0026-895X/01/6002-282–289$3.00
MOLECULAR PHARMACOLOGY
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Mol Pharmacol 60:282–289, 2001

Vol. 60, No. 2
792/918652
Printed in U.S.A.

G Protein-Gated Inwardly Rectifying Potassium Channels Are Targets for Volatile Anesthetics

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Received January 2, 2001; accepted April 25, 2001

This paper is available online at http://molpharm.aspetjournals.org

ABSTRACT

G protein-gated inwardly rectifying potassium channels (GIRKs) are a family of homo- and hetero-oligomeric K^+ channels composed of different subunits (GIRK1 to 4 in mammals). GIRK4 and GIRK1 are found mainly in the atrium, whereas neuronal cells predominantly express the GIRK1, GIRK2, and GIRK3 isoforms. When activated, GIRK channels slow the firing rate of atrial myocytes and neuronal cells. Because of their key role in controlling excitability, we investigated the influence of a prototypic anesthetic, halothane, on GIRK channels of different subunit composition expressed in *Xenopus laevis* oocytes. Halothane enhanced background currents through hetero-oligomeric GIRK1/GIRK4 and homo-oligomeric $GIRK1^{F137S}$ channels but not through homo-oligomeric GIRK2 channels. This activation of basal current did not depend on the presence of coexpressed G protein-coupled receptors but instead required the presence of $G_{\beta/\gamma}$. In contrast to basal GIRK currents, the agonist-induced GIRK current (via coexpressed m_2 muscarinic receptors) was inhibited by halothane. For GIRK1/GIRK4 and $GIRK1^{F137S}$ channels this inhibition was most pronounced at low concentrations of the anesthetic (0.1–0.3 mM) and occurred also when channels had been activated by guanosine-5′-*O*-(3-thio)triphosphate. This inhibition, however, was overridden by high concentrations of halothane (0.9 mM) and augmentation of the agonist-induced current was observed. This increase in agonist-induced current was never seen with GIRK2 homo-oligomeric channels. Agonist-induced currents mediated by GIRK2 channels were always inhibited by halothane with an IC_{50} value of approximately 60 μM. These data suggest a direct interaction of halothane with GIRK channels.

The mechanism of action of general anesthetics is still poorly understood. It is likely, however, that anesthetics change synaptic transmission in the CNS by altering electric excitability of neurons (Kress and Weigl, 1998). On the molecular level, ion channels have been shown to be selective targets for different anesthetics (Mihic et al., 1997), but there are numerous other potential targets, including receptors and G proteins in excitable cells that may contribute to anesthetic action. Hyperpolarization of central nervous system neurons has been attributed to activation of γ-aminobutyric acid A receptor Cl^- channels. In addition, during the last years, K^+ channels, which are determinants for the resting membrane potential, have been found to be important molecular targets for anesthetics (Franks and Lieb, 1988). Trek-1 and TASK, both of which are members of the TOK channel family, were found to be activated by various volatile anesthetics (Patel et al., 1999). Another family of potential targets for anesthetic action in the CNS are GIRK channels, which have been found to be activated by alcohols (Kobayashi et al., 1999; Lewohl et al., 1999). GIRK channels are a family of inwardly rectifying K^+ channels, of which five subunits have been identified so far and designated GIRK1–5 [Kir3.1–3.5; (Dascal, 1997)]. There is strong evidence that GIRK channels are homo- or heteromeric constructs (Spauschus et al., 1996; Corey et al., 1998). The mammalian GIRK1–4 subunits are found differentially distributed in brain and other excitable tissues with virtually all four GIRK isoforms being expressed in the brain and GIRK1 and GIRK4 in cardiac tissue (Wickman et al., 2000). The key event in GIRK activation is the association of $G_{\beta\gamma}$ subunits to intracellular portions of the channel protein. $G_{\beta\gamma}$ is released from heterotrimeric, inactive $G_{\alpha\beta\gamma}$ subunit complexes, which in turn had been activated by agonist binding to a G protein-coupled receptor. Activated GIRK channels drive the membrane potential toward E_{K^+} and thus counteract membrane excitability. Thereby, they slow heart rate in the atrium by acetylcholine, for example. The analgesic effect of opioids and the suppression of firing (Andrade et al., 1986) in the CNS is also believed to be mediated by activation of GIRK channels. Hence, a possible molecular mechanism involved in the an-

This work was supported by research Grants 8266 and 7716 from the OeNB Jubiläumsfonds to L.G.W. and W.S., respectively. W.S. is supported by the Austrian Science Foundation (SFB708, P13724-GEN).

ABBREVIATIONS: CNS, central nervous system; GIRK, G protein-gated inwardly rectifying potassium channel; C-βARK, C-terminal region of β-adrenergic receptor kinase; GTPγS, guanosine-5′-*O*-(3-thio)triphosphate; HK, high potassium

esthetic state could be activation of GIRK channels in the CNS by anesthetics.

We have investigated the action of halothane, a prototypic volatile anesthetic, on G protein-activated potassium channels of different subunit composition in the *Xenopus laevis* oocyte expression system. Halothane is able to exert activatory and inhibitory effects, depending on concentration and on subunit composition of the channels, suggesting that such interactions may play a role in general anesthesia.

