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Interaction of Volatile Anesthetics with Human Kv Channels in Relation to Clinical Concentrations

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Background: **Recent evidence shows that inhibition of human Kv3 channels by intravenous anesthetics occurs at clinical concentrations. The effects of volatile anesthetics on these human ion channels are unknown. This study was designed to establish whether minimum alveolar concentrations (MAC) of halothane, enflurane, isoflurane, and desflurane exhibit effects on Kv3 channels. To obtain an indication whether these findings may be specific to Kv3 channels, the effects of enflurane and isoflurane on human Kv1.1 channels were also investigated.**

Methods: **Kv3 channels natively expressed in SH-SY5Y cells and Kv1.1 channels expressed in HEK293 cells were measured with the whole cell patch clamp technique by standard protocols. Concentrations of volatile anesthetics were determined by gas chromatography.**

Results: **Halothane, enflurane, isoflurane, and desflurane reversibly inhibited Kv3 channels in a concentration-dependent manner. Concentrations at half-maximal effect (IC_{50} values) ranged between 1,800 and 4,600 μM. Hill coefficients were between 1.7 and 2.5. IC_{50} values for inhibition of Kv1.1 channels were 2,800 and 5,200 μM, and Hill coefficients were 3.9 and 5.6 for enflurane and isoflurane, respectively.**

Conclusion: **Volatile anesthetics inhibit human Kv3 channels at clinical concentrations. At 1–3 MAC, inhibition would account on average for 2–12%. Inhibition would be highest with enflurane (between 3% and 22%) and lowest with isoflurane (between 0.2% and 3%). Kv1.1 channels would only be inhibited by enflurane at clinical concentrations (2% at 2 MAC and 8% at 3 MAC). Whether the degree of K channel inhibition by volatile anesthetics may contribute to their clinical action needs further study.**

VOLTAGE-DEPENDENT K channels constitute a heterogeneous group of membrane proteins that are important for various cellular functions.[1,2] Alterations of these ion channels result in complex cellular changes.[3,4] In a previous study, we described the effects of several intravenous anesthetics on human neuronal voltage-dependent Kv3 channels.[5] In contrast to expectations, the intravenous anesthetics inhibited Kv3 channels already at clinically relevant concentrations, and concentrations at half-maximal effect (IC_{50} values) for inhibition of Kv3 channels by intravenous anesthetics correlated with their clinical concentrations.

The action of volatile anesthetics on human voltage-dependent K channels has not been investigated. It is unknown whether these ion channels may be a molecular target of inhalation anesthetics at clinical concentrations. In light of our previous findings, the current study was designed to extend our observations with intravenous anesthetics to the commonly used inhalation anesthetics.

The study examined the action of halothane, enflurane, isoflurane, and desflurane on human Kv3 channels. Concentration-dependent effects were established to relate concentrations of *in vitro* effects to minimum alveolar concentrations (MAC). To obtain an indication of whether these findings may be specific to Kv3 channels, they were repeated for enflurane and isoflurane on human Kv1.1 channels stably transfected in HEK293 cells.

Material and Methods

Cell Culture and Electrophysiologic Recordings

SH-SY5Y cells[6] were grown as previously described.[5] HEK293 cells stably expressing Kv1.1 channels were grown in nonconfluent monolayer using DMEM nutrient mix F12 (Life Technologies, Paisley, Scotland) at 37°C with 95% air and 5% CO_2. Natively expressed voltage-dependent K channels[7] in SH-SY5Y cells and Kv1.1 channels were recorded with the whole cell patch clamp technique[8] as described previously. The holding potential in all experiments was −80 mV; the test potentials were rectangular pulses with a duration of 100 ms increasing from −50 mV to +70 mV in 10-mV steps for Kv3 channels and from −70 mV to +50 mV in 10-mV steps for Kv1.1 channels. The effect of the drugs was recorded during the superfusion of the cells, and washout of drug effect was measured during the superfusion of the cells with drug-free solution. The drugs were purchased from Abbot (Wiesbaden, Germany; halothane, enflurane, isoflurane) and Pharmacia (Erlangen, Germany; desflurane). The recorded signal was filtered at 3 kHz, digitized using an analog-to-digital converter (Digidata 1200; Axon Instruments, Foster City, CA), and stored on a 386 IBM-compatible personal computer with a sampling rate of 10 kHz for later analysis. The concentration of the volatile anesthetic agents in the test solution was measured during each individual experiment by gas chromatography (GC-14B; Shimatzu, Duisburg, Germany).

