Ionic Mechanism of Isoflurane’s Actions on Thalamocortical Neurons

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Ries, Craig R. and Ernest Puil. Ionic mechanism of isoflurane’s actions on thalamocortical neurons. J. Neurophysiol. 81: 1802–1809, 1999. We studied the actions of isoflurane (IFL) applied in aqueous solutions on ventrobasal neurons from thalamic brain slices of juvenile rats. By using the whole cell, patch-clamp method with current- and voltage-clamp recording techniques, we found that IFL increased a noninactivating membrane conductance in a concentration-dependent reversible manner. In an eightfold concentration range that extended into equivalent in vivo lethal concentrations, IFL did not produce a maximal effect on the conductance; this is consistent with a nonreceptor-mediated mechanism of action. TTX eliminated action potential activity but did not alter IFL effects. The effects on the membrane potential and current induced by IFL were voltage-independent but depended on the external [K⁺], reversing near the equilibrium potential for K⁺. External Ba²⁺ or internal Cs⁺ applications, which block K⁺ channels, suppressed the conductance increase caused by IFL. External applications of the Ca²⁺ channel blockers Co²⁺ or Cd²⁺ or internal application of the Ca²⁺ chelator 1,2-bis-(2-aminophenoxy)-ethane-N,N,N',N'-tetracetic acid did not prevent the effects of IFL, implying little involvement of Ca²⁺-dependent K⁺ currents. A contribution of inwardly rectifying K⁺ channels to the increased steady-state conductance seemed unlikely because IFL decreased inward rectification. An involvement of ATP-mediated K⁺ channels also was unlikely because application of the ATP-mediated K⁺ channel blocker glibenclamide (1–80 µM) did not prevent IFL’s actions. In contrast to spiking cells, IFL depolarized presumed glial cells, consistent with an efflux of K⁺ from thalamocortical neurons. The results imply that a leak K⁺ channel mediated the IFL-induced increase in postsynaptic membrane conductance in thalamic relay neurons. Thus a single nonreceptor-mediated mechanism of IFL action was responsible for the hyperpolarization and conductance shunt of voltage-dependent Na⁺ and Ca²⁺ spikes, as reported in the preceding paper. Although anesthetics influence various neurological systems, an enhanced K⁺ leak generalized in thalamocortical neurons alone could account for anesthesia in vivo.

INTRODUCTION

The actions of inhalational anesthetics on neurons enhance endogenous inhibition in the CNS. This augmentation probably results from an increase in ionic conductance through receptor- and nonreceptor-mediated processes in the neuronal membrane (see reviews by Krnjević and Puil 1997; Tanelian et al. 1993). To account for the anesthetic state in vivo, the increased conductance should be observed in neurons that are major participants in generating and maintaining consciousness.

Although anesthetics influence various neurological systems, effects on the corticothalamic system alone could account for anesthesia. In the preceding paper (Ries and Puil 1999) we reported that isoflurane (IFL) decreased the excitability of thalamocortical neurons in vitro through a conductance shunt. We now describe the specific changes in conductance, predominantly a rise in K⁺ leak, that are responsible for the shunt. The ionic mechanism of action appears generalized because of IFL’s hydrophobicity and the ubiquity of the leak conductance in neurons. This generalized mechanism together with the importance of corticothalamocortical excitability in generating conscious states (cf. Ries and Puil 1999) raises the likelihood that IFL’s actions at unified sites widely distributed along somatic and dendritic membranes produce the anesthetic state.

METHODS

Experiments were performed on neurons in slices of the ventral posterior thalamic nucleus at room temperature (~22°C) from juvenile Sprague-Dawley rats (aged P9–P22) of either gender. The preparation and maintenance of the slices as well as electrophysiological and pharmacological techniques are described in the preceding paper (Ries and Puil 1999).

Near steady-state, voltage-current relationships were obtained by applying ramp commands during voltage-clamp conditions. Such commands could be completed within a few seconds and were applied in a hyperpolarizing direction to avoid activation of the Ca²⁺-mediated T-type current. Slow voltage ramps (10 mV/s) started from a holding potential (Vh) of either approximately −30 mV during application of TTX or approximately −60 mV in the absence of TTX. After reaching a final potential as low as −120 mV, the ramp application was repeated such that the currents could be averaged on-line to reduce “noise.” These responses are shown in horizontally mirrored form. In addition, steady-state, voltage-current relationships were obtained by applying hyperpolarizing step voltage commands. Whole cell capacitance was not compensated as currents were measured after the capacitive transient. The conductance of the neuron was calculated from the current response evoked near the resting membrane potential (RMP).