Materials and Methods

Adult female *Xenopus laevis* frogs were anesthetized by placing the frog in 0.15% tricaine methanesulfonate, pH 7.4. The effectiveness of narcosis was checked by pinching the frog with forceps. When narcosis was complete, the frog was posed on ice and a lobe from the ovary removed via an incision (~5 mm) that was sewed afterward with surgical silk. Oocytes were prepared as described previously (Dascal and Lotan, 1992) and 50 nl of cRNA solutions were injected at concentrations yielding optimal current levels for two-electrode voltage-clamp experiments: 30 ng/μl m_2 receptor, 10 ng/μl μ-opioid receptor, 0.3 ng/μl GIRK1, 0.3 ng/μl $GIRK1^{F137S}$, 3 ng/μl GIRK4, 30 ng/μl GIRK2, and 30 ng/μl c-βARK. Endogenous GIRK5 was eliminated by coinjection of 20 ng/μl antisense oligonucleotide (KHA2; 5′-CTGAGGACTTGGTGCCATTCT-3′) together with the cRNAs (Hedin et al., 1996). Plasmids were isolated from bacteria and linearized using standard procedures (Sambrook et al., 1989). cRNA was synthesized as described (Dascal and Lotan, 1992). The following plasmid vectors were used: m_2 receptor (Lim et al., 1995), GIRK1, GIRK4 (Silverman et al., 1996), $GIRK1^{F137S}$ (Vivaudou et al., 1997), c-βARK (Jing et al., 1999), and μ-opioid receptor (Chen and Yu, 1994).

Incubation of oocytes was performed at 19 to 21°C for 4 to 9 days in NDE (96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, 5 mM HEPES, 100 U/ml penicillin, 100 μg/ml streptomycin, and 2.5 mM pyruvate, adjusted with NaOH to pH 7.4). For electrophysiological recordings, oocytes were placed in a recording chamber that allowed superfusion at 19 to 21°C. A virtually complete exchange of bath solution could be reached within 4 s, as judged by changes in offset potentials. Oocytes were constantly rinsed during experiments with ND96 solution (96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 5 mM HEPES, adjusted with NaOH to pH 7.4) or otherwise as indicated. For halothane-containing solutions, a gas-tight superfusion system was used that was made of glass syringes and Teflon tubings to prevent evaporation of the anesthetic. Halothane solutions were prepared from a saturated stock solution (17.8 mM) by dilution to the required working concentrations. The actual concentration of the applied halothane solution was analyzed by gas chromatography using an HP 5890GC (Hewlett Packard, Palo Alto, CA) device. In mock experiments, samples for the gas chromatography analysis were taken from the outlet of the perfusion system and were found to contain 0.86 ± 0.05 mM for 1 mM halothane, 0.321 ± 0.02 mM for 0.3 mM, and 0.112 ± 0.01 mM for 0.1 mM halothane, respectively; in figures and text, these concentrations are referred to as 0.9 mM, 0.3 mM, and 0.1 mM. The method of preparing the solutions did not allow concentrations of more than 1 mM halothane because of excessive loss of the anesthetic.

Currents were recorded with the two-electrode voltage-clamp technique using glass electrodes filled with 1 M KCl (resistance of 0.8 to 1.5 MΩ) and a Geneclamp 500 or Axoclamp 2B amplifier (Axon Instruments, Foster City, CA). Membrane potential was clamped constantly to −70 mV. Current was measured first in regular ND96 solution and then in high-K^+ (HK) extracellular medium (2 mM NaCl, 96 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 5 mM HEPES, adjusted with KOH to pH 7.4). I_{ACh} was induced by superfusion with acetylcholine. In some experiments, the current-voltage relationship of currents was assessed by applying a sawtooth-shaped command potential from −170 to 45 mV within 1 s. The resulting current traces were digitized at 50 Hz using a TL-1–125 interface (Axon Instruments). Analysis of current recordings was performed using Fetchan 6.0 (Axon Instruments).

For inhibition of G proteins, 30 nl of a solution containing 7 ng/μl of the A-protomer of pertussis toxin (Calbiochem, San Diego, CA) was injected 24 to 28 h before the respective experiments (Sharon et al., 1997). Results are given as mean ± S.E. Test for statistical significance was performed with a one-way analysis of variance and the post hoc Scheffé test.

Results

To test whether GIRK channels of different subunit composition are possible targets for the volatile anesthetic halothane, we first expressed GIRK1/GIRK4 subunits together with the muscarinic acetylcholine (m_2) receptor in *X. laevis* oocytes. When cells were rinsed with HK solution oocytes expressing GIRK1/GIRK4 channels produced inward currents (I_{HK}) of 169 ± 15.4 nA (n = 32) at a holding potential of −70 mV. The application of 10 μM acetylcholine evoked additional inward currents (I_{ACh}) of 997 ± 98 nA (n = 31).

When halothane was applied to oocytes expressing GIRK1/GIRK4 channels, a slowly developing inward current was observed in a dose-dependent manner (Fig. 1A). Under the influence of 0.9 mM halothane, the basal GIRK current was enhanced by 90 ± 13% (n = 23). Lower concentrations of halothane were less effective, 0.3 mM halothane showed an increase of 38 ± 6% (n = 11) and 0.1 mM halothane had no effect (n = 10). Uninjected oocytes never showed activation of inward currents because of halothane. On the contrary, uninjected oocytes rather showed a decrease of basal I_{HK} (Fig. 1B).

The time course of current activation caused by halothane application was slow and could generally be best fit with a two-exponential equation. The rise time was determined to be 7.3 ± 1.8 s and 58 ± 22 s for the fast component and the slow component, respectively (n = 6). The slow current component contributed to about 68 ± 7.3% to the total halothane induced current. This slow activation clearly shows that the current did not reach a plateau within 2 min of anesthetic application. Furthermore, the slow washout of the effect seen in Fig. 1A may be caused by the prolonged halothane application and enrichment of the anesthetic in the lipophilic yolk of the oocyte. Thus, to minimize the enrichment of halothane, the application was restricted to 1 min and the degree of current activation was determined at the end of the halothane application.

