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Differential Effects of General Anesthetics on G Protein–coupled Inwardly Rectifying and Other Potassium Channels

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Background: **General anesthetics differentially affect various families of potassium channels, and some potassium channels are suggested to be potential targets for anesthetics and alcohols.**

Methods: **The voltage-gated (ERG1, ELK1, and KCNQ2/3) and inwardly rectifying (GIRK1/2, GIRK1/4, GIRK2, IRK1, and ROMK1) potassium channels were expressed in *Xenopus* oocytes. Effects of volatile agents [halothane, isoflurane, enflurane, F3 (1-chloro-1,2,2-trifluorocyclobutane), and the structurally related nonimmobilizer F6 (1,2-dichlorohexafluorocyclobutane)], as well as intravenous (pentobarbital, propofol, etomidate, alphaxalone, ketamine), and gaseous (nitrous oxide) anesthetics and alcohols (ethanol and hexanol) on channel function were studied using a two-electrode voltage clamp.**

Results: **ERG1, ELK1, and KCNQ2/3 channels were either inhibited slightly or unaffected by concentrations corresponding to twice the minimum alveolar concentrations or twice the anesthetic EC_{50} of volatile and intravenous anesthetics and alcohols. In contrast, G protein–coupled inwardly rectifying potassium (GIRK) channels were inhibited by volatile anesthetics but not by intravenous anesthetics. The neuronal-type GIRK1/2 channels were inhibited by 2 minimum alveolar concentrations of halothane or F3 by 45 and 81%, respectively, whereas the cardiac-type GIRK1/4 channels were inhibited only by F3. Conversely, IRK1 and ROMK1 channels were completely resistant to all anesthetics tested. Current responses of GIRK2 channels activated by μ-opioid receptors were also inhibited by halothane. Nitrous oxide (~0.6 atmosphere) slightly but selectively potentiated GIRK channels. Results of chimeric and multiple amino acid mutations suggest that the region containing the transmembrane domains, but not the pore-forming domain, may be involved in determining differences in anesthetic sensitivity between GIRK and IRK channels.**

Conclusions: **G protein–coupled inwardly rectifying potassium channels, especially those composed of GIRK2 subunits, were inhibited by clinical concentrations of volatile anesthetics. This action may be related to some side effects of these agents.**

POTASSIUM channels play a major role in reducing the excitability of tissues such as the nervous system and heart. In neurons and other excitable cells, these channels set the resting membrane potential, dissipate excitatory influences, and determine the duration and frequency of action potentials.[1] Based on similarity of their functional properties and amino acid sequences and on whether they will coassemble with others, potassium channel subunits are grouped into four families: voltage-gated, calcium-activated, inwardly rectifying, and tandem pore domain families.[1]

Because of their importance in regulating cellular excitability, potassium channels represent potential targets for anesthetic action. In contrast to studies showing that voltage-gated and calcium-activated potassium channels are inhibited by or insensitive to anesthetics,[1,2] some tandem pore domain potassium channels are activated by volatile anesthetics and are suggested to be potential anesthetic targets.[3–5] The function of cardiac adenosine triphosphate–sensitive potassium channels is enhanced by volatile anesthetics, whereas a biphasic effect of volatile anesthetics occurs with cardiac inward rectifier potassium channels.[1,6]

The *ERG1* (eag-related gene) channel is a voltage-gated potassium channel of the *ether-a-go-go* (EAG) family, which is expressed abundantly in the nervous system and heart.[7–9] Mutations in the *ERG1* gene have been shown to be associated with the LQT2 form of the human genetic heart disease long QT syndrome.[8,10] The *ELK1* (eag-like K^+ channel gene) channel is also a member of the EAG family, which is expressed in sympathetic ganglia.[11] The KCNQ2 and KCNQ3 channels are recently cloned voltage-gated potassium channels expressed specifically in the nervous system.[12,13] Mutations in *KCNQ2* or *KCNQ3* gene are associated with benign familial neonatal convulsions, an inherited epilepsy of newborns.[14] KCNQ2/3 and ERG1 channels are suggested to underlie the M current[13,15] that was originally identified as a time- and voltage-dependent potassium current susceptible to inhibition by muscarinic agonists in sympathetic neurons.[16] Previous studies reported that ethanol inhibits the M-like current in hippocampal neurons.[17,18]

Inwardly rectifying potassium channels are distantly related to voltage-gated potassium channels and are regulated by transmitters, hormones, and the cell's internal metabolic state.[19] One subtype of this family, G protein–coupled inwardly rectifying potassium (GIRK) channel, is activated by various G protein–coupled receptors such as m2-muscarinic, α_2-adrenergic, D_2-dopaminergic, δ-, κ-, and μ-opioid, and γ-aminobutyric acid type B receptors.[1] The GIRK1, GIRK2, and GIRK3 subunits are distributed widely as well as differently in most brain regions,[20–22] whereas the cardiac GIRK channels are

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considered to be heteromultimers composed of GIRK1 and GIRK4 subunits.[20,23] Previous studies show that muscarinic-activated potassium channels in atrial myocytes (GIRK1/4 channels) are affected by isoflurane and halothane.[24] Recently, GIRK channels were shown to be potentiated by ethanol[25,26] and to be important for ethanol actions such as analgesia.[26]

Because potassium channels as important regulators of neuronal excitability, we asked if they might be targets for volatile or intravenous anesthetics. In the current study, we evaluated effects of volatile, intravenous, and gaseous anesthetics and alcohols on three voltage-gated (ERG1, ELK1, and KCNQ2/3) and five inwardly rectifying (GIRK1/2, GIRK1/4, GIRK2, IRK1, and ROMK1) potassium channels expressed in *Xenopus* oocytes. Furthermore, we explored the protein regions critical for determining distinct sensitivity to volatile anesthetics between GIRK and IRK channels by examining chimeric channels and multiple amino acid mutations.

Materials and Methods

Construction of Chimeras and Amino Acid Mutants

To construct pBKSGIRK2-087-IRK1-154-GIRK2, we prepared the following four fragments: the 0.2-kilobase (kb) pair *Nco*I-*Sal*I (created at amino acid residues 87–88 of GIRK2 protein with silent mutations) fragment prepared by the polymerase chain reaction (PCR) from pBKSGIRK2[25]; the 0.2-kb *Sal*I-*Pvu*I (created at amino acid residues 142–144 of IRK1 with silent mutations) fragment prepared by PCR from pGEMIRK1[25]; the 1.1-kb *Pvu*I (created at amino acid residues 154–156 of GIRK2 with silent mutations)-*Not*I fragment prepared by PCR from pBKSGIRK2; and the 3.0-kb *Not*I-*Nco*I fragment from pBKSGIRK2. These fragments were ligated to yield pBKSGIRK2-087-IRK1-154-GIRK2 (the source of the N-terminal portion, followed by the amino acid residue number in which the chimeric joint is made, and followed by the source of the C-terminal portion; residue numbering is take from the GIRK2 protein[27]). To construct pBKSGIRK2-154-IRK1-204-GIRK2, the following four fragments were ligated: the 0.4-kb *Nco*I-*Pvu*I fragment prepared by PCR from pBKSGIRK2; the 0.2-kb *Pvu*I-*Spe*I (created at amino acid residues 192–194 of IRK1 with silent mutations) fragment prepared by PCR from pGEMIRK1; the 0.9-kb *Spe*I (created at amino acid residues 204–206 of GIRK2 with silent mutations)-*Not*I fragment prepared by PCR from pBKSGIRK2; and the 3.0-kb *Not*I-*Nco*I fragment from pBKSGIRK2. To construct pBKSGIRK2-087-IRK1, the following three fragments were ligated: the 0.3-kb *Nde*I (created at amino acid residue 1 of GIRK2)-*Sal*I fragment prepared by PCR from pBKSGIRK2; the 1.1-kb *Sal*I-*Not*I fragment from pGEMIRK1; and the 3.0-kb *Not*I-*Nde*I fragment prepared by PCR from pBKSGIRK2. To construct pBKSIRK1-061-GIRK2, the following three fragments were ligated: the 0.2-kb *Nde*I (created at amino acid residue 1 of IRK1)-*Sal*I fragment (composed of amino acid residues 1–49 from IRK1 followed by amino acid residues 61–87 from GIRK2) prepared by a two-step PCR[28] from pGEMIRK1 and pBKSGIRK2; the 1.3-kb *Sal*I-*Not*I fragment prepared by PCR from pBKSGIRK2; and the 3.0-kb *Not*I-*Nde*I fragment from pBKSGIRK2-087-IRK1. The 0.4-kb *Nde*I-*Pvu*I fragment prepared by PCR from pGEMIRK1, the 1.1-kb *Pvu*I-*Not*I fragment from pBKSGIRK2-087-IRK1-154-GIRK2, and the 3.0-kb *Not*I-*Nde*I fragment from pBKSGIRK2-087-IRK1 were ligated to yield pBKSIRK1-154-GIRK2. The amino acid residues 136–143, 152, 159–168, 169–178, 179–188, and 190–197 of pBKSGIRK2 were replaced by the corresponding residues of IRK1 to yield the pBKSGIRK2-MC1, MC2, MC3, MC4, MC5, and MC6, respectively, by using the QuikChange mutagenesis kit (Stratagene, La Jolla, CA) according to the two-stage PCR protocol.[29] All constructs were confirmed by DNA sequencing.