The ionic composition of the external solution, used in conjunction with a gluconate-based internal pipette solution (Ries and Puil 1999), was changed in some experiments. Normally, the calculated equilibrium potentials were as follows (in mV at 25°C); ENa = +46; Ek = −85; Eca = +159 (based on a calculated internal [Ca²⁺] of 10−6 M); and Ec1 = −54. The external [K⁺] was changed from 5.25 to 1.25, and 9.25 mM by adjusting the [KCl]. This change shifted the calculated EK from −85 mV to −123, −106, and −71 mV, respectively. The NaCl also was adjusted in the different K⁺ solutions to maintain the same [Cl⁻]. This procedure avoided changes in the junction potential at the Ag–AgCl reference electrode while changing ENa by only +0.9, +0.7, and −0.5 mV, respectively. In other experiments, choline chloride was partially substituted for NaCl in the artificial cerebrospinal fluid (ACSF). This substitution changed the external [Na⁺] from 124 to 26 mM and shifted ENa from +46 to 0 mV. On occasion sodium isethionate partly replaced NaCl in the

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ACSF, changing the external [Cl\textsuperscript{−}] from 136 to 12 mM, which shifted $E_{Cl}$ from $-54$ to $+10$ mV. When this low [Cl\textsuperscript{−}] was applied the resulting change in junction potential was compensated in part by adjusting the junction-null control to display the same membrane potential; this added voltage to the internal circuitry of the amplifier. During analysis a final correction for the junction potential was made with, as guides, the action-potential threshold and K\textsuperscript{+}-mediated persistent inward rectifier ($I_{K_P}$) (Ries and Puil 1999).

Ionic channel blockers as well as drugs with known ionic actions sometimes were applied in the external solutions. Their concentrations were as follows: TTX, 300 nM; tetraethylammonium (TEA), 10 mM; 4-aminopyridine (4-AP), 3 mM; Cs\textsuperscript{+}, 5 mM; Ba\textsuperscript{2+}, 100 $\mu$M; Cd\textsuperscript{2+}, 50 $\mu$M; and Ni\textsuperscript{2+}, 0.5 mM. In other experiments, substitutions for Ca\textsuperscript{2+} were made with Ba\textsuperscript{2+}, Co\textsuperscript{2+}, and Mg\textsuperscript{2+}. The GABA\textsubscript{A} agonist baclofen (racemate, 10 $\mu$M) and the anticholinesterase agent tacrine (100 $\mu$M), were administered occasionally to verify $E_C$. In addition the ATP-sensitive K\textsuperscript{+} channel blocker glibenclamide (in 0.5% dimethylsulfoxide) (Erdenli and Krnjević 1994), was used to test involvement of $I_{K_{ATP}}$.

The internal pipette solutions also were altered in three types of experiments: 1) CsOH was substituted for KOH; 2) internal Cl\textsuperscript{−} was increased from 12 to 50 mM by substituting KCl for some of the KOH, which shifted $E_{Cl}$ from $-54$ to $-26$ mV; and 3) 1,2-bis-(2-aminophenoxy)-ethane-$N,N,N',N'$-tetraacetic acid (BAPTA, 10 mM) was substituted for EGTA. In the BAPTA experiments CaCl\textsubscript{2} was decreased to 0.5 mM to maintain the same internal [Ca\textsuperscript{2+}]. All substances were obtained from Sigma-Aldrich Canada, except for IFL (Abbott Laboratories, Montreal, Canada) and glibenclamide (Research Biochemicals International, Natick, USA).

Statistical analysis was performed with GraphPad Prism software (version 2.0, San Diego, CA). Results are expressed as means ± SD. Regression lines were plotted with the method of least squares.

RESULTS

The data were obtained from 66 ventral posterior nucleus neurons, exhibiting overshooting action potentials on current pulse injection and the characteristic low-threshold Ca\textsuperscript{2+} spike (LTS) when depolarized from potentials more negative than $-65$ mV. The average RMPs and input resistances were $-66 ± 4$ mV and $240 ± 83 \Omega$, respectively.