With ND96 as the extracellular solution and hence the K^+ reversal potential near the holding potential of −70 mV, halothane did not induce a current (n = 4; data not shown), indicating that the halothane induced current was indeed a potassium current probably mediated by GIRK channels. Because GIRK channels, in contrast to endogenous K^+-channels, are blocked by low concentrations of Ba^{2+} ions, we tested the effect of halothane in the presence of 300 μM Ba^{2+}. Halothane was not able to induce an inward current in GIRK1/GIRK4- or $GIRK1^{F137S}$-expressing oocytes, although prominent GIRK-mediated currents were observed in the same cells in the absence of Ba^{2+} (see Fig. 1C). Moreover, we examined the voltage dependence of the current by applying voltage ramps from −170 to +45 mV. The current showed typical inward rectification and reversed at −25 ± 1 mV in

the presence of HK. The application of halothane increased the inward component of the current without affecting the outward component or the reversal potential. This argues for a specific activation of GIRK currents by halothane (Fig. 2D).

A halothane concentration of 0.9 mM was also able to enhance the acetylcholine-induced current through GIRK1/GIRK4 channels (Fig. 2A). This augmentation of the current retained inward rectification and was additive compared with the effect of halothane on the background current, I_{HK} (compare Fig. 1B). Unexpectedly, when low concentrations of halothane were applied, the agonist-induced currents were inhibited. A concentration of 0.1 mM halothane was more potent than 0.3 mM halothane (Fig. 2, B and C). A concentration of 0.1 mM halothane reduced the acetylcholine induced current by 26 ± 3% (n = 11) whereas 0.3 mM halothane caused a reduction of only 16 ± 8% (n = 4) of the current. When these low concentrations of halothane were applied to acetylcholine-stimulated cells, occasionally the inhibition of the agonist-induced current was most pronounced immediately after the addition of the anesthetic. In these cases, the degree of inhibition of the current was evaluated at the minimum of the current reached after addition of halothane to the bath. Another subunit combination, GIRK1/GIRK2, was tested for possible effects of halothane. In contrast to GIRK1/GIRK4 channels, on the average, halothane slightly reduced basal I_{HK} (data not shown). Accordingly, acetylcholine-induced GIRK1/GIRK2 currents were also inhibited more effectively by halothane than by GIRK1/GIRK4 channels (Fig. 3B). A concentration of 0.1 mM halothane reduced I_{ACh} by 64 ± 18% (n = 5), 0.3 mM halothane only by 54 ± 4% (n = 3), and 0.9 mM halothane by 40 ± 6% (n = 8). Compared with GIRK1/GIRK4 channels, GIRK1/GIRK2 channels showed a higher sensitivity against the inhibiting action of halothane. Surprisingly, high concentrations of the anesthetic were less effective in inhibiting the current compared with lower ones (Fig. 3B).

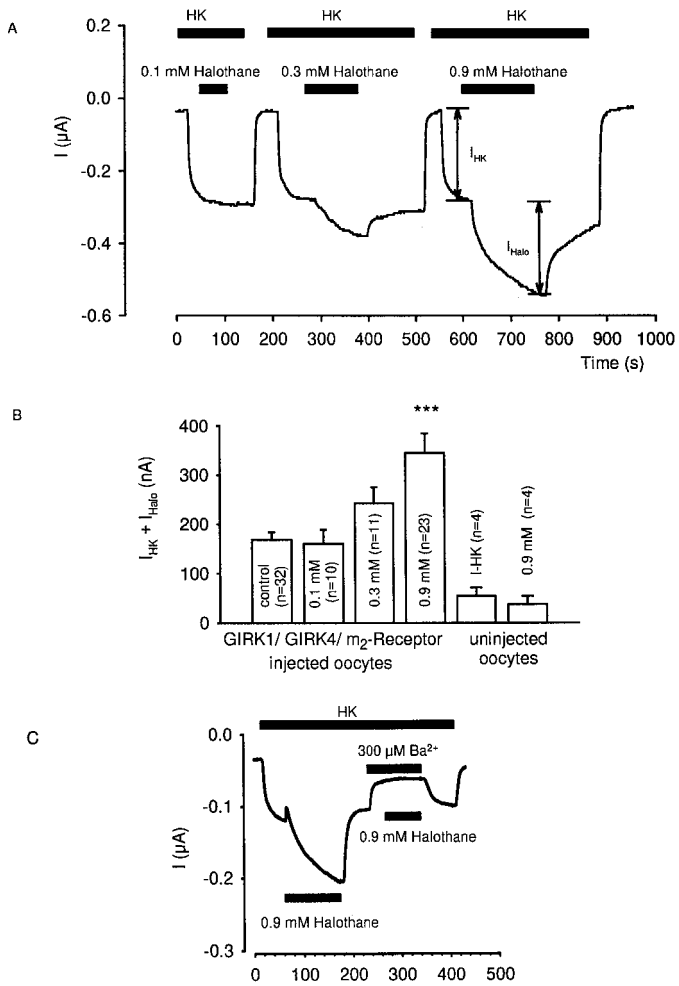

Fig. 1. Halothane increased background currents in GIRK1/GIRK4-expressing oocytes. A, the basal current (I_{HK}) was enhanced upon application of increasing concentrations of halothane (I_{Halo}). On the average, 0.9 mM halothane augmented the basal current by 90% 1 min after the start of application. B, currents induced by change to extracellular high potassium concentration medium (HK, control) and increase of current by different concentrations of halothane (***$p < 0.001$ versus I_{HK}, S.E.M.). In uninjected oocytes, only small basal currents were observed that were not increased by halothane. C, Ba^{2+} sensitivity of the halothane-induced current mediated by $GIRK1^{F137S}$ channels. The halothane-induced current was completely blocked by Ba^{2+}, indicating specific activation of a GIRK-mediated current.

