Comparative Effects of Halogenated Inhaled Anesthetics on Voltage-gated Na\(^+\) Channel Function

Wei Ouyang, Ph.D.,* Karl F. Herold, M.D., Ph.D.,† Hugh C. Hemmings, Jr., M.D., Ph.D.‡

Background: Inhibition of voltage-gated Na\(^+\) channels (Na\(_v\)) is implicated in the synaptic actions of volatile anesthetics. We studied the effects of the major halogenated inhaled anesthetics (halothane, isoflurane, sevoflurane, enfurane, and desflurane) on Na\(_v\), a well-characterized pharmacological model for Na\(_v\) effects.

Methods: Na\(^+\) currents (\(I_{Na}\)) from rat Na\(_v\) \(\alpha\)-subunits heterologously expressed in Chinese hamster ovary cells were analyzed by whole cell voltage-clamp electrophysiological recording.

Results: Halogenated inhaled anesthetics reversibly inhibited Na\(_v\) in a concentration- and voltage-dependent manner at clinical concentrations. At equianesthetic concentrations, peak \(I_{Na}\) was inhibited with a rank order of desflurane > halothane > enfurane > isoflurane > sevoflurane from a physiologic holding potential (–80 mV). This suggests that the contribution of Na\(_v\) channel blockade to anesthesia might vary in an agent-specific manner. From a hyperpolarized holding potential that minimizes inactivation (–120 mV), peak \(I_{Na}\) was inhibited with a rank order of potency for tonic inhibition of peak \(I_{Na}\). Halothane > isoflurane > sevoflurane > enfurane > desflurane. Desflurane produced the largest negative shift in voltage-dependence of fast inactivation consistent with its more prominent voltage-dependent effects. A comparison between isoflurane and halothane showed that halothane produced greater facilitation of current decay, slowing of recovery from fast inactivation, and use-dependent block than isoflurane.

Conclusions: Five halogenated inhaled anesthetics all inhibit a voltage-gated Na\(^+\) channel by voltage- and use-dependent mechanisms. Agent-specific differences in efficacy for Na\(^+\) channel inhibition due to differential state-dependent mechanisms creates pharmacological diversity that could underlie subtle differences in anesthetic and nonanesthetic actions.

Both ligand-gated ion channels, including GABA\(_\text{A}\) (\(\gamma\)-aminobutyric acid, type A) receptors, glycine receptors, neuronal nicotinic acetylcholine receptors, and N-methyl-D-aspartic acid–type glutamate receptors, as well as voltage-gated ion channels, including Ca\(^{2+}\) channels, K\(^+\) channels, and Na\(^+\) channels, represent promising molecular targets for various general anesthetics. Depression of presynaptic action potential amplitude involving Na\(^+\) channel blockade has been implicated in inhibition of neurotransmitter release by the potent inhaled (volatile) anesthetics.\(^3\) The voltage-gated Na\(^+\) channel (Na\(_v\)) superfamily consists of 9 distinct genes that encode for the channel-forming \(\alpha\)-subunit (Na\(_v\)\(_{1.1}\)–1.9), each with tissue-dependent expression and functions.\(^4\) The potent inhaled anesthetics inhibit native neuronal Na\(_v\) channels\(^5\)–\(^7\) as well as heterologously expressed mammalian Na\(_v\) channel \(\alpha\)-subunits.\(^8\)–\(^11\) However, the relative potencies and channel-gating effects of various inhaled anesthetics have not been compared in detail. Although Na\(_v\) channel blockade is of enormous therapeutic importance for cardiac dysrhythmias, acute and chronic pain states, seizure disorders, and possibly general anesthesia, systemic administration of Na\(_v\) channel blockers is associated with severe cardiac and central nervous system side effects.\(^7\) Effects of anesthetics on central nervous system and peripheral Na\(_v\) isoforms are therefore likely to be involved in their anesthetic and some of their agent-specific nonanesthetic effects.

We characterized the effects of five potent inhaled anesthetics on the function and gating of rat Na\(_v\)\(_{1.4}\) \(\alpha\)-subunits heterologously expressed in Chinese hamster ovary cells. The skeletal muscle Na\(_v\) channel isoform Na\(_v\)\(_{1.4}\) is expressed at the neuromuscular junction, where it regulates muscle excitability.\(^12\) Na\(_v\)\(_{1.4}\) function is inhibited by local anesthetic and antidepressant drugs, and it provides a well-characterized model for studies of Na\(_v\) channel pharmacology that is amenable to genetic manipulation for detailed structure-function studies.\(^15\)–\(^17\) The halogenated alkane halothane and methylthyl ethers isoflurane, sevoflurane, enfurane, and desflurane represent the principal volatile anesthetics employed clinically in the modern era. Detailed kinetic characterization of their Na\(_v\) channel blocking effects is important for identifying common and/or distinct features that might contribute to agent-specific pharmacological profiles. We report here that all five anesthetics inhibit Na\(_v\)\(_{1.4}\) at concentrations in the clinical range in proportion to their potencies for producing anesthesia in vivo, which provides additional support for Na\(_v\) channel blockade as a plausible mechanism of inhaled anesthetic action. In addition, agent-specific differences in their relative potencies and involvement of state-dependent mechanisms could contribute to differences in their central and peripheral pharmacodynamic properties.

