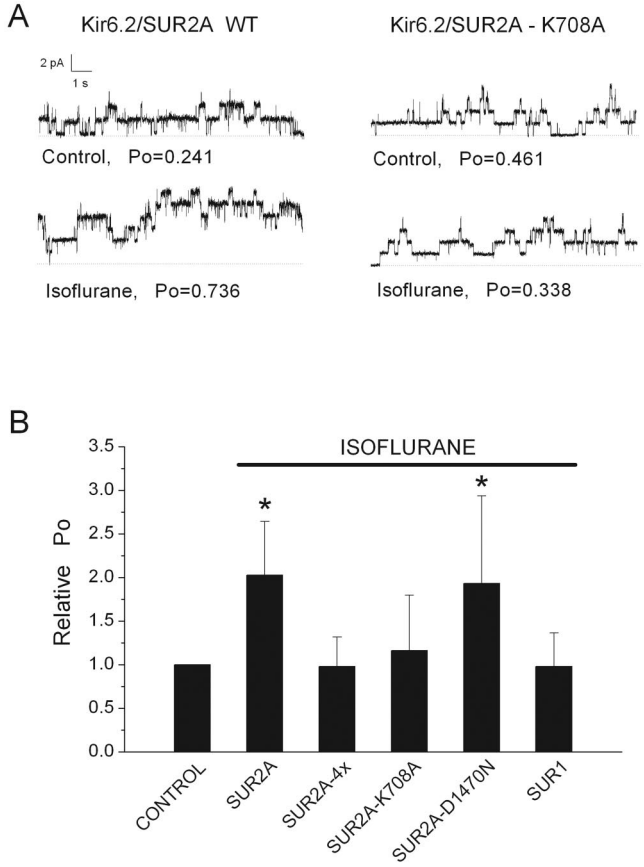

Fig. 6. Mutations in nucleotide binding domain 1 (NBD1) of SUR2A abolish the isoflurane-induced enhancement of channel activity at intracellular pH 6.8. (*A*) Representative recordings of unitary currents through Kir6.2/SUR2A WT and Kir6.2/SUR2A-K708A channels show that isoflurane (0.5 mM) enhanced open probability (Po) of wild-type channels, but this effect was abolished in SUR2A-K708A mutant. (*B*) Summary of isoflurane effects on SUR2A mutant channels at intracellular pH 6.8. *Bars* represent mean relative Po obtained by normalizing Po of each group to respective control. Isoflurane increased Po of channels expressing wild-type SUR2A and SUR2A-D1470N mutant (* $P < 0.05$ *vs.* control). This effect was abolished in channels expressing SUR2A-4X and SUR2A-K708A mutant. Isoflurane had no effect on Po of the SUR1 expressing pancreatic-type channels.

Discussion

It has been established that intracellular protons modulate and increase K_{ATP} channel activity.[24] Three amino acids at the N-terminus, at the C-terminus, and within the second transmembrane domain of Kir6.2 have been identified to be involved in the pH dependence.[16,17] Whether SUR2A participates in pH dependence of K_{ATP} channels has not been reported. The novel finding from our study is that the NBD1 of SUR2A plays a crucial role in isoflurane facilitation of channel activity at moderately acidic pHi 6.8, but not at a more basic pHi of 7.4. This effect occurs under cell-free conditions, in excised membrane patches, suggesting a direct action of the anesthetic on the SUR2A subunit.

We recently demonstrated that isoflurane increases the open probability of native cardiac K_{ATP} channels in the presence of pinacidil, a specific channel opener.[11] This effect was observed only under whole cell patch clamp or in the cell-attached patches, but not under cell-free conditions, suggesting other intracellular mediators might be necessary for enhanced channel activation by isoflurane. However, these findings were obtained under conditions where pHi was set at 7.4. Consequently, it has been demonstrated that under such conditions (pHi 7.4), activation of protein kinase C isoform PKC-ε is required to prime the cardiac K_{ATP} channel to opening by isoflurane.[13] In a recent study, the effect of pHi on isoflurane modulation of native cardiac K_{ATP} channels was investigated under cell-free conditions, in the inside-out configuration of the patch clamp technique.[19] At pHi 6.8, which resembles the intracellular acidosis that is characteristic for early ischemia, isoflurane increased Po

by reducing channel sensitivity to inhibition by intracellular ATP[19] in agreement with increased Po for isoflurane concentrations below 1 mM shown in figure 2. Interestingly, higher concentrations of isoflurane had no effect (1.0 mM) or even inhibited channel activity (1.5 mM isoflurane), possibly due to nonspecific effects on the membrane lipids in the cell-free patches. A pH dependence of the anesthetic effects on ion channels has been established only for certain local anesthetics that contain a tertiary amino group protonated at acidic pH.[25] For example, the local anesthetic lidocaine, a potent blocker of sodium channels, produces a greater depression of conduction in ischemic than in normal myocardium.[26] The pH dependence of halogenated anesthetic actions on cardiac K_{ATP} channels has been described only recently by our group.[19] The molecular basis for this mechanism is still unsolved.