Interestingly, homo-oligomeric GIRK2 channels showed the highest sensitivity against halothane: already, 30 μM halothane was sufficient to block 34 ± 4% (n = 3) of the acetylcholine-induced inward currents (Fig. 3, A and C). In contrast to the findings with heteromeric GIRK1/GIRK4 and even GIRK1/GIRK2 channels, we observed a dose-dependent inhibition of the currents by increasing concentrations of halothane and determined an IC_{50} value of approximately 60 μM. A halothane concentration of 0.9 mM already completely blocked the acetylcholine-induced current through GIRK2 channels. At this concentration, the current was reduced to less than the initial value of I_{HK} (103 ± 3%, n = 5). Of 20 observations, no case of activation of GIRK2 channels by any concentration of halothane was observed.

Thus, it seems that halothane acted specifically on particular GIRK subunits and exerted activating properties on channels containing the GIRK1 subunit but had inhibitory properties on the GIRK2 subunit with an intermediate action on heteromeric channels. Therefore, we wanted to study how homo-oligomeric GIRK1 channels would react to halothane. The expression of homomeric GIRK1 channels does not give conductive channels when the endogenous GIRK5 subunit has been suppressed by coinjection of specific antisense oligonucleotides into the oocyte (Hedin et al., 1996). However, a mutation in the putative pore region of GIRK1 at position 137 from F to S yields a mutant subunit, able to form functional homomeric GIRK1 channels (Chan et al., 1996). $GIRK^{F137S}$ channels coexpressed with the m_2 receptor led to average background currents I_{HK} values of 255 ± 36 nA (n = 38). When activated by acetylcholine, the current was further increased by 558 ± 79 nA (n = 30). When expressed in oocytes, $GIRK1^{F137S}$ channels showed sensitivities against halothane that were comparable with the effects seen with GIRK1/GIRK4 heteromeric channels: a clear induction of basal inward currents at concentrations of more than 0.3 mM (Fig. 4, A and B) was observed. A concentration of 0.9 mM halothane more than doubled the basal current (increase of 112 ± 14%). This high concentration of halothane also augmented the acetylcholine-induced current by 40 ± 14% (n = 10) when the m_2-receptor was coexpressed. In contrast, low anesthetic concentrations inhibited the agonist-induced currents (Fig. 4C) and 0.1 mM halothane decreased I_{ACh} by 43 ± 6% (n = 10), whereas 0.3 mM diminished the current by only 29 ± 7% (n = 7). Hence, similar to GIRK1/GIRK4 channels,

homo-oligomeric GIRK1^{F137S} channels previously activated by agonist were inhibited by low concentrations of halothane. The inhibition was overridden by the activation of currents at higher concentrations of the anesthetic.

To elucidate the mechanism of halothane action on GIRK channels we attempted to clarify whether the receptor, the G protein, or the GIRK channel itself is the target for halothane. Accordingly, we compared the effect of halothane on cells expressing GIRK1^{F137S} channels, either with a different receptor coexpressed (μ-opioid receptor) or in the absence of a coexpressed receptor. In both cases, 0.9 mM halothane induced currents that were not different at the $p = 0.05$ level compared with cells expressing the m_2 receptor (Fig. 5A). Therefore, the activating effect of halothane is neither specific to a certain G protein-coupled receptor, nor is the presence of the receptor even required.

The different modulation of GIRK1- and GIRK2-mediated currents by halothane suggests a target downstream of the G protein. However, when trying to explain the mechanism of halothane action on GIRK channels, it has to be considered that the G protein α subunit, as well as the β/γ dimer, modulate the channel (Dascal, 1997). To inhibit the activation of GIRK channels by $G_{\beta/\gamma}$ and to sequester $G_{\beta/\gamma}$ in vivo, we used heterologously overexpressed C-βARK. C-βARK is a fusion protein comprising the $G_{\beta/\gamma}$ binding domain of the β_2-adrenergic receptor kinase and the transmembrane do-

