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Isoform-selective Effects of Isoflurane on Voltage-gated Na^+ Channels

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Background: **Voltage-gated Na^+ channels modulate membrane excitability in excitable tissues. Inhibition of Na^+ channels has been implicated in the effects of volatile anesthetics on both nervous and peripheral excitable tissues. The authors investigated isoform-selective effects of isoflurane on the major Na^+ channel isoforms expressed in excitable tissues.**

Methods: **Rat $Na_v1.2$, $Na_v1.4$, or $Na_v1.5$ α subunits heterologously expressed in Chinese hamster ovary cells were analyzed by whole cell voltage clamp recording. The effects of isoflurane on Na^+ current activation, inactivation, and recovery from inactivation were analyzed.**

Results: **The cardiac isoform $Na_v1.5$ activated at more negative potentials (peak I_{Na} at −30 mV) than the neuronal $Na_v1.2$ (0 mV) or skeletal muscle $Na_v1.4$ (−10 mV) isoforms. Isoflurane reversibly inhibited all three isoforms in a concentration- and voltage-dependent manner at clinical concentrations (IC_{50} = 0.70, 0.61, and 0.45 mM, respectively, for $Na_v1.2$, $Na_v1.4$, and $Na_v1.5$ from a physiologic holding potential of −70 mV). Inhibition was greater from a holding potential of −70 mV than from −100 mV, especially for $Na_v1.4$ and $Na_v1.5$. Isoflurane enhanced inactivation of all three isoforms due to a hyperpolarizing shift in the voltage dependence of steady state fast inactivation. Inhibition of $Na_v1.4$ and $Na_v1.5$ by isoflurane was attributed primarily to enhanced inactivation, whereas inhibition of $Na_v1.2$, which had a more positive $V_{1/2}$ of inactivation, was due primarily to tonic block.**

Conclusions: **Two principal mechanisms contribute to Na^+ channel inhibition by isoflurane: enhanced inactivation due to a hyperpolarizing shift in the voltage dependence of steady state fast inactivation ($Na_v1.5 \approx Na_v1.4 > Na_v1.2$) and tonic block ($Na_v1.2 > Na_v1.4 \approx Na_v1.5$). These novel mechanistic differences observed between isoforms suggest a potential pharmacologic basis for discrimination between Na^+ channel isoforms to enhance anesthetic specificity.**

BOTH ligand-gated and voltage-gated ion channels are sensitive to general anesthetics through agent- and target-specific actions.[1,2] Voltage-gated ion channels were previously considered to be insensitive to general anesthetics,[3] but it is now evident that clinical concentrations of volatile anesthetics inhibit native neuronal Na^+ channels[4–6] as well as heterologously expressed mammalian Na^+ channel α subunits.[7,8] Presynaptic Na^+ channels have been implicated as important targets for the depressant effects of volatile anesthetics on neurotransmitter release.[9–12] Recent evidence suggests that inhibition of transmitter release by volatile anesthetics involves inhibition of presynaptic action potentials as a result of Na^+ channel blockade.[11,13]

Voltage-gated Na^+ channels are critical to the initiation and conduction of action potentials and determine membrane excitability through amplification of transient potentials in excitable cells. Nine homologous genes for voltage-gated Na^+ channel α subunits have been identified in mammals which encode tetrodotoxin-sensitive and tetrodotoxin-resistant isoforms.[14] The neuronal ($Na_v1.2$), skeletal muscle ($Na_v1.4$), and cardiac ($Na_v1.5$) α-subunit isoforms share more than 60% amino acid sequence identity but have differences in gating and conductance that result in distinct tissue-specific physiologic functions and subtle differences in pharmacologic properties.[14] Changes in Na^+ channel gating properties, especially in activation and/or fast inactivation, can affect the membrane potential and action potential, as seen in a number of Na^+ channelopathies.[15,16] The specificity of general anesthetic effects on the various Na^+ channel isoforms has implications for the central nervous system and peripheral actions of anesthetics, including anesthesia, myocardial depression, cardiac dysrhythmias, and muscle relaxation.[16]

The pore-forming Na^+ channel α subunits generate Na^+ currents with gating properties similar to native channels when expressed in *Xenopus* oocytes[17,18] or mammalian cell lines.[19] Effects of isoflurane on the rat and human tetrodotoxin-sensitive channels $Na_v1.2$, $Na_v1.4$, and $Na_v1.6$ expressed in *Xenopus* oocytes were qualitatively similar in producing voltage-dependent block at clinical concentrations, whereas the tetrodotoxin-resistant channel $Na_v1.8$ was insensitive.[8] The effects of pentobarbital on rat $Na_v1.2$ and human $Na_v1.4$ expressed in mammalian cell lines were also qualitatively similar.[20] However, neither of these studies addressed differential effects on channel gating. To assess isoform-selective effects of general anesthetics on Na^+ channel function, we compared the electrophysiologic effects of the widely used inhaled anesthetic ether isoflurane on the major rat Na^+ channel α-subunit isoforms expressed in brain ($Na_v1.2$), skeletal muscle ($Na_v1.4$), and cardiac muscle ($Na_v1.5$) to eliminate possible species– and expression system–dependent effects.