Materials and Methods

Cell Culture

Chinese hamster ovary cells stably transfected with rat Na\(_v\)\(_{1.4}\) \(\alpha\)-subunit (a gift from S. Rock Levinson, Ph.D.,
INHALED ANESTHETIC EFFECTS ON NAV1.4

Professor, Department of Physiology and Biophysics, University of Colorado Health Sciences Center, Denver, Colorado were cultured in 90% (v/v) Dulbecco’s Modified Eagle Medium, 10% (v/v) fetal bovine serum, 300 μg/ml G418 (Invitrogen, Carlsbad, CA), 100 units/ml penicillin, and 100 μg/ml streptomycin (Biosource, Rockville, MD) under 95% air/5% CO2 at 37°C. Cells were plated on glass coverslips in 35-mm plastic dishes (Becton Dickinson, Franklin Lakes, NJ) 1–3 days before electrophysiological recording.

Electrophysiology
Cells attached to coverslips were transferred to a plastic Petri dish (35 × 10 mm) on the stage of a Nikon ECLIPSE TE300 inverted microscope (Melville, NY). The culture medium was replaced, and cells were superfused at 1.5–2 ml/min with extracellular solution containing (in mM): NaCl 140; KCl 4; CaCl2 1.5; MgCl2 1.5; HEPES 10; d-glucose 5; pH 7.30 with NaOH. Studies were conducted at room temperature (24 ± 1°C) using conventional whole cell patch-clamp techniques.18 Patch electrodes (tip diameter < 1 μm) were made from borosilicate glass capillaries (Drummond Scientific, Broomall, PA) using a micropipette puller (P-97; Sutter Instruments, Novato, CA) and fire polished (Narishige Microforge, Kyoto, Japan). Electrode tips were coated with SYLGARD (Dow Corning Corporation, Midland, MI) to lower background noise and reduce capacitance; electrode resistance was 2–5 MΩ. The pipette electrode solution contained (in mM): CsF 80; CsCl 40; NaCl 15; HEPES 10; EGTA 10; pH 7.35 with CsOH. Currents were sampled at 10 kHz and filtered at 1–3 kHz using an Axon 200B amplifier, digitized via a Digidata 1321A interface, and analyzed using pClamp 8.2 (Axon/Molecular Devices, Sunnyvale, CA). Capacitance and 60–85% series resistance were compensated, and leak current was subtracted using P/4 or P/5 protocols. Cells were held at –80 mV between recordings. Only cells with Na+ series resistance and contributions of endogenous Na+ currents (< 50 pA) occasionally observed in Chinese hamster ovary cells.19

Anesthetics
Thymol-free halothane was obtained from Halocarbon Laboratories (River Edge, NJ); isoflurane and sevoflurane were from Abbott Laboratories (Abbott Park, IL); enflurane was from Anaquest Inc. (Liberty Corner, NJ); desflurane was from Baxter Healthcare Corporation (Deerfield, IL). Anesthetics were diluted from saturated aqueous stock solutions made in extracellular solution (14–16 mM halothane, 10–12 mM isoflurane, 4–6 mM sevoflurane, 10–12 mM enflurane, 8–10 mM desflurane) prepared 12–24 h before experiments into airtight glass syringes and applied locally to attached cells at 50–70 μl/min using an ALA-VM8 pressurized perfusion system (ALA Scientific, Westbury, NY) through a perfusion pipette (diameter, 0.15 mm) positioned 30–40 μm away from patched cells. Concentrations of volatile anesthetics were determined by local sampling of the perfusate at the site of the recording pipette tip and analysis by gas chromatography as described.3