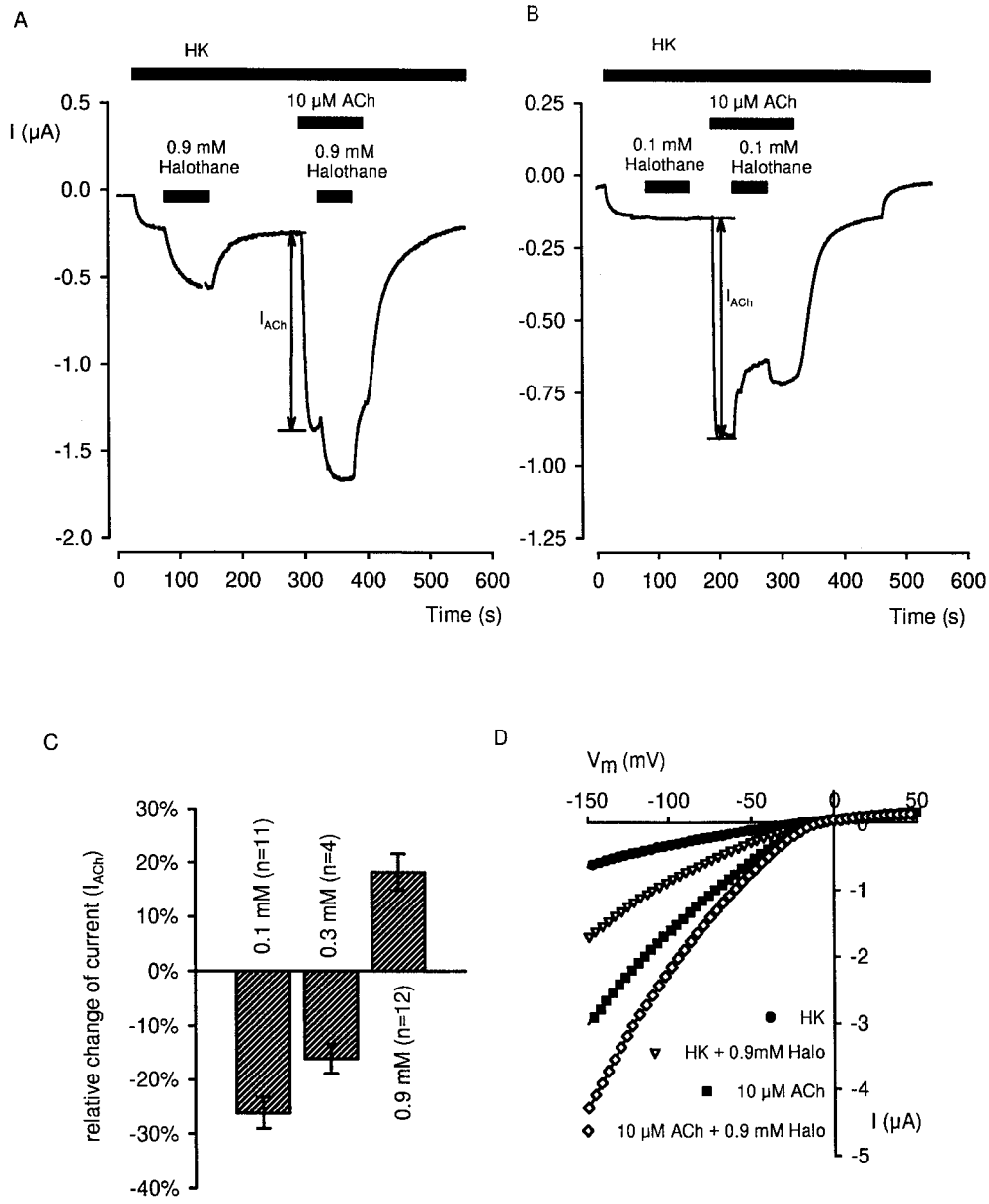

Fig. 2. Effect of halothane on acetylcholine induced GIRK1/GIRK4 mediated currents. A, time course of the halothane effect on GIRK1/GIRK4 acetylcholine-activated currents. High concentrations of halothane induced a current in both the absence and the presence of acetylcholine. The effect of 0.9 mM halothane on background and agonist induced current was additive. B, low concentrations of halothane inhibited I_{ACh} but had no effect on the background current. C, halothane at low concentrations inhibited the GIRK1/GIRK4 mediated current induced by acetylcholine (I_{ACh}) but increased it at high concentrations. Negative values indicate inhibition and positive values indicate augmentation of the agonist-induced current. D, voltage dependence of currents through GIRK1/GIRK4 heteromeric channels. Halothane activated specifically GIRK currents and had no effect on membrane resistance or other ionic conductances within the voltage range tested.

main of *src* for anchoring the construct (Jing et al., 1999) in the plasma membrane. Under these conditions, the background current I_{HK}, as well as the agonist-induced current, were strongly attenuated (Fig. 5B). I_{HK} was reduced from 253 ± 34 nA in control cells to 30 ± 3 nA in C-βARK–expressing cells. The halothane-induced current was diminished correspondingly but was still observable (8 ± 5 nA). Similar results, although less pronounced, were obtained when cells had been injected with the A-protomer of pertussis toxin 24 h before the experiments to inhibit $G_{\alpha i}$ activation by the receptor (Fig. 5B). The incompleteness of block of acetylcholine induced currents in PTX-treated cells was probably caused by promiscuous coupling of heterologous coexpressed m_2-receptors to heterotrimeric G proteins in the *X. laevis* oocytes. These experiments showed that $G_{\beta\gamma}$ was necessary for halothane to activate the GIRK channel.

Halothane clearly had an inhibitory effect on stimulated GIRK channels. This could reflect an interaction with either the muscarinic receptor, the G protein, or the channel protein itself. It has been reported that halothane disrupts receptor-G protein interactions (Dennison et al., 1987; Narayanan et al., 1988). Therefore, one could expect inhibition of acetylcholine-activated GIRK channels by halothane resulting from impaired G protein activation via the receptor. To test whether the attenuation of agonist activated GIRK currents can be traced back to interaction of halothane with the receptor-G protein complex we used the nonhydrolyzable GTP analog GTPγS to activate GIRK currents downstream of the receptor. Injection of 10 nl of a 50 mM GTPγS solution activated an inward current that reached 72% of the absolute current induced by acetylcholine. Similar to the agonist-induced current, the GTPγS-induced current showed time-dependent inactivation (data not shown). When low concentrations of halothane were applied to GTPγS-activated cells, the current was reduced more efficiently with 0.1 mM than with 0.3 mM halothane (Fig. 5C), similar to the effects on I_{ACh}. Hence, as already shown for the activating action of halothane on GIRK channels, the inhibitory actions of halothane on GIRK currents also did not depend on the receptor.