Materials and Methods

Materials and Cell Culture

Isoflurane was obtained from Abbott Laboratories (North Chicago, IL). Tetrodotoxin and antibiotics were

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obtained from Sigma (St. Louis, MO). Chinese hamster ovary cells stably transfected with rat α subunit $Na_v1.2$ and $Na_v1.5$ were generously provided by William A. Catterall, Ph.D. (Professor and Chair, Department of Pharmacology, University of Washington, Seattle, WA), and with rat α subunit $Na_v1.4$ by Simon R. Levinson, Ph.D. (Professor, Department of Physiology and Biophysics, University of Colorado Health Sciences Center, Denver, CO). Cells were cultured in 90% (vol/vol) DMEM/F12, 10% (vol/vol; $Na_v1.2$ and $Na_v1.5$) or 15% (vol/vol; $Na_v1.4$) fetal bovine serum, 100 U/ml penicillin, 100 μg/ml streptomycin, and 200 μg/ml ($Na_v1.2$ and $Na_v1.5$) or 300 μg/ml ($Na_v1.4$) Geneticin® (Invitrogen, Carlsbad, CA), with 5% CO_2–95% O_2 at 37°C. One to 3 days before electrophysiologic recording, cells were plated on glass coverslips in 35-mm plastic dishes (Becton Dickinson, Franklin Lakes, NJ).

Electrophysiologic Recording

Cells attached to coverslips were transferred to a plastic Petri dish (35 × 10 mm) on the stage of a Nikon ECLIPSE TE300 inverted microscope equipped with Hoffmann interference contrast optics (Melville, NY). The culture medium was replaced, and cells were superfused at 2–3 ml/min with an extracellular solution containing 140 mM NaCl, 4 mM KCl, 1.5 mM $CaCl_2$, 1.5 mM $MgCl_2$, 10 mM HEPES, and 5 mM D-glucose, pH 7.30 with NaOH. Currents were analyzed at room temperature (25° ± 1°C) to improve recording stability using conventional whole cell patch clamp techniques.[21] The pipet electrode solution consisted of 80 mM CsF, 40 mM CsCl, 15 mM NaCl, 10 mM HEPES, and 10 mM EGTA, pH 7.35 with CsOH. 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. 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 software (Axon Instruments, Burlingame, 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. To minimize the contribution of endogenous Na^+ currents (< 50 pA) occasionally observed in Chinese hamster ovary cells[22] and increasing series resistance, only cells with Na^+ currents of 0.5–3.5 nA were analyzed.

Isoflurane was diluted from stock solutions (10–12 mM isoflurane, prepared 12–24 h before experiments) in extracellular solution into airtight glass syringes, and applied locally to attached cells at 0.05 ml/min through a 0.15-mm-diameter perfusion pipet positioned 30–40 μm away from patched cells using an ALA-VM8 pressurized perfusion system (ALA Scientific, Westbury, NY). Concentrations of isoflurane were determined by local sampling of the perfusate at the site of the recording pipette tip and analysis by gas chromatography (approximately 85% of the syringe concentration).[4]

Data Analysis

IC_{50} values were obtained by least squares fitting of data to the Hill equation: $Y = 1/(1 + 10((\log IC_{50} - X) * h))$, where Y is the effect, X is the concentration, and h is the Hill slope. Activation curves were fitted to a Boltzmann equation of the form $G/G_{max} = 1/(1 + e(V_{1/2} - V)/k)$, where G/G_{max} is the normalized fractional conductance, G_{max} is maximum conductance, $V_{1/2a}$ is the voltage for half-maximal activation, and k is the slope factor. Na^+ conductance (G_{Na}) was calculated using the equation $G_{Na} = I_{Na}/(V_t - V_r)$, where I_{Na} is the peak Na^+ current, V_t is the test potential, and V_r is the Na^+ reversal potential (E_{Na} = 69 mV). Fast inactivation curves were fitted to a Boltzmann equation of the form $I/I_{max} = 1/(1 + e(V_{1/2} - V)/k)$, where I/I_{max} is the normalized current, I_{max} is the maximum current, $V_{1/2in}$ is the voltage of half-maximal inactivation, and k is the slope factor. Channel recovery from fast inactivation was fitted to a monoexponential function, $Y = A * (1 - \exp(-\tau * X))$, where Y is the fractional current recovery, A is the normalized control amplitude, X is recovery time, and τ is the time constant of recovery. The relative contributions of enhanced inactivation and tonic block to inhibition by isoflurane were expressed as the ratio of isoflurane block due to enhanced inactivation to tonic block. Data were analyzed using pClamp 8.2 (Axon Instruments), Prism 4.0 (Graph-Pad Software Inc., San Diego, CA), and SigmaPlot 6.0 (SPSS Science Software Inc., Chicago, IL). Statistical significance was assessed by analysis of variance with Newman-Keuls *post hoc* test or paired *t* test, as appropriate; $P < 0.05$ was considered statistically significant.