Statistical Analysis
IC50 values were calculated by least squares fitting of data to the Hill equation: Y = 1/(1 + 10(logIC50 – X) × b), where Y is the effect, X is measured anesthetic concentration, and b is Hill slope. Activation curves were fitted to a Boltzmann equation of the form G/Gmax = 1/(1 + e(Vt/2a – V)/k), where G/Gmax is normalized fractional conductance, Gmax is maximum conductance, Vt/2a is voltage for half-maximal activation, and k is the slope factor. Na+ conductance (GNa) was calculated using the equation: GNa = INa/(Vt – VNa), where INa is peak Na+ current, Vt is test potential, and VNa is Na+ reversal potential (VNa = 69 mV). Fast inactivation curves were fitted to a Boltzmann equation of the form II/Imax = 1/(1 + e(Vt/2b – V)/k), where II/Imax is normalized current, Imax is maximum current, Vt/2b is voltage of half maximal inactivation, and k is slope factor. INa current decay was analyzed by fitting the decay phase of the current trace between 90% and 10% of maximal INa to the monoexponential equation I(t) = A·exp(−t/τ) + C, where A is maximal INa amplitude, C is plateau INa, t is time, and τ is time constant of current decay. Channel recovery from fast inactivation was fitted to the monoexponential function Y = A × (1 – exp(−t/τ)), where Y is fractional current recovery constrained to 1.0 at infinity time, A is normalized control amplitude, X is recovery time, and τ is time constant of recovery, and the goodness of fit was compared to that of a biexponential function. The effects of anesthetics were compared to control using sum-of-squares F test between curve fits of mean data. The time course of use-dependent decay of normalized INa was compared to fitting by the monoexponential equation I(t) = exp(−t/τuse · n) + C, where n is pulse number, C is plateau INa, and τuse is time constant of use-dependent decay. Data were analyzed using pClamp 8.2 (Axon/Molecular Devices), Prism 4.0 (GraphPad Software Inc., San Diego, CA), and SigmaPlot 6.0 (SPSS Science Software Inc., Chicago, IL). Curve fits were compared by sum-of-squares F test. Statistical significance was assessed by analysis of variance with Newman-Keuls post hoc test or paired or unpaired t tests, as appropriate; P < 0.05 was considered statistically significant.

Results
Inhibition of Peak INa
Average peak Na+ current (INa) in Chinese hamster ovary cells transfected with the Na1.4 α-subunit was −3.0 ± 0.2 nA (n = 11) from a holding potential of −120 mV. Peak INa was rapidly (onset < 1.5 min) and reversibly inhibited by all five inhaled anesthetics tested (fig. 1) and by the specific Na+ channel blocker tetrodo-
Fig. 1. Inhibition of Na\textsubscript{a},1.4 by equipotent concentrations of various inhaled anesthetics. Na\textsuperscript{+} currents (I\textsubscript{Na}) were recorded from a holding potential of –80 mV by 25-ms test steps to \(V_{\text{max}}\) (–10 or –20 mV) as shown in the inset. The effects of halothane (A, 0.40 mM; 1.1 minimum alveolar concentration [MAC]), isoflurane (B, 0.42 mM; 1.2 MAC), and sevoflurane (C, 0.46 mM; 1.0 MAC) from a holding potential of –80 mV (open bars) or –120 mV (filled bars) are shown in these representative traces (summary data are given in Results). The time-course of I\textsubscript{Na} inhibition expressed as fractional I\textsubscript{Na} (I\textsubscript{Na}/I\textsubscript{Na control}) during application of isoflurane (0.43 mM, 1.2 MAC) or halothane (0.39 mM, 1.1 MAC) for 1.5 min is shown in F. I\textsubscript{Na} was repetitively activated from a holding potential of –120 mV by 25-ms test pulses to –10 mV at 0.5-s intervals.

Fig. 2. Voltage-dependent inhibition of Nav1.4 by inhaled anesthetics. Equipotent concentrations (1 minimum alveolar concentration [MAC]) of inhaled anesthetics differentially inhibited \(I_{\text{Na}}\) from a holding potential of –80 mV (open bars) or –120 mV (filled bars). The measured concentrations of halothane (Halo), isoflurane (Iso), sevoflurane (Sevo), enflurane (Enf), and desflurane (Des) were 0.42 ± 0.05 mM (1.2 MAC), 0.46 ± 0.03 mM (1.3 MAC), 0.44 ± 0.03 mM (1.0 MAC), 0.82 ± 0.04 mM (1.1 MAC), and 0.83 ± 0.03 mM (1.0 MAC), respectively, from a holding potential of –80 mV; they were 0.38 ± 0.05 mM (1.1 MAC), 0.41 ± 0.04 mM (1.1 MAC), 0.45 ± 0.05 mM (1.0 MAC), 0.80 ± 0.05 mM (1.1 MAC), and 0.83 ± 0.04 mM (1.0 MAC), respectively, from a holding potential of –120 mV. Data are expressed as mean ± SEM (n = 4–12). ** P < 0.01 by unpaired t test.

