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Central Nervous System Sodium Channels Are Significantly Suppressed at Clinical Concentrations of Volatile Anesthetics

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Background: **Although voltage-dependent sodium channels have been proposed as possible molecular sites of anesthetic action, they generally are considered too insensitive to be likely molecular targets. However, most previous molecular studies have used peripheral sodium channels as models. To examine the interactions of volatile anesthetics with mammalian central nervous system voltage-gated sodium channels, rat brain IIA sodium channels were expressed in a stably transfected Chinese hamster ovary cell line, and their modification by volatile anesthetics was examined.**

Methods: **Sodium currents were measured using whole cell patch clamp recordings. Test solutions were equilibrated with the test anesthetics and perfused externally on the cells. Anesthetic concentrations in the perfusion solution were determined by gas chromatography.**

Results: **All anesthetics significantly suppressed sodium currents at clinical concentrations. This suppression occurred through at least two mechanisms: (1) a potential-independent suppression of resting or open sodium channels, and (2) a hyperpolarizing shift in the voltage-dependence of channel inactivation resulting in a potential-dependent suppression of sodium currents. The voltage-dependent interaction results in IC_{50} values for anesthetic suppression of sodium channels that are close to clinical concentrations at potentials near the resting membrane potential.**

Conclusions: **Contrary to the hypothesis that sodium channels are insensitive to general anesthetics, the results presented here indicate that current through central nervous system sodium channels examined at physiologic membrane potentials is significantly blocked by clinical concentrations of volatile anesthetics. This anesthetic interaction with sodium channels is voltage-dependent, consistent with a state-dependent modulated receptor model in which anesthetics more strongly affect the inactive state of the channel than the resting state. (Key words: Anesthetic mechanisms: anesthetic-protein interaction. Anesthetics, volatile: desflurane; diethylether; enflurane; halothane; isoflurane; sevoflurane. Brain, central nervous system: sodium channel inactivation, sodium channels. Measurement techniques: patch clamp.)**

VOLTAGE-GATED sodium channels are essential elements of neuronal function, mediating the rising phase of depolarization during the propagation of action potentials. Because of their central role in neuronal communication and integration, these proteins have been proposed as possible anesthetic targets,[1–5] but experimental evidence has not provided strong support for this proposition. Sodium channels have been found to be relatively insensitive to clinical doses of anesthetics. However, some of these previous studies used peripheral nerve preparations, and during the past decade, there has been increasing evidence of functional and pharmacologic differences between sodium channels, not only among different tissues, but within the same tissue.[6–8] Additionally, most previous studies used hyperpolarizing potentials to remove channel inactivation before examining anesthetic interactions[9,10]; if anesthetics strongly interact with the inactive state of the channel, such interactions could be missed under these conditions. Therefore, to understand these anesthetic interactions more fully, it is important to examine sodium channels of central nervous system (CNS) origin under a wider range of electrophysiologic conditions.

To investigate these interactions with CNS sodium channels, we previously examined the effects of various classes of anesthetics on human brain synaptosomal sodium channels incorporated into planar lipid bilayers.

This article is accompanied by a Highlight. Please see this issue of ANESTHESIOLOGY, page 27A.

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In these experiments, we found that anesthetics blocked sodium current through these channels with varying potencies, obeying the Meyer-Overton correlation.[5] Clinical concentrations of anesthetics blocked about 10% of sodium channel currents, similar to what was reported previously for the peripheral nerve preparations.[10] However, the bilayer experiments required the use of sodium channel activators to remove or alter channel inactivation, and possible interactions of anesthetics with the inactive state of the channel were minimized under these conditions.

To examine the effects of anesthetics on sodium channels of CNS origin with unmodified inactivation properties, we investigated the anesthetic modification of rat brain sodium channels stably transfected into Chinese hamster ovary cells. The sodium channels expressed in this system have been examined thoroughly both electrophysiologically and pharmacologically[11,12] and were found to have similar functionality to sodium channels *in situ*. Furthermore, these channels are greater than 97% structurally identical to human brain sodium channels.[13] We used varying voltage-clamp protocols to examine state-dependent anesthetic effects on these channels. The relevance of these results to understanding anesthetic actions is discussed.

Materials and Methods

Cell Culture

A stably transfected Chinese hamster ovary cell line (CNaIIA-1) was used. The vector used for transfection contained a gene conferring resistance to the aminoglycoside antibiotic G418.

The cells were grown in RPMI medium (Gibco, Grand Island, NY) containing 10% fetal bovine serum and 1% penicillin/streptomycin mixture (Gibco) as well as 200 μg/ml G418 to select for transfected cells. Cells were cultured in 25 cm^2 polystyrene culture flasks (Corning, Corning, NY) at 37°C in room air containing 5% CO_2. For electrophysiologic recordings, cells were transferred to 60 mm Petri dishes (Becton Dickinson, Lincoln Park, NJ).

Electrophysiology

Cells were used 2–3 days after the transfer, before the cell layer became confluent. For electrophysiologic measurements, the culture medium was replaced by an extracellular solution containing 130 mM NaCl, 4 mM KCl, 1.5 mM $CaCl_2$, 1.5 mM $MgCl_2$, 5 mM glucose, and 5 mM HEPES, adjusted to *p*H 7.4 with CsOH. All solutions were filtered through 0.22 μm-filters (Millipore, Bedford, MA) before use.

Recordings were made at room temperature (24.4 ± 1.2°C). Because we expressed anesthetic potencies not as minimum alveolar concentration (MAC) values (strongly temperature-dependent) but as aqueous concentrations (relatively temperature-insensitive[14]), the concentrations can be extrapolated to body temperature.