cRNA Preparation and Expression in Oocytes

The cDNA encoding the human ERG1 subunit[8] in pSP64 vector, cDNA encoding the rat ELK1 subunit[11] in pBluescript SK vector, cDNAs encoding the human KCNQ2 and rat KCNQ3 subunits[13] in pBluescriptII SK vector, and cDNA encoding the rat μ-opioid receptor[30] in pRc/CMV vector were used for cRNA synthesis. *In vitro* transcripts were prepared using the mRNA capping kit (Stratagene, La Jolla, CA). The cRNAs for GIRK1, GIRK2, GIRK4, IRK, GIRK2-IRK1 chimeras and multiple amino acid mutants, and ROMK1 potassium channels were synthesized as described previously.[25]

The use of experimental animals (frogs) was approved by the Animal Care and Use Committees of University of Texas. Isolation of *Xenopus laevis* oocytes and microinjection of the cRNA was performed as described previously.[25] Isolated oocytes were placed in modified Barth saline (MBS) containing 88 mM NaCl, 1 mM KCl, 2.4 mM $NaHCO_3$, 0.82 mM $MgSO_4$, 0.91 mM $CaCl_2$, 0.33 mM $Ca(NO_3)_2$, and 10 mM HEPES adjusted to pH 7.5. The cRNAs (5–30 ng/oocyte, in a 1:1 molar ratio for heteromeric channels) were injected into the cytoplasm of oocytes. The injected oocytes were singly placed in Corning cell wells (Corning Glass Works, Corning, NY) containing incubation medium (sterile MBS supplemented with 10 mg/l streptomycin, 10,000 U/l of penicillin, 50 mg/l of gentamicin, 90 mg/l theophylline, and 220 mg/l pyruvate) and incubated at 15–19°C. Two to 5 days after injection, oocytes were used in electrophysiologic recording.

Electrophysiologic Recording

Oocytes expressing the potassium channels were placed in a rectangular chamber (approximately 100-μl volume) and perfused (2 ml/min) with MBS. The animal poles of oocytes were impaled with two glass electrodes (0.5–10 MΩ) filled with 3 M KCl and voltage clamped at

−80 mV, unless stated otherwise, using a Warner Instruments model OC-725A (Hamden, CT) oocyte clamp. For voltage-gated potassium channels, current responses were evoked by a 2-s voltage step to +20 mV from a holding potential of −80 mV, with 10-s interpulse intervals. During the voltage step protocol, anesthetics were applied for 1 min, since a maximal effect of anesthetics occurred within 1 min. Data collection and analysis were performed with pClamp software (Axon Instruments, Foster City, CA). For the inwardly rectifying potassium channels, oocytes were first bathed in MBS, then switched to a high potassium (hK) solution containing 2 mM NaCl, 96 mM KCl, 1.5 mM $CaCl_2$, 1 mM $MgCl_2$, and 5 mM HEPES at pH 7.5 to elicit an inward current through basally active channels.[31,32] After establishing a stable response, anesthetics were applied in the hK solution for 1 min before changing back to the hK solution.

Solutions of anesthetics were prepared as described previously.[33] The anesthetic concentrations in the text represent the final bath concentrations quantified by gas chromatography as described previously.[33] For nitrous oxide, concentrations tested were the maximal concentrations that could be achieved in our perfusion system. Data were obtained from three to nine oocytes taken from at least two different frogs. All experiments were performed at room temperature (23°C).

Compounds

Halothane was obtained from Halocarbon Laboratories (River Edge, NJ); isoflurane was obtained from Anaquest Co. (Madison, WI); enflurane was obtained from Ohio Medical Products (Madison, WI); F3 (1-chloro-1,2,2-trifluorocyclobutane) and F6 (1,2-dichlorohexafluorocyclobutane) were obtained from Lancaster Synthesis, Inc. (Windham, NH); ethanol was obtained from Aaper Alcohol and Chemical Co. (Shelbyville, KY); 2,6-diisopropylphenol (propofol) was obtained from Aldrich Chemical Co. (Milwaukee, WI); etomidate was obtained from Janssen Pharmaceutica (Beerse, Belgium); alphaxalone was obtained from Research Biochemicals International (Natick, MA); nitrous oxide, xenon, and nitrogen were obtained from Airgas-Southwest, Inc. (San Antonio, TX); and 1-hexanol, pentobarbital sodium, ketamine hydrochloride, [D-Ala^2,*N*-Me-Phe^4,Gly^5-ol]enkephalin (DAMGO), and other reagents were purchased from Sigma Co. (St. Louis, MO). Propofol and alphaxalone were first dissolved in dimethyl sulfoxide and then diluted in MBS or hK solution. The highest final dimethyl sulfoxide concentration (0.01%) did not affect current responses of potassium channels tested.

Statistical Analysis

The results obtained were statistically analyzed by *t* tests or one-way analysis of variance followed by Scheffé multiple comparison tests. *P* values < 0.05 were considered significant. Data are represented as mean ± SD.

Results

Effects of Anesthetics on Voltage-gated Potassium Channels

The ERG1 channels were activated by a 2-s voltage step to +20 mV from a holding potential of −80 mV. A step back to the holding potential of −80 mV elicited a large tail current (fig. 1A). Effects of anesthetics on ERG1 channels were evaluated by measuring amplitudes of the tail currents during perfusion of the anesthetics. Enflurane at 1.2 mM (twice minimum alveolar concentration [MAC]) slightly inhibited the tail current of ERG1 channels (fig. 1A). Figure 1B shows effects of 2 MAC or twice the anesthetic EC_{50} of various anesthetics[2,34–36] on ERG1 channels. Volatile anesthetics (halothane, isoflurane, enflurane, and F3) only slightly (4–11%) inhibited the ERG1 channels. Ethanol (380 mM) inhibited ERG1 channels by 24%, whereas hexanol (1.1 mM) inhibited them by 6%. A nonimmobilizer F6 and intravenous anesthetics (pentobarbital, propofol, etomidate, alphaxalone, and ketamine) barely affected the ERG1 channels. The current responses of KCNQ2/3 channels were evoked by the same voltage step protocol, and the effects of anesthetics on the tail currents were evaluated (fig. 1C). The KCNQ2/3 channels were also relatively insensitive to the anesthetics tested. ELK1 channels were activated by a 3-s voltage step from a holding potential of −80 mV over the range −70 to +40 mV in 10-mV increments (fig. 1D). Enflurane (1.2 mM) produced little effect on the current responses. Figure 1E shows effects of 2 MAC of anesthetics on ELK1 channels. ELK1 channels were resistant to all anesthetics tested.