IFL induced a postsynaptic, net outward current

By using TTX application (300 nM) we first abolished Na\textsuperscript{+}-dependent rectification and action potentials. We then determined voltage-current relationships by applying hyperpolarizing ramp commands in neurons held at a $V_N$ of approximately $-30$ mV (see METHODS). A high concentration (4%) of IFL was applied for 1–2 min to obtain a rapid, yet submaximal effect. This led to an increased conductance, enhancing the outward current at voltages >RMP ($n = 12$, Fig. 1A). The magnitude and relative linearity of the IFL-induced outward current were evident from subtraction of the control and IFL-induced currents (Fig. 1B). It seemed likely then that postsynaptic voltage-dependent Na\textsuperscript{+} currents did not distort the outward current.

We performed experiments to assess possible Ca\textsuperscript{2+} contributions to the net outward current. First, we applied external solutions with nominally zero [Ca\textsuperscript{2+}] and high [Mg\textsuperscript{2+}] (4 mM MgCl\textsubscript{2}, 0 mM CaCl\textsubscript{2}), which also would diminish Ca\textsuperscript{2+}-dependent transmitter release. This procedure eliminated the postsynaptic LTS but did not cause detectable changes in the IFL-induced hyperpolarization and conductance increase ($n = 5$). Second, coapplication of IFL with the Ca\textsuperscript{2+} channel blockers Cd\textsuperscript{2+} (CdCl\textsubscript{2}, 50 $\mu$M, $n = 2$) or Co\textsuperscript{2+} (1 mM CoCl\textsubscript{2}, 0 mM CaCl\textsubscript{2} and 3 mM MgCl\textsubscript{2}, $n = 2$, Fig. 1C) had no effect on the rate of onset and peak amplitude of the conductance increase induced by IFL. Although we have not completely dismissed a presynaptic contribution, a postsynaptic influx of Ca\textsuperscript{2+} did not distort the IFL-induced outward current.

Concentration-effect relationship

We assessed the concentration-dependence of IFL effects with hyperpolarizing step commands of long duration ($≥1$ s) in a voltage range between $-70$ mV and $-100$ mV from a $V_N$ of approximately $-60$ mV. In addition to TTX, coapplications of TEA (10 mM) and 4-AP (3 mM) were used to block rectifying currents. The magnitudes of the conductance increase were approximately the same in the absence of the Na\textsuperscript{+} and K\textsuperscript{+} blockers (Ries and Puil 1999). In a concentration-dependent manner, cumulative applications of IFL (0.5–2%) produced noninactivating currents ($n = 8$, Fig. 2A). As with ramp-com
mands, the voltage-current plots demonstrated that the increases in conductance caused by IFL were voltage independent (Fig. 2B). The results, pooled with single 4% IFL applications, were used to construct a concentration-effect relationship for the conductance increase (Fig. 2C). This relationship showed a linearity over an eightfold concentration range (IFL 0.5 to 4%).

Principal ionic conductance in IFL actions

By changing the K\(^{+}\) driving force with different external K\(^{+}\) concentrations (1.25, 2.45, 5.25, and 9.25 mM), we assessed a K\(^{+}\) involvement in the IFL response. From a V\(_{RMP}\) at or near RMP, a change in K\(^{+}\) driving force was verified by the increase in outward holding current with low [K\(^{+}\)] perfusion and an inward shift in the holding current with high [K\(^{+}\)] perfusion (Fig. 3, A and B). Application of IFL then induced outward currents during all changed external [K\(^{+}\)] (Fig. 3, A). However, the current magnitude was larger during low [K\(^{+}\)] and smaller during high [K\(^{+}\)] conditions. These observations were consistent with a K\(^{+}\) involvement in IFL actions.

By using hyperpolarizing voltage ramps we next determined the reversal potential for IFL actions (E\(_{IFL}\)) at various external K\(^{+}\) concentrations. For example, when the extracellular [K\(^{+}\)] was decreased, E\(_{IFL}\) shifted with the change in E\(_{K}\) to a more negative value (Fig. 3, B and C). At all external K\(^{+}\) concentrations, however, E\(_{IFL}\) was at a positive value relative to the calculated E\(_{K}\). Figure 3D shows E\(_{IFL}\) as a function of log E\(_{K}\). The number in parentheses represents n.
$E_{m}/K_{m}$, which behaves like the Nernst potential for $K^{+}$. A slope of 39 mV for the experimental data compares approximately to a slope of 60 mV for calculated $E_{K}$ at 25°C. The increasing disparity of the relationship of $E_{IFL}$ to $E_{K}$ as external $[K^{+}]$ decreases implies a systematic experimental error or a contribution of ions other than $K^{+}$ to IFL’s effects.