Sodium currents were studied using the whole-cell configuration of the patch-clamp recording technique,[15] using a standard patch-clamp amplifier (Axopatch 200, Axon Instruments, Foster City, CA) controlled by commercially available software (pCLAMP, Axon Instruments) on a standard personal computer. Currents were filtered at 5 kHz, digitized, and recorded to hard disk. Capacitive transients and series resistance were measured and compensated using the amplifier's internal compensation circuitry; active series resistance compensation was used to compensate 60–85% of the series resistance. Average series resistance before compensation was 3.5 ± 1.5 MΩ; the average cell capacitance was 18.1 ± 8.6 pF.

Patch-clamp pipettes were pulled from micropipette glass (Drummond, Broomall, PA) and filled with an intracellular solution containing 10 mM NaCl, 90 mM CsF, 60 mM CsCl, and 6 mM HEPES, adjusted to *p*H 7.4 with CsOH.

Cells with currents larger than 6 nA and smaller than 0.5 nA were excluded because of increasing series resistance error or possible contamination by possible small endogenous sodium currents in Chinese hamster ovary cells.[11] The average current of the 75 cells included in this study was 2.4 ± 1.5 nA.

Anesthetic Solutions

Volatile anesthetic solutions were prepared by injection of defined amounts of anesthetics into PTFE-capped glass vials filled with extracellular solution. The vials were vigorously vortexed for 2 min and sonicated in a bath sonicator for 1 h, yielding consistent anesthetic concentrations. The anesthetic solutions were applied *via* a glass-PTFE-perfusion system and a superfusion pipette (flow rate 0.5–0.8 ml/min) close to the cell. Anesthetic concentrations of halothane, isoflurane, enflurane, desflurane, and sevoflurane were measured in some experiments in the perfusion reservoir at the start of the perfusion and after 3 and 7 min using gas chromatography. The degree of evaporation from the open-

perfusion reservoir is different for each anesthetic (largest for desflurane with a decrease in aqueous concentration of 25% after 7 min). No correction was made for this differential evaporation; all concentrations reported are those at the beginning of perfusion, and actual anesthetic concentrations at the cells will be lower.

Solutions of diethylether and ethanol were prepared in the same way, but concentrations were calculated from the amount of anesthetic injected into the glass vial.

Statistics

Curve fits were computed using a least-squares algorithm (Marquardt-Levenberg) of commercially available software (Sigmaplot, Jandel Scientific, Corte Madera, CA). Data are ±SEM, unless noted otherwise.

Results

Suppression of Brain Sodium Channels Follows the Meyer-Overton Correlation

A standard way to calculate suppression of sodium currents by drugs is to determine peak inward current from current-voltage plots.[9,10] Sodium currents are elicited by voltage steps to various test potentials (here −60 to +100 mV) from a hyperpolarized holding potential (here −120 mV), at which channel inactivation is removed. Sodium currents elicited with this protocol are inhibited by volatile anesthetics, as exemplified by desflurane (fig. 1). The suppression occurs within seconds and can be reversed by washing out the anesthetic with anesthetic-free extracellular solution. Reversal was complete in most experiments, even after repeated drug application. Higher concentrations of anesthetic tended to lead to incomplete reversal and, therefore, were applied last in experiments in which multiple concentrations were applied. The voltage dependence of sodium current activation was unchanged by the anesthetics.

Concentration-response curves were established from averaged suppression data from at least six cells for each anesthetic (fig. 2). Suppression was calculated as the reduction of the maximum inward current (which occurs, depending on the cell, in the test potential range of −10 to +10 mV), expressed as percent of control. Data for all anesthetics were fit with simple hyperbolic curves, assuming 100% maximum suppression. The logarithm of the IC_{50} values obtained (table 1) correlates to the logarithm of the octanol/water partition coefficients[16] in the same linear manner as the Meyer-Overton correlation of MAC values (compare to fig. 6). Ethanol, which can be used as an (intravenous) anesthetic adjuvant,[17] was included in the experiments to extend the range of partition coefficients. For the anesthetics studied, linear regression fits to the data yielded slopes of −1.05 ($r^2 = 0.956$) for peak sodium current suppression and −0.99 ($r^2 = 0.996$) for concentrations yielding anesthesia (represented as MAC values, converted to aqueous concentrations[16]). On average for the anesthetics studied, 50% block of peak sodium current requires threefold higher concentrations than human MAC values.

Volatile Anesthetics Induce a Hyperpolarizing Shift in Steady-State Sodium Channel Inactivation

Steady-state inactivation is a physiologically important property of sodium channels determining the availability of the channels at a certain membrane potential. The standard voltage protocol used to measure steady-state inactivation comprises prepulses to varying potentials (here, 500 ms to potentials between −150 and −10 mV) and a constant test pulse (here, −10 mV). Before application of an anesthetic, currents (shown for an exemplary cell in fig. 3) decrease at prepulse potentials positive to about −80 mV. This phenomenon can be explained by a voltage-dependent transition from closed to inactivated channels, the latter being unavailable for opening. The voltage-dependent distribution between the two states represents steady-state inactivation and is described by a Boltzmann function. Data were fitted to the function $f(V) = I_{max} - \{I_{max}/\exp[-z^*F^*(V - V_h)/RT]\}$, which is characterized by three parameters, with I_{max} maximum current at hyperpolarized potentials, z the slope parameter, and V_h the midpoint potential at which the function assumes its half-maximal value (average control value for all experiments was −54.0 mV); F is the Faraday constant, R the gas constant, and T the absolute temperature. Currents are normalized to I_{max} before addition of the anesthetic.