Effects of Anesthetics on Inwardly Rectifying Potassium Channels

Current responses of inwardly rectifying potassium channels were obtained by perfusion of a hK solution that elicits an inward current through basally active channels.[31,32] After establishing a stable response, effects of anesthetics were evaluated by application of the anesthetics in the hK solution. The currents of GIRK2 channels were markedly inhibited by 2 MAC of halothane (fig. 2A). Although currents of GIRK2 channels were inhibited immediately after application of volatile anesthetics, recovery from inhibition was slow and often incomplete. Conversely, currents of IRK1 channels were not affected by halothane. Application of anesthetics in MBS did not change the baseline currents in MBS for both GIRK2 and IRK1 channels. The effects of 2 MAC or twice the anesthetic EC_{50} of various anesthetics on GIRK1/2, GIRK1/4, and GIRK2 channels were examined (figs. 2B, 2C, and 2D). Halothane, isoflurane, and enflurane inhibited GIRK1/2 and GIRK2 channels, whereas they only slightly affected the GIRK1/4 channels. F3 prominently inhibited GIRK1/2, GIRK1/4, and GIRK2 channels, whereas a structurally related nonimmobilizer

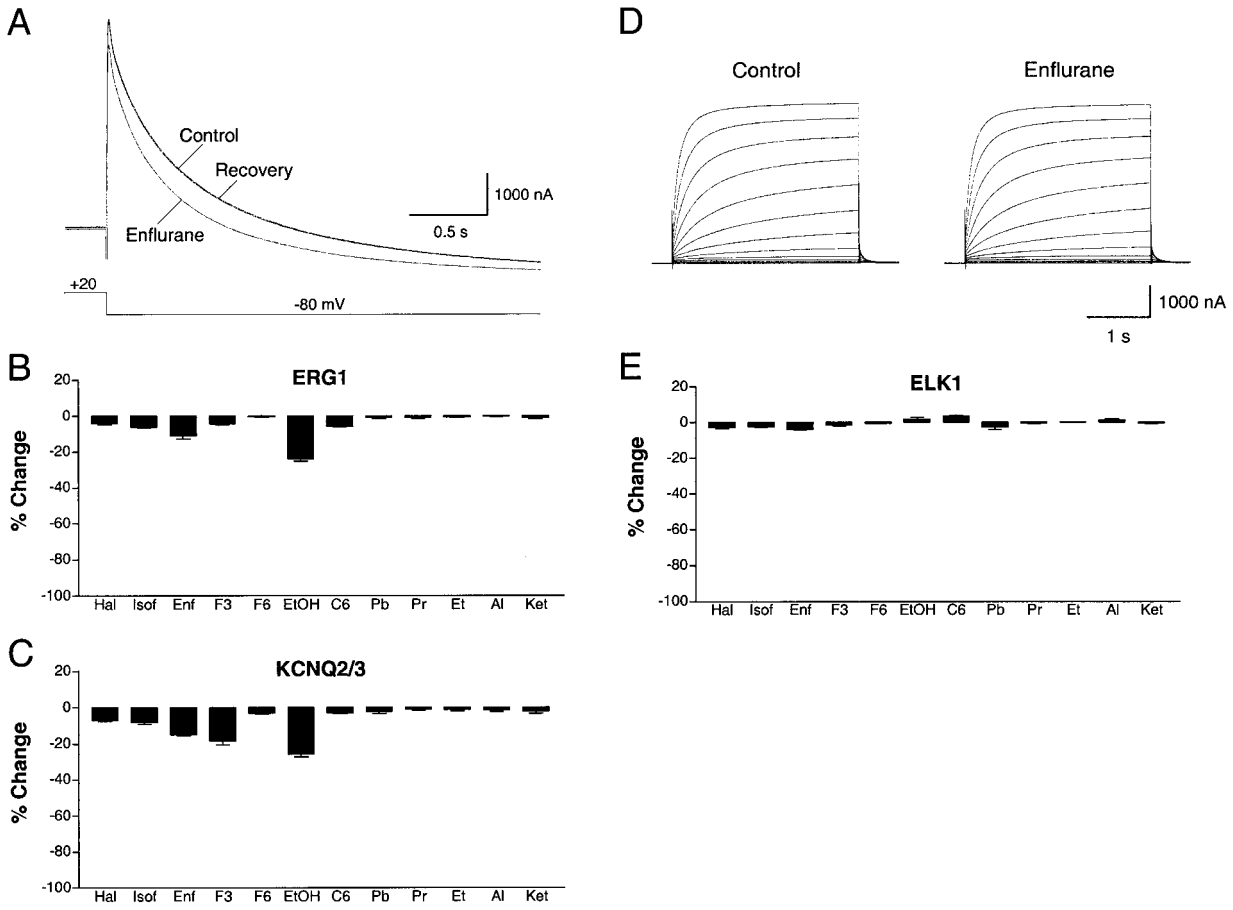

Fig. 1. Effects of anesthetics on voltage-gated potassium channels. (*A*) Representative tracings of the tail currents of ERG1 channels before, during, and after perfusion of 1.2 mM enflurane. Inward current is downward. The tail currents in response to a step back to the holding potential were evaluated for anesthetic effects. (*B*) Effects of anesthetics on ERG1 channels (n = 3–6). Anesthetics were applied at a concentration corresponding to 2 MAC, *i.e.*, 0.5 mM for halothane (Hal), 0.6 mM for isoflurane (Isof), 1.2 mM for enflurane (Enf), 1.6 mM for F3, 35.6 μM (predicted 2 MAC) for F6, 380 mM for ethanol (EtOH), 1.1 mM for hexanol (C6), 100 μM for pentobarbital (Pb), 2 μM for propofol (Pr), 10 μM for etomidate (Et), 10 μM for alphaxalone (Al), and 20 μM for ketamine (Ket).[2,34–36] (*C*) Effects of 2 MAC of anesthetics on the tail currents of KCNQ2/3 channels (n = 30-5). (*D*) Representative tracings of current responses of ELK1 channels to a 3-s voltage step from a holding potential of −80 mV over the range −70 to +40 mV in 10-mV increments with 3.5-s interpulse intervals before (*left*) and during (*right*) perfusion of 1.2 mM enflurane. Inward current is downward. (*E*) Effects of 2 MAC of anesthetics on ELK1 channels (n = 3–4).

F6 only slightly inhibited the GIRK channels. Ethanol markedly potentiated the currents of GIRK channels as reported previously,[25,26] whereas hexanol slightly inhibited GIRK channels. Not only ethanol, but also methanol and propanol, are reported to prominently potentiate the currents of GIRK channels.[25,26] All GIRK channels were insensitive to intravenous anesthetics. To assure that GIRK1/2 channels were actually expressed by heteromeric assembly of GIRK1 and GIRK2 subunits, the amount of GIRK2 subunit cRNA was reduced to one tenth of the original amount, and the current amplitude was compared between the GIRK1 (~10 ng of GIRK1 subunit cRNA/oocyte), GIRK1/2 (~10 and ~1 ng of GIRK1 and GIRK2 subunit cRNAs/oocyte, respectively), and GIRK2 (~1 ng of GIRK2 subunit cRNA/oocyte) channels. During these conditions, current responses of GIRK1/2 channels were very significantly larger than those of GIRK1 and GIRK2 channels, indicating heteromeric assembly for the GIRK1/2 channels, which is consistent with previous reports.[37] In contrast to GIRK channels, IRK1 and ROMK1 channels were completely resistant to volatile anesthetics, alcohols, and intravenous anesthetics (figs. 2E and 2F).