Fig. 3. Concentration dependence of Na\textsubscript{a},1.4 inhibition by inhaled anesthetics. Normalized peak \(I_{\text{Na}}\) values from a holding potential of –120 mV were fitted to the Hill equation to yield IC\textsubscript{50} values in the millimolar range and Hill slopes of 2, except for halothane, which had a Hill slope of 1 (fig. 3); this suggests the possibility of two sites of interaction with Na\textsubscript{a},1.4 for the ethers versus a single site of interaction for the alkane.

Effects on Channel Gating

None of the anesthetics tested altered the current-voltage relationship or reversal potential for \(I_{\text{Na}}\) (fig. 4); data not investigated using a holding potential of –120 mV to maintain channels in the resting state, allowing assessment of resting channel block with minimal interference from voltage-dependent inactivation. All five anesthetics exhibited concentration-dependent inhibition of peak \(I_{\text{Na}}\) with IC\textsubscript{50} values in the millimolar range and Hill slopes of 2, except for halothane, which had a Hill slope of 1 (fig. 3); this suggests the possibility of two sites of interaction with Na\textsubscript{a},1.4 for the ethers versus a single site of interaction for the alkane.

Voltage-gated Na\textsuperscript{+} channels have at least three distinct conformational states: resting, open, and inactivated.\(^{13}\) The potency of each anesthetic for tonic inhibition was...
shown). At concentrations equivalent to ~1 MAC, all five anesthetics produced no significant shift in the voltage dependence of activation (fig. 5), with minor effects on slope factors (data not shown). The voltage dependence of fast inactivation was determined for the prototypical anesthetic isoflurane and for desflurane and halothane, which exhibited extremes in voltage sensitivity (fig. 2). Representative current traces, which reflect channel availability at various holding potentials, obtained using a protocol designed to minimize the influence of slow inactivation are shown in figure 6A. Isoflurane, halothane, and desflurane strongly enhanced inactivation in a concentration-dependent manner. Desflurane produced a greater negative shift in \( V_{1/2} \) than isoflurane or halothane as seen in curve fits of the mean data (fig. 6B). At concentrations of ~2 MAC, isoflurane, halothane, and desflurane shifted \( V_{1/2} \) by ~7 mV, ~9 mV, and ~15 mV, respectively (fig. 6B). Similar effects were evident when the data were analyzed by calculating the mean values of \( V_{1/2} \) derived from curve fits of the individual data sets (table 1). Slope factors, which reflect the voltage sensitivity of the inactivation gate, \( \tau_{in} \) were not significantly affected by isoflurane or halothane, but they were slightly increased by desflurane (table 1). Macroscopic current inactivation was examined by fitting the rate of decay of current elicited by depolarization from ~120 mV to \( V_{max} \) to a mono-exponential equation. Time constants of current decay (\( \tau_m \)) were reduced by desflurane > halothane > isoflurane (table 2). The effects of isoflurane and halothane on recovery of \( I_{Na} \) from fast inactivation were evaluated by a two-pulse protocol with varying time intervals (fig. 7). Both anesthetics slowed recovery from inactivation by increasing the time constant of recovery (\( \tau_r \), ms) derived from monoeexponential fits of the fractional current (fig. 7).

**Use-dependent Block**

Use-dependent block of \( \text{Nav}_{1.4} \) was evident as a reduction in normalized \( I_{Na} \) relative to the peak of the first pulse evaluated in a series of rapid depolarizing pulses (fig. 8). In the absence of anesthetic, repetitive pulses produced only small reductions in peak \( I_{Na} \). Both halothane and isoflurane at ~2 MAC reduced the time constant of use-dependent decay (\( \tau_{use} \)). Halothane produced a greater reduction in steady-state normalized \( I_{Na} \) amplitude than isoflurane (\( P < 0.05 \) by paired \( t \) test, \( n = 3 \)). These results are consistent with contributions of open channel block and/or enhanced inactivation to inhibition of \( \text{Nav}_{1.4} \).