After application of an anesthetic (demonstrated for ether in fig. 3B), currents are reduced at all potentials as expected from the experiments described above, and additionally, steady-state inactivation is shifted in the hyperpolarizing direction (leftward).

These results reveal two effects of volatile anesthetics on sodium currents: (1) a voltage-independent current suppression at hyperpolarized potentials and (2) a hy-

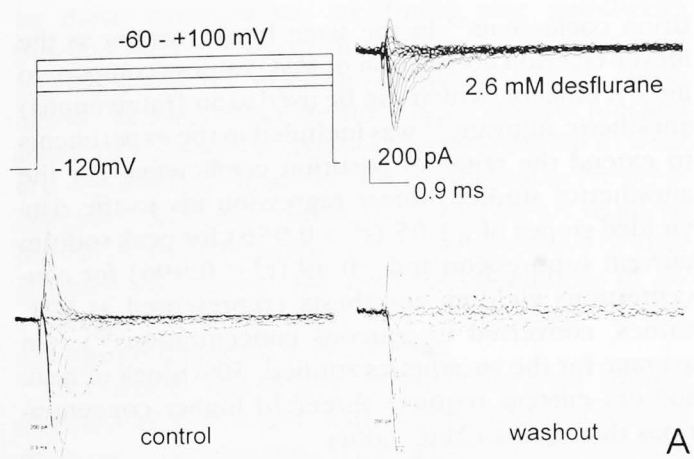


Fig. 1. Effect of desflurane on whole-cell sodium currents. (*A*) Currents elicited by voltage steps from a holding potential of −120 mV to test potentials varying from −60 to +100 mV. Interpulse duration was 1.5 s. Filter frequency 5 kHz. Traces are before (*left*), during (*upper right*) superfusion with 2.6 mM desflurane, and after washout (*lower right*) with desflurane-free extracellular solution from a cell expressing the rat brain IIA sodium channel α subunits (cell B4522). A sustained outward current at positive potentials was sometimes observed after prolonged perfusion with high anesthetic concentrations (*lower right*). Calibration bars are 300 pA and 0.9 ms. (*B*) Peak current-voltage relationship for the current traces shown in *A* (filled circles = control; triangles = superfusion with 2.6 mM desflurane; open circles = washout).

perpolarizing (leftward) shift in steady-state inactivation, which leads to an additional, voltage-dependent current reduction in the voltage range of channel inactivation.

The concentration-dependence of the shift in V_h is shown for the six anesthetics studied in figure 4. For sevoflurane, the effect appears to saturate at higher concentrations (fig. 4B). In most experiments, however, currents at high anesthetic doses became too small for accurate determination of V_h, and it was not possible to determine whether the effect saturated for all anesthetics.

In most experiments, no complete reversal of the shift could be achieved during washout, probably because of the limited time of washout (5–10 min) or irreversible anesthetic effects. A hyperpolarizing shift of V_h in the absence of drugs has been reported for other whole-cell preparations.[18,19] However, in our cells, after the first 5 min of the experiment, no shift of steady-state inactivation with time (up to 30 min, data not shown) was found in control experiments without anesthetic application.

Potential-Dependence of Anesthetic Potency Leads to IC_{50} Values at Resting Membrane Potentials Comparable to Clinical Anesthetic Concentrations

The hyperpolarizing shift of channel inactivation induced by volatile anesthetics leads to a voltage-dependence of anesthetic potency. From channel inactivation plots normalized to I_{max} before addition of the anesthetic (fig. 3B), concentration-response curves at each potential can be calculated. Plotting the IC_{50} values yielded by those calculations *versus* the prepulse potential (fig. 5) demonstrates an increase in anesthetic potency in the voltage region of channel inactivation. The data in figure 5 imply that the voltage dependence is similar for all anesthetics studied.

IC_{50} values at −120 mV, at which no channels are inactivated, and at −60 mV, near the resting potential and firing threshold of neuronal cells, are compared in table 1 with concentrations yielding anesthesia. At −120 mV, IC_{50} values for sodium channel suppression are much larger than clinical anesthetic potencies, whereas at −60 mV, the values are similar.

Both at −60 and at −120 mV, the IC_{50} values correlate well with octanol/water partition coefficients on logarithmic scales (fig. 6); slopes for the linear regression fits are −1.05 (r^2 = 0.918) at −60 mV and −1.00 (r^2 = 0.933) at −120 mV.

Use-dependent Block by Volatile Anesthetics

Under physiologic conditions, stimulation of a cell rarely occurs as a single signal but rather as trains of

