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Suppression of Central Nervous System Sodium Channels by Propofol

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Background: **Previous studies have provided evidence that clinical levels of propofol alter the functions of voltage-dependent sodium channels, thereby inhibiting synaptic release of glutamate. However, most of these experiments were conducted in the presence of sodium-channel activators, which alter channel inactivation. This study electrophysiologically characterized the interactions of propofol with unmodified sodium channels.**

Methods: **Sodium currents were measured using whole-cell patch-clamp recordings of rat brain IIa sodium channels expressed in a stably transfected Chinese hamster ovary cell line. Standard electrophysiologic protocols were used to record sodium currents in the presence or absence of externally applied propofol.**

Results: **Propofol, at concentrations achieved clinically in the brain, significantly altered sodium channel currents by two mechanisms: a voltage-independent block of peak currents and a concentration-dependent shift in steady-state inactivation to hyperpolarized potentials, leading to a voltage dependence of current suppression. The two effects combined to give an apparent concentration yielding a half-maximal inhibitory effect of 10 μM near the threshold potential of action potential firing (about −60 mV). Propofol inhibition was also use-dependent, causing a further block of sodium currents at these anesthetic concentrations.**

Conclusions: **In these experiments with pharmacologically unaltered sodium channels, propofol inhibition of currents occurred at concentrations about eight-fold above clinical plasma levels and thus at brain concentrations reached during clinical anesthesia. Therefore, the results indicate a possible role for sodium-channel suppression in propofol anesthesia.**

(Key words: Anesthetic mechanisms; anesthetic–protein interaction; brain.)

THE molecular mechanisms of action of the widely used anesthetic agent propofol (2,6-diisopropylphenol) are not completely understood. Most research has focused on enhanced γ-aminobutyric acid (GABA)–mediated synaptic inhibition.[1–5] However, a recent study with selectively bred mice found no difference in sensitivity of $GABA_A$ receptors in two distinct breeds of mice, despite differences in their clinical sensitivity to propofol.[6] These results suggest that additional mechanisms of propofol action besides the $GABA_A$ receptor may exist.

In addition to the effects of propofol on $GABA_A$-activated chloride channels, propofol has been shown to have several molecular actions on various receptors.[7–11] Recent evidence points to additional presynaptic mechanisms, including modification of voltage-dependent sodium,[12,13] potassium,[14] and calcium channels.[15] For example, propofol has been found to inhibit glutamate release primarily by blocking current through sodium channels.[13] However, the mechanism of this sodium current suppression needs to be further elucidated.

Previous electrophysiologic studies on propofol suppression of neuronal sodium currents have used synaptosomal preparations with toxin-treated sodium channels having altered channel inactivation.[12] Examination of propofol interactions with sodium channels using ion-flux measurements and toxin-binding studies, however, indicated that propofol suppressed glutamate release by blocking sodium currents both in the presence and absence of these activators.[13,16] These studies also indicated that propofol interacts, either specifically or allosterically, with the binding sites of the toxins used in the previous studies.[16]

Because no electrophysiologic examination of the effects of propofol on mammalian central nervous system sodium channels in the absence of modifiers has been described, we investigated the actions of propofol on rat-brain sodium channels expressed in a mammalian cell line using the whole-cell patch-clamp technique in volt-

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age-clamp mode. The results were compared with previous studies, as well as with the actions of other intravenous and volatile anesthetics on sodium channel function.[17]

Material and Methods

Cell Culture

A stably transfected Chinese hamster ovary cell line (CNaIIA-1; gift from Dr. William Catterall, University of Washington, Seattle, WA), expressing the rat brain IIa sodium channel,[18] was used. The vector used for transfection contained a gene conferring resistance to the aminoglycoside antibiotic G418 (GIBCO, Grand Island, NY).

The cells were grown in RPMI 1640 medium (GIBCO) 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 2–4 days after 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.

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. All solutions were filtered through 0.22-μ filters (Millipore, Bedford, MA) before use.

Sodium currents were studied using the whole-cell configuration of the patch-clamp recording technique,[19] using a standard patch-clamp amplifier (Axopatch 200, Axon Instruments, Foster City, CA) controlled by commercially available software (pCLAMP 5, Axon Instruments) on a standard personal computer. Currents were filtered at 5 kHz, digitized (at 50 kHz for the first 5 ms of each pulse, than 10 kHz), and recorded to hard disk. Capacitative transients and series resistance were measured and compensated using the internal compensation circuitry of the amplifier; active series resistance compensation was used to compensate for 60–70% of the series resistance. Average series resistance before compensation was 3.1 ± 1.2 MΩ (n = 7); average cell capacitance was 19.2 ± 11.0 pF (n = 7).