Nitrous Oxide and Potassium Channels

Effects of nitrous oxide on voltage-gated and inwardly rectifying potassium channels were examined (fig. 3). Nitrous oxide (0.58 atmosphere [atm]) did not affect ERG1, ELK1, and KCNQ2/3 channels. Conversely, nitrous oxide potentiated GIRK1/2, GIRK1/4, and GIRK2 channels by 8–9%. Perfusion of a hK solution bubbled with 100% nitrogen produced no measurable effects on GIRK1/4 and GIRK2 channels (% change of 1 ± 1% and 0 ± 2%, respectively; n = 4), indicating that the observed potentiation of GIRK channels by nitrous oxide is not caused by displacement of oxygen from the solutions. IRK1 and ROMK1 channels were insensitive to nitrous oxide.

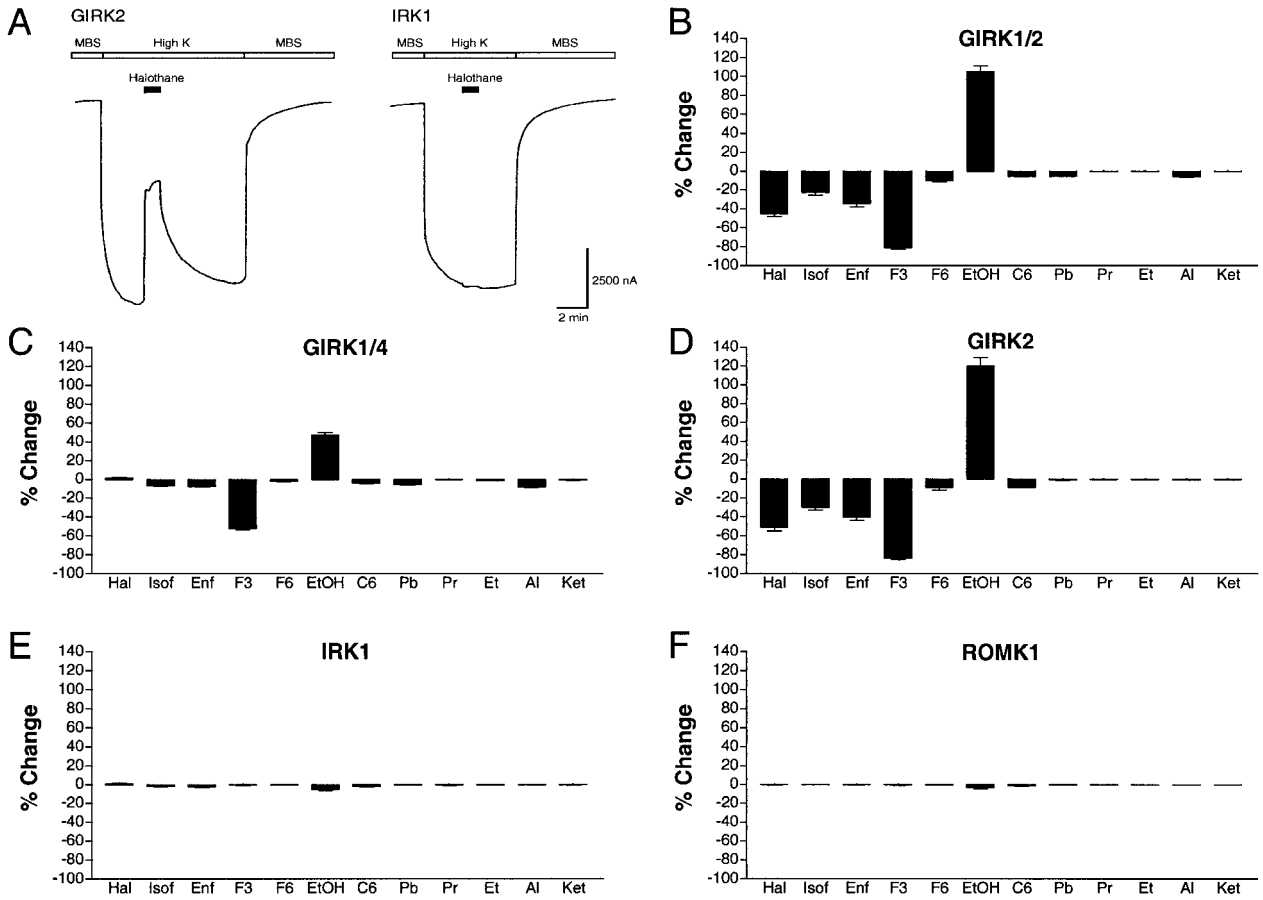

Fig. 2. Effects of anesthetics on inwardly rectifying potassium channels. (*A*) Representative current tracings showing the effects of 0.5 mM halothane on GIRK2 and IRK1 channels. Inward current is downward. The period of treatment with drugs is indicated by bars. (*B–F*) Effects of 2 MAC of anesthetics on GIRK1/2 (*B*), GIRK1/4 (*C*), GIRK2 (*D*), IRK1 (*E*), and ROMK1 (*F*) channels (n = 3–9). Hal = halothane; Isof = isoflurane; Enf = enflurane; EtOH = ethanol; C6 = hexanol; Pb = pentobarbital; Pr = propofol; Et = etomidate; Al = alphaxalone; Ket = ketamine.

Concentration–Response and Current–Voltage Relations of Inwardly Rectifying Channels for Anesthetic Inhibition

To examine differences in sensitivity to volatile anesthetics among inwardly rectifying potassium channels, we evaluated the dose–inhibition relations of GIRK1/2, GIRK1/4, GIRK2, and IRK1 channels for halothane and F3. GIRK1/2 and GIRK2 channels were inhibited by halothane in a dose-dependent manner (fig. 4A). Halothane at 0.25 mM (1 MAC) inhibited GIRK1/2 and GIRK2 channels by 31 ± 3% and 39 ± 4%, respectively (n = 4–7). Conversely, GIRK1/4 and IRK1 channels were not inhibited by halothane even at 5 MAC. F3 potently inhibited GIRK1/2, GIRK1/4, and GIRK2 channels in a dose-dependent manner, and GIRK1/4 channels were less sensitive to F3 than GIRK1/2 and GIRK2 channels (fig. 4B). F3 at 0.8 mM (1 MAC) inhibited GIRK1/2 and GIRK2 channels by 78 ± 5% and 78 ± 3%, respectively (n = 4–7), whereas it inhibited GIRK1/4 channels by 41 ± 3% (n = 6). In contrast, IRK1 channels were completely resistant to F3. We next examined the voltage dependence of inhibition of GIRK2 channels by halothane. The GIRK2 currents elicited by a hK solution showed inward rectification, a characteristic of GIRK channels (fig. 4C). Halothane inhibition of GIRK2 channels was not different among membrane potential tested (fig. 4C, $P > 0.92$). Similarly, inhibition of GIRK2 channels by 0.08 mM F3 was not different for the membrane potentials tested (data not shown). These results demonstrate that inhibition of GIRK channels by volatile anesthetics is not voltage-dependent.

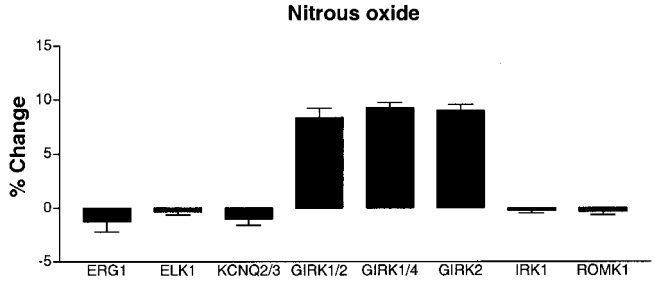

Fig. 3. Effects of nitrous oxide on voltage-gated and inwardly rectifying potassium channels. Nitrous oxide (0.58 atm) was applied for 1 min (n = 3–4).

