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Isoflurane and Propofol Inhibit Voltage-Gated Sodium Channels in Isolated Rat Neurohypophysial Nerve Terminals

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ABSTRACT

Mounting electrophysiological evidence indicates that certain general anesthetics, volatile anesthetics in particular, depress excitatory synaptic transmission by presynaptic mechanisms. We studied the effects of representative general anesthetics on voltage-gated Na^+ currents (I_{Na}) in nerve terminals isolated from rat neurohypophysis using patch-clamp electrophysiological analysis. Both isoflurane and propofol inhibited I_{Na} in a dose-dependent and reversible manner. At holding potentials of −70 or −90 mV, isoflurane inhibited peak I_{Na} with IC_{50} values of 0.45 and 0.56 mM, and propofol inhibited peak I_{Na} with IC_{50} values of 4.1 and 6.0 μM, respectively. Isoflurane (0.8 mM) did not significantly alter the $V_{1/2}$ of activation; propofol caused a small positive shift. Isoflurane (0.8 mM) or propofol (5 μM) produced a negative shift in the voltage dependence of inactivation. Recovery of I_{Na} from inactivation was slower from a holding potential of −70 mV than from −90 mV; isoflurane and propofol further delayed recovery from inactivation. In conclusion, the volatile anesthetic isoflurane and the intravenous anesthetic propofol inhibit voltage-gated Na^+ currents in isolated neurohypophysial nerve terminals in a concentration- and voltage-dependent manner. Marked effects on the voltage dependence and kinetics of inactivation and minimal effects on activation support preferential anesthetic interactions with the fast inactivated state of the Na^+ channel. These results are consistent with direct inhibition of oxytocin and vasopressin release from the neurohypophysis by isoflurane and propofol. Inhibition of voltage-gated Na^+ channels may contribute to the presynaptic effects of general anesthetics on nerve terminal excitability and neurotransmitter release.

The mechanisms by which general anesthetics produce the triad of unconsciousness, amnesia and immobility have yet to be defined despite over a century of research. Depression of excitatory transmission and/or facilitation of inhibitory transmission are the principal neurophysiological effects of general anesthetics on synaptic transmission, but the cellular and molecular targets for these actions have not been established (Perouansky and Hemmings, 2003). Considerable evidence indicates that potentiation of $GABA_A$ receptors is involved in the facilitation of inhibitory transmission by anesthetics (Yamakura et al., 2001). General anesthetics also modulate other members of the ligand-gated ion channel superfamily, including neuronal nicotinic acetylcholine receptors and *N*-methyl-D-aspartate-type glutamate receptors (Yamakura et al., 2001). General anesthetics also affect voltage-gated Na^+, K^+, and Ca^{2+} channels (Topf et al., 2003), cell signaling mechanisms such as protein kinase C (Hemmings, 1998) and G protein-coupled receptor pathways (Ishizawa et al., 2002), vesicular exocytotic mechanisms (van Swinderen et al., 1999), and transmitter uptake mechanisms (Shahani et al., 2002). The contributions of these and possibly other targets to the synaptic actions of general anesthetics have not been clearly defined.

Mounting electrophysiological evidence indicates that general anesthetics depress excitatory synaptic transmission by presynaptic mechanisms (MacIver et al., 1996; Kirson et al., 1998; Perouansky and Hemmings, 2003). At the neurochemical level, volatile anesthetics and propofol inhibit depolarization-evoked glutamate release from isolated rat cortical nerve terminals (Schlame and Hemmings, 1995; Lingamaneni et al., 2001). To investigate the possible ion channel targets for these presynaptic actions, we used isolated rat neurohypophysial (NHP) nerve terminals as a system amenable to patch-clamp electrophysiological analysis. Nerve terminals from supraoptic and periventricular magnocellular neurons terminate in the neurohypophysis and contain large dense-core synaptic vesicles filled with oxytocin or arginine vasopressin. These nerve terminals are of sufficient size (5–16 μm in diameter) for patch-clamp analysis (Lemos and Nordmann, 1986). Previous studies indicate that NHP nerve terminals express voltage-gated Na^+, K^+, and Ca^{2+} channels, which are involved in the control of nerve terminal excitability and peptide release (Lemos and Nowycky, 1989; Bielefeldt et al., 1992; Lindau et al., 1992; Turner and Stu-

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ABBREVIATIONS: NHP, neurohypophysial; ANOVA, analysis of variance; I-V, current-voltage.

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enkel, 1998; Wang et al., 1999). We now report that the widely used general anesthetics isoflurane and propofol at clinically relevant concentrations inhibit voltage-gated Na^+ channels in isolated NHP nerve terminals.

Materials and Methods

Materials. Isoflurane was obtained from Abbott Laboratories (North Chicago, IL) and propofol from Sigma-Aldrich (St. Louis, MO). Amphotericin B, tetraethylammonium chloride, and tetrodotoxin were purchased from Sigma-Aldrich. Male Sprague-Dawley rats (260–300 g) were from Charles River Laboratories, Inc. (Wilmington, MA).

Nerve Terminal Preparation. Experimental protocols were approved by the Institutional Animal Care and Use Committee of Weill Medical College of Cornell University. NHP terminals were prepared as described previously (Wang et al., 1999) with minor modifications. Male Sprague-Dawley rats were anesthetized with 80% CO_2, 20% O_2 and decapitated; this technique avoids hypoxemia and exposure to clinically used anesthetic drugs (H. C. Hemmings, Jr., unpublished observations). The neurohypophysis was separated from pars anterior and pars intermedia of the pituitary, and gently homogenized in a solution containing 270 mM sucrose, 10 mM HEPES-Tris, and 0.01 mM K-EGTA, pH 7.25, using a 0.5-ml Teflon/glass homogenizer. The NHP homogenate was pipetted into a plastic Petri dish (35 × 10 mm) and allowed to settle for 5 to 8 min. The Petri dish with dissociated NHP nerve terminals was placed onto the stage of an ECLIPSE TE300 inverted microscope (Nikon, Melville, NY) equipped with interference contrast optics (Hoffmann, Melville, NY) and superfused (2–3 ml/min) with Locke's solution consisting of 145 mM NaCl, 5 mM KCl, 2.2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM Na-HEPES, and 15 mM D-glucose, pH 7.30. Each preparation contained many structures less than 3 μm in diameter, as well as a small number of terminals with diameters of 5 to 16 μm. Terminals were identified by their bright refraction, smooth spherical shape, and absence of a nucleus. Isolated NHP terminals were readily distinguished from pars intermedia cells, which are larger and nucleated. In the present study, we selected terminals of 5- to 10-μm diameter because of their relative abundance and space clamp characteristics for Na^+ current recording.