**Discussion**

We compared the actions of five potent halogenated inhaled anesthetics on a single \( \text{Na}^+ \) channel isoform (\( \text{Nav}_{1.4} \)) expressed in a uniform mammalian cellular environment to enhance detection of possible agent-specific
effects on state-dependent inhibition. All five of these clinically used inhaled anesthetics, with representatives from both alkane and ether subclasses, inhibited currents conducted by the α-subunit of the Na\textsubscript{v1.4} voltage-gated Na\textsuperscript{+} channel isoform at clinically relevant concentrations consistent with a role for blockade of Na\textsubscript{v} in anesthetic immobilization.\textsuperscript{1} There were differences between agents in their potencies for inhibition of Na\textsubscript{v1.4} relative to their anesthetic potencies, and there were differences in the voltage-dependence of their inhibition. For example, at equianesthetic concentrations, desflurane was the most effective inhibitor of peak $I_{\text{Na}}$ from a near physiologic holding potential of -80 mV, and halothane was most effective from a hyperpolarized holding potential of -120 mV. Members of the same drug class have agent-specific differences in their effects on a single target with potential pharmacological implications. A clinical implication of these findings is that inhibition of Na\textsubscript{v1.4} could contribute to skeletal muscle-relaxing effects of anesthetics given the high density of Na\textsubscript{v1.4} at the neuromuscular junction.\textsuperscript{12} Indeed the greater inhibition of Na\textsubscript{v1.4} by desflurane at 1 MAC correlates with its relatively greater enhancement of nondepolarizing neuromuscular blocking drug potency during anesthesia in vivo in human subjects.\textsuperscript{22}

**Fig. 6.** Effects of isoflurane, halothane, and desflurane on voltage dependence of Na\textsubscript{v1.4} fast inactivation. (A) Representative traces show inhibition of $I_{\text{Na}}$ by isoflurane (left), halothane (middle), and desflurane (right) using a fast inactivation protocol (inset) involving a conditioning pulse of 30 ms followed by a test pulse of 25 ms to minimize slow inactivation. Normalized data were fitted to the Boltzmann equation to yield voltage of 50% inactivation ($V_{1/2}$) and slope factor for $-1$ minimum alveolar concentration (MAC) (B) or $-2$ MAC (C). Each anesthetic significantly shifted the $V_{1/2}$, in the negative direction as determined by sum-of-squares F test comparison between curve fits of mean data ($P<0.05$). Parameters derived from analysis of independent curve fits are presented in table 1. Anesthetic concentrations were 0.46 ± 0.09 mM and 0.82 ± 0.07 mM for isoflurane, 0.40 ± 0.06 mM and 0.77 ± 0.10 mM for halothane, and 0.82 ± 0.06 mM and 1.61 ± 0.07 mM for desflurane. Data are expressed as mean ± SEM, n = 5-7.
Table 1. Inhaled Anesthetic Effects on Na\textsubscript{v} \textsubscript{1.4} Inactivation

<table>
<thead>
<tr>
<th>Anesthetic</th>
<th>(V_{1/2}\text{in}, \text{mV})</th>
<th>(k)</th>
<th>(n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isoflurane</td>
<td>-49.1 ± 1.4</td>
<td>6.5</td>
<td>4</td>
</tr>
<tr>
<td>Control</td>
<td>-55.4 ± 2.6</td>
<td>6.9</td>
<td>4</td>
</tr>
<tr>
<td>Halothane</td>
<td>-48.8 ± 2.5</td>
<td>7.0</td>
<td>5</td>
</tr>
<tr>
<td>Desflurane</td>
<td>-57.8 ± 2.5(\dagger)</td>
<td>7.2</td>
<td>4</td>
</tr>
<tr>
<td>Control</td>
<td>-57.2 ± 1.1</td>
<td>6.6</td>
<td>5</td>
</tr>
<tr>
<td>Desflurane</td>
<td>-69.7 ± 3.0(\dagger)$|</td>
<td>7.4</td>
<td>3*</td>
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<table>
<thead>
<tr>
<th>Anesthetic</th>
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<td>Isoflurane</td>
<td>-53.2 ± 2.3</td>
<td>7.1</td>
<td>5</td>
</tr>
<tr>
<td>Control</td>
<td>-63.3 ± 2.1*</td>
<td>8.4</td>
<td>5</td>
</tr>
<tr>
<td>Halothane</td>
<td>-54.1 ± 2.2</td>
<td>7.3</td>
<td>4</td>
</tr>
<tr>
<td>Desflurane</td>
<td>-64.9 ± 2.8(\dagger)</td>
<td>7.9</td>
<td>5</td>
</tr>
<tr>
<td>Control</td>
<td>-58.7 ± 1.2</td>
<td>6.7</td>
<td>3</td>
</tr>
<tr>
<td>Desflurane</td>
<td>-83.2 ± 1.6(\dagger)$|</td>
<td>7.5</td>
<td>4*</td>
</tr>
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</table>

Data for each experiment were fitted to a standard Boltzmann equation, and \(V_{1/2}\text{in}\) and slope values for each experiment were averaged and displayed as mean ± SEM. \(P \leq 0.05\), \(\dagger P \leq 0.01\), \(\| P \leq 0.001\) vs. respective control by paired \(t\) test. \(\dagger P \leq 0.05\) vs. halothane by one-way analysis of variance with Newman-Keuls post hoc test. \(k\) = slope factor; MAC = minimum alveolar concentration; \(V_{1/2}\text{in}\) = voltage of half-maximal inactivation.