Cells with currents larger than 5 nA and smaller than 0.5 nA were excluded because of increasing series resistance error or possible contamination by small endogenous sodium currents in Chinese hamster ovary cells.[18] The average current of the seven cells included in this study was 2.1 ± 1.3 nA. Recordings were made at room temperature (22–25°C).

2,6-Diisopropylphenol (Sigma, St. Louis, MO) was dissolved directly in the extracellular solution. Concentrations were calculated from the amount injected into the glass vials. The vials were vigorously vortexed for 2 min and sonicated in a bath sonicator for 30 min. The solution was filtered before use and applied *via* a glass-polytetrafluoroethylen perfusion system and a glass superfusion pipette (flow rate 0.5–0.8 ml/min) close to the cell. During the experiment, the anesthetic solution was continually perfused over the cell.

Statistics

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

Results

Suppression of Sodium Currents by Propofol

Sodium currents elicited by stepping the membrane potential from a holding value of −120 mV to test potentials ranging from −60 to +100 mV (fig. 1A) were reversibly suppressed by propofol in a concentration-dependent manner (fig. 1B). Suppression occurred within seconds (the response time of the perfusion system). In contrast, currents reached only about 80% of control values after a 2-min washout. The voltage dependence of sodium current amplitude was unchanged by the anesthetic.

Maximum inward currents (minima of the current-voltage relations) were used to calculate current suppression, expressed as percentage of control. The data from all experiments (n = 7) were averaged to establish a concentration–response plot (fig. 1C). Data are reported as percentage current suppression of the baseline value. Because sequences of increasing propofol concentrations with a washout only after 50 and 125 mM propofol were used throughout the experiments, using the

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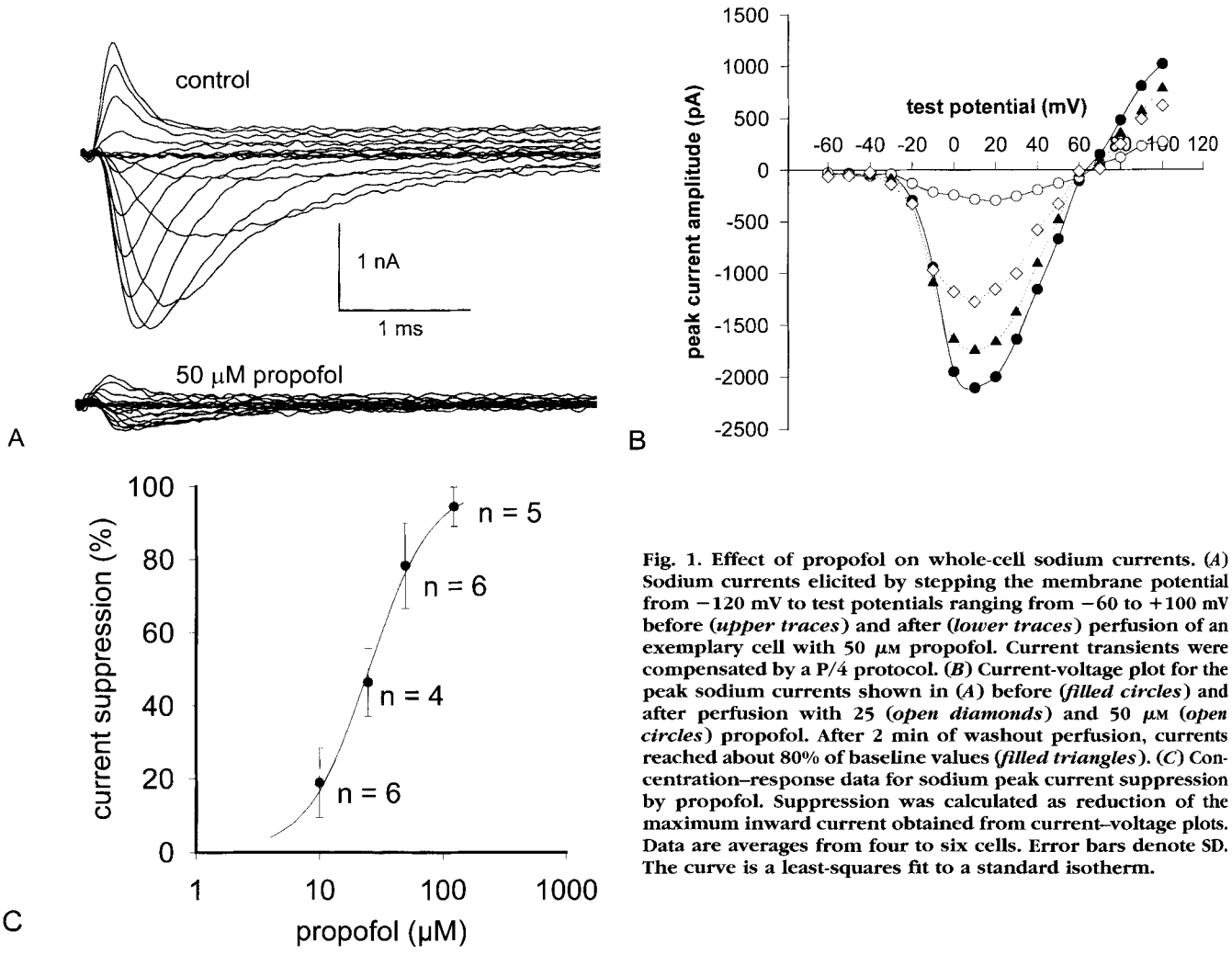