Electrophysiological Recording. Recording pipettes (tip diameter <1 μm) were made from borosilicate glass capillaries (Drummond Scientific, Broomall, PA) using a micropipette puller (Sutter Instruments, Novato, CA) and fire polished (Narishige Microforge, Kyoto, Japan). The perforated patch-clamp technique (Wang et al., 1999) was used to record Na^+ currents at room temperature (23–25°C). Terminals with access resistances of <10 MΩ were selected for study. Pipettes were filled with a solution containing amphotericin B (240–300 μg/ml) in 145 mM Cs-glutamate, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, 5 mM D-glucose, and 10 mM tetraethylammonium chloride, pH 7.25. Capacitance and 60 to 80% series resistance were compensated. Whole-terminal currents were sampled at 10 kHz and filtered at 1 to 3 kHz using an Axon 200B amplifier and pClamp 8 software (Axon Instruments, Inc., Burlingame, CA). Locke's solution perfused the chamber at 0.10 to 0.15 ml/min. Anesthetics were introduced from glass syringes and applied locally to attached terminals at 0.05 ml/min through a 0.15-mm-diameter perfusion pipette (30–40 μm away from patched terminals) using an ALA-VM8 pressurized perfusion system (ALA Scientific, Westbury, NY). Isoflurane and propofol were diluted into Locke's solution from stock solutions (10–12 mM isoflurane in Locke's solution, prepared 12–24 h before experiments; or 10 mM propofol in dimethyl sulfoxide). Concentrations of isoflurane or propofol in the recording chamber were determined by local sampling of the perfusate at the site of the recording pipette tip and analysis by gas chromatography (Ratnakumari and Hemmings 1998) or high-performance liquid chromatography (Lingamaneni et al., 2001), respectively.

Data Analysis. IC_{50} values were obtained by least-squares fitting of data to the Hill equation: $Y = 1/(1 + 10[(\log IC_{50} - X) \times n_H])$, where X is the concentration, Y is the response, and n_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 the maximum conductance, $V_{1/2}$ 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} = 67 mV). Steady-state 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, $V_{1/2}$ is the voltage of half-maximal inactivation, and k is the slope factor. Data for I_{Na} recovery from inactivation were fitted to a single exponential rising function of the form $I/I_{max} = 1 - A\,e(-t/\tau)$, where I/I_{max} is the normalized current, A is the amplitude, τ is the time constant for I_{Na} recovery, and t is the interpulse interval. Data were analyzed using pClamp 8 (Axon Instruments, Inc.), Prism 3.02 (GraphPad Software Inc., San Diego, CA) and SigmaPlot 6.0 (SPSS Science, Chicago, IL). Data are expressed as mean ± S.E.M. Statistical significance was assessed by ANOVA or paired or unpaired t test, as appropriate; $p < 0.05$ was considered statistically significant.

Results

Basic Properties of I_{Na}. Representative Na^+ current traces determined in neurohypophysial terminals (5–10 μm diameter) using the perforated patch-clamp recording technique are shown in Fig. 1. The perforated-patch technique was used because conventional whole-terminal patch recording led to greater rundown of Na^+ current (data not shown) and this complicated repeated measurements essential to pharmacological analysis. We took two measures to improve space clamp conditions for Na^+ current recording using the perforated-patch method: 1) relatively small nerve terminals were selected for recording and 2) larger pipette tips were used.

Voltage-activated Na^+ currents were blocked completely by 0.5 μM tetrodotoxin (Fig. 1). I_{Na} amplitudes evoked from a holding potential of −90 mV (conventionally used to fully activate Na^+ current) were larger compared with a holding potential of −70 mV (closer to physiological resting potential) because fewer channels are in the inactivated state at −90 mV (Ruben et al., 1992). At a holding potential of −90 mV, mean peak I_{Na} amplitude was −1370 ± 380 pA (n = 57); at a holding potential of −70 mV, mean peak I_{Na} amplitude was −1090 ± 210 pA (n = 39).

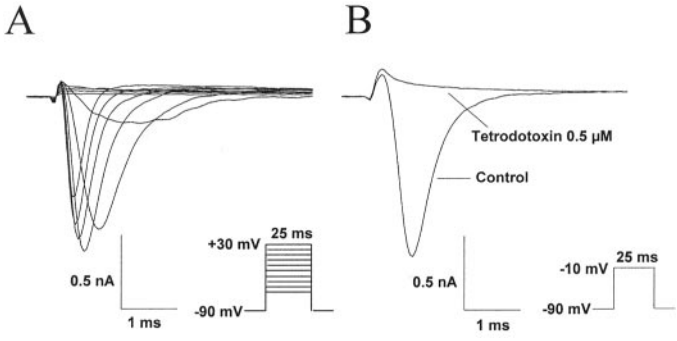

Fig. 1. A, representative I_{Na} traces in a neurohypophysial terminal (8 μm) using the perforated-patch-clamp technique. B, voltage-activated I_{Na} was inhibited completely by tetrodotoxin.

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Anesthetic Effects on Peak I_{Na}. Isoflurane and propofol inhibited I_{Na} in a reversible and dose-dependent manner (Fig. 2). Onset of inhibition of I_{Na} by isoflurane or propofol was rapid (less than 1 min of application) and rapidly reversed upon washing (within 1 min) using focal pipette perfusion of the patched terminals (data not shown). In the presence of 0.4 or 0.8 mM isoflurane, peak I_{Na} amplitude was reduced to 58 ± 2% ($p < 0.01$) and 39 ± 4% ($p < 0.01$) of control, respectively. In the presence of 2 or 5 μM propofol, peak I_{Na} amplitude was reduced to 75 ± 3% ($p < 0.05$) and 56 ± 5% ($p < 0.01$) of control, respectively (Fig. 2B).