Table 2. Inhaled Anesthetic Effects on Na\textsubscript{v} \textsubscript{1.4} Current Decay

<table>
<thead>
<tr>
<th>Anesthetic</th>
<th>(\tau_m), \text{ms}</th>
<th>Anesthetic Concentration, (\text{mm})</th>
<th>(n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isoflurane</td>
<td>0.54 ± 0.08</td>
<td>0.47 ± 0.08*</td>
<td>0.46 ± 0.05</td>
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<td>Halothane</td>
<td>0.52 ± 0.09</td>
<td>0.43 ± 0.08*</td>
<td>0.85 ± 0.04</td>
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<td>Desflurane</td>
<td>0.51 ± 0.07</td>
<td>0.42 ± 0.04*</td>
<td>0.43 ± 0.03</td>
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<tr>
<td></td>
<td>0.55 ± 0.07</td>
<td>0.39 ± 0.06$\dagger$</td>
<td>0.81 ± 0.04</td>
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<tr>
<td></td>
<td>0.65 ± 0.10</td>
<td>0.40 ± 0.05$\dagger$</td>
<td>0.80 ± 0.06</td>
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<tr>
<td></td>
<td>0.54 ± 0.09</td>
<td>0.34 ± 0.08$\dagger|</td>
<td>1.65 ± 0.14</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. \(\dagger P \leq 0.01\), \(\| P \leq 0.001\) vs. respective control by paired \(t\) test. \(\dagger P \leq 0.05\) vs. isoflurane, \(\| P \leq 0.005\) vs. halothane by one-way analysis of variance with Newman-Keuls post hoc test. \(\tau_m\) = time constant of current decay calculated for a prepulse potential of -120 mV.

Inhaled anesthetics are known to inhibit various isoforms of \(Na_v\) \(\alpha\)-subunits heterologously expressed in Chinese hamster ovary cells (rat \(Na_{v,1.2}\), \(Na_{v,1.4}\), and \(Na_{v,1.5}\)),\textsuperscript{11} human embryonic kidney cells (HEK 293, human \(Na_{v,1.5}\)),\textsuperscript{9} and \textit{Xenopus} oocytes (rat \(Na_{v,1.2}\) and 1.6, human \(Na_{v,1.4}\)).\textsuperscript{10} Small differences in potencies reported between various studies probably result from differences in isoform sensitivity, species, expression systems, \(\beta\)-subunit coexpression, recording conditions, stimulation protocols, anesthetic concentration determinations, etc. Isoflurane at clinically relevant concentrations inhibits rat neuronal (\(Na_{v,1.2}\), skeletal muscle (\(Na_{v,1.4}\), and cardiac muscle (\(Na_{v,1.5}\) voltage-gated \(Na^+\) channel \(\alpha\)-subunits studied under identical conditions with isoform-dependent differences in state-dependent block.\textsuperscript{11} Significantly lower \(IC_{50}\) values for isoflurane were observed at more physiologic holding potentials\textsuperscript{11} due to marked voltage-dependent effects on channel-gating compared to the hyperpolarized potential used to characterize tonic block in the current study. The \(IC_{50}\) for inhibition of \(Na_{v,1.4}\) by isoflurane reported previously for a holding potential of -100 mV (\(IC_{50} = 0.99\text{ mm}\))\textsuperscript{10} compares well with the value obtained in the present study for a holding potential of -120 mV (\(IC_{50} = 1.16\text{ mm}\)). Rat \(Na_{v,1.8}\) in \textit{Xenopus} oocytes has been reported to be insensitive to isoflurane,\textsuperscript{10} but recent evidence indicates that rat \(Na_{v,1.8}\) expressed in a mammalian neuroblastoma cell line is inhibited by isoflurane at concentrations comparable to those effective on other isoforms (unpublished data, 2008; Karl F. Herold, M.D., and Hugh C. Hemmings, M.D., Ph.D., New York NY). All mammalian \(Na_v\) isoforms tested so far are susceptible to inhibition by the prototypical inhaled anesthetic isoflurane with minor isoform-specific differences in relative potency and mechanism.