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Table 1. Clinical, Physicochemical, and Experimental Data of Volatile Anesthetics

G_{max}	CC (μM)	Oct	IC_{50} (μM)	Hill Coefficient	IC_{50}/CC	n
Halothane	190	315	1,850 ± 206	2.2 ± 0.4	10	31
Isoflurane	240	156	2,830 ± 283	2.5 ± 0.7	12	24
Enflurane	520	122	2,930 ± 235	2.0 ± 0.3	6	32
Desflurane	530	28.5	4,450 ± 1,000	1.7 ± 0.3	8	11

Clinical concentrations (CC, 1 minimum alveolar concentration), octanol–water coefficients (oct), Hill parameters, and the ratios of IC_{50} values and clinical concentrations of the volatile anesthetics (IC_{50}/CC). Concentration for half-maximal inhibition (IC_{50} values) and Hill coefficients resulted from the fit of the concentration-dependent effects on G_{max} of Kv3 channels according to the Hill function.

Data and Statistical Analysis

The peak outward potassium current in a trace was determined by fitting the current to a two-exponential time course (activation and inactivation) and determining the maximal amplitude of the fit. Peak currents were converted to conductances using the Nernst potential for potassium (−85 mV for the standard K^+ concentration gradient), and voltage was corrected using the measured series resistance and compensation level. The conductance-voltage data were fit to a Boltzmann function of the form:

$$G(V) = (G_{max} - G_{min})/(1 + \exp(-z_aF(V - V_{mid})/RT)) + G_{min} \quad (1)$$

where G_{max} and G_{min} are the maximum and minimum conductances measured for the dataset; V_{mid} is the voltage corresponding to 50% of maximum conductance; z_a is the effective gating charge; and R, T, and F have their usual meanings. Inhibition was defined as 1 minus the ratio of G_{max} in the presence of the drug to the mean of the current before and after application of the drug. The concentration–response curves were fitted with the equation

$$e/e_{max} = c^\gamma/[IC_{50}^\gamma + c^\gamma] \quad (2)$$

using nonlinear regression analysis (SAS Institute, Cary, NC). Here, e = effect, e_{max} = maximal effect, c = anesthetic concentration, and γ = Hill coefficient. Concentration–response data for inhibition of G_{max} were fitted with $e_{max} = 1$. The number is always the number of experiments. IC_{50} values and Hill coefficients are given as mean ± standard error of the mean as specified by the Sigma-Plot software (Kandel GmbH, Erkrath, Germany). All other data are given as mean ± SD unless stated otherwise. The values of minimum alveolar concentrations and octanol–water coefficients (table 1) were taken from literature sources.[9,10]

Results

The Kv3 current traces in figure 1 show the effects of the drugs investigated in this study. All volatile anesthetics reversibly inhibited these potassium channels natively expressed in human neuroblastoma SH-SY5Y cells. The halogenated ethers enflurane, isoflurane, and desflurane, but not the halogenated alkane halothane, accelerated macroscopic current decline (fig. 1A). The effects of the anesthetics on Kv3 channels were measured as inhibition of the maximal whole cell conductance (G_{max}; for details see Methods). During control conditions, G_{max} was 9.1 ± 3.9 nanosiemens (n = 89). Inhibition of G_{max} was concentration dependent and reversible at all concentrations. Inhibition was described mathematically by Hill functions (fig. 1B, table 1). The IC_{50} values for inhibition of G_{max} by the volatile anesthetics were between 1,800 μM for halothane and 4,450 μM for desflurane. The Hill coefficients were between 1.7 and 2.5.