**Baclofen and tacrine effects serve as markers for $E_{K}$**

Independently from IFL application, we obtained measurements of reversal potentials for two agents that have actions that involve mostly $K^{+}$ conductances. First, the effects of the GABA$_{A}$ agonist baclofen (Crunelli et al. 1988) and its reversal potential were compared with those of IFL. As with IFL baclofen application (10 μM) induced a persistent outward current when $V_{h}$ was near rest (Fig. 4A). Unlike IFL, however, the outward current caused by baclofen reversed polarity at $-84 ± 1$ mV ($n = 3$; Fig. 4B and C), i.e., close to the calculated $E_{K}$ ($-85$ mV). In another test for $E_{K}$ in two neurons, we applied the anticholinesterase tacrine (100 μM) (Stevens and Cotman 1987). Tacrine decreased the resting outward current when $V_{h}$ was near rest with a reversal potential identical to $E_{K}$. We concluded then that errors such as junction potentials were probably not responsible for the difference between $E_{IFL}$ and $E_{K}$. Because RMP also differed from $E_{K}$, it seemed reasonable to explore a relationship between $E_{IFL}$ and RMP. Indeed the variation in $E_{IFL}$ correlated to the variation in RMP, measured before IFL application ($r = 0.74$, $n = 20$).

**Contributions of ions other than $K^{+}$ to IFL actions**

Manipulations in the ionic environment were performed to investigate a secondary role for ions other than $K^{+}$. $E_{IFL}$ did not change during blockade of a theoretical steady-state window T current (Hutcheon et al. 1994) by either a nominally zero external $[Ca^{2+}]$ with replacement of external $Ca^{2+}$ with $Mg^{2+}$ (4 mM, $n = 4$) or with $Ni^{2+}$ (0.5 mM, $n = 3$). In other experiments we reduced the external $[Na^{+}]$ from 124 to 26 mM by replacement with the impermeant cation choline ($n = 3$) and external $[Cl^{-}]$ from 136 to 12 mM by replacement with the impermeant anion isethionate ($n = 3$). We also raised the internal $[Ca^{2+}]$ from 12 to 50 mM ($n = 3$). Although liquid junction potentials could still be a complicating factor in some of these experiments (see METHODS), we did not obtain evidence for a contribution of transmembrane $Ca^{2+}$, $Na^{+}$, or $Cl^{-}$ fluxes to $E_{IFL}$ and hence to IFL’s effects (see also previous results on $Ca^{2+}$ channel blockers).

**Contributions of $K^{+}$ conductances to IFL**

Although the IFL-induced current appeared resistant to blockade by 4-AP and TEA, other $K^{+}$ channel blockers were used to identify the increase in $K^{+}$ conductance. External Ba$^{2+}$ ($2$ and $0$ mM $Ca^{2+}$) depolarized neurons held near rest by decreasing conductance and inward rectification; a subsequent application of IFL (1%) produced little or no change in membrane conductance and potential. In these neurons, this total blockade was surmountable by application of a higher concentration of IFL (4%, $n = 4$). The reversal potential for Ba$^{2+}$ application ($-77 ± 3$ mV) was similar to $E_{IFL}$ ($-76 ± 3$ mV) under control $K^{+}$ conditions. In another set of experiments the usual effects of IFL (1 or 2%) were not observed when internal Cs$^{+}$ was used to replace $K^{+}$ in the internal pipette solution. Like the external Ba$^{2+}$ blockade, this apparently total blockade by internal Cs$^{+}$ was surmountable in three neurons ($n = 4$) with higher concentrations of IFL (4%). These results also were consistent with an IFL-induced increase in $K^{+}$ conductance.

We investigated a possible involvement of a $Ca^{2+}$-dependent $K^{+}$ current ($I_{K(Ca)}$). Consistent with the results obtained previously with nominally zero external $[Ca^{2+}]$, TEA (10 mM, $n = 8$) blockade of $I_{K(Ca)}$ or blockade of $Ca^{2+}$ channels with $Co^{2+}$ (1 mM, $n = 2$) or $Cd^{2+}$ (50 μM, $n = 2$) did not affect either the increase in conductance induced by IFL application or $E_{IFL}$. The influence of internal $Ca^{2+}$ buffering on IFL actions also was tested by applying internal BAPTA (10 mM, same internal $[Ca^{2+}]$), a faster $Ca^{2+}$ chelator than EGTA. In the BAPTA-applied neurons, the increase in conductance during IFL application was unchanged in magnitude ($n = 4$) compared with other neurons recorded with EGTA in the internal pipette solution. With the caution that internal gluconate may inhibit $I_{K(Ca)}$ (Velumian et al. 1997), we tentatively concluded that IFL did not greatly activate $I_{K(Ca)}$.