Human \(Na_{v,1.4}\) heterologously expressed in \textit{Xenopus} oocytes is inhibited by isoflurane and halothane,\textsuperscript{10} whereas rat \(Na_{v,1.4}\) in the same expression system was reported to be insensitive to halothane unless coexpressed with protein kinase C (PKC).\textsuperscript{24} Our results indicate that rat \(Na_{v,1.4}\) expressed in a mammalian cell line is inhibited by multiple inhaled anesthetics in the absence of overexpression or pharmacological activation of PKC, indicating that PKC activation is apparently not required for inhibition. A requirement for activation of endogenous PKC, which can be activated by halogenated inhaled anesthetics,\textsuperscript{25} cannot be excluded, however. Attempts to test this possibility by inhibition of endogenous PKC using small-molecule PKC inhibitors have been unsuccessful because the PKC inhibitors themselves inhibit \(Na^+\) channels.\textsuperscript{26}
Inhaled anesthetics negatively shift the voltage dependence of \( I_{\text{Na}} \) fast inactivation. Similar shifts in inactivation of \( I_{\text{Na}} \) are produced in ventricular cardiomyocytes by isoflurane and halothane\(^9\) and in rat neurohypophysial nerve terminals\(^6\) and heterologously expressed Na\(_v\) isoforms by isoflurane.\(^10,11\) Negative shifts in the voltage dependence of fast inactivation suggest greater anesthetic binding affinity and selective stabilization of inactivated states. The large negative shift in \( V_{1/2}\text{in} \) by desflurane contributes to its greater potency compared to isoflurane and halothane for inhibition of Na\(_v\)1.4 from the more positive (physiologic) holding potential of \(-80\) mV vs. \(-120\) mV. Preferential anesthetic interaction with the nonconducting inactivated state of Na\(_v\)1.4 is also consistent with anesthetic slowing of recovery from fast inactivation. The slightly greater slowing effect of halothane compared to isoflurane is consistent with a greater shift in \( V_{1/2}\text{in} \) and hence stronger interaction with the fast inactivated state for halothane.

Enhanced inactivation is critical to inhibition of Na\(_v\)1.4 by inhaled anesthetics at more positive membrane potentials. This has classically been attributed to fast inactivation, but recent evidence implicates slow inactivation, but recent evidence implicates slow inactivation. This has classically been attributed to fast inactivation, but recent evidence implicates slow inactivation.

The possible role of slow inactivation in the Na\(_v\) channel-blocking effects of inhaled anesthetics is not clear, but it is an important area for future investigation that will be facilitated using mutations in Na\(_v\)1.4 that enhance or remove inactivation.\(^27\) Possible mechanisms underlying state-dependent effects include facilitated transitions from the closed to inactivated state (tonic block), open to inactivated state (enhanced inactivation), and/or stabilization of inactivated states (delayed recovery). Small differences between anesthetics in their state-dependent effects on various Na\(_v\) isoforms\(^11\) could underlie anesthetic- and isoform-specific differences in pharmacological profiles of specific anesthetics in vivo, e.g., differential effects on brain, heart, and skeletal muscle.

All five inhaled anesthetics tested produced insignificant shifts in the voltage dependence of Na\(_v\)1.4 activation, similar to the findings of Stadnicka et al.\(^6\) but in contrast to those of Weigt et al.,\(^25\) who found small negative shifts for Na\(_v\)1.5. These findings suggest isoform-selective interactions between volatile anesthetics and the activation process similar to those observed for the local anesthetic lidocaine.\(^28\) Another class of anesthetics, the n-alkanols, also inhibits voltage-gated Na\(^+\) channels at anesthetic concentrations, but with somewhat distinct mechanisms from those of inhaled anesthetics that involve primarily open channel block with relatively small effects on both activation and inactivation.\(^29\) Thus the n-alkanols and inhaled anesthetics both inhibit Na\(^+\) channels, but they differ in the relative involvement of open channel block and activation (greater for the alkanols) versus activation mechanisms (greater for inhaled anesthetics).

Both halothane and isoflurane exhibited use-dependent block with repetitive stimuli. The fraction of open versus inactivated channels increases during fast repetitive depolarizations; therefore, the presence of use-dependent block suggests a possible role for open-channel block and/or slow inactivation mechanisms by both anesthetics.\(^17,21\) Halothane was more efficacious than isoflurane in inhibiting normalized current amplitude with repetitive stimuli consistent with its greater tonic \( I_{\text{Na}} \) blocking effect. Open-channel block is particularly important in pathologic conditions such as myotonia and periodic paralysis, which involve Na\(_v\)1.4 inactivation gating defects,\(^30\) inflammatory and neuropathic pain states that involve repetitive activation of Na\(_v\)1.7 and Na\(_v\)1.8,\(^31,32\) and ischemia, which leads to resting membrane depolarization.\(^33\) Accessory subunits can have important effects on the pharmacological and gating properties of voltage-gated Na\(_v\) channels.