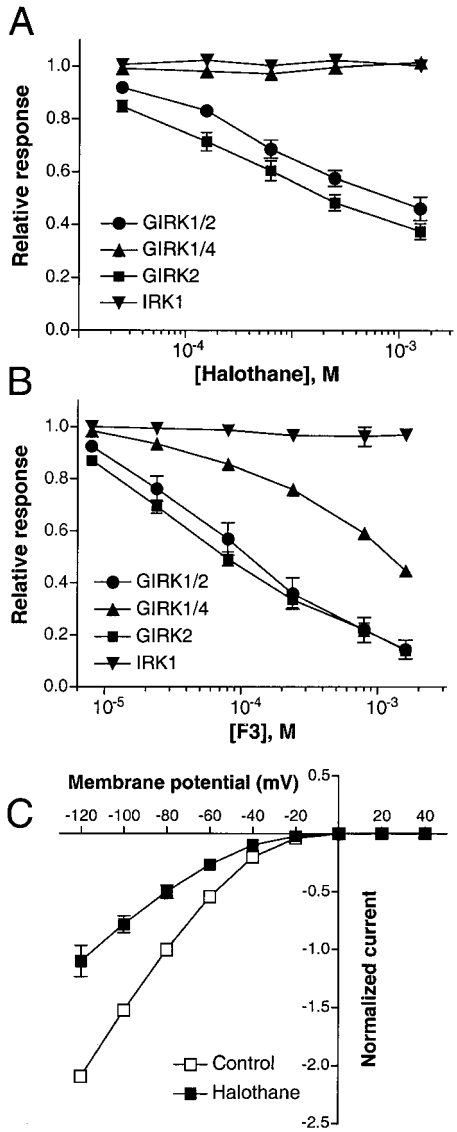

Fig. 4. Dose–inhibition and current–voltage relations of inwardly rectifying channels for inhibition by volatile anesthetics. (*A*) Dose–response relations of GIRK1/2, GIRK1/4, GIRK2, and IRK1 channels for halothane. Each point represents the mean ± SD of measurement of three to seven oocytes. Error bars not visible are smaller than symbols. (*B*) Dose–response relations of GIRK1/2, GIRK1/4, GIRK2, and IRK1 channels for F3 (n = 3–7). (*C*) Current–voltage relations of GIRK2 channels for halothane (0.5 mM) inhibition (n = 3). The measured currents were normalized to the control currents at −80 mV.

Effects of Anesthetics on G Protein–Coupled Inwardly Rectifying Potassium Channels Activated by Opioid Receptors

To examine whether anesthetics inhibit GIRK channels activated by G protein–coupled receptors, GIRK channels were coexpressed with μ-opioid receptors, and anesthetic effects on GIRK channel currents activated by μ-opioid receptors were evaluated. In oocytes expressing both GIRK2 channels and μ-opioid receptors, application of a selective μ-opioid receptor agonist DAMGO evoked an inward current in hK solution that is mediated by GIRK2 channels activated by μ-opioid receptors. Effects of halothane were evaluated by measuring current responses to DAMGO during incubation of halothane. Halothane inhibited the DAMGO-evoked currents of GIRK2 channels in a reversible manner (fig. 5A). Halothane (0.5 mM) inhibited DAMGO-evoked currents of GIRK2 channels by 53 ± 3% (fig. 5B). In contrast, 0.5 mM halothane did not affect DAMGO-evoked currents of GIRK1/4 channels. Propofol (2 μM) and ketamine (20 μM) barely affected DAMGO-evoked currents of GIRK2 channels, whereas nitrous oxide (0.58 atm) potentiated them by 12 ± 2% (n = 6; 100% nitrogen did not affect them [2 ± 2% inhibition, n = 5]). Therefore, effects of anesthetics on GIRK channels was similar between basally active and receptor-activated GIRK channels.

G Protein–Coupled Inwardly Rectifying Potassium and Inwardly Rectifying Potassium Chimeric Channels

Because GIRK2 channels were markedly inhibited by volatile anesthetics but IRK1 channels were completely insensitive to them, and both GIRK2 and IRK1 subunits form homomeric channels, we constructed chimeric subunits between GIRK2 and IRK1 subunits to identify the subunit region determining the distinct anesthetic

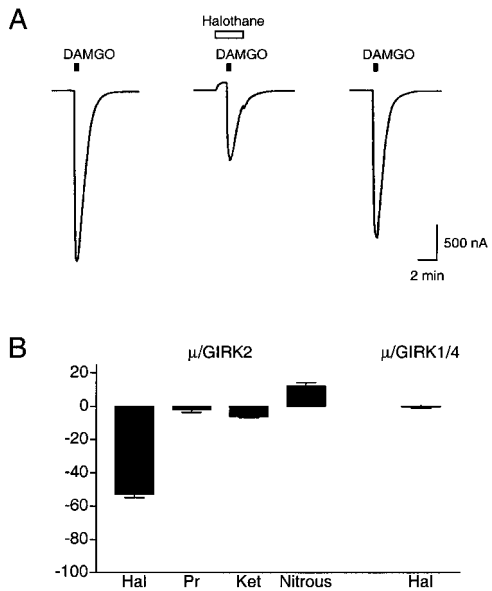

Fig. 5. Effects of anesthetics on GIRK channels activated by G protein–coupled receptors. (*A*) Representative tracing of current responses of GIRK2 channels activated by μ-opioid receptors in response to 100 nM DAMGO in a high-potassium solution before (*left*), during (*middle*), and after (*right*) incubation of 0.5 mM halothane. Inward current is downward. The period of treatment with drugs is indicated by bars. Exposure to halothane alone produced the outward current in a high-potassium solution as a result of inhibition of basally active GIRK2 channels. (*B*) Effects of 2 MAC of anesthetics or 0.58 atm nitrous oxide (Nitrous) on current responses of GIRK2 or GIRK1/4 channels activated by μ-opioid receptors in response to 100 nM DAMGO (n = 5–9).

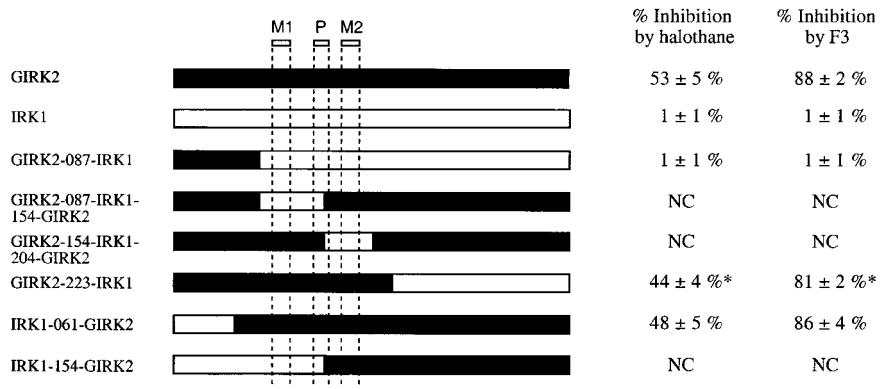

Fig. 6. Structure of GIRK-IRK chimeric subunits and effects of volatile anesthetics. The sequences of GIRK2 and IRK1 subunits are shown by filled and open columns, respectively. The putative transmembrane (M1 and M2) and pore-forming (P) domains are indicated by dotted lines. Interfaces between filled and open columns indicate chimera junction sites. The numbers in the right represent the percentage inhibition by 0.5 mM halothane and 1.6 mM F3 of current responses to a high-potassium solution. Values are mean ± SD of measurement of three to five oocytes. Current responses (μA) obtained were 4.4–12 for GIRK2, 6.2–9.8 for IRK1, 9.5–13 for GIRK2-087-IRK1, 1.3–7.9 for GIRK2-223-IRK1, and 1.5–7.2 for IRK1-061-GIRK2 channels. *Coexpressed with GIRK1 subunits. NC = no measurable current.