Fig. 1. Effect of propofol on whole-cell sodium currents. (*A*) Sodium currents elicited by stepping the membrane potential from −120 mV to test potentials ranging from −60 to +100 mV before (*upper traces*) and after (*lower traces*) perfusion of an exemplary cell with 50 μM propofol. Current transients were compensated by a P/4 protocol. (*B*) Current-voltage plot for the peak sodium currents shown in (*A*) before (*filled circles*) and after perfusion with 25 (*open diamonds*) and 50 μM (*open circles*) propofol. After 2 min of washout perfusion, currents reached about 80% of baseline values (*filled triangles*). (*C*) Concentration–response data for sodium peak current suppression by propofol. Suppression was calculated as reduction of the maximum inward current obtained from current–voltage plots. Data are averages from four to six cells. Error bars denote SD. The curve is a least-squares fit to a standard isotherm.

average of baseline and washout will underestimate the effect of lower concentrations of propofol. Using only the baseline value, a sigmoid function for the concentration (c) dependence of inhibition I ($I = 100 \cdot c^n/(IC_{50}{}^n + c^n)$) fit to the data yields an IC_{50} value (concentration yielding a half-maximal inhibitory effect) of 25.4 ± 1.1 μM (± SE of the fit), and a slope parameter (n) of 1.7 ± 0.1. For comparison, a fit to data calculated as percentage suppression of the average of baseline and washout values yielded an IC_{50} value of 28.7 ± 1.0 μM, and a slope parameter of 1.9 ± 0.1.

Effect of Propofol on Sodium-channel Steady-state Inactivation

Sodium-channel steady-state inactivation is a physiologically important property determining the availability of the channels at various membrane potentials. Steady-state inactivation was assessed with a two-pulse protocol comprising a 500-ms prepulse to potentials ranging from −150 to −10 mV followed by a constant test pulse to −10 mV. During the prepulse, a voltage-dependent equilibrium distribution (steady-state) is reached between resting and inactivated states of the channels, the latter being unavailable for opening during the subsequent test pulse.

Under control conditions, currents (shown for an exemplary cell in fig. 2A) decrease at prepulse potentials positive to about −80 mV because of an increasing ratio of inactivated *versus* resting channels. Peak amplitudes of the currents can be plotted *versus* the prepulse potential to yield steady-state inactivation curves (fig. 2B).

