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

L. G. WEIGL and W. SCHREIBMAYER

Department for Anaesthesia and General Intensive Care Medicine, University Hospital Vienna, Austria (L.G.W.); and Department for Medical Physics and Biophysics, Graz University, Graz, Austria (W.S.)

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 Gbg7. In contrast to basal GIRK currents, the agonist-induced GIRK current (via coexpressed m2 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 IC50 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 Gbg subunits to intracellular portions of the channel protein. Gbg is released from heterotrimeric, inactive Gαβγ 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 EK⁺ 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-

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 methane sulfonate, 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 m3 receptor, 10 ng/μl μ-opioid receptor, 0.3 ng/μl GIRK1, 0.3 ng/μl GIRK1F137S, 3 ng/μl GIRK4, 30 ng/μl GIRK2, and 30 ng/μl c-βARK. Endogenous GIRK5 was eliminated by co-injection of 20 ng/μl antisense oligonucleotide (KH2A; 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 synthesis was described as (Dascal and Lotan, 1992). The following plasmid vectors were used: m3 receptor (Lem et al., 1995), GIRK1, GIRK4 (Silverman et al., 1996), GIRK1F137S (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 MgCl2, 1.8 mM CaCl2, 5 mM HEPES, 100 μM 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 potentials. Oocytes were constantly rinsed during experiments with superfusion at 19 to 21°C. A virtually complete exchange of bath solutions. Oocytes were superfused with regular ND96 solution (96 mM NaCl; 2 mM KCl, 1 mM MgCl2, 1 mM CaCl2, 5 mM HEPES, adjusted with NaOH to pH 7.4) or otherwise as necessary for inactivation of fast inward currents (IHK) of 16.9 ± 3.2 nA (n = 23) at a holding potential of −70 mV. The application of 10 μM acetylcholine evoked additional inward currents (IACH) 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 IHK (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 Ba2+ ions, we tested the effect of halothane in the presence of 300 μM Ba2+. Halothane was not able to induce an inward current in GIRK1/GIRK4- or GIRK1F137S-expressing oocytes, although prominent GIRK-mediated currents were observed in the same cells in the absence of Ba2+ (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 $26 \pm 3\%$ ($n = 11$) whereas 0.3 mM halothane caused a reduction of only $16 \pm 8\%$ ($n = 4$) 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 $64 \pm 18\%$ ($n = 5$), 0.3 mM halothane only by $54 \pm 4\%$ ($n = 3$), and 0.9 mM halothane by $40 \pm 6\%$ ($n = 8$).

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).

Interestingly, homo-oligomeric GIRK2 channels showed the highest sensitivity against halothane: already, $30 \mu M$ halothane was sufficient to block $34 \pm 4\%$ ($n = 3$) 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 $\mu M$. 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 $I_{HK}$ ($103 \pm 3\%$, $n = 5$). 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 $I_{BG}$ values of $255 \pm 36\, nA$ ($n = 38$). When activated by acetylcholine, the current was further increased by $558 \pm 79\, nA$ ($n = 30$). 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 $112 \pm 14\%$). This high concentration of halothane also augmented the acetylcholine-induced current by $40 \pm 14\%$ ($n = 10$) 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 $43 \pm 6\%$ ($n = 10$), whereas 0.3 mM diminished the current by only $29 \pm 7\%$ ($n = 7$). Hence, similar to GIRK1/GIRK4 channels,
homo-oligomeric GIRK1\textsuperscript{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\textsuperscript{F137S} channels, either with a different receptor coexpressed (\(\mu\)-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 \(\alpha\) subunit, as well as the \(\beta\gamma\) 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-\(\beta\)ARK. C-\(\beta\)ARK is a fusion protein comprising the G\(_{\beta\gamma}\) binding domain of the \(\beta_2\) adrenergic receptor kinase and the transmembrane do-

![Figure 2](https://molpharm.aspetjournals.org/article-pdf/285/285/285/2222319/285-285.pdf)

**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 back-
ground 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 dimin-
ished 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 $G_{ai}$ activation by
the receptor (Fig. 5B). The incompleteness of block of acetyl-
choline induced currents in PTX-treated cells was probably
caused by promiscuous coupling of heterologous coexpressed
m2-receptors to heterotrimeric G proteins in the X. laevis
oocytes. These experiments showed that $G_{bg}$ 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 recep-
tor-G protein interactions (Dennison et al., 1987; Narayanan
et al., 1988). Therefore, one could expect inhibition of acetyl-
choline-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 re-
ceptor-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-in-
duced current, the GTPγS-induced current showed time-de-
pendent inactivation (data not shown). When low concentra-
tions 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_{AC}$$.

Hence, as already shown for the activating action of halo-
thane 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-con-
taining channels. A, time course of a GIRK2-mediated current and inhi-
bition by different concentrations of halothane. A, concentration of 30 μM
halothane markedly decreased $I_{AC}$ through GIRK 2 channels. B, inhibition
of acetylcholine activated currents through GIRK1/GIRK2 hetero-
oligomeric 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. $I_{AC}$ was inhibited by micromolar concen-
trations of the anesthetic.

Fig. 4. Halothane effects on GIRK1F137S channels. A, time course of the
GIRK-mediated K+ current in a cell expressing GIRK1F137S homomeric
channels together with the m2 receptor. Halothane inhibited the acetyl-
choline-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 GIRK1F137S together with the m2 receptor. A
0.9 mM halothane concentration increased $I_{HK}$ by about 112%; lower
concentrations were less effective. C, effect of halothane on the acetyl-
choline induced inwardly rectifying K+ current. Halothane consistently
acted as an antagonist at low concentrations but was able to increase the
current at higher concentrations.
Our results clearly demonstrate that halothane, a prototypical 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 adenyl cyclase of rat hearts by attenuation of the muscarinic inhibition (Narayan 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_{ai}. 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 reacted quite similarly to G_{ai}. 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_{ai} from G_{ai} 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_{ai} by halothane (Narayan 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_{ai}, because sequestration of G_{ai} by C-βARK greatly diminished the effect. So far, at least two G_{ai} 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
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\(_{\text{KAC}}\) in atrial myocytes without simultaneous stimulation by either acetylcholine or GTP\(_\gamma\)S, because in myocytes, the concentration of free G\(_{\beta\gamma}\) is low. Magyar and Szabo (1996) further showed that activation of I\(_{\text{KAC}}\) in atrial myocytes 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 GTP\(_\gamma\)S 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 \(\mu M\) 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.

References


Address correspondence to: L. G. Weigl, Dept. for Anesthesia and General Intensive Care Medicine, University Hospital Vienna, Waehringer Guertel 18–20, A-1090 Vienna, Austria. E-mail: lukas.weigl@univie.ac.at