Results

Basic Properties of Na^+ Currents

Na^+ currents in Chinese hamster ovary cells expressing $Na_v1.2$, $Na_v1.4$, and $Na_v1.5$ α subunits were completely blocked by 0.5 μM tetrodotoxin (data not shown). After membrane rupture, I_{Na} was allowed to stabilize 5 min before recording.[23] Peak I_{Na} amplitudes (mean ± SD) were −1,470 ± 130 pA for $Na_v1.2$ (n = 21), −2,820 ± 160 pA for $Na_v1.4$ (n = 28), and −2,340 ± 170 pA for $Na_v1.5$ (n = 35). Currents activated at −60 mV or greater for all isoforms; peak activation occurred at 0 mV for $Na_v1.2$, −10 mV for $Na_v1.4$, and −30 mV for

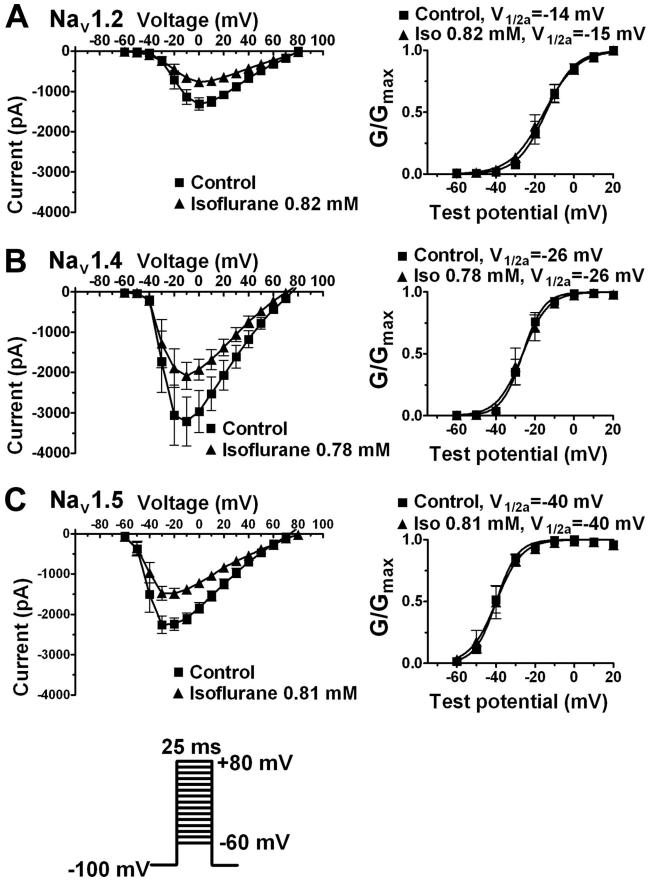

Fig. 1. Effects of isoflurane on Na^+ channel current–voltage (I-V) relations and activation. Chinese hamster ovary cells expressing various rat Na^+ channel α-subunit isoforms were subjected to whole cell patch clamp recording. Voltage of peak I_{Na} activation (V_{max}) was 0 mV for $Na_v1.2$ (*A*), −10 mV for $Na_v1.4$ (*B*), and −30 mV for $Na_v1.5$ (*C*). Isoflurane (0.8 mM, approximately 2.2 times minimum alveolar concentration[28]) inhibited all three isoforms. Data were fitted to a Boltzmann equation yielding voltage of 50% maximal activation ($V_{1/2a}$) and slope factor (k) values. Isoflurane had no effect on $V_{1/2a}$. Protocol: 100-ms prepulse from a holding potential of −100 mV, 25-ms test potential from −60 to +80 mV in 10-mV increments, pulses applied at 5-s intervals. Mean isoflurane concentrations were 0.82 ± 0.06 mM for $Na_v1.2$, 0.78 ± 0.06 mM for $Na_v1.4$, and 0.81 ± 0.05 mM for $Na_v1.5$ (mean ± SEM, n = 4–7).

$Na_v1.5$ (fig. 1). These results are consistent with previously reported differences in the voltages of peak activation for $Na_v1.2$, $Na_v1.4$, and $Na_v1.5$.[24,25–27] $Na_v1.2$ and $Na_v1.4$ currents decayed more rapidly than $Na_v1.5$ currents. The measured reversal potential was near the predicted value (69 mV at 25°C) for I_{Na}.

Isoflurane Inhibits Peak I_{Na}

Isoflurane inhibited I_{Na} through all three Na^+ channel isoforms in a concentration-dependent manner (figs. 1 and 2A). Onset of inhibition was rapid (< 1 min) using focal pipet application, and reversed within 2 min upon washout (return to 98 ± 0.6% of control amplitude in 1.7 ± 0.5 min, n = 4). The potency of inhibition was greater from a holding potential of −70 mV than from −100 mV, especially for $Na_v1.4$ and $Na_v1.5$ (fig. 2). The concentration-dependent inhibition of I_{Na} by isoflurane was well fitted by the Hill equation; IC_{50} values varied significantly between isoforms. $Na_v1.5$ was the most sensitive isoform from a holding potential of −70 mV (IC_{50} = 0.45 mM) and the least sensitive from a holding potential of −100 mV (IC_{50} = 1.1 mM; fig. 2A). The Hill slope of the concentration–response curve was unchanged for $Na_v1.2$ at a holding potential of −70 or −100 mV (1.5 ± 0.14 *vs.* 1.4 ± 0.09, respectively, n = 4–12), but slightly increased for $Na_v1.4$ and $Na_v1.5$ ($Na_v1.4$: 1.4 ± 0.13 *vs.* 2.0 ± 0.14, respectively, n = 4–9; $Na_v1.5$, 1.4 ± 0.13 *vs.* 1.8 ± 0.13, respectively, n = 4–17). Significant inhibition (30–46% from a holding potential of −70 mV) of all three isoforms occurred at the minimum alveolar concentration (MAC) of isoflurane, a clinically relevant concentration (equivalent to the aqueous EC_{50} for general anesthesia in rat of 0.35 mM [28]).