Our results suggest a possibility of direct, endogenous mediators not requiring actions of isoflurane on the K_{ATP} channel. Activation by halogenated anesthetics independent of second-messenger pathways has been reported for some potassium channels. The tandem-pore KCNK2 and KCNK3 (TREK-1 and TASK) channels are reversibly opened by halothane and isoflurane, and the anesthetic actions have been assigned to specific domains of the pore-forming α-subunit of these channels.[27] We report here a novel finding that the actions of isoflurane on potassium channels could be mediated also through interaction with the channel β subunit, which in our study is SUR2A, the regulatory subunit of the K_{ATP} channel.

The K_{ATP} channels are metabolic sensors that link cellular excitability to metabolism. Because the K_{ATP} channel gating is integrated with the cellular energetic network, they are capable of adjusting membrane excitability under ischemic or hypoxic stress. The intracellular adenine nucleotides, ATP and adenosine diphosphate (ADP), are principal physiologic regulators of K_{ATP} channels. However, although ADP, which binds to the SUR subunit, is an established endogenous activator of the K_{ATP} channels, changes in the cytosolic ADP concentration that occur during ischemia are not sufficient to account for channel opening in the presence of millimolar concentrations of intracellular ATP.[28] Other physiologic modulators of channel gating such as phosphatidylinositol-4,5-bisphosphate (PIP_2),[29] coenzyme-linked fatty acids,[30] and phosphotransfer coupling enzymes[31,32] have been described. The phosphotransfer enzymes such as creatine kinase or adenylate kinase provide a mechanism to communicate changes in the energetic state of the cell to the NBDs of SUR by modulating the activity of adenosine triphosphatase at NBD2.[33] In the K_{ATP} channel complex, cooperative interaction of both NBD1 and NBD2 is crucial for coupling the nucleotide-bound states and the functional state of the channel pore. In fact, ATP binding at NBD1, cooperatively supported by ATP hydrolysis, and MgADP binding at NBD2 are required for the proper structural state of SUR that translates into positive gating of the K_{ATP} channel.[34]

Regulation of the K_{ATP} channel by nonnucleotides, the sulfonylureas and the potassium channel openers, also relies on NBDs of the SUR subunit, although binding sites of nonnucleotides are located distal to the NBDs.[35–37] The binding sites for sulfonylureas and potassium channel openers on SUR subunit have been well characterized within the recent years,[38] but similar data are not available for halogenated anesthetics. In our study, the isoflurane-induced enhanced channel activation is abolished by mutations in NBDs, specifically in NBD1. We speculate, therefore, that the mechanism of isoflurane actions shows some similarity to the actions of the openers, because both depend on the intactness of the NBDs. However, lack of antagonistic effects of mutations in NBD2 and much lower efficacy of isoflurane activation suggest important differences in the activation mechanism by isoflurane and the potassium channel openers.

Interestingly, isoflurane facilitation was absent in the K_{ATP} channels formed by Kir6.2 and SUR1, the pancreatic β-cell isoform of SUR. This, in part, could have resulted from differences at the molecular level between the pancreatic SUR1 and the cardiac SUR2A. With approximately 80% of their amino acids conserved and 67% identical, SUR1 and SUR2A differ substantially in their sensitivity to nucleotides (ATP and ADP), which has the impact on physiologic activity of pancreatic *versus* cardiac K_{ATP} channels.[39,40] These two SUR isoforms also exhibit different affinities toward glibenclamide and the potassium channel openers.[41,42] In our study, isoflurane preferentially enhanced opening of the cardiac K_{ATP} channel (Kir6.2/SUR2A), suggesting a unique role of SUR2A in modulation of K_{ATP} channel gating by this halogenated anesthetic.

The limitation of our study is that the K_{ATP} channel was investigated in a noncardiac environment. While the use of neonatal or adult cardiomyocytes would provide the most natural environment for expression of K_{ATP} channel in terms of membrane composition and cellular metabolism, the mammalian cell line (HEK293) allowed us to study Kir6.2 in the absence of SUR2A and to express the mutated K_{ATP} channel subunits without confounding endogenously expressed wild-type channel subunits. Another limitation is that the design of our study does not allow us to make a definite link between our findings and the cardioprotective role of the K_{ATP} channel opening. More studies are required to demonstrate this connection.

While speculating on the mechanism of isoflurane-induced channel opening, we cannot yet explain the underpinnings of isoflurane interaction with the channel. One possibility is that isoflurane modulates the intrinsic adenosine triphosphatase activity of SUR2A. Thus far, adenosine triphosphatase activity of SUR2A was only measured in

isolated purified NBDs, mainly NBD2. However, similar to the K_{ATP} channel openers, isoflurane may very well require interaction of NBD1 and NBD2 and/or other protein domains of SUR2A, and this must be addressed in future studies. Further, the effects of isoflurane and other general inhalation anesthetics on channel activity must be compared. This is particularly warranted because differences have been reported in the modulation of native K_{ATP} channels by isoflurane and halothane.[12]

In conclusion, results of our study support a direct and pHi-dependent interaction of isoflurane with the nucleotide binding domain NBD1 of SUR2A, the regulatory subunit of the cardiac sarcolemmal K_{ATP} channel. This interaction could contribute to the mechanism by which isoflurane facilitates opening of the K_{ATP} channel under conditions of moderate intracellular acidosis that occurs during early myocardial ischemia.

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