From a holding potential of −90 mV, peak I_{Na} activation was elicited at −10 mV (Fig. 3A). Low concentrations of either anesthetic had minimal effects on the current-voltage (I-V) relationship, other than reductions in peak I_{Na} amplitude. At holding potentials of −70 or −90 mV, IC_{50} values for peak I_{Na} inhibition by isoflurane were 0.45 and 0.56 mM (Fig. 3B) with Hill slopes of 1.7 and 1.8, respectively. At holding potentials of −70 or −90 mV, IC_{50} values for propofol were 4.1 and 6.0 μM ($p < 0.01$) with Hill slopes of 1.6 and 1.8, respectively. These results indicate slightly more potent inhibition of I_{Na} at physiological resting membrane potential. The effects of both drugs were well fitted by the Hill equation with efficacies of 100% inhibition. Both anesthetics were effective at concentrations observed during clinical anesthesia: the EC_{50} for general anesthesia for isoflurane is 0.35 mM (Taheri et al., 1991) and for propofol is 2.2 μM (Tonner et al., 1992).

Anesthetic Effects on I_{Na} Activation and Inactivation. The effects of anesthetics on the voltage dependence of Na^+ channel activation are shown in Fig. 4. Isoflurane (0.8 mM) did not significantly alter the $V_{1/2}$ of activation of Na^+ conductance from holding potentials of −70 or −90 mV, consistent with no shift in the I-V curve (Fig. 3A). At a holding potential of −70 mV, 5 μM propofol produced a slight positive shift in the activation curve ($p < 0.05$, $n = 5$; Table 1), consistent with the small shift in the I-V curve apparent from −90 mV at 10 μM propofol (Fig. 3A). Both anesthetics slightly increased the slope factor (k) at −70 mV, and propofol also increased the slope factor at −90 mV.

Steady-state inactivation curves for I_{Na} in the absence or presence of anesthetics were determined with standard two-pulse protocols (Figs. 5 and 6). Isoflurane (0.8 mM) produced a negative shift in the voltage dependence of inactivation with no effect on the slope factor (Table 1). Propofol (5 μM) produced a negative shift in the voltage dependence of inactivation and increased the slope factor (Table 1). Although not statistically significant, isoflurane and propofol had greater effects on inactivation curves when using a short (30 ms) compared with a long prepulse protocol (Fig. 6; Table 1). These observations are consistent with drug binding to and stabilization of the inactivated state of Na^+ channels.

Anesthetic Effects on I_{Na} Recovery from Inactivation. The kinetics of I_{Na} recovery from the inactivated state are crucial in regulating NHP terminal excitability and repetitive firing, and hence neuropeptide release. Inactivation

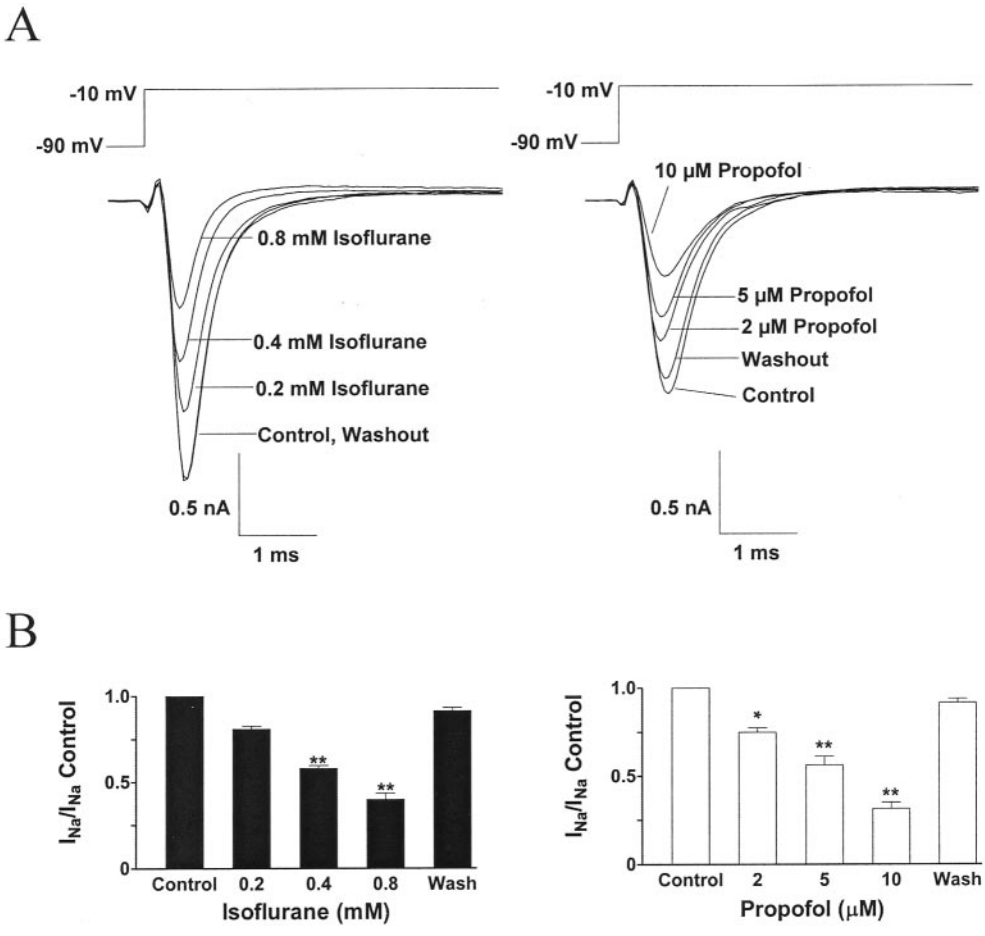

Fig. 2. Reversible inhibition of I_{Na} by isoflurane and propofol in neurohypophysial terminals. A, representative current traces from two terminals. B, concentration-dependent inhibition of peak I_{Na} by isoflurane or propofol. Data are shown as mean ± S.E.M. (n = 7–13). Washout data were obtained 2 min after a 0.5-min exposure to 0.8 mM isoflurane or 10 μM propofol. *, $p < 0.05$ versus control; **, $p < 0.01$ versus control, by ANOVA with Dunnett's post hoc test. Holding potential −90 mV, 25-ms test potential to −10 mV.