Effects of Isoflurane on Gating Parameters

Isoflurane did not alter the current–voltage relation or change I_{Na} reversal potential (fig. 1). Isoflurane did not affect the voltage dependence of activation, but slightly increased k values for all three Na_v isoforms (fig. 1 and table 1). Isoflurane produced a hyperpolarizing shift in the voltage dependence of steady state fast inactivation for all three Na_v isoforms; k values for $Na_v1.2$ and $Na_v1.5$ were also increased (fig. 3 and table 1). Isoflurane shifted the $V_{1/2in}$ in the hyperpolarizing direction by approximately 8 mV for $Na_v1.2$ (n = 8), by approximately 10 mV for $Na_v1.4$ (n = 6), and by approximately 11 mV for $Na_v1.5$ (n = 7; fig. 3 and table 1).

Mechanisms of Na^+ Channel Inhibition by Isoflurane

Na^+ channel availability varies with holding potential because the ratio of inactivated to resting channels increases at more depolarized membrane potentials.[29] To estimate the relative contributions of enhanced inactivation *versus* tonic blockade to inhibition of I_{Na} by isoflurane, we analyzed the effects of isoflurane using a two-pulse protocol consisting of a prepulse to −100 or −70 mV followed by a test pulse to peak activation voltage (fig. 4). Effects on inactivation were assessed using a ratio subtraction method, in which all channels are assumed to be resting and available at the −100 mV holding potential, as indicated by their voltage dependence of inactivation (fig. 3). The difference between the fractional isoflurane block from a holding potential of −70 *versus* −100 mV reflects enhanced inactivation due to isoflurane (fig. 4B). Isoflurane enhanced channel inactivation in all three Na_v isoforms, but the magnitude of the effect varied significantly between isoforms. Enhanced channel inactivation was more prominent for $Na_v1.4$ and $Na_v1.5$ than for $Na_v1.2$. At a concentration of 0.82 mM

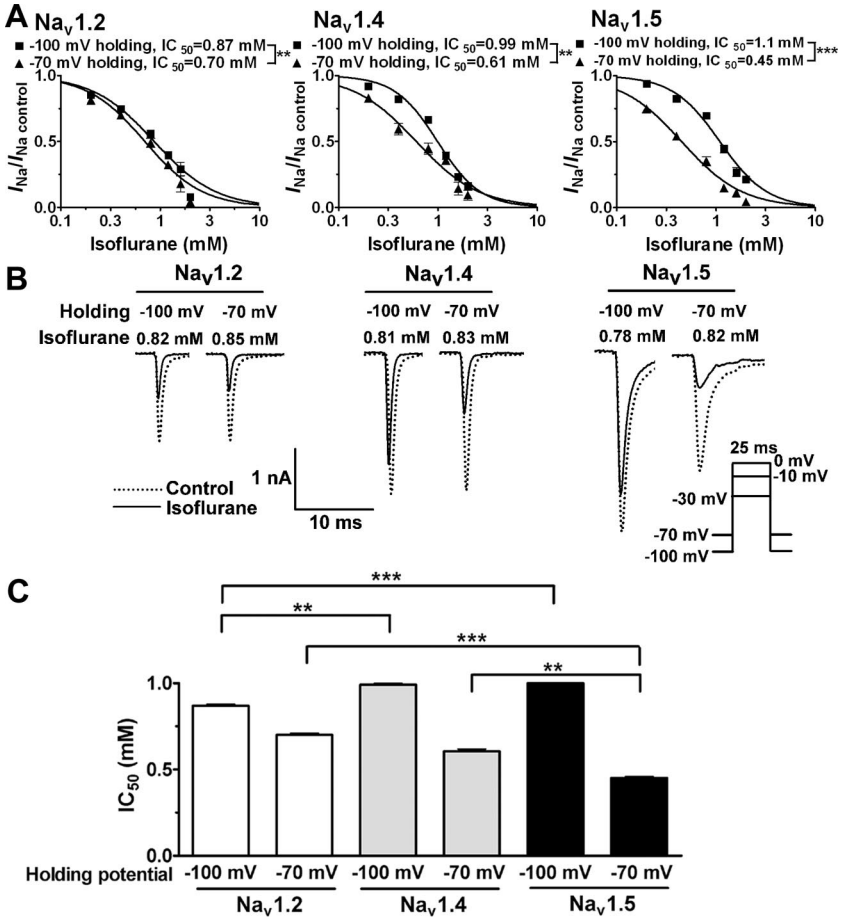

Fig. 2. Effect of holding potential on inhibition by isoflurane of three major voltage-gated Na^+ channel isoforms. Na^+ currents were evoked from holding potentials of −100 or −70 mV by 25-ms test steps to V_{max}: 0 mV for $Na_v1.2$, −10 mV for $Na_v1.4$, and −30 mV for $Na_v1.5$. (*A*) Isoflurane (approximately 0.8 mM) significantly inhibited all three isoforms from both holding potentials, but to different degrees. Normalized peak I_{Na} was fitted to the Hill equation. All three isoforms were more sensitive to isoflurane from a holding potential of −70 mV, which is near normal resting potential. (*B*) Representative current traces. (*C*) $Na_v1.5$ was most sensitive to isoflurane at a holding potential of −70 mV, but least sensitive to isoflurane at −100 mV, whereas $Na_v1.2$ was most sensitive to isoflurane at −100 mV and least sensitive at −70 mV. Data are expressed as mean ± SEM, n = 3–17. ** $P < 0.01$; *** $P < 0.001$ by one-way analysis of variance with Newman-Keuls *post boc* test (*C*) or by unpaired *t* test (*A*).

