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 (Nav) is implicated in the synaptic actions of volatile anesthetics. We studied the effects of the major halogenated inhaled anesthetics (halothane, isoflurane, sevoflurane, enflurane, and desflurane) on Na$\textsubscript{1.4}$, a well-characterized pharmacological model for Nav effects.

Methods: Na$^+$ currents (I$\textsubscript{Na}$) from rat Na$\textsubscript{1.4}$ $\alpha$-subunits heterologously expressed in Chinese hamster ovary cells were analyzed by whole cell voltage-clamp electrophysiological recording.

Results: Halogenated inhaled anesthetics reversibly inhibited Na$\textsubscript{1.4}$ in a concentration- and voltage-dependent manner at clinical concentrations. At equiphasiological concentrations, peak I$\textsubscript{Na}$ was inhibited with a rank order of desflurane $>$ halothane $>$ isoflurane $>$ sevoflurane $>$ enflurane $>$ desflurane. Desflurane produced the largest negative shift in voltage-dependent inactivation (−120 mV), peak I$\textsubscript{Na}$ was inhibited with a rank order of potency for tonic inhibition of peak I$\textsubscript{Na}$ of halothane $>$ isoflurane $>$ sevoflurane $>$ enflurane $>$ 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 create pharmacologic diversity that could underlie subtle differences in anesthetic and nonanesthetic actions.

Materials and Methods

Cell Culture

Chinese hamster ovary cells stably transfected with rat Na$\textsubscript{1.4}$ $\alpha$-subunit (a gift from S. Rock Levinson, Ph.D.,...
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, Midland, MI) to lower background noise and electrode resistance was 2–5 MΩ. 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⁺ currents of 0.5–3.5 nA were analyzed to minimize increasing series resistance and contributions of endogenous Na⁺ currents (< 50 pA) occasionally observed in Chinese hamster ovary cells.₁⁹

Anaesthetics Thymol-free halothane was obtained from Halocarbon Laboratories (River Edge, NJ); isofluorane and sevoflurane were from Abbott Laboratories (Abbott Park, IL); enfu- ranes was from Anaquest Inc. (Liberty Corner, NJ); desflurane was from Baxter Healthcare Corporation (Deerfield, IL). Anaesthetics were diluted from saturated aqueous stock solutions made in extracellular solution (14–16 mM halothane, 10–12 mM isofluorane, 4–6 mM sevoflurane, 10–12 mM enfurane, 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 pi- pette (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.₅

Statistical Analysis IC₅₀ values were calculated by least squares fitting of data to the Hill equation: Y = 1/(1 + 10(exp(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(V₁/₂ₐ - Vₐ)/k), where G/Gₘₐₓ is normalized fractional conductance, Gₘₐₓ is maximum conductance, V₁/₂ₐ is voltage for half-maximal activation, and k is the slope factor. Na⁺ conductance (Gₙₐ) was calculated using the equation: Gₙₐ = Iₙₐ/(Vₐ - Vₑ), where Iₙₐ is peak Na⁺ current, Vₑ is test potential, and Vₐ is Na⁺ reversal potential (Eₙₐ = 69 mV). Fast inactivation curves were fitted to a Boltzmann equation of the form I/Iₘₐₓ = 1/(1 + e(V₁/₂₂ₐ - V)/k), where I/Iₘₐₓ is normalized current, Iₘₐₓ is maximum current, V₁/₂₂ₐ 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 monoeponential equation Iₙₐ = A · exp(-t/τₐ) + C, where A is maximal Iₙₐ amplitude, C is plateau Iₙₐ, t is time, and τₐ is time constant of current decay. Channel recovery from fast inactivation was fitted to the monoeponential function Y = A × (1 - exp(-t/τₑ) × X), where Y is fractional current recovery constrained to 1.0 at infinity time, A is normalized control amplitude, X is recovery time, and τₑ 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 analyzed by fitting the monoexponential equation Iₙₐ = exp(-t/τₑ - n), where n is pulse number, C is plateau Iₙₐ, and τₑ 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 Naᵥ1.4 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-
inhibited from a holding potential of –120 mV by 25-ms test steps to V\text{max} (–10 or –20 mV) as shown in the inset. The effects of halothane (Δ, 0.40 mM; 1.1 minimum alveolar concentration [MAC]), isoflurane (B, 0.42 mM; 1.2 MAC), and desflurane (C, 0.46 mM; 1.0 MAC), enflurane (D, 0.81 mM; 1.1 MAC), and sevoflurane (E, 0.85 mM; 1.1 MAC) at –1 MAC are shown in these representative traces (summary data are given in Results). The time-course of \( I_{\text{Na}} \) inhibition expressed as fractional \( I_{\text{Na}} (I_{\text{Na}}/I_{\text{Na}} \text{ control}) \) 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. \( I_{\text{Na}} \) was repetitively activated from a holding potential of –120 mV by 25-ms test pulses to –10 mV at 0.5-s intervals. 