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was investigated using a standard two-pulse protocol with varying interpulse intervals from holding potentials of −70 or −90 mV (Fig. 7). I_{Na} recovery from inactivation was fitted by a single exponential function. Recovery was slower from a holding potential of −70 mV and was further slowed by application of isoflurane or propofol (Fig. 7; Table 2). Propofol had a greater effect on recovery from inactivation from a holding potential of −70 mV compared with −90 mV, whereas isoflurane had similar effects at either potential (Table 2). The slower rate of recovery from inactivation in the presence of propofol suggests slower dissociation of propofol from inactivated channels compared with isoflurane.

Discussion

The structurally distinct general anesthetics isoflurane and propofol inhibited voltage-gated Na^+ currents in isolated neurohypophysial nerve terminals in a concentration- and voltage-dependent manner. Both anesthetics blocked nerve terminal voltage-gated Na^+ currents at concentrations observed during clinical anesthesia. Inhibition was more potent from a holding potential of −70 mV, a physiological resting membrane potential, than from −90 mV. This is consistent with the marked effects of both anesthetics on the voltage dependence and kinetics of Na^+ channel inactivation.

The actions of isoflurane and propofol on I_{Na} varied with holding potential. The $V_{1/2}$ of activation was shifted slightly in a positive direction by propofol at a holding potential of −70 mV (by ~5 mV), but isoflurane had no significant effect on $V_{1/2}$ of activation. The $V_{1/2}$ of inactivation was consistently shifted in a negative direction by both isoflurane (−8 mV for 800-ms prepulse protocol; −14 mV for 30-ms prepulse protocol) and propofol (−11 mV for 800-ms prepulse protocol; −14 mV for 30-ms prepulse protocol). Both anesthetics also delayed recovery from inactivation. These results suggest that anesthetics exert distinct effects on Na^+ channel gating, which may involve multiple target sites on Na^+ channels and/or on their modulators. Inhibition of Na^+ currents by isoflurane is caused by enhanced channel inactivation, whereas inhibition by propofol can be attributed primarily to enhanced inactivation with some contribution of less effective activation apparent at higher concentrations. The greater effects of isoflurane and propofol on inactivation after a short prepulse (30 ms), which induces mainly fast inactivation, suggest that these general anesthetics primarily affect fast inactivation, as do the local anesthetics (Ragsdale et al., 1994). The marked effects of isoflurane and propofol on channel inactivation are consistent with greater anesthetic affinity for the inactivated state of the channel, analogous to the actions of local anesthetics and anticonvulsants (Catterall, 2002). The slowed rate of recovery from inactivation may reflect either slow anesthetic dissociation from the inactivated or resting state or slowed conversion of channels from the inactivated to resting states.

The interaction between a drug and its receptor involves a specific intermolecular interaction that yields a sigmoidal concentration-effect response curve with a Hill slope related to the stoichiometry of the interaction. Despite efforts to obtain satisfactory space-clamp conditions for Na^+ current