(approximately 2 times MAC[28]), isoflurane enhanced block due to inactivation by 6% for $Na_v1.2$ (n = 12), 22% for $Na_v1.4$ (n = 9), and 25% for $Na_v1.5$ (n = 4) from a holding potential of −70 mV (fig. 4B). These results are consistent with the data in figure 3 obtained with a different protocol, in which isoflurane enhanced block due to inactivation by 7% for $Na_v1.2$ (n = 8), 20% for $Na_v1.4$ (n = 6), and 28% for $Na_v1.5$ (n = 6). Tonic block by isoflurane, indicated by fractional block from a holding potential of −100 mV, was greater for $Na_v1.2$ than for $Na_v1.4$ and $Na_v1.5$. From a holding potential of −70 mV, the ratio of isoflurane block due to enhanced inactivation *versus* tonic block was 0.11 for $Na_v1.2$, 1.44 for $Na_v1.4$, and 1.96 for $Na_v1.5$ (at 0.38 mM isoflurane, approximately 1 MAC).

Isoflurane significantly slowed recovery of all three Na_v isoforms from inactivation from a holding potential of either −70 or −100 mV, but to a greater degree for $Na_v1.2$ (fig. 5 and table 2). Channel recovery from inactivation was slower from a holding potential of −70 mV compared with −100 mV because of greater inactivation at –70 mV.

Discussion

Isoflurane inhibited heterologously expressed rat neuronal ($Na_v1.2$), skeletal muscle ($Na_v1.4$), and cardiac

Table 1. Effects of Isoflurane on Na^+ Current Activation and Inactivation

	Activation					Inactivation				
	Control		Isoflurane			Control		Isoflurane		
Isoform	$V_{1/2a}$, mV	k	$V_{1/2a}$, mV	k	Isoflurane, mM	$V_{1/2in}$, mV	k	$V_{1/2in}$, mV	k	Isoflurane, mM
$Na_v1.2$	−14.4 ± 0.1	7.4 ± 0.1	−15.3 ± 0.1	8.5 ± 0.2	0.82 ± 0.06	−35.4 ± 0.1	8.2 ± 0.1	−42.5 ± 0.3†	11.3 ± 0.3†	0.81 ± 0.05
$Na_v1.4$	−26.2 ± 0.2	5.4 ± 0.2	−26.3 ± 0.2	6.5 ± 0.2	0.78 ± 0.06	−53.9 ± 0.1	6.9 ± 0.1	−64.0 ± 0.1†	7.3 ± 0.1	0.84 ± 0.06
$Na_v1.5$	−39.8 ± 0.2	5.5 ± 0.2	−39.9 ± 0.2	6.6 ± 0.2	0.81 ± 0.05	−59.8 ± 0.1	7.7 ± 0.1	−70.9 ± 0.2†	9.9 ± 0.2*	0.81 ± 0.04

* $P < 0.05$; † $P < 0.01$ *vs.* respective control by paired *t* test (n = 4–8).

k = slope factor; $V_{1/2a}$ and $V_{1/2in}$ = voltage of half-maximal activation or inactivation, respectively.