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**Figure 7.** Effects of isoflurane and halothane on recovery of Na\(_v\)1.4 from fast inactivation. The protocol (upper right inset) involved 12 depolarizing test steps at various recovery times (\( t \)) in 2.5-ms intervals. Representative current traces obtained for the effects of isoflurane (2.3 minimum alveolar concentration [MAC]) and halothane (2.2 MAC) are shown on the right. The time-course of channel recovery from fast inactivation was best fitted by a monoexponential function in all cases to yield a recovery time constant (\( \tau_r \)). The rate of recovery, expressed as current normalized to initial control current, was slowed by isoflurane (\( A \)) and halothane (\( B \)). The recovery time constant was greater for halothane than for isoflurane at the higher concentrations as determined by sum-of-squares F test between curve fits of mean data (\( P < 0.001 \)). Mean isoflurane concentrations were \( 0.44 \pm 0.06 \) mm and \( 0.82 \pm 0.08 \) mm; mean halothane concentrations were \( 0.41 \pm 0.07 \) ms and \( 0.78 \pm 0.10 \) mm. Data are expressed as mean \( \pm \) SEM, \( n = 5–8 \). **versus** control.
Na\(^+\) channels that must be considered in pharmacological studies, although \(\alpha\)-subunit expression is sufficient to mimic native Na\(^+\) channel-gating properties.\(^4\) Modulation of Na\(^+\) function by \(\beta\)-subunits depends on both the \(\alpha\)-subunit isoform and the cell type used for expression. Coexpression of the \(\beta\)-1 subunit has no effect on inhibition by isoflurane of Na\(^+\), Na\(^+\), Na\(^+\), or Na\(^+\) in Xenopus oocytes.\(^10\) In mammalian expression systems, \(\beta\)-1 subunit coexpression has no major effects on local anesthetic sensitivity, current kinetics, or activation and inactivation properties of tetrodotoxin-sensitive currents in ND7/23 cells,\(^34\) but it produces positive shifts of channel activation and inactivation of Na\(^+\), Na\(^+\), and Na\(^+\) in human embryonic kidney 293t cells.\(^36\) Although we have not ruled out possible effects of \(\beta\)-subunit coexpression on inhaled anesthetic sensitivity of Na\(^+\), Na\(^+\), and Na\(^+\) in Chinese hamster ovary cells, these findings make a major effect unlikely.

The role of voltage-gated Na\(^+\) channels in the mechanisms of inhaled anesthetics is an important and unresolved question, but a number of factors impede its resolution.\(^1\) Correlations between in vitro effects on Na\(^+\) currents and anesthetic potencies in vivo are limited by our ignorance regarding the specific cells, networks, and molecular targets involved in the behavioral effects of inhaled anesthetics (immobilization in the case of MAC). We are thus unable to define the degree of Na\(^+\) channel inhibition critical for an anesthetic effect that must be taken into consideration when evaluating correlations between potencies measured in vitro and in vivo. Interestingly, experiments with local anesthetics suggest that relatively small degrees of Na\(^+\) blockade (10–20% inhibition of peak current amplitude) can have profound effects on neuronal firing rate.\(^37\) Moreover, determination of anesthetic effects on ion channels in vitro is subject to numerous experimental variables, including the species and isoform of the channel, expression system used, accessory subunits, modulation by cellular signaling pathways, temperature, experimental conditions, including holding potentials, and stimulus protocols, etc. These and other factors can have profound effects on channel function and pharmacological sensitivity. Given these reservations, the observation that all five inhaled anesthetics tested inhibit Na\(^+\), Na\(^+\) supports, but does not prove, an important role for Na\(^+\) inhibition in anesthesia. Additional support for this hypothesis is provided by the recent observation that intrathecal administration of the Na\(^+\), agonist veratridine increases MAC in rats.\(^38\)

In summary, halogenated inhaled anesthetics all inhibit heterologously expressed Na\(^+\), Na\(^+\), at clinical concentrations for inhaled anesthetic action. Small agent-specific differences in relative potency and gating effects are consistent with subtle interagent variability in pharmacodynamic profiles, such as skeletal muscle relaxant effects. Agent-specific differences in potency for Na\(^+\), Na\(^+\), inhibition at normal resting membrane potential were determined primarily by differences in state-dependent block reflected in effects on inactivation gating. These gating effects of inhaled anesthetics are remarkably similar to those of local anesthetics, and they suggest the possibility of overlapping binding sites,\(^13\)–\(^17\) an interesting hypothesis that can now be tested by detailed structure-function studies in Na\(^+\), using mutations that affect gating mechanisms and local anesthetic sensitivity.

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