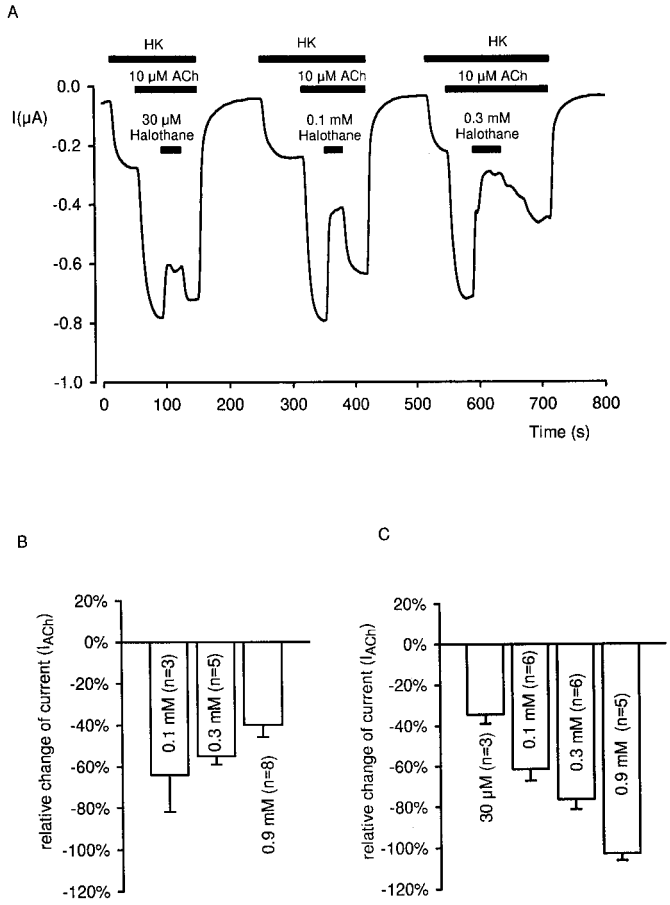

Fig. 3. Effect of halothane on hetero- and homo-oligomeric GIRK2-containing channels. A, time course of a GIRK2-mediated current and inhibition by different concentrations of halothane. A, concentration of 30 μM halothane markedly decreased I_{ACh} through GIRK 2 channels. B, inhibition of acetylcholine activated currents through GIRK1/GIRK2 heterooligomeric channels. The inhibitory potency of halothane decreased with increasing concentrations of the anesthetic. C, homooligomeric channels comprising solely the GIRK2 subunit were more sensitive to halothane than all other subunits tested. I_{ACh} was inhibited by micromolar concentrations of the anesthetic.

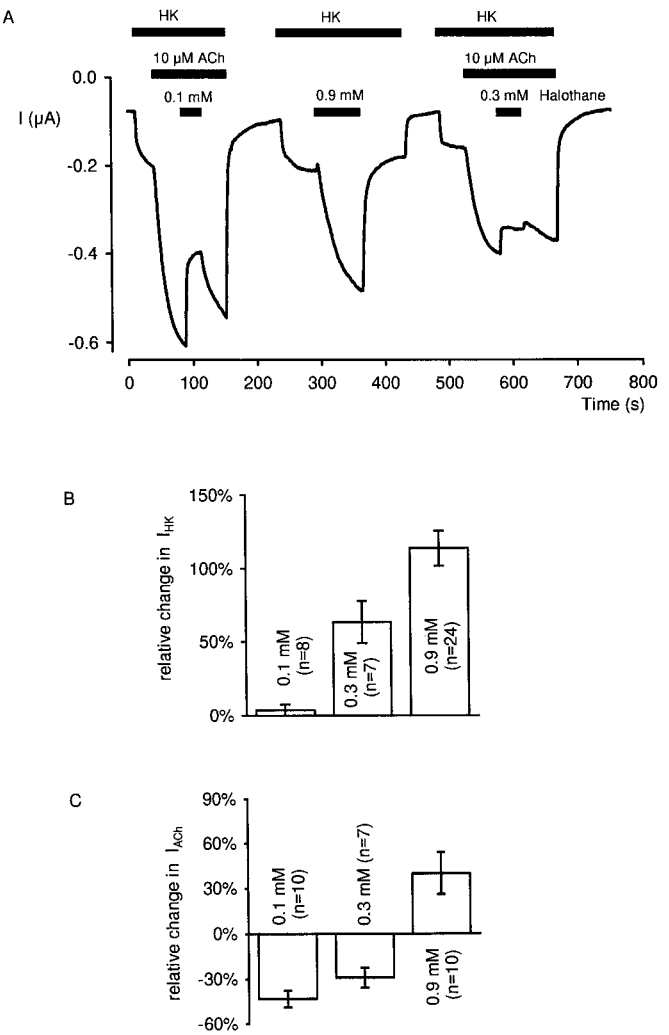

Fig. 4. Halothane effects on $GIRK1^{F137S}$ channels. A, time course of the GIRK-mediated K^+ current in a cell expressing $GIRK1^{F137S}$ homomeric channels together with the m_2 receptor. Halothane inhibited the acetylcholine-induced current at low concentrations, but it increased the basal current in the absence of acetylcholine. B, effect of halothane on basal currents of cells expressing $GIRK1^{F137S}$ together with the m_2 receptor. A 0.9 mM halothane concentration increased I_{HK} by about 112%; lower concentrations were less effective. C, effect of halothane on the acetylcholine induced inwardly rectifying K^+ current. Halothane consistently acted as an antagonist at low concentrations but was able to increase the current at higher concentrations.