We then investigated a possible activation of $I_{K(ATP)}$ by IFL. For example, glibenclamide, a specific blocker of $I_{K(ATP)}$, prevents much of the conductance change caused by anoxia (Politi and Rogawski 1991). During current-clamp recordings and manual clamping at $V_{h}$ of approximately $-60$ mV, we monitored input conductance on injection of small intermittent hyperpolarizing current pulses. A standardized 2-min application of IFL (4%) was applied before, during, and after glibenclamide application ($1–80$ μM, $n = 7$). Application of glibenclamide at 40 and 80 μM ($n = 4$) tended to increase input resistance and slightly decreased a positive holding current (implicating a depolarizing action). However, glibenclamide application did not significantly change the IFL-induced in-
Effects of IFL on nonspiking cells, consistent with \( K^+ \) efflux from neurons

Five cells were presumed to be glia because of the following characteristics: 1) relatively negative RMP (−79 ± 1 mV), 2) low input resistance (4 ± 3 M\(\Omega\)), 3) short membrane time constant (1.5 ± 1.1 ms), and 4) absence of action potentials despite injections of depolarizing pulses of large amplitude. During voltage clamping of these “unresponsive” cells at RMP, applications of a high IFL concentration (4%) produced an inward current that was both reversible and reproducible on several repeated applications (\( n = 5 \), Fig. 6A). The application of a high concentration of \( K^+ \) (9.25 mM) similarly evoked an inward current (\( n = 1 \), Fig. 6A). Because glial cells behave as a \( K^+ \)-selective electrode, the inward current may have resulted from a decreased steady-state outward current. Consistent with a decrease in \( K^+ \) efflux, both the IFL- and the \( K^+ \)-induced inward currents were associated with depolarization.

Voltage ramps were used during IFL applications to investigate conductance changes in nonspiking cells. Along with the depolarization, IFL application decreased conductance in a

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**FIG. 5.** Effects of IFL on \( K_{ATP} \) current and \( K^+ \)-mediated inward rectification. A: chart record of voltage response from a manual voltage clamp of −64 mV (current-clamp recording) with IFL applications before and during perfusion of the ATP-sensitive \( K^+ \) channel blocker glibenclamide. The repetitive downward deflections are tests for input resistance, whereas the upward deflections represent mostly action potentials before and after IFL application. Glibenclamide did not prevent IFL from increasing conductance. B: voltage-clamp recordings show current responses to hyperpolarizing voltage ramps (from −64 to −124 mV; top) before and during IFL application. The IFL-induced current was shown (bottom) by subtracting the control from the IFL response. Although increasing conductance at potentials positive to \( E_K \), IFL decreased inward rectification at potentials negative to \( E_K \).

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**FIG. 6.** Effects of IFL on nonspiking cells. A: voltage-clamp recordings showing chart record of membrane current (top) in a nonspiking cell held at −78 mV. IFL applications decreased an outward current before and during perfusion of high \( K^+ \) (9.25 mM). \( V_h \) (bottom trace) was gradually changed to more positive levels to maintain stability during high \( K^+ \). The vertical deflections represent hyperpolarizing voltage ramps (in duplicate; averaged and enlarged in B). B: currents evoked by hyperpolarizing voltage ramps (from −64 to −94 mV) before (1) and during (2 and 3) IFL application in A. Current at bottom was obtained by subtracting the control (1) from the final IFL (3) response. In contrast to neurons, IFL decreased conductance and depolarized RMP. Note the similarity between \( E_{K_{ATP}} \) and \( E_K \) in this nonspiking cell.
voltage-independent manner throughout a hyperpolarized voltage range (from $-65$ to $-95$ mV, $n = 5$, Fig. 6B). This action had a reversal potential of $-83 \pm 1$ mV, close to $E_K$. A small positive separation in these values was consistent with a postulated increase in external $[K^+]$ and a corresponding depolarizing shift in the equilibrium potential. These results were consistent then with a hypothesis that the IFL-induced depolarization of nonspiking cells was due to $K^+$ efflux from neurons.