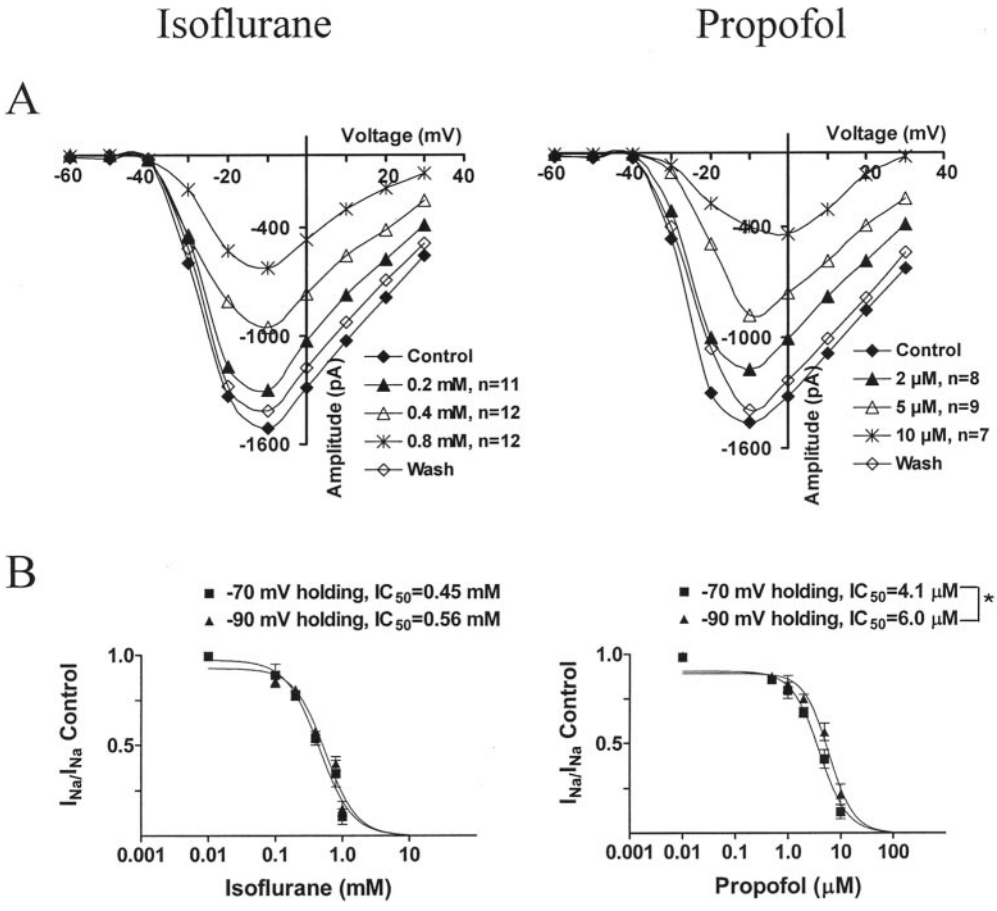

Fig. 3. A, effects of isoflurane or propofol on representative I-V relationships of I_{Na} in isolated neurohypophysial terminals. Holding potential −90 mV, 25-ms test potential from −60 to +30 mV in 10-mV increments. B, concentration-effect curves for inhibition of peak I_{Na} by isoflurane or propofol. Data (mean ± S.E.M.; n = 6–9) were fitted to the Hill equation. Currents elicited from a holding potential of −70 mV were more sensitive to propofol than those elicited from −90 mV (*, $p < 0.01$ by unpaired t test).

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recording using the perforated-patch method, atypically steep activation of Na^+ current was observed in some recordings. Such imperfect voltage control would tend to increase the steepness of the concentration-effect curves, which would lead to artificially high Hill slope values. This limitation makes it difficult to infer the stoichiometry of the interaction of general anesthetics with the Na^+ channel from Hill slope values alone. Thermodynamic parameters of the binding equilibrium of general anesthetics to Na^+ channels could provide more detailed information on binding interactions. For example, the temperature dependence of binding of anesthetics could be analyzed using van't Hoff plots made by comparing IC_{50} values at various temperatures. However, such experiments in this preparation are limited by the instability of isolated nerve terminals at temperatures above 25°C.

Voltage-gated Na^+ channels are hetero-oligomers composed of α, β_1, and β_2 subunits. At least nine different Na^+ channel α subunit isoforms have been cloned and identified in human and rat: Nav1.1, Nav1.2, Nav1.3, and Nav1.6 are expressed in the central nervous system; Nav1.7, Nav1.8, and Nav1.9 are expressed in the peripheral nervous system; and Nav1.4 and Nav1.5 are expressed in skeletal and cardiac muscle, respectively (Goldin, 2002). The α subunit alone is sufficient to produce functional channels (Catterall, 2002); it contains four homologous domains, each containing six transmembrane segments. Local anesthetics bind to receptor sites in segment S6 of domains III (Catterall, 2002) and IV (Ragsdale et al., 1994; Catterall, 2002). The intracellular loop between domains III and IV may function as the inactivation gate to mediate fast inactivation. Transmembrane segment S6 in domain I also contributes to the local anesthetic binding site (Yarov-Yarovoy et al., 2002). Our findings that general anesthetics shift the steady-state inactivation curve toward more negative membrane potentials and slow recovery from inactivation support an interaction with the inactivated state of the Na^+ channel similar to that of local anesthetics. Whether general anes-

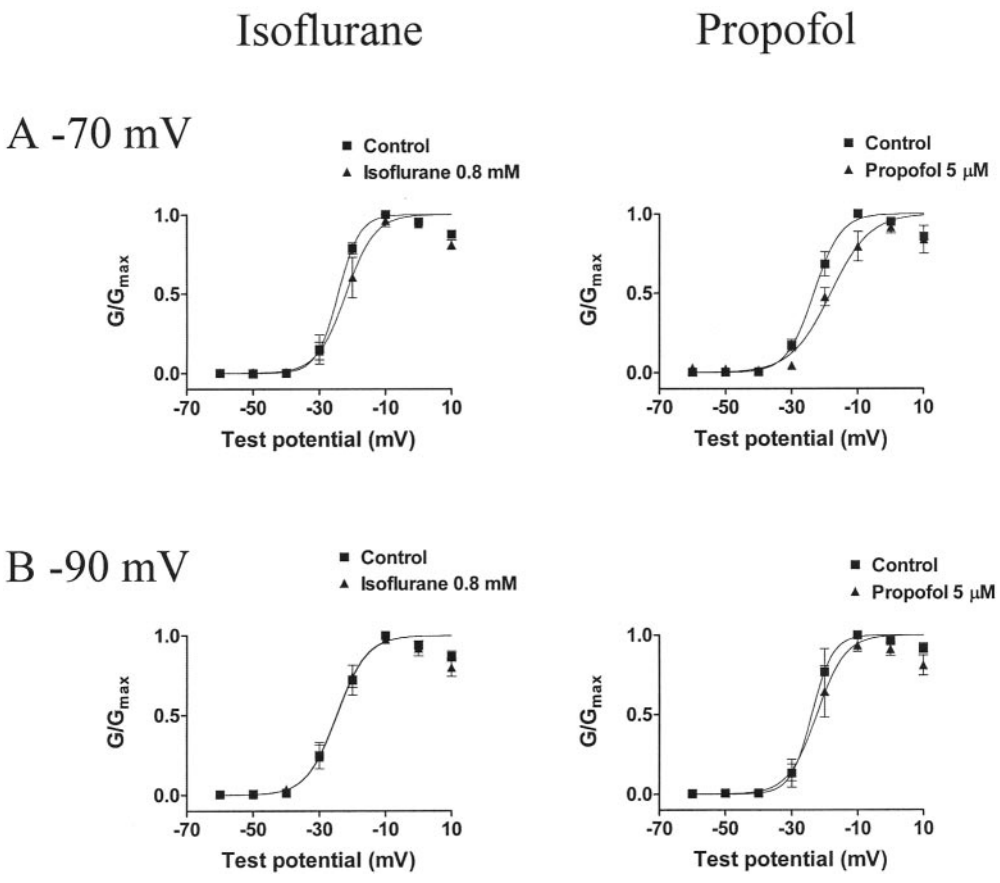

Fig. 4. Effects of isoflurane (left) or propofol (right) on the voltage dependence of activation of peak I_{Na} in neurohypophysial terminals from holding potentials of −70 mV (A) or −90 mV (B). Data shown as mean ± S.E.M. (n = 5–9).