Discussion

Our results clearly demonstrate that halothane, a prototypic volatile anesthetic, exerts multiple effects on GIRK channels, depending on their subunit composition and molecular state. In biochemical studies, halothane has been found to increase the basal activity of adenylyl cyclase of rat hearts by attenuation of the muscarinic inhibition (Narayanan et al., 1988). Further, it has been postulated that halothane prevents the dissociation of the G protein from the receptor (Aronstam et al., 1986) in rat brain or that halothane inhibits GDP-GTP exchange (Böhm et al., 1994, Pentyala et al., 1999). These findings are consistent with the observation that halothane decreases the activity of the inhibitory G protein in human heart preparations (Schmidt et al., 1995). Therefore, the available biochemical data indicate that halothane hampers G protein signaling via inhibition of $G_{\alpha i}$. In addition, Magyar and Szabo (1996) reported a decrease in the rate of muscarinic K^+ channel activation when 0.9 mM halothane was coapplied with acetylcholine to bullfrog atrial myocytes. The authors concluded that halothane slowed but did not eliminate the receptor-G protein coupling. In our experiments, inhibition of $GIRK1^{F137S}$-mediated currents by low concentrations of halothane was observed when channels were activated by the agonist or GTPγS. Also, homo-oligomeric GIRK2 and hetero-oligomeric GIRK1/GIRK2 channels were inhibited by low doses of halothane. Hence, this inhibition at low doses of the anesthetic may be attributable to attenuation of G protein activation by the anesthetic. High concentrations of the anesthetic selectively activated currents mediated by GIRK1-containing channels. Coexpression of the GIRK1 subunit rendered the channel complex less sensitive to this inhibition; at higher doses of the anesthetic, the inhibitory action was overridden by activation of the current. This additional inward current induced by halothane was caused by selective activation of GIRK channels because the current-voltage relation showed 1) inward rectification, 2) sustained ion-selectivity and 3) block by micromolar amounts of Ba^{2+} ions. Several lines of evidence indicate that this activatory action of halothane was caused by direct interaction of the anesthetic with the channel protein: 1) the activation by halothane was subunit specific. This behavior would not be expected if halothane acted on the level of the G protein or upstream thereof, because all subunit compositions tested react quite similarly to $G_{\beta/\gamma}$. 2) Activation of GIRK currents by halothane did not require the presence of heterologous coexpressed G protein-coupled receptors. 3) Activation of GIRK via dissociation of $G_{\beta\gamma}$ from $G_{\alpha i}$ would require G protein activation by halothane. This assumption is in clear contradiction to findings of other laboratories, which quite consistently showed the inhibition of $G_{\alpha i}$ by halothane (Narayanan et al., 1988; Böhm et al., 1994; Pentyala et al., 1999). Hence, we conclude that activation of GIRK currents by halothane is a direct consequence of GIRK1/halothane interaction. On the other hand, as demonstrated in the present study, activation by halothane requires the presence of free available $G_{\beta/\gamma}$, because sequestration of $G_{\beta/\gamma}$ by C-βARK greatly diminished the effect. So far, at least two $G_{\beta/\gamma}$ binding sites on the GIRK channel have been identified (He et al., 1999). There is one high-affinity binding site that is thought to be permanently occupied in channels expressed in *X. laevis* oocytes, thus producing the basal current and a second low-affinity binding site that is responsible for agonist-induced activation of the channel. In our experiments, the background current was augmented, whereas the agonist-induced current was either diminished or increased depending on the applied halothane concentrations. The most straightforward explanation for this dualistic