Toxin (data not shown). Inhibition was greater from the more physiologic holding potential of –80 mV than from the hyperpolarized potential of –120 mV (fig. 2), indicative of significant voltage-dependent inhibition. At aqueous concentrations equivalent to 1 minimum alveolar concentration (MAC) for rat (0.35 mM for halothane and isoflurane, 0.46 mM for sevoflurane, 0.75 mM for enflurane, and 0.80 mM for desflurane), desflurane showed the greatest inhibition of peak \( I_{\text{Na}} \) from a holding potential of –80 mV. The rank order of inhibition was desflurane (57 ± 6.2% at 0.83 ± 0.06 mM, \( n = 4 \)) > halothane (32 ± 3.5% at 0.42 ± 0.05 mM, \( n = 6 \)) > enflurane (32 ± 6.7% at 0.82 ± 0.06 mM, \( n = 5 \)) > isoflurane (19 ± 1.9% at 0.46 ± 0.04 mM, \( n = 4 \)) > sevoflurane (17 ± 3.3% at 0.44 ± 0.04 mM, \( n = 4 \); mean ± SEM). 

The degree of voltage-dependent inhibition (difference between efficacy at –80 mV vs. –120 mV) varied between anesthetics and was greatest for desflurane and least for halothane (fig. 2).

Voltage-gated \( \text{Na}^+ \) channels have at least three distinct conformational states: resting, open, and inactivated.\(^\text{13} \)

The potency of each anesthetic for tonic inhibition was 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 \( IC_{50} \) 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 \( Na_{\text{,1.4}} \) 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 shown).

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Fig. 1. Inhibition of \( Na_{\text{,1.4}} \) by equipotent concentrations of various inhaled anesthetics. \( \text{Na}^+ \) currents (\( I_{\text{Na}} \)) 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 (Δ, 0.40 mM; 1.1 minimum alveolar concentration [MAC]), isoflurane (B, 0.42 mM; 1.2 MAC), and desflurane (C, 0.46 mM; 1.0 MAC), enflurane (D, 0.81 mM; 1.1 MAC), and sevoflurane (E, 0.85 mM; 1.1 MAC) at –1 MAC are shown in these representative traces (summary data are given in Results). The time-course of \( I_{\text{Na}} \) inhibition expressed as fractional \( I_{\text{Na}} (I_{\text{Na}}/I_{\text{Na}} \text{ control}) \) 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. \( I_{\text{Na}} \) 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 \( Na_{\text{,1.4}} \) 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 (1.0 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 \( Na_{\text{,1.4}} \) 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 \( IC_{50} \) values (± SE) and Hill slopes. \( IC_{50} \) values differed from each other by sum-of-squares F test (\( P < 0.05 \)). □ = halothane; △ = isoflurane; ▽ = sevoflurane; ◇ = enflurane; ○ = desflurane.
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 $V_{1/2i}$ than isoflurane or halothane as seen in curve fits of the mean data (fig. 6B). At concentrations of ~1 MAC, isoflurane, halothane, and desflurane shifted $V_{1/2i}$ by ~7 mV, ~9 mV, and ~13 mV, respectively (fig. 6B). Similar effects were evident when the data were analyzed by calculating the mean values of $V_{1/2i}$ derived from curve fits of the individual data sets (table 1). Slope factors, which reflect the voltage sensitivity of the inactivation gate, 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 $V_{\text{max}}$ to a mono-exponential equation. Time constants of current decay ($\tau_i$) were reduced by desflurane > halothane > isoflurane (table 2). The effects of isoflurane and halothane on recovery of $I_{\text{Na}}$ 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 monoexponential fits of the fractional current (fig. 7).

Use-dependent Block

Use-dependent block of Na$_{\text{v}1.4}$ was evident as a reduction in normalized $I_{\text{Na}}$ 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 $I_{\text{Na}}$. Both halothane and isoflurane at ~2 MAC reduced the time constant of use-dependent decay ($\tau_{\text{use}}$). Halothane produced a greater reduction in steady-state normalized $I_{\text{Na}}$ 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 Na$_{\text{v}1.4}$.