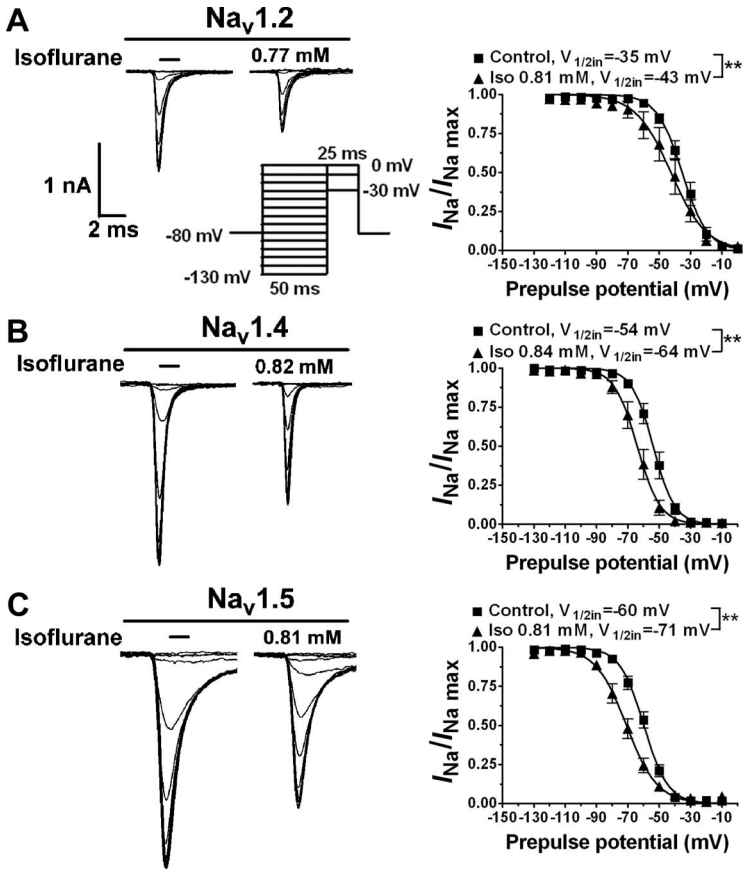

Fig. 3. Effects of isoflurane on Na^+ channel fast inactivation. (*A–C*) Superimposed traces elicited by V_{max} test pulses to 0 mV for $Na_v1.2$, −10 mV for $Na_v1.4$, and −30 mV for $Na_v1.5$ after a 50-ms prepulse of −120 to 0 mV for $Na_v1.2$, or −130 to −10 mV for $Na_v1.4$ and $Na_v1.5$ applied at 5-s intervals. Traces shown were recorded from single representative Chinese hamster ovary cells. Data were fitted to the Boltzmann equation yield to voltage of 50% inactivation ($V_{1/2in}$). Isoflurane significantly shifted the voltage dependence of steady state fast inactivation in the hyperpolarizing direction. Mean isoflurane concentrations were 0.81 ± 0.05 mM for $Na_v1.2$, 0.84 ± 0.06 mM for $Na_v1.4$, and 0.81 ± 0.04 mM for $Na_v1.5$. Data are expressed as mean ± SEM, n = 6–8. ** P < 0.01 by paired t test.

muscle ($Na_v1.5$) voltage-gated Na^+ channel α subunits in an isoform-selective manner. From a normal resting membrane potential, $Na_v1.5$ was most sensitive to isoflurane, whereas from a hyperpolarized potential with minimal inactivation, $Na_v1.5$ was least sensitive. The other isoforms demonstrated intermediate sensitivities, but all were significantly inhibited at clinically relevant concentrations. Isoflurane negatively shifted the voltage dependence of steady state fast inactivation for all three isoforms in the negative direction. There were significant differences between isoforms in the relative importance of enhanced inactivation *versus* tonic blockade in the inhibitory effects of isoflurane.

The potency of isoflurane for inhibition of all three isoforms was greater from a physiologic *versus* hyperpolarized holding potential, as previously reported for the effects of isoflurane on Na^+ currents in isolated rat neurohypophysial nerve terminals[5] and cultured rat dorsal root ganglion neurons,[6] and on heterologously expressed Na^+ channels.[7,8,30] These results agree with previous findings that isoflurane inhibited rat $Na_v1.2$ and human $Na_v1.4$ expressed in *Xenopus* oocytes more potently from a holding potential of −60 mV (approximately 50% channel inactivation) *versus* −90 mV.[8] This study did not identify any marked differences in the sensitivity of tetrodotoxin-sensitive Na_v α-subunit isoforms to a variety anesthetics in the absence or presence of β-subunit coexpression, although the tetrodotoxin-insensitive rat isoform $Na_v1.8$ was remarkable in its insensitivity to volatile anesthetics.[8] Small differences in the potency of the intravenous anesthetic pentobarbital in blocking $Na_v1.2$, $Na_v1.4$, and neuroblastoma Na^+ channels have been reported previously, although possible species and expression system effects could not be resolved.[20]

Mammalian Na_v channels undergo rapid voltage-dependent gating between closed and open states and then enter inactivated states from which they do not readily reopen.[29] Inhibition by isoflurane involved both enhanced channel inactivation and tonic blockade in which isoflurane binding is assumed during the closed resting state and open state (open channel block). The different sensitivities to isoflurane of the three Na_v isoforms are due in part to differential effects of isoflurane on channel gating. From a holding potential of –70 mV, enhanced inactivation was the predominant mechanism for inhibition of $Na_v1.5$ and $Na_v1.4$, whereas open channel block dominated effects on $Na_v1.2$. From a hyperpolarized holding potential of −100 mV, at which minimal inactivation is present, inhibition of all three isoforms involved primarily tonic open channel block. These differences between isoflurane effects on $Na_v1.2$ *versus* $Na_v1.4$ and $Na_v1.5$ suggests a potential mechanism for pharmacologic discrimination between desired anes-