The voltage dependence of the distribution between

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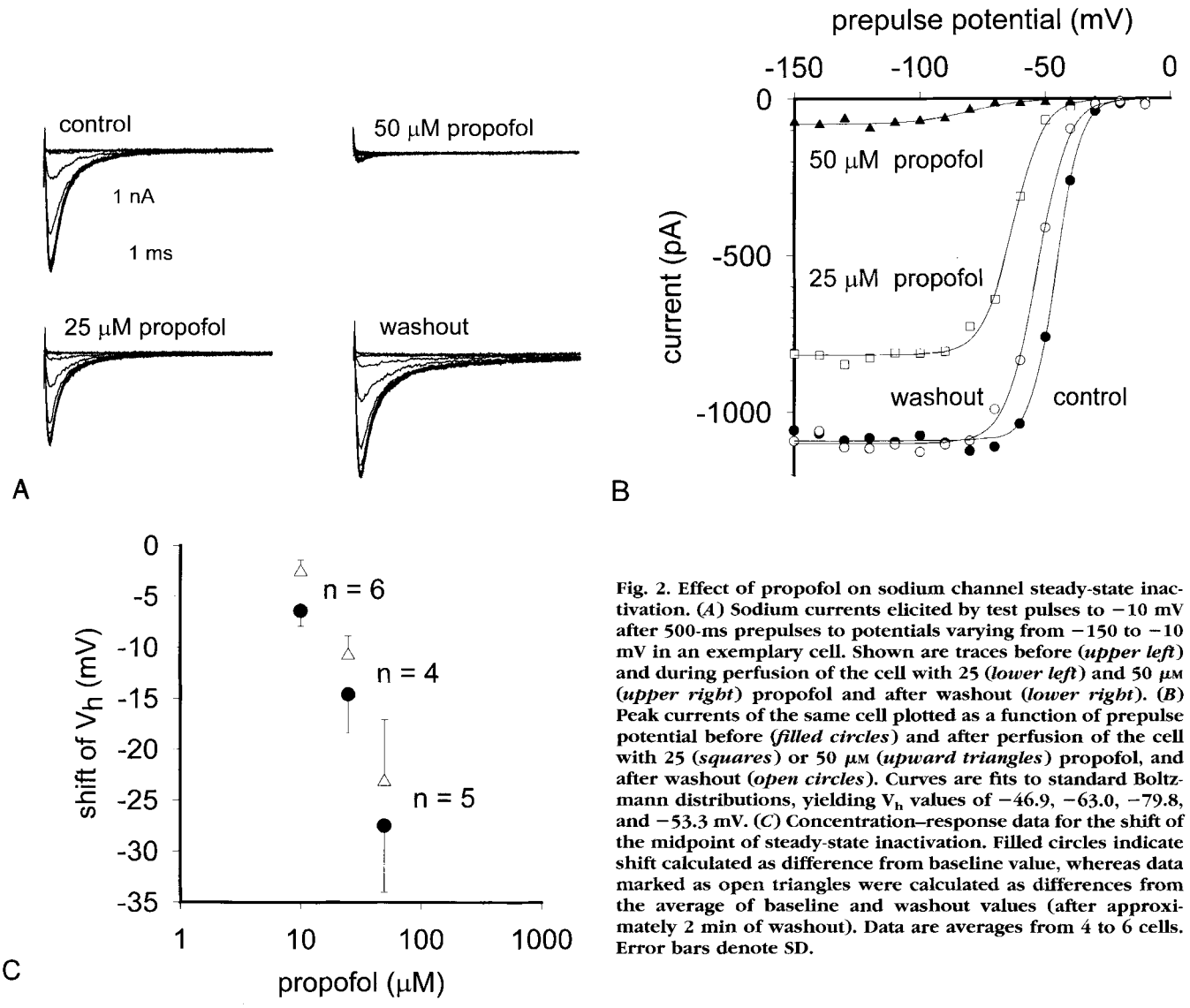

Fig. 2. Effect of propofol on sodium channel steady-state inactivation. (*A*) Sodium currents elicited by test pulses to −10 mV after 500-ms prepulses to potentials varying from −150 to −10 mV in an exemplary cell. Shown are traces before (*upper left*) and during perfusion of the cell with 25 (*lower left*) and 50 μM (*upper right*) propofol and after washout (*lower right*). (*B*) Peak currents of the same cell plotted as a function of prepulse potential before (*filled circles*) and after perfusion of the cell with 25 (*squares*) or 50 μM (*upward triangles*) propofol, and after washout (*open circles*). Curves are fits to standard Boltzmann distributions, yielding V_h values of −46.9, −63.0, −79.8, and −53.3 mV. (*C*) Concentration–response data for the shift of the midpoint of steady-state inactivation. Filled circles indicate shift calculated as difference from baseline value, whereas data marked as open triangles were calculated as differences from the average of baseline and washout values (after approximately 2 min of washout). Data are averages from 4 to 6 cells. Error bars denote SD.

inactivated and resting channels is described by a Boltzmann function: $I(V) = I_{max}\text{-}I_{max}/\{1+\exp[-z \cdot F \cdot (V - V_h)/RT]\}$. This function is characterized by three parameters, with I_{max} being the maximum current at hyperpolarized potentials (before any inactivation occurs), z the equivalent gating charge, and V_h the midpoint potential at which the function reaches its half-maximal value (control value for the seven cells was −54.0 (5.8 mV); F is the Faraday constant, R the gas constant, and T the absolute temperature.