**DISCUSSION**

These investigations have shown that, in a concentration-dependent manner, IFL application to thalamocortical neurons increased a postsynaptic conductance that was linear throughout a range of membrane voltages that occur during wakefulness and sleep. As described in the preceding paper (Ries and Puil 1999), the increased conductance produced a hyperpolarization and inhibited neurons by shunting voltage-dependent $Na^+$ and $Ca^{2+}$ currents. If IFL were to have similar actions on the nerve terminal membranes, i.e., a possibility that seems likely, we would expect an additional decrease in synaptic activity.

Along with the increased conductance, IFL application to neurons at the RMP increased the positive holding current, implicating an outward $K^+$ current. Several observations confirmed a major involvement of a $K^+$ conductance in the IFL actions: 1) the magnitude of the steady-state outward current increased during low $[K^+]_o$ perfusion and decreased during high $[K^+]_o$ perfusion; 2) $E_{\text{IFL}}$ was close to the $E_K$ calculated for different external $[K^+]_o$ conditions; 3) during application of IFL at high concentrations glia cells depolarized in association with a decrease in membrane conductance, presumably caused by IFL-induced $K^+$ leakage from thalamocortical neurons; and 4) applications of external $Ba^{2+}$ or internal $Cs^+$, which block leak and other $K^+$ channels but not external TEA or 4-AP, which block voltage-dependent $K^+$ channels, suppressed the increased conductance caused by application of IFL. It seemed likely then that the IFL-induced increase in conductance involved $K^+$ leak channels. The ability of IFL to influence electrical signaling and produce an anesthetic state likely relates to the abundance of nonligand gated $K^+$ leak channels and their important role in determining the RMP.

**Is an ionic conductance other than $K^+$ involved in IFL actions?**

The increased disparity of $E_{\text{IFL}}$ and $E_K$ with decreases in extracellular $[K^+]_o$ and the correlation of $E_{\text{IFL}}$ to the initial RMP implied a minor contribution of ionic currents other than $K^+$. Yet the results of experiments where we altered $Na^+$, $Cl^-$, or $Ca^{2+}$ concentrations did not implicate an involvement of ions other than $K^+$. A lack of $Cl^-$ involvement in IFL actions may relate to a higher concentration dependence for $Cl^-$ channel activation as well as conflicting reports of anesthetic-induced potentiation (Jones et al. 1992) and depression (El-Beheiry and Puil 1989a) of inhibitory postsynaptic potentials (mediated by GABA). Although experimental limitations may have contributed to an underestimation of $E_{\text{IFL}}$, the accuracy of voltage-clamp estimations should be greater for a steady-state conductance evoked throughout the soma and dendrites than for a transient dendritic conductance (Spruston et al. 1993; see Ries and Puil 1999). By acting unspecifically on both somatic and dendritic membranes, however, IFL application would have caused spatial control to diminish during the voltage-clamp recording. In such a case, an electrotonically distant $K^+$ conductance would have shifted the apparent $E_{\text{IFL}}$ to values more negative than $E_K$ and resulted in an overestimation of $E_{\text{IFL}}$ negativity. By using baclofen and tacrine to verify the calculated $E_K$, it was apparent that errors resulting from a reduced external $[K^+]_o$ or a junction potential were not responsible for the difference between $E_{\text{IFL}}$ and $E_K$. Thus the results are consistent with a major role for an increased $K^+$ leak conductance, with or without a role for an increased conductance to other ions.

**Nonleak $K^+$ conductances**

Another possibility is that anesthetics affect a $Ca^{2+}$-mediated $K^+$ conductance (Krnenvic ´ 1974). Steroid anesthetics such as althesin or IFL block slow afterhyperpolarizations (AHPs) and spike-train AHPs activated by $Ca^{2+}$ entry into neocortical neurons (El-Beheiry and Puil 1989b). This contrasts with observations on hippocampal and cerebellar neurons where ethanol, pentobarbital, and the benzodiazepines midazolam and clonazepam increase such $Ca^{2+}$-mediated $K^+$ conductances (Carlen et al. 1985). We found that external $I_{K(Ca)}$ blockers and chelation of internal $Ca^{2+}$ by BAPTA did not suppress IFL actions. Considering these results together with the experiments on altered $Ca^{2+}$ concentrations, it does not seem likely that $Ca^{2+}$ release or entry contributed significantly to the actions of IFL.