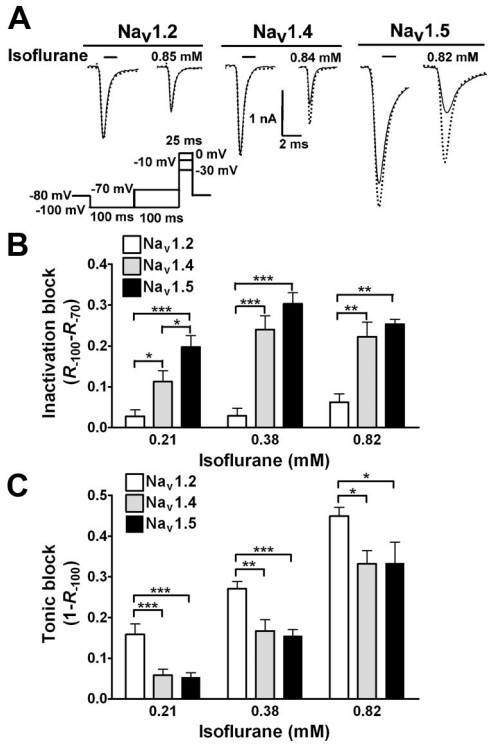

Fig. 4. Differential block of Na^+ channel isoforms by isoflurane. (*A*) $Na_V1.2$, $Na_V1.4$, and $Na_V1.5$ were recorded using a two-pulse protocol. Na^+ currents were elicited by 25-ms V_{max} voltage steps to peak activation voltages (fig. 1) from holding potentials of −70 mV (*solid lines*, 100-ms prepulse) or −100 mV (*dotted lines*, 200-ms prepulse) or at 5-s intervals. Control traces in the absence of isoflurane are on the *left*. Curves shown are from single representative cells. (*B*) Enhanced inactivation by isoflurane. Isoflurane reduced channel availability of all three isoforms reflected in the difference in fractional block at −100 mV (R_{-100}) *versus* −70 mV (R_{-70}). $R_{-100} - R_{-70} = (I_{Na}/I_{Na}$ control from −100 mV holding) − (I_{Na}/I_{Na} control from −70 mV holding). (*C*) Tonic block by isoflurane. The effects of isoflurane due to tonic block were determined as ($1 - R_{-100}$). Mean isoflurane concentrations in *B* and *C* were 0.21 ± 0.06, 0.38 ± 0.04, and 0.82 ± 0.05 mM. Data are expressed as mean ± SEM, n = 3–14. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ by one-way analysis of variance with Newman-Keuls *post hoc* test.

thetic effects on $Na_V1.2$ in the central nervous system and undesired peripheral anesthetic effects on $Na_V1.4$ and $Na_V1.5$ in heart and skeletal muscle.[31]

$Na_V1.1$, $Na_V1.2$, $Na_V1.3$, $Na_V1.5$, and $Na_V1.6$ are differentially expressed in various brain regions and at various developmental stages such that modulation of neuronal function by effects on Na^+ channels varies with developmental stage and brain region.[14] For example, $Na_V1.2$ expression increases during the third postnatal week and continues to increase to adulthood, with greatest expression in the rostral brain regions.[14] Although $Na_V1.5$ is expressed most highly in cardiac tissue, it is also found in the piriform cortex and subcortical limbic nuclei, which show similar Na^+ current gating properties as the cardiac Na^+ current.[14] $Na_V1.5$ is also detectable in neonatal skeletal muscle and after denervation of adult skeletal muscle.[14] $Na_V1.1$, $Na_V1.3$, and $Na_V1.6$ have also been detected in ventricular cardiomyocytes and contribute to depolarization and excitation-contraction coupling in the heart.[32] Therefore, effects on multiple voltage-gated Na^+ channel isoforms likely contribute to both central nervous system and peripheral effects of isoflurane.

The effects of volatile anesthetics on neuronal Na^+ channels resemble those of the local anesthetic lidocaine in several respects: a hyperpolarizing shift in the voltage dependence of steady state fast inactivation,[25–27] stabilization of fast and slow inactivated states of the channel,[30,33] specific inhibition of neurotoxin binding to toxin site 2, but not sites 1 or 5,[4] and use-dependent block.[25] These similar pharmacologic effects suggest that isoflurane, a hydrophobic halogenated ether anesthetic, and lidocaine, a hydrophobic tertiary amine local anesthetic, share common or overlapping binding sites and/or convergent allosteric mechanisms in modulating Na_V channel gating. The greater potency of isoflurane for cardiac $Na_V1.5$ channels is analogous to previous reports that cardiac Na^+ channels are more sensitive to local anesthetics than nerve and skeletal muscle channels.[25–27]

Isoflurane shifted the voltage dependence of steady state fast inactivation in the hyperpolarized direction, but did not significantly shift the voltage dependence of steady state activation. These results agree with previous reports that isoflurane does not affect the voltage dependence of activation, but shifts inactivation in the hyperpolarizing direction, for $Na_V1.2$,[7,8] $Na_V1.4$,[8,34] and $Na_V1.5$.[30] Maximal effects on fast inactivation occurred with $Na_V1.5$, which is consistent with a previous report.[30] Inactivation slope factors were significantly increased for $Na_V1.2$ and $Na_V1.5$, consistent with an effect on voltage sensitivity. Because the voltage sensor is the main factor coupling activation and inactivation,[35] parallel effects on activation and inactivation suggest that interactions with the voltage sensor contribute to Na^+ channel inhibition by isoflurane. These effects are similar to those of lidocaine on cardiac Na^+ channels.[36,37] The S1–S2 extracellular loop of domain IV has been implicated in channel activation kinetics and lidocaine block.[24] The similar actions between local and general anesthetics on Na^+ channel electrophysiologic properties suggest the possibility of a conserved molecular mechanism involving a similar site.

Isoflurane increased the time constant for recovery from inactivation of all three Na_V isoforms ($Na_V1.2 > Na_V1.5 \approx Na_V1.4$) such that slowed channel recovery contributes to isoflurane inhibition of repetitive stimuli. Isoflurane inhibition of $Na_V1.4$ and $Na_V1.5$ primarily involves mechanisms before channel recovery, such as enhanced gating from closed and open states to inactivated states,[30] whereas effects on recovery from inactivation are greater for $Na_V1.2$. These data provide further support for two membrane potential dependent mecha-