After application of propofol, currents were reduced at all potentials, as expected from the experiments described previously. Additionally, the voltage dependence of steady-state inactivation was shifted in the hyperpolarizing (leftward) direction. This effect can be shown by plotting the shift in the midpoint potential V_h versus propofol concentration (fig 2C). At the highest concentration used (125 μM), currents were too small for accurate determination of V_h. Therefore, it was not possible to determine whether this hyperpolarizing shift saturated.

The parameter z of the inactivation curve (equivalent gating charge) was reduced slightly by increasing propofol concentrations, from 4.2 ± 1.0 before propofol application to 3.8 ± 0.9 at 10 μM, 3.2 ± 0.7 at 25 μM, and 3.0 ± 0.7 at 50 μM propofol.

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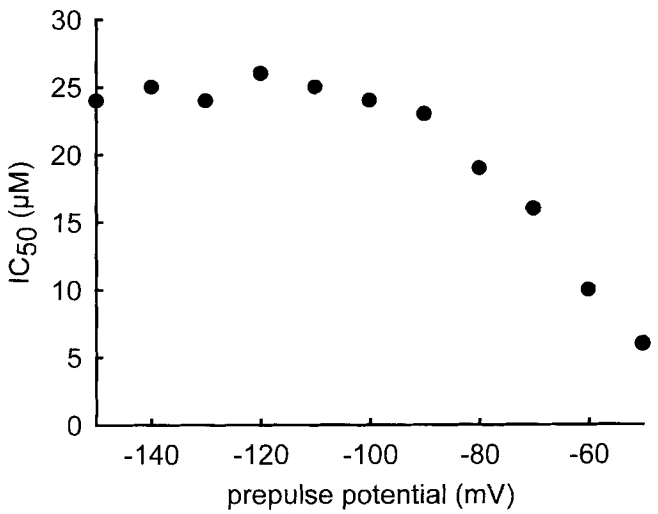

Fig. 3. IC_{50} of propofol plotted as a function of prepulse potential. IC_{50} values were calculated from currents elicited by a voltage step to −10 mV from different prepulse potentials (same pulse protocol as in fig. 2, sigmoid functions with maximum effect fixed at 100% were fitted to data averaged from four to six cells for each concentration of propofol). Data at 10, 25, and 50 μM propofol were used.

Voltage Dependence of Propofol Inhibition

The results of the above experiments reveal two effects of propofol on sodium currents: a voltage-independent current suppression observed alone at hyperpolarized prepulse potentials, and a hyperpolarizing shift of steady-state inactivation. This hyperpolarizing shift led to an additional voltage-dependent current reduction in the voltage range of channel inactivation (potentials more positive than −80 mV). Thus, propofol potency is voltage-dependent. For quantification of this voltage dependence, the concentration-response curves at each potential were calculated from steady-state inactivation curves (fig. 2B). Plotting the IC_{50} values yielded by these calculations *versus* the prepulse potential demonstrates the increase in potency in the voltage range of sodium-channel inactivation (fig. 3). At −60 mV, close to the action potential firing threshold of neuronal cells, the IC_{50} value is only 10 μM (or 14 μM when calculated as the suppression of the average of control and washout data), compared with about 25–30 μM at potentials negative to the voltage range of channel inactivation.

Use-dependent Inhibition by Propofol

Under physiologic conditions, action potentials and the opening of sodium channels rarely occur as single events but rather as trains of frequent stimuli. It has been shown for volatile anesthetics that a hyperpolarizing shift in steady-state inactivation coincides with a slowed recovery of the channels from the inactivated state to the resting state.[17] This results in use-dependent block if the intervals between depolarizing pulses are too short to allow for complete recovery of channels from the inactivated state.

We assessed use-dependent block with trains of 20 depolarizations from −85 to 0 mV applied at 5 Hz (pulse length 28.5 ms). Currents were reduced with concentration-dependent magnitudes and time constants (fig. 4A).

To further investigate the mechanism underlying this use-dependent block, we varied the pulse protocol in terms of pulse frequency (fig. 4B) and pulse duration (fig. 4C). A higher pulse frequency and a longer pulse duration both increased the time the channels spent in the inactive state, and both manipulations thus increased use-dependent block.