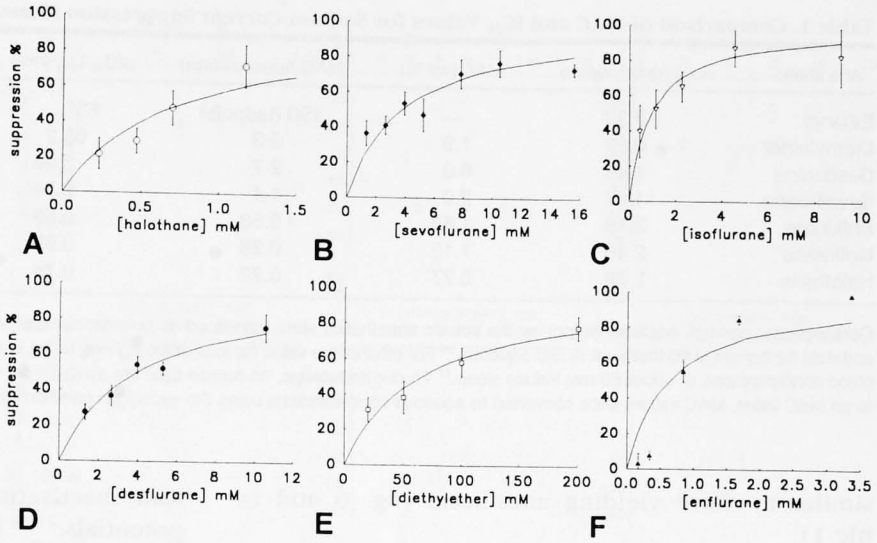


Fig. 2. Concentration-response curves for sodium peak current block by different volatile anesthetics. Block was calculated as reduction of the maximum inward current obtained from I-V-plots such as shown in figure 1B. (*A*) Halothane (averages of 5–6 cells), (*B*) sevoflurane (averages of 5–16 cells), (*C*) isoflurane (averages of 3–10 cells), (*D*) desflurane (averages of 3–11 cells), (*E*) diethylether (averages of 3–10 cells), and (*F*) enflurane (averages of 6 cells). Note different concentration scales. Error bars denote SEM.

frequent stimuli. Pharmacologic block of these frequent stimuli can be quantitatively different from stationary block if channel recovery from inactivation is slowed by a drug that interacts with the inactive state of the channel. As indicated in figure 7 for halothane, volatile anesthetics cause such a slowing of channel recovery (*i.e.,* returning to the resting channel conformation) after a depolarization, consistent with fast association and dissociation rates of anesthetic binding to the inactive state of the channel. This effect was the same for all anesthetics studied at comparable concentrations (data not shown). Channel recovery without anesthetics and at low anesthetic concentrations could be fitted well with single exponential functions, whereas at higher concentrations, multiple exponentials were necessary to fit the data.

Prolonged channel recovery after a depolarization, such as shown in figure 7, leads to use-dependent block if the intervals between depolarizing pulses become too short to allow for complete recovery to the resting state of the channel from the inactive state. We assessed use-dependent block with trains of 20 depolarizations from −85 to 0 mV applied at 5 Hz. All examined anesthetics caused use-dependent block; figure 8B shows desflurane as an example: Increasing anesthetic concentrations caused more pronounced use-dependent current reduction and a decrease in the time constant of this use-dependent block.

To further investigate the mechanism underlying use-dependent block, we studied its dependence on the parameters of the pulse protocol (fig. 9). Higher pulse frequency (fig. 9A), longer pulse duration (fig. 9B), more depolarized holding potential, and more depolarized pulse potential all lead to more pronounced use-dependent block. These manipulations increase the fraction of time sodium channels spend in the inactivated state, and the more pronounced block is therefore consistent with a preferential interaction of volatile anesthetics with inactivated sodium channels.

Discussion

Mechanisms of Anesthetic Suppression of Sodium Channel Currents

The results presented here indicate that volatile anesthetics can significantly suppress sodium currents through voltage-gated sodium channels at clinically relevant concentrations. There are at least two distinct mechanisms by which these anesthetics suppress CNS sodium channel currents: (1) a potential-independent suppression of resting or open sodium channels and (2) a hyperpolarizing shift in the voltage-dependence of sodium channel inactivation. This latter modification of sodium channel function causes channels to inactivate at more hyperpolarized potentials, resulting in a voltage-dependent anesthetic suppression of sodium channel currents. As a consequence of these combined interactions, IC_{50} values for sodium channel suppression are much lower at potentials close to neuronal resting membrane potentials (−60 mV[20]) than at the more hyperpolarized potentials used to determine peak current reduction. At the depolarized potentials, current suppression occurs at anesthetic concentrations

Table 1. Comparison of MAC and IC_{50} Values for Sodium Current Suppression Measured with Different Protocols

Anesthetic	Log pc(oct/water)	MAC (vol %)	MAC Aqueous (mM)	IC_{50} I_{peak} (mM)	IC_{50} at −120 mV (mM)	IC_{50} at −60 mV (mM)
Ethanol	−0.32	—	190 (tadpole)	635	690	292
Diethylether	0.89	1.9	9.3	65.2	63.7	19.9
Desflurane	1.46	6.0	2.7	3.98	4.97	1.79
Sevoflurane	1.67	2.0	1.4	3.74	3.48	0.56
Enflurane	2.19	1.68	0.58	0.68	0.75	0.34
Isoflurane	2.46	1.15	0.28	0.85	1.45	0.25
Halothane	2.70	0.77	0.22	0.75	1.13	0.46

Octanol/water partition coefficients (pc) for the volatile anesthetics were calculated as pc(octanol/water) = pc(octanol/gas)/pc(water/gas)[16,38]; value for ethanol[39] and data for human MAC (both vol % and aqueous.[38] For ethanol the value for loss of the righting reflex in tadpoles was used for comparison.[39] In humans, ethanol blood concentrations of about 50 mM induce sleep.[17] To our knowledge, no human data are available for the loss of response to a surgical stimulus, comparable to an MAC value. MAC values were converted to aqueous concentrations using the water/gas partition coefficients ($MAC_{aqu} = MAC^{*}_{gas}pc(water/gas)^{*}10/22.4$).[16]

similar to those yielding anesthesia (fig. 6 and table 1).