Other noninactivating $K^+$ currents did not contribute to the IFL-induced increase in steady-state conductance in our studies. In fact, IFL decreased inward rectification caused by $I_{KIR}$ probably by shunting this voltage-dependent current. In addition an action of IFL on GABA$_B$-activated $K^+$ channels seemed unlikely here because 4-AP, which reportedly blocks baclofen effects (Inoue et al. 1985; see also Sugiyama et al. 1992), did not reduce the hyperpolarization induced by IFL. More likely are possibilities that anesthetics act on ligand-gated, voltage-independent $K^+$ channels, especially the muscarine-sensitive $K^+$ leak conductance (Puil and El-Beheiry 1990) or the serotonin-sensitive $K^+$ channel (Winegar et al. 1996).

**Comparison with anoxic effects**

We considered the analogy that unconsciousness rapidly occurs during induction of either anesthesia or anoxia in vivo. Like IFL application to thalamocortical neurons, anoxic insults hyperpolarize CNS neurons by increasing conductance to $K^+$ (Krnenvic ´ and Leblond 1989). In part $I_{K(\text{ATP})}$ is responsible for these effects during energy depletion, as demonstrated by the effects of applications of specific ATP-sensitive $K^+$ channel blockers. Glibenclamide, for example, prevents much of the conductance change during acute anoxia (Politi and Rogawski 1991). Although glibenclamide inhibits IFL-induced coronary artery vasodilation (Cason et al. 1994) and myocardial protection (Kersten et al. 1996), glibenclamide does not affect the minimum alveolar concentration for IFL in rats (Zucker 1992). Whereas glibenclamide occasionally is ineffective in some...
hippocampal neurons (Erdemli and Krnjević 1994), TEA application blocks their anoxic responses (Krnjević and Leblond 1989). In contrast we observed intact IFL responses during glibenclamide or TEA application and hence found no evidence for an involvement of I_{K(ATP)}.

Previous studies on inhalational anesthetics

The pioneering studies of Nicoll and Madison (1982) showed that anesthetics other than IFL increase membrane conductance, probably to K\(^+\), and hyperpolarize hippocampal and spinal neurons of in vitro preparations. These effects occur despite bicuculline blockade of GABA\(_\text{A}\)-activated Cl\(^-\) channels and under low or high external Cl\(^-\) conditions. Their observations included the effects of the inhalational agents diethylether and halothane during TTX blockade of action potentials. More recent studies confirmed such pharmacological properties for IFL and halothane in neocortical, hippocampal, and intralaminar thalamic neurons (Berg-Johnsen and Langmoen 1990; El-Beheyry and Puil 1989b; Sugiyama et al. 1992). There are some inconsistencies, however, because inhalational agents can have insignificant effects on membrane potential of hippocampal neurons (Miu and Puil 1989), depending on the concentration or type of agent (MacIver and Kendig 1991). While hippocampal neurons probably participate in consciousness, coherent activity of neurons in the corticothalamocortical system is essential for conscious behavior. Hence the blunted membrane excitability (Ries and Puil 1999) and disrupted oscillatory activity (Tennigk et al. 1997) caused by an increased K\(^-\) leak conductance in thalamocortical neurons provide a likely mechanism of IFL anesthesia in humans.

Significance

The IFL actions are consistent with the historical hydrophobic mechanism involving the lipid membrane and hydrophobic channel proteins. In the current investigations, an eightfold range of IFL concentrations increased the K\(^+\) leak conductance without producing a maximal effect. From a pharmacological viewpoint this suggests a nonspecific (i.e., nonreceptor-mediated) mechanism for IFL.

Likely generalized, the enhanced leak conductance caused by IFL shunted the effectiveness of somatic injected current for eliciting voltage transfer to the axon hillock and dendrites. The leak enhancement mechanism accounted for the ability of IFL to annihilate action potentials as well as low- and high-threshold Ca\(^{2+}\) spikes in thalamocortical neurons. The apparently widespread reduction in their membrane excitability, the hypodromicity of IFL, and its K\(^+\) mechanism of action are consistent with the classical viewpoint of “general” (i.e., nonreceptor-mediated) actions for anesthetics.

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REFERENCES


LITTLE, H. J. How has molecular pharmacology contributed to our understanding of the mechanism(s) of general anaesthesia? Pharmacol. Ther. 69: 37–58, 1996.