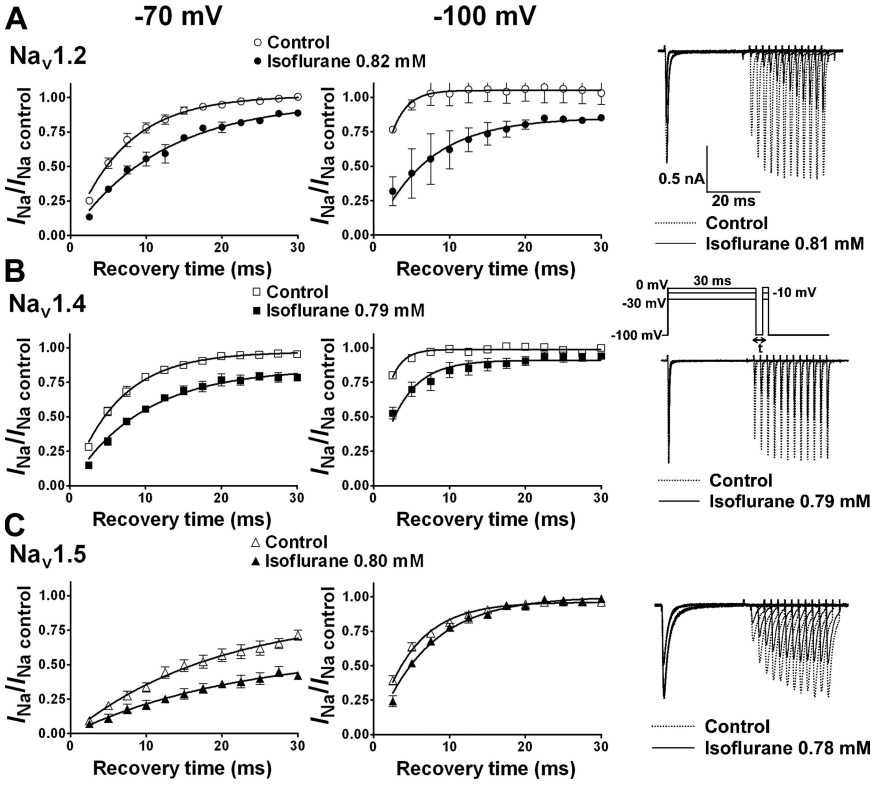

Fig. 5. Effects of isoflurane on channel recovery from fast inactivation. Recovery of channel availability was assessed using a two-pulse protocol with a 30-ms conditioning pulse followed by a variable recovery interval of up to 30 ms, and then a 5-ms test pulse to peak activation voltages (fig. 1). The time course of channel recovery from fast inactivation was well fitted by a monoexponential function. Time course of recovery of I_{Na} from fast inactivation from a holding potential of −70 mV (*left*) or −100 mV (*middle*) is shown for $Na_v1.2$ (*A*), $Na_v1.4$ (*B*), and $Na_v1.5$ (*C*), expressed as current normalized to initial control pulse. Representative current traces for a holding potential of −100 mV are shown (*right*).

nisms (enhanced inactivation and tonic blockade) for isoflurane inhibition of Na^+ channels with variable contributions between different isoforms. The marked effects of isoflurane on channel inactivation are consistent with greater anesthetic affinity for inactivated channels, as observed for local anesthetics and anticonvulsants[38,39] and as suggested previously for halothane.[4,40] This provides a potential mechanism for cardioprotection and neuroprotection from ischemia by selective Na^+ channel blockade in depolarized cells.[41]

Local anesthetics produce tonic block by interacting with a binding site in the inner pore region that inhibits ion permeation through the hydrophilic pore, in addition to effects on gating.[42] Local anesthetics also interact with the selectivity filter region.[43,44] A similar mechanism may pertain to volatile anesthetics that decrease channel availability by producing a hyperpolarizing shift in the voltage dependence of inactivation and produce tonic block, probably due to binding or a conformational change that narrows and/or occludes the ion channel pore. The specific structural and mechanistic differences that determine the differential effects of isoflurane on $Na_v1.2$, $Na_v1.4$, and $Na_v1.5$, possibly involving more than one binding site, will require detailed structure–function investigations.

Isoflurane has two major effects on voltage-gated Na^+ channels: preferential interaction with inactivated states to produce a hyperpolarizing shift in the voltage dependence of inactivation, which reduces channel availability and slows recovery, and interaction with the open and/or resting state to produce tonic block. At physiologic resting membrane potentials, these two mechanisms make variable isoform-selective contributions to isoflurane inhibition of $Na_v1.2$, $Na_v1.4$, and $Na_v1.5$. Further work should clarify the pharmacologic significance of these isoform-selective effects of isoflurane, and possibly other volatile anesthetics, on central nervous system and peripheral tissues.

Table 2. Effects of Isoflurane on Na^+ Channel Recovery from Inactivation

	$Na_v1.2$		$Na_v1.4$		$Na_v1.5$	
Holding	−70 mV	−100 mV	−70 mV	−100 mV	−70 mV	−100 mV
Control	4.9 ± 0.1	1.4 ± 0.1	4.4 ± 0.1	1.1 ± 0.1	13.4 ± 0.1	3.4 ± 0.1
Isoflurane (0.8 mM)	8.3 ± 0.1†	4.8 ± 0.2*	6.6 ± 0.1†	2.4 ± 0.1*	15.5 ± 0.1†	4.8 ± 0.1†

Data are expressed as mean ± SEM, τ in milliseconds, n = 3–6. Actual isoflurane concentration in the $Na_v1.2$ group was 0.82 ± 0.06 mM, in the $Na_v1.4$ group was 0.79 ± 0.04 mM, and in the $Na_v1.5$ group was 0.80 ± 0.06 mM.

* $P < 0.01$; † $P < 0.001$ *vs.* control by unpaired Student *t* test.

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