Several factors need to be considered when interpreting the anesthetic concentrations used in the present experiments. These experiments were conducted at room temperature, allowing a direct comparison with previous pharmacologic examinations of these channels,[12] as well as with other anesthetic studies of sodium channels that generally have been carried out at room or lower temperatures.[1,3–5,9,10] Although we reported aqueous anesthetic concentrations, which are relatively temperature-insensitive,[14] sodium channel properties may shift with temperature. For example, it has been shown in peripheral and/or nonmammalian sodium channel preparations that sodium channel kinetics change with higher temperatures and that steady-state inactivation is shifted toward more depolarized potentials.[21,22] However, these experiments were conducted with animals whose body temperature is lower than mammals, and thus, it is difficult to predict how such changes might affect the anesthetic interactions reported here.

The voltage-independent anesthetic suppression of sodium channels at hyperpolarized potentials is similar to that found in previous studies examining peak current reduction[9,10] and occurs at similar anesthetic concentrations. The volatile anesthetic modification of sodium channels found in the current experiments is consistent with a state-dependent interaction best described by the modulated receptor hypothesis of drug interaction.[23,24] In this model, different channel states (at least three for sodium channels: resting, open, and

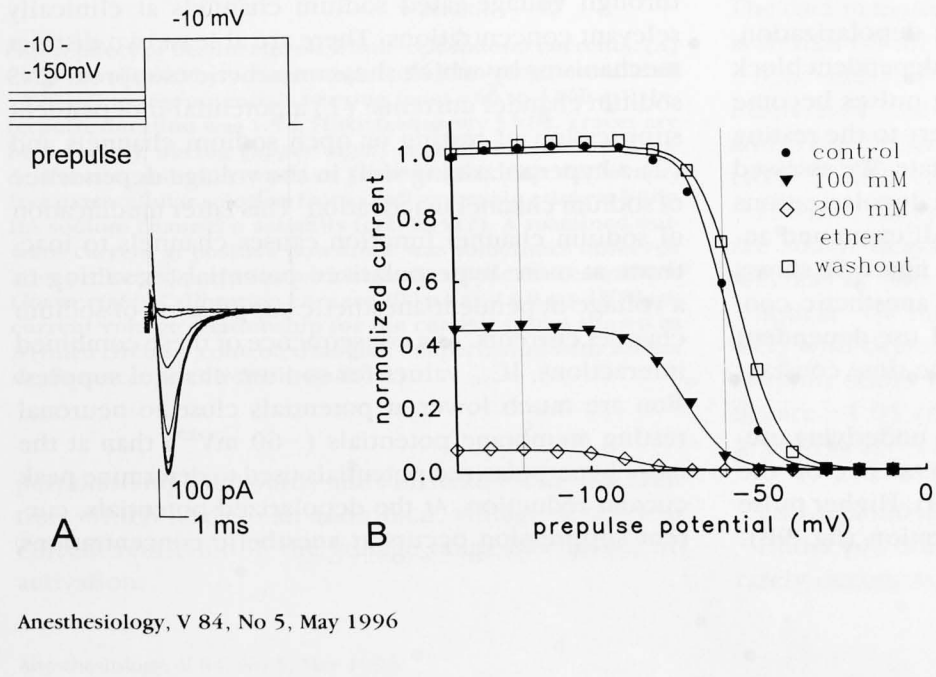


Fig. 3. Effect of volatile anesthetics (here diethylether as an example, cell R4343) on sodium channel steady-state inactivation. (*A*) Sodium currents were elicited by test pulses to −10 mV after 500 ms-prepulses to potentials varying from −150 to −10 mV. (*B*) Normalized peak currents are plotted as a function of prepulse potential (circles = control; triangles = −100 mM ether, diamonds = −200 mM ether; squares = washout). Lines are fits to two-level Boltzmann distributions. Currents were normalized to the maximum current before addition of the anesthetic, obtained from the Boltzmann-fits. The midpoint of channel inactivation, obtained from the Boltzmann-fits, shifted from −58.8 mV (control—circles) to −72.0 and −89.1 mV during perfusion with 100 mM (triangles) and 200 mM (diamonds) diethylether, respectively. After washout (squares), it returned to a value of −55.0 mV.

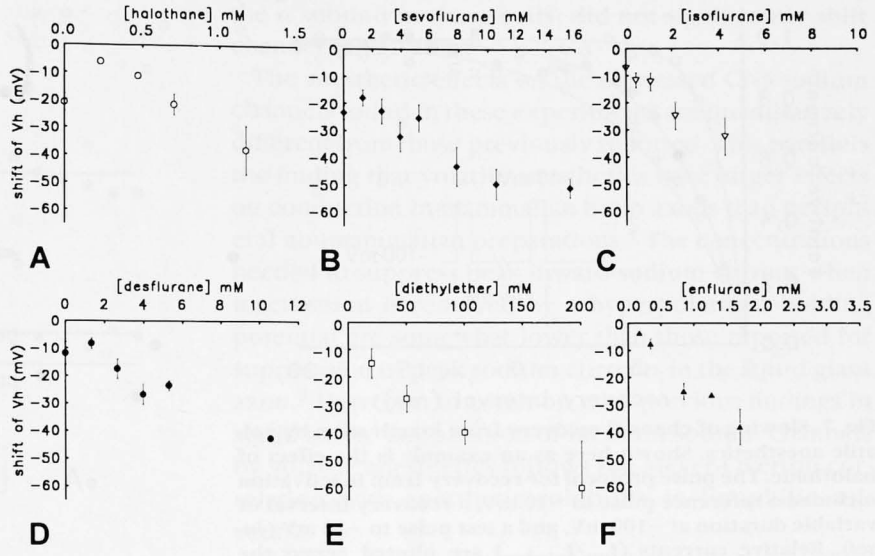


Fig. 4. Concentration-dependent shift of the midpoint of steady-state channel inactivation (obtained from Boltzmann fits as shown in fig. 4) by volatile anesthetics. Concentration scales are same as in figure 2. (*A*) Halothane (averages of 5–6 cells), (*B*) sevoflurane (averages of 3–9 cells), (*C*) isoflurane (averages of 3–10 cells, datapoint at 8.5 mM single cell), (*D*) desflurane (averages of 6–9 cells, datapoint at 10.6 mM from 2 cells), (*E*) diethylether (averaged of 3–10 cells), and (*F*) enflurane (averages of 3–4 cells, datapoint at 1.26 mM from single cell). Values for washout are shown at 0 mM. Error bars denote SEM (range for datapoints with n = 2).