TABLE 1
Effects of isoflurane and propofol on Na^+ current activation and inactivation

	Activation				Inactivation			
	Holding: −70 mV		Holding: −90 mV		300-ms Prepulse		30-ms Prepulse	
	$V_{1/2}$	k	$V_{1/2}$	k	$V_{1/2}$	k	$V_{1/2}$	k
	mV		mV		mV		mV	
Control	−24.3 ± 0.9	3.3 ± 0.6	−24.6 ± 1.0	4.6 ± 0.8	−59.2 ± 0.4	4.8 ± 0.4	−61.4 ± 0.8	5.5 ± 0.8
Isoflurane 0.8 mM	−21.9 ± 1.3	4.4 ± 1.2	−24.8 ± 1.5	4.6 ± 1.2	−67.5 ± 1.0**	4.9 ± 0.9	−75.3 ± 0.6**	5.0 ± 0.5
Control	−23.3 ± 1.0	4.1 ± 0.8	−23.9 ± 0.6	3.5 ± 0.6	−54.5 ± 0.3	5.4 ± 0.2	−58.7 ± 0.7	5.6 ± 0.6
Propofol 5 μM	−18.2 ± 1.4*	6.1 ± 1.3	−22.4 ± 1.4	4.1 ± 1.2	−65.1 ± 0.6**	11.1 ± 0.6**	−72.9 ± 1.2**	10.5 ± 1.0**

* $p < 0.05$; ** $p < 0.01$ versus respective control by unpaired t test (n = 4–9).

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thetics also interact with segment S6 will require further investigation.

A voltage sensor characterized by a series of positively charged amino acids in segment S4 is critically involved in Na^+ channel activation (Catterall, 2002; Goldin et al., 2002). The actions of isoflurane on the voltage dependence of activation were unremarkable, and the small effect of propofol was evident only at a holding potential of −70 mV. In a previous report, volatile anesthetics shifted the voltage dependence of both activation and steady-state inactivation of cardiac Na^+ currents toward more hyperpolarized potentials (Weigt et al., 1997). This may represent a subtle difference in anesthetic actions on neuronal (Nav1.2, Nav1.3, and Nav1.6) versus cardiac (Nav1.5) voltage-gated Na^+ channels.

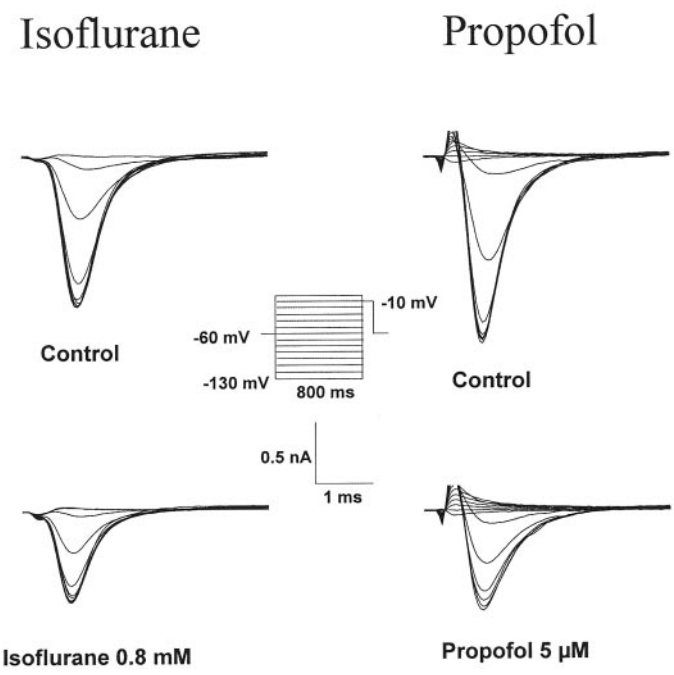

Fig. 5. Representative traces showing effects of isoflurane (left) and propofol (right) on I_{Na} inactivation in neurohypophysial terminals.

In an influential review, Franks and Lieb (1994) concluded that effects of general anesthetics on voltage-gated ion channels occurred only at concentrations irrelevant to clinical anesthesia. This conclusion was based largely on studies conducted in molluscan axons (Haydon and Urban, 1983) and has not been supported by more recent studies of mammalian Na^+ channels. Thus, isoflurane (IC_{50} = 0.85 mM at −120 mV holding potential; Rehberg et al., 1996) and propofol (IC_{50} = 25.4 μM at −120 mV holding potential; Rehberg and Duch, 1999) inhibited cloned rat brain (type IIa) Na^+ channel α subunits (Nav1.2) heterologously expressed in Chinese hamster ovary cells; both anesthetics also shifted the voltage dependence of inactivation in a negative direction and delayed recovery from inactivation. The IC_{50} values for inhibition of peak I_{Na} by isoflurane and propofol in NHP terminals reported here are lower than those reported for the isolated Nav1.2 α subunit. These differences suggest possible effects of specific Na^+ channel isoforms, post-translational modification, and/or the presence of auxiliary subunits on Na^+ channel sensitivity to anesthetics. For example, β subunit coexpression increases local anesthetic sensitivity of heterologously expressed rat Nav1.2 α subunits (Bonhaus et al., 1996).