Discussion

We compared the actions of five potent halogenated inhaled anesthetics on a single Na$^+$ channel isoform (Na$_{\text{v}1.4}$) 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{a,1.4} voltage-gated Na\textsuperscript{+} channel isoform at clinically relevant concentrations consistent with a role for blockade of Na\textsubscript{a} in anesthetic immobilization.\textsuperscript{1} There were differences between agents in their potencies for inhibition of Na\textsubscript{a,1.4} 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 $I_{Na}$ 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{a,1.4} could contribute to skeletal muscle-relaxing effects of anesthetics given the high density of Na\textsubscript{a,1.4} at the neuromuscular junction.\textsuperscript{12} Indeed the greater inhibition of Na\textsubscript{a,1.4} by desflurane at 1 MAC correlates with its relatively greater enhancement of nondepolarizing neuromuscular blocking drug potency during anesthesia in \textit{vivo} in human subjects.\textsuperscript{22}

**Fig. 6.** Effects of isoflurane, halothane, and desflurane on voltage dependence of Na\textsubscript{a,1.4} fast inactivation. (A) Representative traces show inhibition of $I_{Na}$ 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 ($V_{1/2in}$) and slope factor for −1 minimum alveolar concentration (MAC) (B) or −2 MAC (C). Each anesthetic significantly shifted the $V_{1/2in}$ 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{\textit{v}}.1.4 Inactivation

<table>
<thead>
<tr>
<th>Anesthetic</th>
<th>(V_{1/2,mV})</th>
<th>(k)</th>
<th>(n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>49.1 ± 1.4</td>
<td>6.5 ± 0.2</td>
<td>4</td>
</tr>
<tr>
<td>Isoflurane (0.46 ± 0.09 mm)</td>
<td>55.4 ± 2.6</td>
<td>6.9 ± 0.1</td>
<td>4</td>
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<tr>
<td>Halothane (0.40 ± 0.06 mm)</td>
<td>57.8 ± 2.5$\dagger$</td>
<td>7.2 ± 0.4</td>
<td>7</td>
</tr>
<tr>
<td>Control</td>
<td>57.2 ± 1.1</td>
<td>6.6 ± 0.1</td>
<td>5</td>
</tr>
<tr>
<td>Desflurane (0.82 ± 0.06 mm)</td>
<td>69.7 ± 3.0$\dagger$$\dagger$</td>
<td>7.4 ± 0.3$\star$</td>
<td>5</td>
</tr>
</tbody>
</table>

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

Closed resting state.\textsuperscript{21} Our results are comparable to those reported for the cardiac isoform Na\textsubscript{\textit{v}}.1.5, for which equianesthetic concentrations of halothane are more potent than isoflurane in tonic blockade of human\textsuperscript{9} and guinea pig\textsuperscript{23} cardiac \(I_{Na^+}\). Isoflurane, halothane, and desflurane, which were analyzed in more detail, exhibited state-dependent block and a negative shift in the voltage dependence of inactivation, consistent with enhancement of inactivation at physiologic holding potentials. State-dependent block has been reported previously for isoflurane effects on \(I_{Na^+}\) in rat neurohypophysial nerve terminals,\textsuperscript{7} guinea pig cardiomyocytes,\textsuperscript{23} and heterologously expressed Na\textsubscript{\textit{v}}.1.2, Na\textsubscript{\textit{v}}.1.4, Na\textsubscript{\textit{v}}.1.5, and Na\textsubscript{\textit{v}}.1.6\textsuperscript{8-11}. Moreover, isoflurane and halothane affected channel-gating, evident as accelerated current decay and use-dependent block (halothane > isoflurane). The similarity of these effects of inhaled anesthetics to the effects of local anesthetics, antidepressants, and anticonvulsants on Na\textsubscript{\textit{v}}.1.2 and Na\textsubscript{\textit{v}}.1.4\textsuperscript{13-17} currents suggests conserved or overlapping drug binding sites and/or allosteric conformational mechanisms for these chemically diverse \(Na^+\) channel antagonists. The relative contributions of open state block and enhanced fast and/or slow inactivation to use-dependent block by inhaled anesthetics, which differs between various \(Na^+\) channel blockers, should be resolvable by examining anesthetic effects on fast inactivation-deficient \(Na^+\) channels.