inactivated) are assumed to have different affinities for drug binding. The hyperpolarizing shift of steady-state inactivation (fig. 4), the use-dependent block of the channels (figs. 8 and 9), and the slowing of the rate of recovery from inactivation (fig. 7) can be explained qualitatively by assuming that anesthetics bind more strongly to the inactivated state of the channel rather than to its resting state.[25] The observed use-dependent block could be due to binding to the open state of the channel, but the increasing inhibition found when increasing the time the channel spends in the inactivated state (longer depolarizations, fig. 9B) indicates preferential binding to the inactivated state. The modulated receptor model has been proposed to explain the interactions of other drugs, such as local anesthetics, antiarrhythmics, and anticonvulsants, with the sodium channel,[26] and these agents may share common or overlapping binding sites.

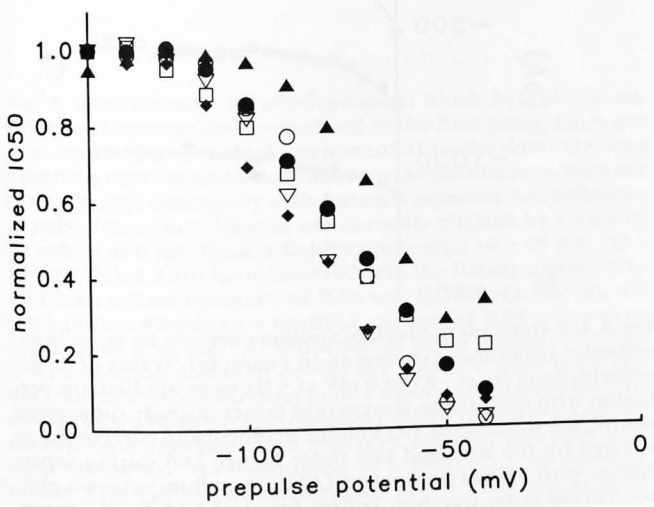


Fig. 5. IC_{50} of volatile anesthetics plotted as a function of prepulse potential. Values are normalized to the IC_{50} at −140 mV for comparison of the voltage-dependent changes between anesthetics. Averaged data of 3–6 cells for halothane (open circles), 3–11 cells for sevoflurane (solid diamonds), 4–7 cells for isoflurane (open downward triangles), 3–8 cells for desflurane (filled circles), 3–9 cells for diethylether (open squares), and 3–5 cells for enflurane (filled upward triangles) were used.

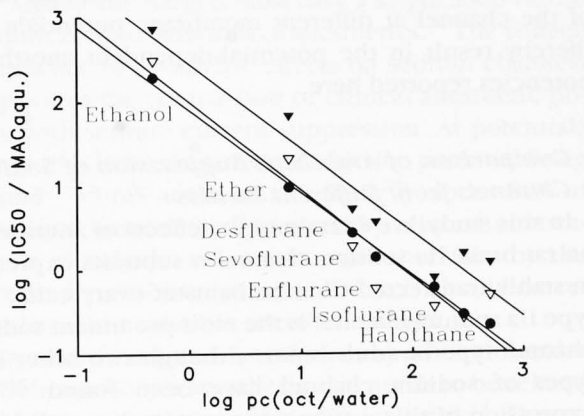


Fig. 6. Linear correlation of the logarithm of the IC_{50} values for sodium current block at two different prepulse potentials (−120 mV = solid triangles; −60 mV = open triangles) and the logarithm of the octanol/water partition coefficients. For comparison, human MAC values converted to aqueous concentrations are plotted on the same scale (filled circles). Lines are linear regression fits to the data.

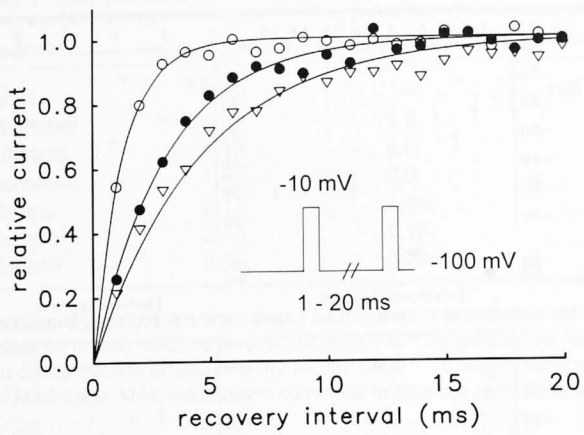
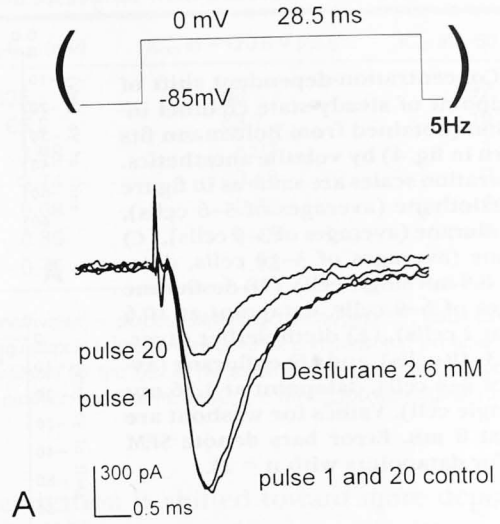