Discussion

Mechanisms of Propofol Suppression of Sodium Currents

In this study we demonstrated that propofol suppresses sodium currents mediated by voltage-activated neuronal sodium channels expressed in a mammalian cell line. Propofol suppressed sodium channels by at least two distinct mechanisms: a direct suppression of resting or open channels that was voltage-independent at hyperpolarized prepulse potentials, and an interaction with inactivated channels leading to a hyperpolarizing shift in sodium-channel inactivation and a voltage-dependent potency of propofol. IC_{50} values for propofol are therefore lower at depolarized potentials and reach 10 μM in the range of the threshold potential of action potential firing (*e.g.*, around −60 mV).[20] These two mechanisms are qualitatively comparable to the effects of volatile anesthetics[17] and pentobarbital[21] on neuronal sodium channels. For these latter drugs, the effects were described in terms of a modulated receptor model, in which different channel states (for sodium channels at least three: resting, open, and inactivated) are assumed to have different affinities for drug binding.

In the present experiments, both the hyperpolarizing shift of steady-state inactivation and the use-dependent block can be explained by assuming that propofol binds more strongly to the inactivated state of the channel than to its resting state. The observation that use-dependent block is increased by increasing the time the channel spends in the inactivated state (longer depolarizations,

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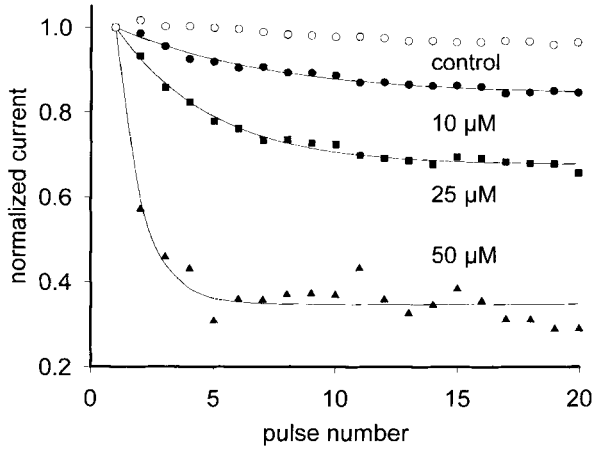

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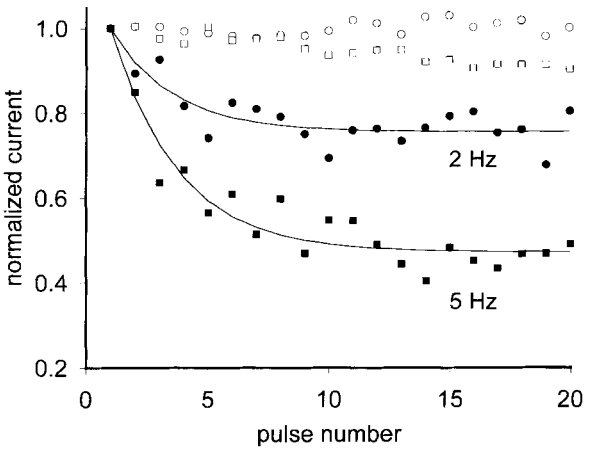

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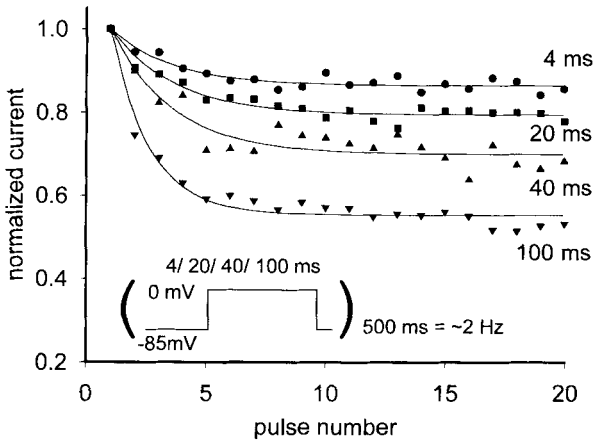

C

Fig. 4. Use-dependent block induced by propofol. Plotted are currents elicited by trains of 20 pulses to 0 mV. Data are from separate exemplary cells. Currents are normalized to the first pulse. Curves are fits of a single exponential function to the data. (*A*) Concentration dependence of the use-dependent block at 5 Hz (*open circles*, control; *filled circles*, 10 μM; *squares*, 25 μM; *triangles*, 50 μM propofol). Pulse length was 28.5 ms. Fits yielded time constants of 1.2, 0.8, and 0.2 s, and amplitudes of 0.84, 0.68, and 0.35 at 10, 25, and 50 μM propofol, respectively. (*B*) Frequency dependence of the use-dependent block induced by 50 μM propofol (*open symbols*, controls; *circles*, 2 Hz; *squares*, 5 Hz). Pulse length was 28.5 ms. Fits yielded time constants of 1.3 and 0.5 s, and amplitudes of 0.76 and 0.47 at 2 and 5 Hz, respectively. (*C*) Influence of pulse duration on the use-dependent block induced by 50 μM propofol. Depolarizing pulse duration was varied between 4 (*circles*), 20 (*squares*), 40 (*upward triangles*), and 100 ms (*downward triangles*); the recovery interval was kept constant at 500 ms, corresponding to a frequency of approximately 2 Hz. Fits yielded time constants of 1.3, 1.3, 1.3, and 0.8 s, and amplitudes of 0.87, 0.80, 0.70, and 0.55 with 4, 20, 40, and 100 ms pulses, respectively.

fig. 4C) is strong evidence that propofol alters the distribution between resting and inactivated channels.