Inhaled anesthetics are known to inhibit various isoforms of Na\textsubscript{\textit{v}}.1.4,\textsubscript{11} which heterologously expressed in Chinese hamster ovary cells (rat Na\textsubscript{\textit{v}}.1.2, Na\textsubscript{\textit{v}}.1.4, and Na\textsubscript{\textit{v}}.1.5),\textsuperscript{11} human embryonic kidney cells (HEK 293, human Na\textsubscript{\textit{v}}.1.5),\textsuperscript{9} and Xenopus oocytes (rat Na\textsubscript{\textit{v}}.1.2 and 1.6, human Na\textsubscript{\textit{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\textsubscript{\textit{v}}.1.2), skeletal muscle (Na\textsubscript{\textit{v}}.1.4), and cardiac muscle (Na\textsubscript{\textit{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\textsubscript{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\textsubscript{50} for inhibition of Na\textsubscript{\textit{v}}.1.4 by isoflurane reported previously for a holding potential of –100 mV (IC\textsubscript{50} = 0.99 mM)\textsuperscript{11} compares well with the value obtained in the present study for a holding potential of –120 mV (IC\textsubscript{50} = 1.16 mM). Rat Na\textsubscript{\textit{v}}.1.8 in Xenopus oocytes has been reported to be insensitive to isoflurane,\textsuperscript{10} but recent evidence indicates that rat Na\textsubscript{\textit{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\textsubscript{\textit{v}}.1 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\textsubscript{\textit{v}}.1.4 heterologously expressed in Xenopus oocytes is inhibited by isoflurane and halothane,\textsuperscript{10} whereas rat Na\textsubscript{\textit{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\textsubscript{\textit{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\textsubscript{\textit{v}}.1.4 channels.\textsuperscript{26}

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

<table>
<thead>
<tr>
<th>Anesthetic</th>
<th>(\tau_m)</th>
<th>Concentration, m M</th>
<th>(n)</th>
</tr>
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<tbody>
<tr>
<td>Isoflurane</td>
<td>0.54 ± 0.08</td>
<td>0.47 ± 0.08$\dagger$</td>
<td>0.46 ± 0.05</td>
</tr>
<tr>
<td>Halothane</td>
<td>0.52 ± 0.09</td>
<td>0.43 ± 0.08$\dagger$</td>
<td>0.85 ± 0.04</td>
</tr>
<tr>
<td>Desflurane</td>
<td>0.51 ± 0.07</td>
<td>0.42 ± 0.04$\dagger$</td>
<td>0.43 ± 0.03</td>
</tr>
<tr>
<td>Control</td>
<td>0.55 ± 0.07</td>
<td>0.39 ± 0.06$\dagger$</td>
<td>0.81 ± 0.04</td>
</tr>
<tr>
<td>Anesthetic</td>
<td>0.55 ± 0.10</td>
<td>0.40 ± 0.05$\dagger$</td>
<td>0.80 ± 0.06</td>
</tr>
<tr>
<td>Desflurane</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. * \(P < 0.01\), † \(P < 0.001\) vs. respective control by unpaired \(t\) test. $P < 0.05$ vs. isoflurane, $\dagger P < 0.05$ 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 negatively shift the voltage dependence of $I_{Na}$ fast inactivation. Similar shifts in inactivation of $I_{Na}$ are produced in ventricular cardiomyocytes by isoflurane and halothane,9 and in rat neurohypophysial nerve terminals7 and heterologously expressed Na, 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 $V_{1/2in}$ by desflurane contributes to its greater potency compared to isoflurane and halothane for inhibition of Na,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,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 $V_{1/2in}$ and hence stronger interaction with the fast inactivated state for halothane.

Enhanced inactivation is critical to inhibition of Na,1.4 by inhaled anesthetics at more positive membrane potentials. This has classically been attributed to fast inactivation, but recent evidence implicates slow inactivation mechanisms. This has classically been attributed to fast inactivation mechanisms. 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 $I_{Na}$ blocking effect. Open-channel block is particularly important in pathologic conditions such as myotonia and periodic paralysis, which involve Na,1.4 inactivation gating defects,30 inflammatory and neuropathic pain states that involve repetitive activation of Na,1.7 and Na,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, channels at anesthetic concentrations, but with some distinct mechanistic differences 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 inactivation mechanisms (greater for inhaled anesthetics).

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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\(_{1.2}\), Na\(_{1.4}\), Na\(_{1.6}\), or Na\(_{1.8}\) \(\alpha\)-subunits expressed 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,\(^3\) but it produces positive shifts of channel activation and inactivation of Na\(_{1.2}\) in tsA-201 cells\(^5\) and positive shifts of inactivation but no change in cocaine affinity of Na\(_{1.4}\) and Na\(_{1.5}\) in human embryonic kidney 293t cells.\(^6\) Although we have not ruled out possible effects of \(\beta\)-subunit coexpression on inhaled anesthetic sensitivity of Na\(_{1.4}\) 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.\(^5\) 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\(_{1.4}\) 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.\(^5\)

In summary, halogenated inhaled anesthetics all inhibit heterologously expressed Na\(_{1.4}\) at clinical concentrations by state-dependent mechanisms. These findings support inhibition of Na\(^+\), as a common mechanism 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\(_{1.4}\) 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\(_{1.4}\) using mutations that affect gating mechanisms and local anesthetic sensitivity.

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