Fig. 7. Slowing of channel recovery from inactivation by volatile anesthetics. Shown here as an example is the effect of halothane. The pulse protocol for recovery from inactivation included a reference pulse to −10 mV, a recovery interval of variable duration at −100 mV, and a test pulse to −10 mV (*inset*). Relative currents ($I_{test}/I_{reference}$) are plotted *versus* the length of the recovery interval. In this example, the time constant of recovery is increased from a control value of 1.26 ms (open circles) to 3.09 ms by 0.5 mM halothane (filled circles) and to 4.55 ms by 1.1 mM halothane (open triangles).

A less specific type of interaction also could take place. For example, a change in the physicochemical properties of the cellular lipid membrane could result in an alteration of the free energy differences between the inactivated state of the channel and either its resting or its open state. In this case, the steady-state equilibrium between the channel conformations would be altered. With either explanation, anesthetic potency would depend on the available conformational states of the channel at different membrane potentials and thereby result in the potential-dependent anesthetic potencies reported here.

Comparison of Anesthetic Suppression of Sodium Channels from Different Tissues

In this study, we examined the effects of anesthetics on rat brain IIa sodium channel α subunits expressed in stably transfected Chinese hamster ovary cells. The type IIa sodium channel is the most prominent sodium channel type in adult brain, although two other subtypes of sodium channel have been found.[12] The expression of this α-subunit in mammalian cell lines, without coexpression of β subunits, is sufficient to obtain the normal physiologic[11] and pharmacologic[12] behavior of sodium channels found *in situ*. Further, this rat brain sodium channel has greater than 97% structural identity with the parallel human brain sodium



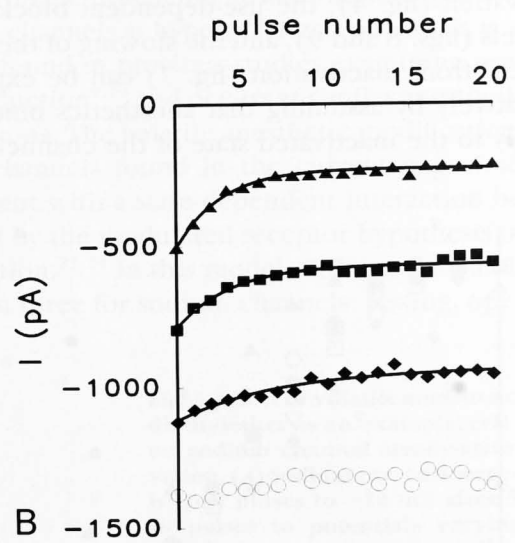


Fig. 8. Use-dependent block of sodium currents by volatile anesthetics (desflurane shown as an example). Trains of 20 depolarizations from −85 to 0 mV at 5 Hz were applied. On perfusion with desflurane, the current is increasingly suppressed during the pulse train. (*A*) Voltage protocol and current traces elicited by the first and last pulse before and during superfusion with 2.6 mM desflurane. (*B*) Peak sodium currents plotted *versus* pulse number, before (circles) and during superfusion with 1.3 mM (diamonds), 2.6 mM (squares), and 5.3 mM (triangles) desflurane. Same cell as in (*A*). Lines are least-squares fits to a single exponential function: $I_{(t)} = I_\infty + \{(I_1 - I_\infty)^*\exp(-[t - 1]/\tau)\}$, yielding the parameters I_1, the current of the first pulse; I_∞, the limiting current at the n-th pulse; and τ, the time constant. Time constants for use-dependent block at 1.3, 2.6, and 5.3 mM desflurane are 6.0, 3.2 and 2.7 ms, respectively. The I_∞/I_1-ratios for the respective concentrations are 0.84, 0.72, and 0.45.