sensitivity. The GIRK2-087-IRK1 subunit was composed of the GIRK2 subunit sequence from the amino terminus to the junction site (residue 087 of GIRK2) and the IRK1 subunit sequence from the junction site to the carboxyl terminus (fig. 6). The currents of the GIRK2-087-IRK1 channels were resistant to both 0.5 mM halothane and 1.6 mM F3, indicating that anesthetic sensitivity is dependent on the carboxyl terminal side of the residue 087. The GIRK2-087-IRK1-154-GIRK2 and GIRK2-154-IRK1-204-GIRK2 channels produced little current response to an hK solution even after coexpression with GIRK1 subunits. The GIRK2-223-IRK1 channels[25] coexpressed with GIRK1 subunits were markedly inhibited by halothane and F3, indicating that the responsible site resides on the amino terminal side of the residue 223. The IRK1-061-GIRK2 channels were inhibited by volatile anesthetics, suggesting the involvement of the carboxyl terminal side of the residue 061. The IRK1-154-GIRK2 channels did not produce current responses even after coexpression with GIRK1 subunits. Data obtained from these chimeras suggest that anesthetic sensitivity is determined by the region of residues 087-223 that contains the transmembrane (M1 and M2) and pore-forming (P) domains.

GIRK2 Multiple Amino Acid Mutants

Because there is evidence of involvement of channel-lining residues in anesthetic inhibition of ligand-gated ion channels,[38,39] and the P and M2 domains correspond to the channel-lining regions of potassium channel pore,[40] we constructed multiple amino acid mutants around the P and M2 domains of GIRK2 channels and examined whether this region determines anesthetic sensitivity. The region encompassing the P and M2 domains were divided into six segments containing 8–15 amino acid residues (MC1–MC6; fig. 7A), and each segment of GIRK2 subunits was replaced by the corresponding segment of IRK1 subunits to create GIRK2-

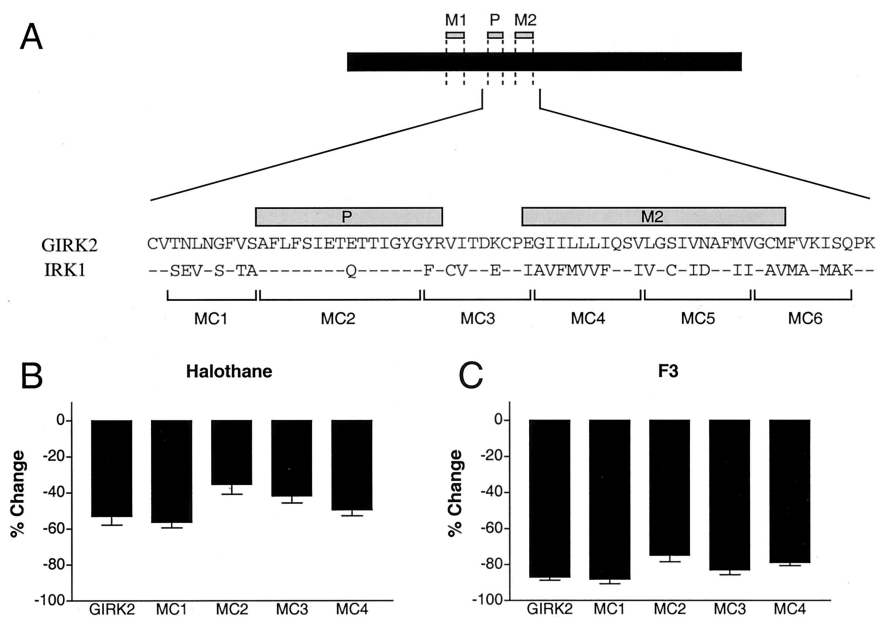

Fig. 7. Effects of multiple amino acid mutations of GIRK2 channels on sensitivity to volatile anesthetics. (*A*) Schematic representation of the region and amino acid sequences encompassing P and M2 domains of GIRK2 and IRK1 subunits. Bars in sequences represent residues identical to those above. Segments in which residues of GIRK2 channels were replaced by corresponding residues of IRK1 channels were shown (MC1–MC6). (*B*) Effects of multiple amino acid mutations of GIRK2 channels on inhibition by 0.5 mM halothane (n = 4–8). (*C*) Effects of multiple amino acid mutations of GIRK2 channels on inhibition by 1.6 mM F3 (n = 4–7). Current responses (μA) obtained were 3.5–7.5 for GIRK2-MC1, 3.1–8.7 for GIRK2-MC2, 6.6–12 for GIRK2-MC3, and 2.5–7.0 for GIRK2-MC4 channels. The GIRK2-MC5 and GIRK2-MC6 channels produced no measurable current, even after coexpression with GIRK1 subunits.

MC1, GIRK2-MC2, GIRK2-MC3, GIRK2-MC4, GIRK2-MC5, and GIRK2-MC6 mutant subunits. The GIRK2-MC1, GIRK2-MC2, GIRK2-MC3, and GIRK2-MC4 channels produced currents in response to an hK solution, but GIRK2-MC5 and GIRK2-MC6 channels produced little current even after coexpression with GIRK1 subunits. The GIRK2-MC1, GIRK2-MC2, GIRK2-MC3, and GIRK2-MC4 channels were inhibited by 0.5 mM halothane to the extents that were not significantly different from the wild-type GIRK2 channels (fig. 7B). F3 (1.6 mM) also inhibited GIRK2-MC1, GIRK2-MC2, GIRK2-MC3, and GIRK2-MC4 channels to the extents similar to the wild-type channels (fig. 7C). Thus, the regions MC1–MC4 may not be involved in determining differences in anesthetic sensitivity between GIRK and IRK channels.

Discussion

In the current investigation, we showed that voltage-gated potassium channels, ERG1, ELK1, and KCNQ2/3 channels, are insensitive to clinical concentrations of volatile, intravenous, and gaseous anesthetics. Thus, these voltage-gated potassium channels are not likely to be involved in anesthetic mechanisms. Conversely, among inwardly rectifying potassium channels, GIRK channels were sensitive to volatile anesthetics, ethanol, and nitrous oxide. GIRK channels were inhibited by clinical concentrations of volatile anesthetics, whereas nitrous oxide and ethanol selectively potentiated GIRK channels.

GIRK1/2 channels were markedly inhibited by halothane, isoflurane, enflurane, and F3. In contrast, GIRK1/4 channels were resistant to halothane, isoflurane, and enflurane but were inhibited by F3. The brain GIRK channels are considered to be heteromultimers composed of GIRK1 and GIRK2 subunits, whereas the cardiac GIRK channels are heteromultimers composed of GIRK1 and GIRK4 subunits.[20–23] Thus, our results suggest that brain GIRK channels, but not cardiac GIRK channels, are inhibited by volatile anesthetics used in clinical practice. In bullfrog atrial myocytes, however, it is reported that muscarinic-activated potassium current ($I_{K(ACh)}$), which is considered to be mediated by heteromultimers of GIRK1 and GIRK4 subunits,[23] is affected by isoflurane and halothane.[24] The activation rate and rapid desensitization of $I_{K(ACh)}$ elicited by acetylcholine were reduced by isoflurane (0.8 mM) and halothane (0.9 mM), whereas the magnitude of $I_{K(ACh)}$ was slowly reduced by isoflurane but rapidly augmented by halothane.[24] Thus, although effects of isoflurane and halothane on steady state amplitudes of GIRK1/4 channels were small in our studies, it is possible that channel gating kinetics of GIRK1/4 channels is somewhat altered by the volatile anesthetics.