One possible explanation for the qualitatively similar mechanisms of sodium current suppression by propofol, volatile anesthetics, and pentobarbital would be common or overlapping binding sites. This explanation is supported by the fact that quantitatively, sodium-channel suppression for all these drugs correlates well with the octanol-water partition coefficient (for propofol a value of 4300 was used[22]) in a double logarithmic representation (fig. 5). However, this correlation does not allow any inferences on whether the interaction between the drugs and the sodium channel is protein- or lipid-mediated. In contrast to these similarities, the concentration-response curve for the reduction of peak sodium current by propofol exhibits a slope parameter of 1.7, whereas the data for all volatile anesthetics and pentobarbital were well fitted with a slope parameter of 1.[17,21] Additional interactions may be involved in propofol action, which may also explain the incomplete washout of the propofol modification. The latter phenomenon may be caused by a slow washout of the lipophilic drug from the membrane, or related to irreversible effects of the drug on the cell.

Comparison with Results from Previous Propofol Studies

For this study we used rat brain IIa sodium channel α subunits expressed in a stably transfected Chinese hamster ovary cell line to study the effects of propofol on brain sodium channels. The suitability of this preparation for these anesthetic studies has been discussed previously in detail.[17] Briefly, this preparation, despite the lack of β subunits, has been shown not to deviate from normal physiologic[23] and pharmacologic[24] behavior of sodium channels *in situ*. Moreover, the β_1 subunit,

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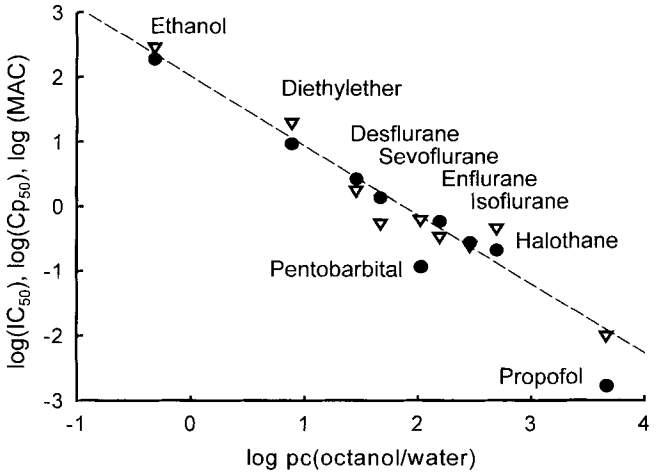

Fig. 5. Correlation of IC_{50} values for sodium-channel block at −60 mV (*open triangles*) and clinical effect concentrations (human minimum alveolar concentration converted to aqueous concentrations or Cp_{50}, *filled circles*) with octanol–water partition coefficients for volatile anesthetics, pentobarbital, and propofol. The line is a linear regression fit to the data for sodium channel block with a slope of −1.08. Data for the volatile anesthetics and ethanol are from reference 17, and for pentobarbital from reference 21. Free propofol concentration at Cp_{50} was calculated as 1.3 μM,[16] and an octanol—water partition coefficient for propofol of 4300 was used.[22]

when coexpressed in this preparation, does not significantly shift channel inactivation.[25] The type IIa sodium channel is the most prominent subtype present in adult brain, and the rat brain IIa sodium-channel subtype has greater than 97% structural identity with the equivalent human-brain sodium channel.[26]

The effect of propofol on human-brain sodium channels has been studied previously in a lipid bilayer preparation.[12] In lipid bilayers, sodium-channel inactivation was removed by batrachotoxin, and therefore the propofol effect on channel inactivation could not be studied. However, the IC_{50} value for propofol obtained in the lipid bilayer preparation is comparable with what has been found in this study at hyperpolarized prepulse potentials (about 25 μM at potentials negative to the voltage range of channel inactivation).