We previously reported indirect neurochemical evidence that volatile anesthetics and propofol inhibit presynaptic Na^+ channels and Na^+ channel-dependent glutamate re-

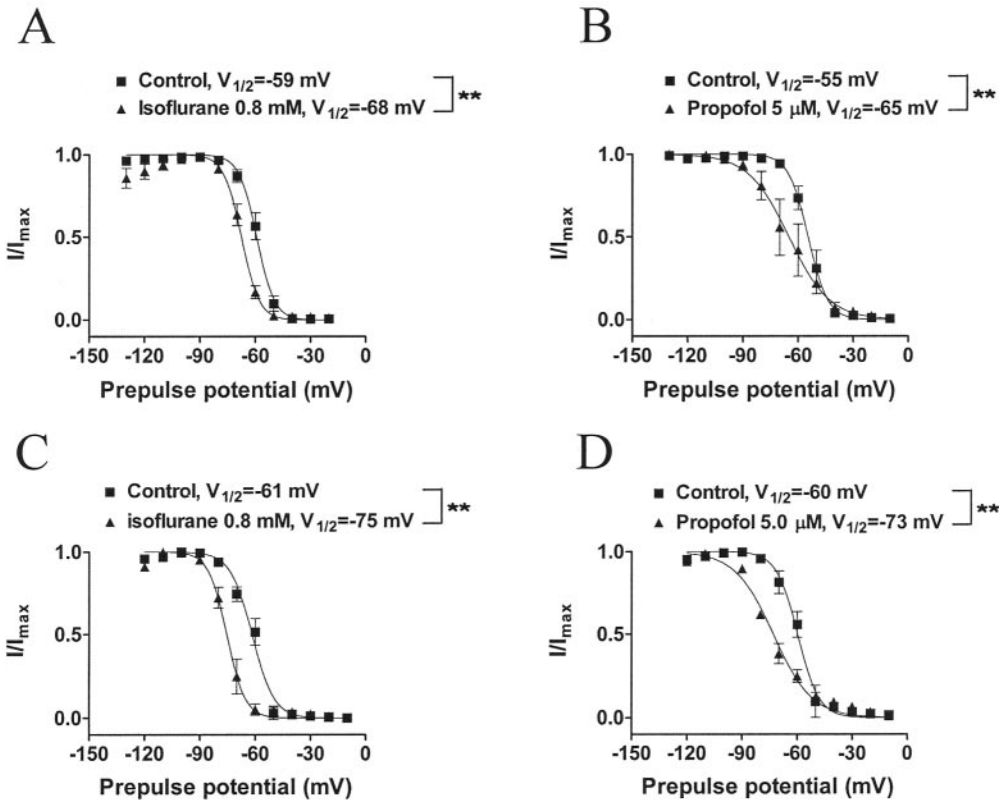

Fig. 6. Effects of isoflurane and propofol on I_{Na} inactivation curves with short (800 ms; A and B) and long (30 ms; C and D) prepulses. Currents were elicited by a 25-ms test pulse to −10 mV after a prepulse ranging from −130 or −120 mV to 0 mV in 10-mV increments. Data shown as mean ± S.E.M. (n = 4–6). **, $p < 0.01$ by unpaired t test.

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lease in isolated rat cerebrocortical nerve terminals (Schlame and Hemmings, 1995; Ratnakumari et al., 2000; Lingamaneni et al., 2001; Westphalen and Hemmings, 2003). Volatile anesthetics also inhibit voltage-gated Na^+ currents in isolated rat dorsal root ganglion neurons (Ratnakumari et al., 2000). We now provide direct evidence that isoflurane and propofol block voltage-gated Na^+ channels in nerve terminals at concentrations achieved clinically during general anesthesia. In a study of nerve terminals isolated from rat cerebral cortex, potency of isoflurane for inhibition of veratridine-evoked glutamate release (IC_{50} = 0.41–0.50 mM) was comparable with that for inhibition of NHP Na^+ channels,