In the central nervous system, activation of GIRK channels results in the outward flow of potassium ions, which causes a decrease in neuronal excitability.[1,32] Thus, inhibition of brain GIRK channels by volatile anesthetics would increase excitability and is not likely to be involved in mechanisms producing anesthesia. This suggestion is supported by the finding that equipotent concentrations (in terms of immobility) of volatile anesthetics inhibited GIRK channels by different degrees. For example, 2 MAC of isoflurane inhibited GIRK1/2 channels by only 22%, whereas 2 MAC of F3 inhibited them by 81%. Because F3 inhibited GIRK channels but structurally related nonimmobilizer F6 did not, inhibition of GIRK channels may be related to some excitatory, rather than inhibitory, behavioral actions induced by volatile anesthetics.

G protein–coupled inwardly rectifying potassium channels are activated by many G protein–coupled receptors.[1] The inhibition of GIRK2 channels by volatile anesthetics may imply that a part of function of G protein–coupled receptors that is mediated by GIRK channels in the nervous system is inhibited by volatile anesthetics. In the current study, we showed that current responses of μ-opioid receptors to DAMGO, mediated by GIRK2 channels, were inhibited by halothane. This inhibition of μ-opioid receptor function by halothane is likely caused by inhibition of GIRK channels, not opioid receptors, because the extent of inhibition by halothane was similar to that of basally active GIRK2 channels, and current responses of μ-opioid receptors coupled with GIRK1/4 channels were not inhibited. Although signal transduction pathways of opioid receptors include not only activation of GIRK channels but also alteration of Ca^{2+} channel function and levels of second messengers such as cyclic adenosine monophosphate,[41] it is possible that some actions of opioids might be inhibited by volatile anesthetics. Interestingly, halothane antagonizes suppressive effects of morphine on the heart rate increase to noxious stimulation, but not on the purposeful movement to noxious stimulation.[42]

G protein–coupled inwardly rectifying potassium channels were inhibited by volatile anesthetics, but homologous IRK channels were completely resistant to volatile anesthetics. Although GIRK channels are activated by binding of the G protein β and γ subunits,[43] inhibition of GIRK channels by volatile anesthetics may not be caused by inhibition of activity or binding of G proteins, but may be caused by inhibition of potassium channels themselves, because GIRK1/4 channels were insensitive to halothane, isoflurane, and enflurane at concentrations that markedly inhibited GIRK2 channels. Previous studies showed the involvement of cytoplasmic domains of various potassium channel subunits in actions of anesthetics[3] and alcohols.[25,44,45] As for ethanol actions on GIRK channels, the carboxyl terminus is identified to be critical for enhancement of GIRK channels by ethanol.[25] Our data from chimeric GIRK-IRK channels, however, suggest that cytoplasmic amino and carboxyl

termini are not involved in determining anesthetic sensitivity of GIRK channels. Thus, not only the direction of effects (inhibition or potentiation), but also mechanisms or sites of actions on GIRK channels may be different between volatile anesthetics and ethanol. The multiple amino acid mutations in the channel-lining region did not affect sensitivity of GIRK2 channels to halothane and F3. Because GIRK2-MC5 and GIRK2-MC6 channels did not produce measurable currents, we cannot exclude the possibility of involvement of this region in determining anesthetic sensitivity. However, our results suggest that the region around the P domain may not be important for determining differences in anesthetic sensitivity between GIRK and IRK channels.

Previous studies reported that, in hippocampal neurons, ethanol in low concentrations (22–44 mM) inhibits the M current.[17,18] This current was originally identified as a time- and voltage-dependent potassium current susceptible to inhibition by muscarinic agonists in sympathetic neurons.[16] Analogous currents (M-like currents) have subsequently been identified in a variety of other neuronal and nonneuronal cells, and the M-like current plays a prominent role in inhibiting the excitability of the cell. Recently, the M current was proposed to be composed of a heteromeric assembly of KCNQ2 and KCNQ3 subunits.[13] Furthermore, two components of the M-like current in neuronal cells are reported to be mediated by two distinct molecular species of potassium channel: KCNQ2/3 and ERG1 channels.[15] In the current study, low concentrations of ethanol (10–50 mM) did not affect KCNQ2/3 and ERG1 channels (data not shown), and even high concentrations (380 mM) of ethanol only slightly inhibited KCNQ2/3 and ERG1 channels. Therefore, ethanol inhibition of the M current in hippocampal neurons may not be caused by direct inhibition of the potassium channels, but may involve other target components.

In conclusion, we determined anesthetic sensitivity of various voltage-gated and inwardly rectifying potassium channels. Among the potassium channels tested, GIRK channels, especially those composed of GIRK2 subunits, were markedly inhibited by clinical concentrations of volatile anesthetics. These results suggest that function of G protein–coupled receptors, which is mediated by GIRK channels in the nervous system, may be inhibited by volatile anesthetics. Although inhibition of GIRK channel function by anesthetics is unlikely to account for the anesthetic actions, it may contribute to some excitatory side effects. Results from chimeras and multiple amino acid mutants between GIRK2 and IRK1 subunits suggest that mechanisms or sites of actions on GIRK channels are different between volatile anesthetics and ethanol, and that the region containing the transmembrane domains, but not the P domains, may be involved in determining differences in anesthetic sensitivity between GIRK and IRK channels.