With the intention to clarify whether the results reported may be unique to Kv3 channels, the actions of enflurane and isoflurane on the whole cell conductance of human Kv1.1 channels were also examined (fig. 2A). These ion channels were stably transfected in HEK293 cells. During control conditions, G_{max} was 23 ± 13 nanosiemens (n = 77). Both anesthetics reversibly inhibited Kv1.1 channels and accelerated macroscopic current decline (fig. 2A). Inhibition was concentration dependent (fig. 2B) and reversible at all concentrations. IC_{50} values for inhibition of G_{max} were 2,760 ± 369 (n = 22) for enflurane and 5,160 ± 219 (n = 29) for isoflurane. Hill coefficients were 3.9 ± 1.2 and 5.6 ± 0.8 for enflurane and isoflurane, respectively (fig. 2B).

Discussion

The volatile anesthetic agents halothane, enflurane, isoflurane, and desflurane reversibly inhibit human neuronal Kv3 channels in a concentration-dependent manner. The current study allows extending our observations with intravenous anesthetics[5] to the commonly used inhalation anesthetics.

Although the volatile anesthetics and the intravenous anesthetics both inhibit Kv3 channels in a concentration-dependent and reversible manner, the concentration–response curves and the ratios of IC_{50} values to clinical concentrations differ between both groups of anesthetic