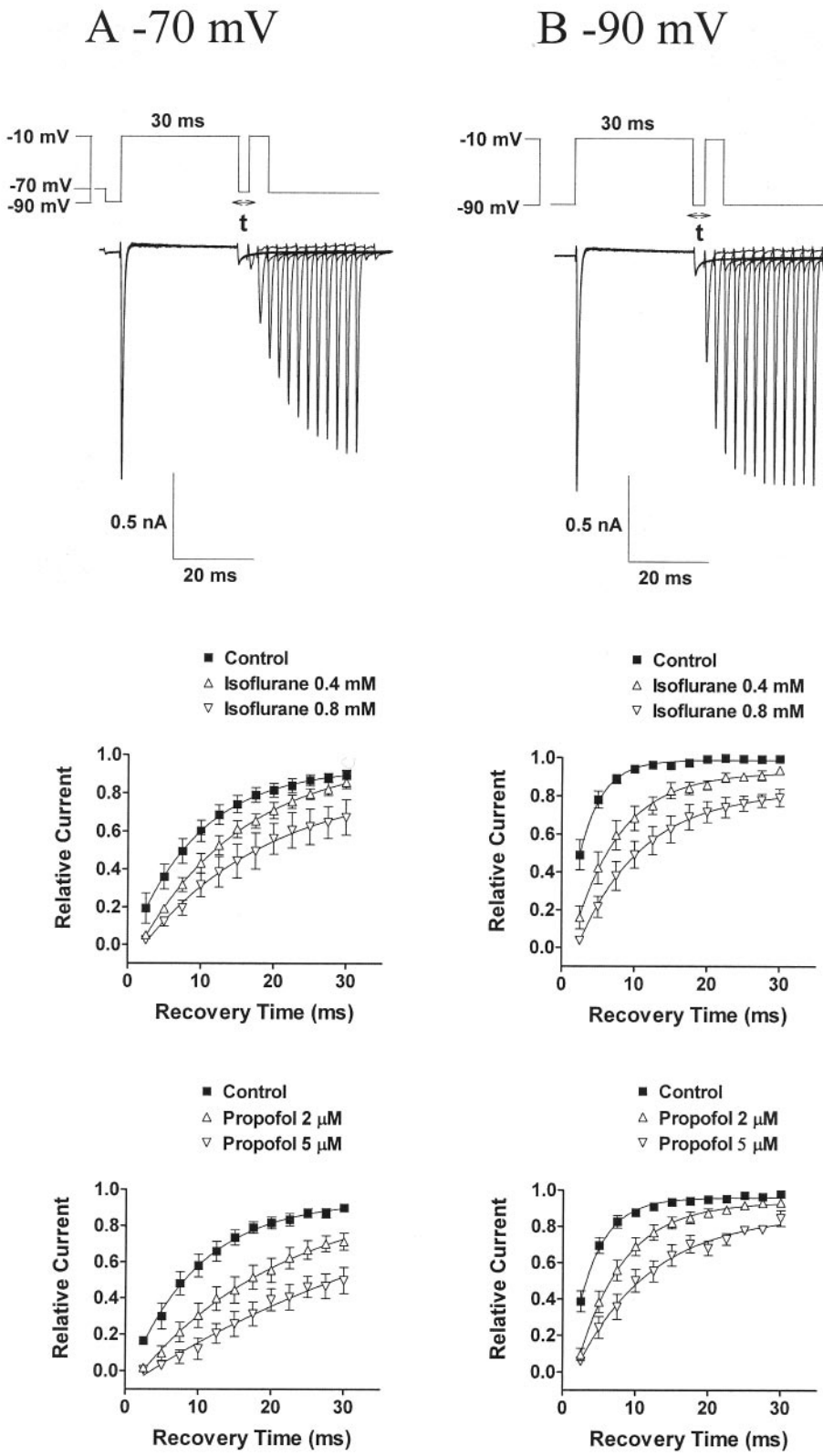

Fig. 7. Effects of isoflurane or propofol on the recovery from inactivation of I_{Na} at holding potentials of −70 mV (A) or −90 mV (B). Test pulses (5 ms) followed a 30-ms conditioning step to −10 mV with recovery times (t) of 2.5 to 30 ms. Relative currents were calculated as peak I_{Na} elicited by test pulse versus that elicited by conditioning pulse from −90 mV in each sweep; these values were normalized to control values in the absence of anesthetics. Data shown as mean ± S.E.M. (n = 5–8).

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TABLE 2
Effects of isoflurane and propofol on of Na^+ current recovery from inactivation

Holding Potential	−90 mV		−70 mV	
	τ	Ratio	τ	Ratio
	ms		*ms*	
Control	3.4 ± 0.2		9.3 ± 0.2††	
Isoflurane 0.4 mM	6.6 ± 0.3	2.1 ± 0.5	14.3 ± 0.4	1.7 ± 0.8
Isoflurane 0.8 mM	8.8 ± 0.2**	2.4 ± 0.8	17.1 ± 1.4*	1.9 ± 0.4
Control	3.9 ± 0.2		8.5 ± 0.3†	
Propofol 2 μM	6.4 ± 0.1	1.8 ± 0.4	20.5 ± 1.7*	2.8 ± 0.7‡
Propofol 5 μM	10.5 ± 0.9**	2.9 ± 0.9	53.6 ± 15.9**	5.7 ± 1.6‡‡

* $p < 0.05$; ** $p < 0.01$ versus respective control by ANOVA with Dunnett's post hoc test.
† $p < 0.05$; †† $p < 0.01$ for control τ at −70 versus −90 mV by unpaired t test (n = 5–8).
‡ $p < 0.05$; ‡‡ $p < 0.01$ for τ ratio (drug effect versus control) at −70 versus −90 mV by unpaired t test (n = 5–8).

whereas propofol was more potent in inhibiting NHP Na^+ channels than glutamate release (IC_{50} = 11–18 μM; Lingamaneni et al., 2001). This difference may be caused by effects of Na^+ channel modification by veratridine, the presence of different Na^+ channel isoforms in cerebral cortex, and/or effects of different modulator proteins or signaling pathways on sensitivity to isoflurane versus propofol.

Voltage-gated Na^+ channels are essential to the generation and propagation of action potentials (Catterall, 2002) and for nerve terminal depolarization leading to activation of voltage-gated Ca^{2+} channels, Ca^{2+} entry, and Ca^{2+}-dependent neurotransmitter release from nerve terminals (Tibbs et al., 1989). Depolarization results in Na^+ influx and increases in $[Na^+]_i$ in neurohypophysial nerve terminals (Turner and Stuenkel, 1998), which is also an important factor in the control of NHP peptide secretion (Toescu and Nordmann, 1991). Increases in $[Na^+]_i$ can elicit Ca^{2+}-independent vasopressin release from NHP nerve terminals (Stuenkel and Nordmann, 1993); this may be an important alternative pathway to the Ca^{2+}-dependent vesicular release pathway coupled to voltage-gated Ca^{2+} channel activation (Lee et al., 1992; Lindau et al., 1992). Inhibition of voltage-gated Na^+ channels is predicted to inhibit transmitter release by both pathways. Ethanol has been shown to inhibit peptide release and voltage-gated Ca^{2+} channels in isolated rat NHP terminals (Wang et al., 1991). Our evidence suggests that general anesthetics exert a direct inhibitory effect on NHP terminal excitability and presumably peptide release. Future studies will investigate the effects of anesthetics on Ca^{2+}-dependent versus Ca^{2+}-independent peptide release and the possible involvement of voltage-gated Ca^{2+} channels.

Inhibition of synaptic transmission by general anesthetics (Wakasugi et al., 1999) may result from depressed action potential firing (Antkowiak, 1999) or nerve terminal excitability caused by inhibition of presynaptic Na^+ channels. Although the Na^+ channel subtype(s) present in NHP nerve terminals has not been identified, native channels seem to be somewhat more sensitive to anesthetics than heterologously expressed rat brain Nav1.2 α subunits. Magnocellular neurons in the supraoptic nucleus, which send axons to the neurohypophysis, express both Nav1.2 and Nav1.6 (α-II and α-Na6) and βI and βII subunits (Tanaka et al., 1999), which suggests that these isoforms may also be the isoforms present in NHP nerve terminals.

Postsynaptic ligand-gated ion channels such as $GABA_A$ receptors and/or *N*-methyl-D-aspartate receptors participate in the actions of most general anesthetics (Yamakura et al., 2001). The presynaptic effects of general anesthetics on neurotransmitter release are gaining recognition for their role in the synaptic actions of general anesthetics (Richards, 1998; Perouansky and Hemmings, 2003). Our data support previous findings that general anesthetics influence synaptic transmission via presynaptic mechanisms, specifically via inhibition of presynaptic voltage-gated Na^+ channels through enhanced inactivation.

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