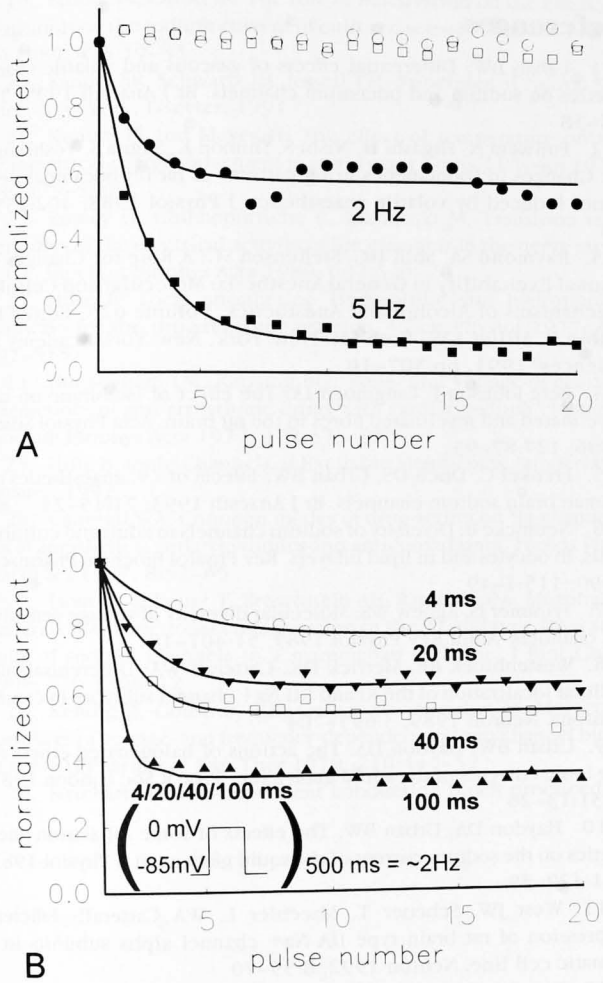


Fig. 9. Determinants of use-dependent block by volatile anesthetics. Currents are normalized to the first pulse. Lines are least-square fits of a single exponential to the data, yielding a limiting current and a time constant as parameters. Data are from separate exemplary cells for each protocol. (*A*) Influence of pulse frequency. Plotted are currents elicited by trains of 20 pulses to 0 mV from a holding potential of −85 mV. At 2 Hz (circles), 1.2 mM halothane reduces the limiting current to 0.50 with a time constant of 0.66 ms. At 5 Hz (squares), 1.2 mM halothane leads to a limiting current of 0.09 and a time constant of 0.34 ms. (*B*) Influence of pulse duration. Pulses from a holding potential of −85 mV to 0 mV were used, the duration of the depolarizing pulses was varied from 4 ms (circles) to 20 ms (downward triangles), 40 ms (squares), and 100 ms (upward triangles). The recovery interval was kept constant at 500 ms, corresponding to a frequency of approximately 2 Hz. The cell was superfused with 1.2 mM sevoflurane.

channel,[13] indicating that the results obtained should be pertinent to the human CNS channel and making it a viable experimental model for examining anesthetic interactions with sodium channels. A recent report[27] indicated that the $\beta 1$ subunit, when coexpressed with the α subunit in these cells, did not significantly shift channel inactivation.

The anesthetic effects on the expressed CNS sodium channels found in these experiments are quantitatively different from those previously reported. This parallels the finding that volatile anesthetics have larger effects on conduction in mammalian brain axons than peripheral nonmammalian preparations.[4] The concentrations needed to suppress peak inward sodium current when inactivation is removed by a hyperpolarized holding potential are somewhat lower than those reported for suppression of peak sodium currents in the squid giant axon.[9] However, in common with previous findings in squid axon,[1] suppression of rat brain sodium channels by volatile anesthetics follows the Meyer-Overton correlation with anesthetic solubility in lipophilic solvents.

The anesthetics studied also had a much larger effect on the voltage-dependence of inactivation of brain sodium channels compared to that reported for the squid giant axon[9] or the toad sciatic nerve.[28] In this latter preparation, diethylether exhibited no use-dependent block,[28] whereas halothane did so only at high stimulus frequencies.[29] These differences may reflect species variability in anesthetic modification of channel inactivation, as has been reported for n-alkanols.[19]

Clinical Significance of Anesthetic Block of Sodium Channels

In determining the contributions of potential molecular targets to general anesthesia, it is generally assumed that "significant" targets must have a $k_{1/2}$ of action close the clinical concentrations of anesthetics.[14] The voltage dependence of anesthetic effects on sodium channels complicates the comparison of clinical anesthetic potency with sodium current suppression. At potentials close to the threshold potential of action potential firing (around −55 mV at the axon hillock[20]), about 50% of the sodium current is suppressed by clinical concentrations of volatile anesthetics, and therefore by the criterion given above, sodium channels should be reconsidered as a possible anesthetic target.

However, the relevance of this or any other molecular anesthetic action to anesthesia also needs to be related to changes occurring at the cellular level.[14] The assumption of a correlation between a 50% effect on a given target and its relevance to anesthesia may not be correct.

Further complicating the interpretation of molecular anesthetic actions on sodium channels, these

proteins have several cellular physiologic functions that may be differentially affected by a common molecular anesthetic modification. Despite the significant anesthetic suppression of sodium channel current found in the present study, it has been established at the cellular level that anesthetics have only minor effects on axonal conduction at these same concentrations.[30,31] This may be due to the "safety factor," defined as the ratio of current supplied by incoming action potentials to that required to sustain propagation, which is normally quite high.[32] It has been estimated that more than 70% of sodium channels must be blocked to prevent action potential firing.[33] Further, a block of sodium channels in nerve and muscles would be fatal, as found in cases of puffer fish poisoning.[34] Nonetheless, the use-dependent block of sodium channels at higher stimulation frequencies leads to anesthetic impairment of axonal conduction.[27] At lower stimulus frequencies, use-dependent block would increase the threshold for action potential firing, with concomitant changes in neuronal firing patterns and frequencies.

Additionally, suppression of sodium currents should lead to an elevation in neuronal firing threshold,[35] and consistent with our results, volatile anesthetics have been shown to elevate the threshold potential of neurons at clinical concentrations.[2,36,37] Changes in threshold are probably most effective in disrupting neuronal function in the region of the axon hillock, although changes in the excitability of the axon have been implicated in general anesthesia.[3]

Finally, information in the CNS is encoded not as the amplitude or duration of action potentials but as their frequency,[20] and thus, increasing the threshold potentials needed to fire an action potential, added to use-dependent anesthetic block, could disrupt the flow of information in the CNS and thereby contribute to the clinical state of anesthesia.

In summary, we have presented evidence that clinical concentrations of volatile anesthetics significantly suppress currents through sodium channels at physiologic membrane potentials. The relevance of these molecular interactions to the clinical state of anesthesia needs to be further examined at the cellular level.

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