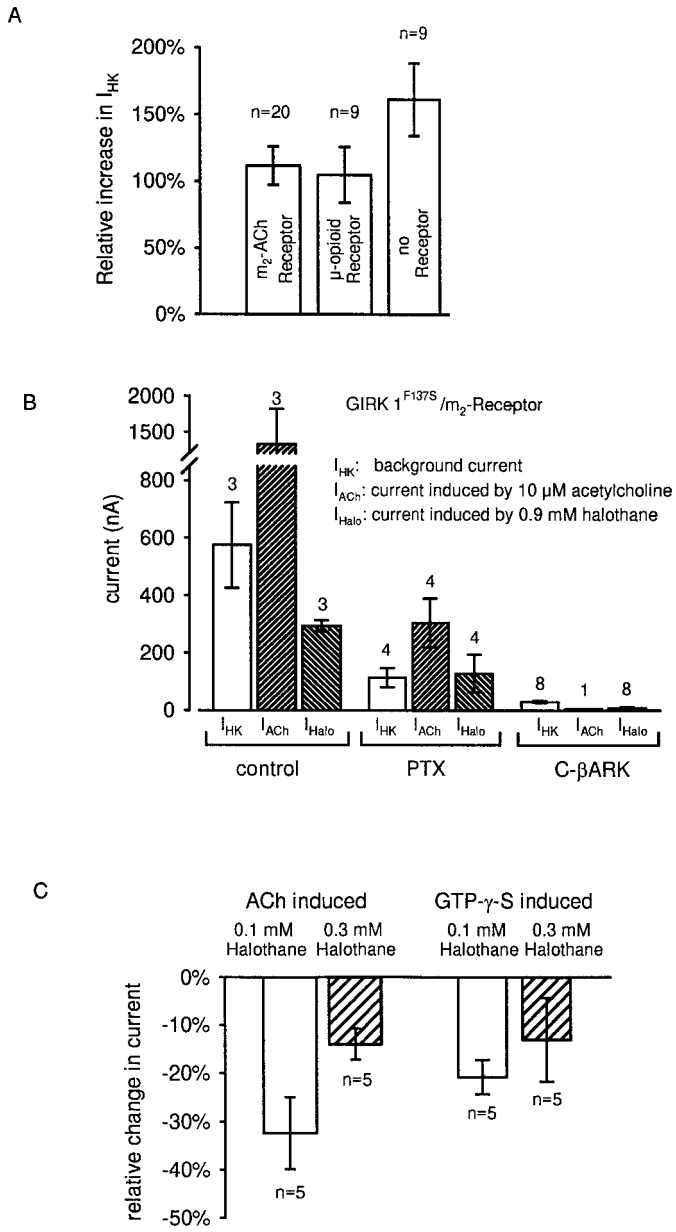

Fig. 5. Involvement of receptor and G protein in the halothane effect. A, 0.9 mM halothane increased the $GIRK1^{F137S}$ background currents regardless which receptor subtype was coexpressed. Activation of GIRK-mediated currents also occurred in the absence of a receptor. B, activation of GIRK-mediated currents required the presence of $G_{\beta\gamma}$. Coexpression of C-βARK reduced the basal currents as well as the response to 10 μM acetylcholine and 0.9 mM halothane. A similar effect was observed when cells had been injected with the A protomere of pertussis toxin (PTX), although here the inhibition was less pronounced. Control and PTX-treated cells were from the same batch of oocytes and experiments were done on the same day. Shown are current values induced by the respective treatment. C, inhibition of agonist-induced currents by halothane happened downstream of the receptor. Acetylcholine-induced currents (I_{HK} + I_{ACh}), as well as currents activated by injection of GTPγS were reduced by low concentrations of halothane. The difference in reduction seen with acetylcholine and GTPγS-induced currents was statistically insignificant.

effect seen with high and low concentrations of halothane would be an inhibition of the activated G protein by low doses of halothane. At high doses, a direct effect on the GIRK1 subunit occurs via allosteric promotion of $G_{\beta/\gamma}$ association. Such a change in $G_{\beta/\gamma}$ affinity would also explain the slow time course of the halothane-induced channel activation: the channel is not opened because of halothane binding but still has to be activated by free $G_{\beta/\gamma}$. Halothane, therefore, would represent an inverse agonist with partial agonistic properties at high concentrations. In the case of complete absence of $G_{\beta/\gamma}$, no activation with halothane is possible because the high-affinity binding site for $G_{\beta/\gamma}$ is not occupied. The most striking difference between the subunits tested was the complete absence of any halothane induced activation of GIRK2 channels compared with GIRK1 channels. On the molecular level, the most pronounced difference between these two subunits is a stretch of 65 amino acid residues near the putative low-affinity $G_{\beta/\gamma}$ binding site lacking in the GIRK2 subunit. Therefore, it is tempting to speculate that the site of action of halothane on the channel is within this region of GIRK1. However, further experimental work will be necessary to clarify this question.

The hypothesis that halothane changes $G_{\beta/\gamma}$ affinity is also in line with the findings of Magyar and Szabo (1996) that halothane is not able to increase the I_{KACh} in atrial myocytes without simultaneous stimulation by either acetylcholine or GTPγS, because in myocytes, the concentration of free $G_{\beta\gamma}$ is low. Magyar and Szabo (1996) further showed that activation of I_{KACh} in atrial myocytes by halothane was caused by an increased number in channel openings and not because of an increase in channel conductance or prolongation of mean channel life time. They concluded, therefore, that halothane has little effect on the open channel but that it changes the channel activation kinetics. Such changes in channel kinetics could indeed occur if halothane were to interfere with $G_{\beta/\gamma}$ binding as described above. Our findings generally corroborate the observations of Magyar and Szabo (1996) that halothane has intricate effects on G protein-activated K^+ channels. They found a rapid inhibition of channel activation, which they interpreted as an effect on coupling process, and that halothane is also able to induce the K^+ current at stimulatory GTPγS concentrations. However, a direct comparison of their observations with our findings is not easily possible, because they used bullfrog atrial myocytes in which G protein activity is rather fast and some features of the channels, such as fast desensitization, are not observed in oocytes.

Is Modulation of GIRK Channels by Halothane a Mechanism Relevant to Anesthesia? General anesthesia with halothane occurs at concentrations of 0.75% atm in humans to 1.03% atm in rats (Franks and Lieb, 1993). These are the minimum alveolar concentration values, which are expressed in partial pressures of an anesthetic in the gaseous phase and correspond to aqueous concentrations of about 0.2 to 0.3 mM. The depressing effect of halothane on GIRK2 channels was observed with an IC_{50} value of about 60 μM and is therefore well within the concentrations reached during general anesthesia. The agonist-dependent activation of heteromeric GIRK1/GIRK2 (neuronal form) and GIRK1/GIRK4 channels was inhibited preferentially by low clinical concentrations of halothane. It may be that disturbance of the inhibitory action, ascribed to GIRK channels, contributes to anesthesia. However, our knowledge of the complex functioning of the CNS still does not allow an exact assessment of molecular effects for anesthesia. Whether activation of GIRK channels by concentrations of 0.3 to 0.9 mM is important for anesthesia remains questionable, because concentrations of halothane 2 to 4 times greater than the minimum alveolar concentration cause deleterious side effects, such as respiratory and cardiovascular depression (Franks and Lieb, 1994). In contrast to GIRK1/GIRK4 channels, the hetero-oligomeric GIRK1/GIRK2 and homo-oligomeric GIRK2 isoforms proved rather resistant to activation by halothane. The activation of GIRK1-containing channels occurred already at clinically relevant concentrations and could therefore directly explain not only effects relevant for anesthesia, but also some cardiovascular side effects, such as the occurrence of bradycardia.

In summary, we find that GIRK channels are targets for anesthetics and that halothane shows complex and subunit-dependent interaction with these channels. Although activation of GIRK channels would have been expected to be a more relevant mechanism for anesthesia, it cannot be ruled out that inhibition of GIRK channels also contributes to the state of anesthesia.

Acknowledgments

We are grateful to Dr. Martin Hohenegger for helpful discussion and comments on the manuscript.

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