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References

1. Yost CS: Potassium channels: Basic aspects, functional roles, and medical significance. ANESTHESIOLOGY 1999; 90:1186–203
2. Franks NP, Lieb WR: Molecular and cellular mechanisms of general anaesthesia. Nature 1994; 367:607–14
3. Patel AJ, Honore E, Lesage F, Fink M, Romey G, Lazdunski M: Inhalational anesthetics activate two-pore-domain background K^+ channels. Nature Neurosci 1999; 2:422–6
4. Gray AT, Zhao BB, Kindler CH, Winegar BD, Mazurek MJ, Xu J, Chavez RA, Forsayeth JR, Yost CS: Volatile anesthetics activate the human tandem pore domain baseline K^+ channel KCNK5. ANESTHESIOLOGY 2000; 92:1722–30
5. Sirois JE, Lei Q, Talley EM, Lynch III C, Bayliss DA: The TASK-1 two-pore domain K^+ channel is a molecular substrate for neuronal effects of inhalation anesthetics. J Neurosci 2000; 20:6347–54
6. Stadnicka A, Bosnjak ZJ, Kampine JP, Kwok WM: Modulation of cardiac inward rectifier K^+ current by halothane and isoflurane. Anesth Analg 2000; 90:824–33
7. Warmke JW, Ganetzky B: A family of potassium channel genes related to *eag* in *Drosophila* and mammals. Proc Natl Acad Sci U S A 1994; 91:3438–42
8. Sanguinetti MC, Jiang C, Curran ME, Keating MT: A mechanistic link between an inherited and an acquired cardiac arrhythmia: *HERG* encodes the I_{Kr} potassium channel. Cell 1995; 81:299–307
9. Shi W, Wymore RS, Wang HS, Pan Z, Cohen IS, McKinnon D, Dixon JE: Identification of two nervous system-specific members of the *erg* potassium channel gene family. J Neurosci 1997; 17:9423–32
10. Curran ME, Splawski I, Timothy KW, Vincent GM, Green ED, Keating MT: A molecular basis for cardiac arrhythmia: *HERG* mutations cause long QT syndrome. Cell 1995; 80:795–803
11. Shi W, Wang HS, Pan Z, Wymore RS, Cohen IS, McKinnon D, Dixon JE: Cloning of a mammalian *elk* potassium channel gene and EAG mRNA distribution in rat sympathetic ganglia. J Physiol (Lond) 1998; 511:675–82
12. Schroeder BC, Kubisch C, Stein V, Jentsch TJ: Moderate loss of function of cyclic-AMP-modulated KCNQ2/KCNQ3 K^+ channels causes epilepsy. Nature 1998; 396:687–90
13. Wang HS, Pan Z, Shi W, Brown BS, Wymore RS, Cohen IS, Dixon JE, McKinnon D: KCNQ2 and KCNQ3 potassium channel subunits: Molecular correlates of the M-channel. Science 1998; 282:1890–3
14. Biervert C, Schroeder BC, Kubisch C, Berkovic SF, Propping P, Jentsch TJ, Steinlein OK: A potassium channel mutation in neonatal human epilepsy. Science 1998; 279:403–6
15. Selyanko AA, Hadley JK, Wood IC, Abogadie FC, Delmas P, Buckley NJ, London B, Brown DA: Two types of K^+ channel subunit, erg1 and KCNQ2/3, contribute to the M-like current in a mammalian neuronal cell. J Neurosci 1999; 19:7742–56
16. Brown DA, Adams PR: Muscarinic suppression of a novel voltage-sensitive K^+ current in a vertebrate neurone. Nature 1980; 283:673–6
17. Moore SD, Madamba SG, Siggins GR: Ethanol diminishes a voltage-dependent K^+ current, the M-current, in CA1 hippocampal pyramidal neurons in vitro. Brain Res 1990; 516:222–8
18. Madamba SG, Hsu M, Schweitzer P, Siggins GR: Ethanol enhances muscarinic cholinergic neurotransmission in rat hippocampus in vitro. Brain Res 1995; 685:21–32
19. Jan LY, Jan YN: Cloned potassium channels from eukaryotes and prokaryotes. Annu Rev Neurosci 1997; 20:91–123
20. Lesage F, Guillemare E, Fink M, Duprat F, Heurteaux C, Fosset M, Romey G, Barhanin J, Lazdunski M: Molecular properties of neuronal G-protein-activated inwardly rectifying K^+ channels. J Biol Chem 1995; 270:28660–7
21. Kobayashi T, Ikeda K, Ichikawa T, Abe S, Togashi S, Kumanishi T: Molecular cloning of a mouse G-protein-activated K^+ channel (mGIRK1) and distinct distributions of three GIRK (GIRK1, 2 and 3) mRNAs in mouse brain. Biochem Biophys Res Commun 1995; 208:1166–73
22. Karschin C, Dissmann E, Stuhmer W, Karschin A: IRK(1-3) and GIRK(1-4) inwardly rectifying K^+ channel mRNAs are differentially expressed in the adult rat brain. J Neurosci 1996; 16:3559–70
23. Krapivinsky G, Gordon EA, Wickman K, Velimirovic B, Krapivinsky L, Clapham DE: The G-protein-gated atrial K^+ channel I_{KACh} is a heteromultimer of two inwardly rectifying K^+-channel proteins. Nature 1995; 374:135–41
24. Magyar J, Szabo G: Effects of volatile anesthetics on the G protein-regulated muscarinic potassium channel. Mol Pharmacol 1996; 50:1520–8
25. Lewohl JM, Wilson WR, Mayfield RD, Brozowski SJ, Morrisett RA, Harris RA: G-protein-coupled inwardly rectifying potassium channels are targets of alcohol action. Nature Neurosci 1999; 2:1084–90
26. Kobayashi T, Ikeda K, Kojima H, Niki H, Yano R, Yoshioka T, Kumanishi T: Ethanol opens G-protein-activated inwardly rectifying K^+ channels. Nature Neurosci 1999; 2:1091–7

27. Lesage F, Duprat F, Fink M, Guillemare E, Coppola T, Lazdunski M, Hugnot JP: Cloning provides evidence for a family of inward rectifier and G-protein coupled K^+ channels in the brain. FEBS Lett 1994; 353:37–42

28. Ho SN, Hunt HD, Horton RM, Pullen JK, Pease LR: Site-directed mutagenesis by overlap extension using the polymerase chain reaction. Gene 1989; 77:51–9

29. Wang W, Malcolm BA: Two-stage PCR protocol allowing introduction of multiple mutations, deletions and insertions using QuikChange site-directed mutagenesis. Biotechniques 1999; 26:680–2

30. Chen Y, Mestek A, Liu J, Hurley JA, Yu L: Molecular cloning and functional expression of a m-opioid receptor from rat brain. Mol Pharmacol 1993; 44:8–12

31. Kovoor A, Henry DJ, Chavkin C: Agonist-induced desensitization of the mu opioid receptor-coupled potassium channel (GIRK1). J Biol Chem 1995; 270: 589–95

32. Dascal N: Signalling via the G protein-activated K^+ channels. Cell Signal 1997; 9:551–73

33. Yamakura T, Harris RA: Effects of gaseous anesthetics nitrous oxide and xenon on ligand-gated ion channels: Comparison with isoflurane and ethanol. ANESTHESIOLOGY 2000; 93:1095–101

34. Mihic SJ, McQuilkin SJ, Eger EI II, Ionescu P, Harris RA: Potentiation of g-aminobutyric acid type A receptor-mediated chloride currents by novel halogenated compounds correlates with their abilities to induce general anesthesia. Mol Pharmacol 1994; 46:851–7

35. Alifimoff JK, Firestone LL, Miller KW: Anaesthetic potencies of primary alkanols: Implications for the molecular dimensions of the anaesthetic site. Br J Pharmacol 1989; 96:9–16

36. Krasowski MD, Harrison NL: General anaesthetic actions on ligand-gated ion channels. Cell Mol Life Sci 1999; 55:1278–303

37. Duprat F, Lesage F, Guillemare E, Fink M, Hugnot JP, Bigay J, Lazdunski M, Romey G, Barhanin J: Heterologous multimeric assembly is essential for K^+ channel activity of neuronal and cardiac G-protein-activated inward rectifiers. Biochem Biophys Res Commun 1995; 212:657–63

38. Forman SA, Miller KW, Yellen G: A discrete site for general anesthetics on a postsynaptic receptor. Mol Pharmacol 1995; 48:574–81

39. Pratt MB, Husain SS, Miller KW, Cohen JB: Identification of sites of incorporation in the nicotinic acetylcholine receptor of a photoactivatible general anesthetic. J Biol Chem 2000; 275:29441–51

40. Doyle DA, Morais Cabral J, Pfuetzner RA, Kuo A, Gulbis JM, Cohen SL, Chait BT, MacKinnon R: The structure of the potassium channel: Molecular basis of K^+ conduction and selectivity. Science 1998; 280:69–77

41. Loh HH, Smith AP: Molecular characterization of opioid receptors. Annu Rev Pharmacol Toxicol 1990; 30:123–47

42. Kissin I, Kerr CR, Smith LR: Morphine-halothane interaction in rats. ANESTHESIOLOGY 1984; 60:553–61

43. Reuveny E, Slesinger PA, Inglese J, Morales JM, Iniguez-Lluhi JA, Lefkowitz RJ, Bourne HR, Jan YN, Jan LY: Activation of the cloned muscarinic potassium channel by G protein bg subunits. Nature 1994; 370:143–6

44. Covarrubias M, Vyas TB, Escobar L, Wei A: Alcohols inhibit a cloned potassium channel at a discrete saturable site: Insights into the molecular basis of general anesthesia. J Biol Chem 1995; 270:19408–16

45. Harris T, Shahidullah M, Ellingson JS, Covarrubias M: General anesthetic action at an internal protein site involving the S4-S5 cytoplasmic loop of a neuronal K^+ channel. J Biol Chem 2000; 275:4928–36