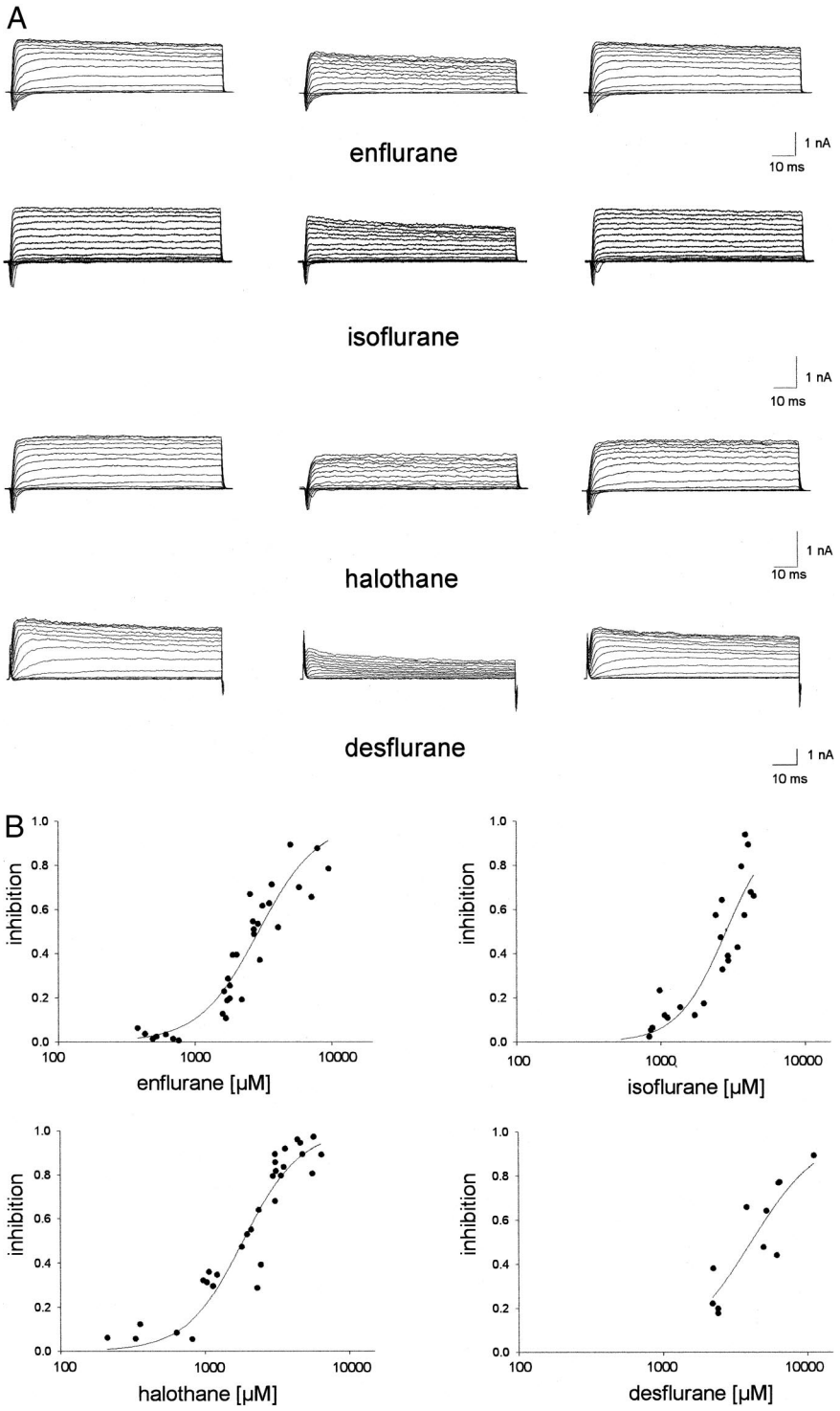

Fig. 1. (*A*) Superimposed traces of voltage-dependent Kv3 channels activated by depolarizing steps from a holding potential of −80 mV to test potentials ranging from −50 mV to +70 mV. The interpulse duration was 1 s. Shown are the current traces during control conditions, during the influence of the anesthetic agents on the K currents, and after the drugs were washed out. The concentrations used were 1,800 μM enflurane, 1,980 μM isoflurane, 1,100 μM halothane, and 6,100 μM desflurane. (*B*) Concentration–response curves for inhibition of maximal Kv3 whole cell conductance (G_{max}) by enflurane, isoflurane, halothane, and desflurane. The concentration of each volatile anesthetic agent was determined by gas chromatography and plotted against the inhibitory effect. IC_{50} values and Hill coefficients are given in table 1.

agents. Whereas IC_{50} values of intravenous anesthetics are on average 22-fold greater than of the MAC equivalent of intravenous anesthetics (CP_{50}),[5] half-maximal inhibition of Kv3 channels by volatile anesthetics occurs at concentrations on average 9-fold more than 1 MAC. Whereas intravenous anesthetics inhibit Kv3 channels with Hill coefficients close to unity (mean value 1.1, n = 7),[5] the Hill coefficient for inhibition by volatile anesthetics is 2.1 (mean value, n = 4). Despite these differences, inhibition of Kv3 channels by both groups of agents is on average similar at clinical concentrations. As calculated from the Hill functions at 1 MAC, human Kv3 channels would be inhibited by the volatile anesthetics by 2% (2.9% by intravenous anesthetics at CP_{50} values[5]). At 1 MAC or more, inhibition of Kv3 channels would be highest with enflurane (between 3% and 22% between 1 and 3 MAC) and lowest with isoflurane (between 0.2% and 3% between 1 and 3 MAC).