In the study of propofol effects on glutamate release, propofol inhibited sodium flux with an IC_{50} of about 9 μM.[13] This concentration is similar to what we found at depolarized potentials (IC_{50} about 10 μM at −60 mV). Therefore our results agree with, and support, the conclusions of the previous studies using central nervous system sodium channels. Comparable qualitative and quantitative data have been reported for sodium currents in rat ventricular myocytes.[27] In contrast, for sodium currents in myelinated axons of *Xenopus laevis* a much higher IC_{50} value for propofol has been reported.[28] Similar differences in the sensitivity of peripheral nerve and central nervous system sodium channels have been previously found for volatile anesthetics.[17]

Clinical Significance of Sodium Channel Suppression by Propofol

In the present study we found that propofol blocked sodium-channel current with an IC_{50} of about 10 μM in the range of the threshold potential of action potential firing (positive to −60 mV). For propofol, the plasma concentration at which 50% of the patients do not respond to skin incision (Cp_{50}) has been determined as 85 μM in whole blood,[29] which corresponds to a free propofol concentration in plasma of 1.3 μM (assuming protein-bound fraction of 98% and a plasma:whole blood concentration ratio of 0.78[30]). Thus the effects we observed occur at about eight-fold higher concentrations than the reported clinical propofol levels.

When comparing the propofol concentrations necessary to block sodium channels, however, several complicating factors must be considered. First, a concentration at which 50% inhibition occurs at the molecular level may not translate directly into a half-maximal effect at the tissue or organismal levels. For example, small reductions in sodium-channel conductance should result theoretically in substantial changes of action potential firing threshold.[31] This effect has been demonstrated for volatile anesthetics as a result of the hyperpolarizing shift in steady-state inactivation.[32] Second, the use-dependent block of sodium channels observed in our experiments should lead to anesthetic impairment of axonal conduction in regions with low safety factors for conduction (ratio of the current supplied by the incoming action potentials to that required to sustain propagation), such as neuronal bifurcations[33] or dendrites.[34] Finally, as mentioned previously, a recent study has demonstrated that propofol impairs release of glutamate in rat-brain synaptosomes by inhibition of sodium channels.[13] These effects occurred in the same concentration range as the sodium channel suppression found in this study.

Comparison with Volatile Anesthetics and Pentobarbital

As discussed previously, the clinical effect of propofol occurs at about eight-fold lower concentrations than a 50% sodium channel suppression at −60 mV

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(IC_{50} value of 10 μM). In contrast, for volatile anesthetics, sodium-channel suppression at neuronal threshold potentials has been shown to correlate well with clinical minimum alveolar concentrations.[17] Thus, minimum alveolar concentrations and IC_{50} values for sodium-channel suppression at −60 mV for the volatile anesthetics are similar (fig. 5). Similar to propofol, however, another intravenous anesthetic, pentobarbital, inhibits sodium currents at concentrations about five-fold above clinical levels.

As discussed previously, all anesthetics examined cause qualitatively similar effects on sodium channels: a voltage-independent effect on open or resting sodium-channel states, and a shift in steady-state inactivation that results in a voltage-dependent block of sodium channels. All anesthetics, volatile and intravenous, have similar quantitative interactions with the closed or resting states, with IC_{50}s for voltage-independent block about 5-10-fold above clinical levels.[17,21] However, volatile anesthetics cause a much greater shift in steady-state inactivation than either propofol or pentobarbital. For example, at IC_{50} for the voltage-independent block, halothane caused about a −30-mV shift in inactivation and isoflurane about a −20-mV shift. In contrast, equivalent concentrations of propofol and pentobarbital caused only about a −5- to −8-mV shift. Therefore it is the change in the distribution between resting and inactive channels that appears to differ significantly between the volatile and intravenous anesthetics examined. Nonetheless, examination of the Meyer-Overton plot in figure 5 indicates that all of the anesthetics inhibit sodium channels at concentrations that correlate well with anesthetic hydrophobicity. Whereas for the volatile anesthetics clinical effects occur at the same concentrations, propofol and pentobarbital fall below this Meyer-Overton regression line.

One possible conclusion is that sodium-channel suppression is more important in anesthesia with volatile anesthetics than with propofol or pentobarbital, as these anesthetics may have more sensitive receptors. It needs to be considered, however, that plasma concentrations for intravenous anesthetics may not be identical to effect-site concentrations in the brain. It has been suggested that brain concentrations of propofol are actually about eight-fold higher than plasma concentrations,[35] corresponding to a brain: plasma partition coefficient of propofol of around 8,[36] and thus sodium-channel suppression may indeed have a role in propofol anesthesia.

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