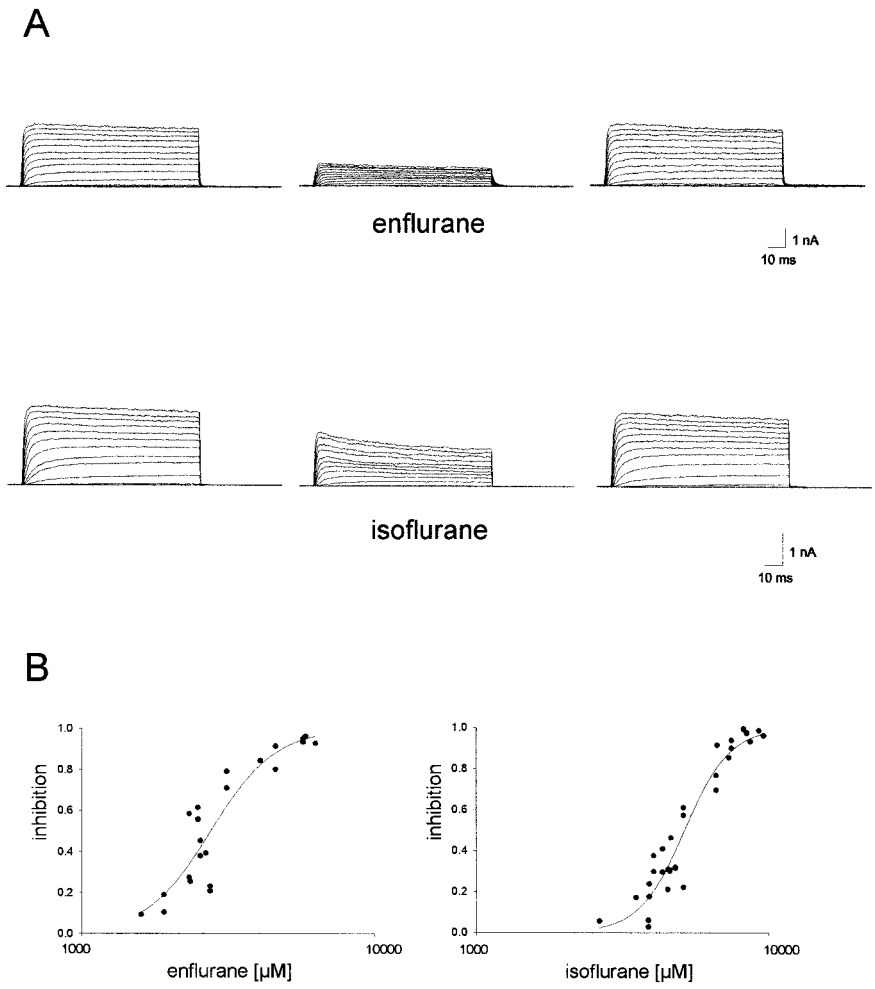

Fig. 2. (*A*) Superimposed traces of human voltage-dependent Kv1.1 currents evoked by depolarizing steps from a holding potential of −80 mV to test potentials ranging from −70 mV to +50 mV. The interpulse duration was 1 s. Shown are the current traces during control conditions, during the influence of enflurane (3,124 μM) and isoflurane (4,037 μM), and after the drug was washed out. (*B*) Concentration response curves for inhibition of maximal Kv1.1 whole cell conductance (G_{max}) by enflurane and isoflurane. The concentration of each volatile anesthetic agent was determined by gas chromatography and plotted against the inhibitory effect. IC_{50} values and Hill coefficients are given in the Results.

This potency difference between the structural isomers also is observed with Kv1.1 channels. Enflurane inhibits Kv1.1 and Kv3 channels with nearly identical IC_{50} values, whereas isoflurane inhibits Kv1.1 channels with an IC_{50} value of twice the concentration necessary for half-maximal inhibition of Kv3 channels. Hill coefficients differ between Kv3 channels and Kv1.1 channels for inhibition by both anesthetics. As a consequence, inhibition of Kv1.1 channels at clinical concentrations would only become obvious with enflurane (2% at 2 MAC and 8% at 3 MAC).

The smaller than half-maximal effects of the volatile anesthetics at clinical concentrations may suggest that inhibition of voltage-dependent K channels does not contribute to the pharmacologic action of these drugs during clinical anesthesia. However, a concentration–response curve at the cellular level needs not to be identical with the concentration–response curve of an intact neuronal network.[11,12] A recent mutational study, for example, shows that voltage-dependent K channels determine the response of a specific brain circuit of drosophila to halothane.[13] Therefore, the relative lack of sensitivity does not preclude voltage-dependent K channels from constituting a relevant biophysical target of anesthetic agents.

In this context, it may be interesting that inhibition of all 12 general anesthetics investigated on Kv3 channels (this study and that of Friederich and Urban[5]) correlates with clinical concentrations. This correlation is even better than the correlation of K channel inhibition and octanol–water coeffcients[14–16] ($r = 0.97$ *vs.* $r = 0.75$, $n = 12$). Therefore, lipophilicity alone seems not to predict the molecular actions of these drugs on Kv channels. Consequently, additional factors, such as specific polar interactions, must be involved in the observed inhibitory effects.[17]

In summary, volatile anesthetics inhibit human Kv3 and Kv1.1 channels in a concentration-dependent and reversible manner. Pharmacologic effects already occur at clinical concentrations. Whether inhibition of this class of ion channels by volatile anesthetics may contribute to clinically observed anesthetic drug effects needs further study.

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