British Journal of Pharmacology (1996) 119, 1498-1504

Inhibition by propofol of [^{3}H]-batrachotoxinin-A 20-α-benzoate binding to voltage-dependent sodium channels in rat cortical synaptosomes

L. Ratnakumari & [1]H.C. Hemmings, Jr.

Departments of Anesthesiology & Pharmacology, Cornell University Medical College, New York, NY 10021, U.S.A.

1 Propofol (2,6 di-isopropylphenol), an intravenous general anaesthetic, blocks voltage-dependent Na^+ channels (Na^+ channels). In this study the interaction between propofol and Na^+ channels was analysed by examining its effects on neurotoxin binding to various receptor sites of the Na^+ channel in rat cerebrocortical synaptosomes.

2 Propofol (10-200 μM) exhibited concentration-dependent inhibition of equilibrium binding of [^{3}H]-batrachotoxinin-A 20-α-benzoate ([^{3}H]-BTX-B) to receptor site 2 of the Na^+ channel (mean IC_{50} = 26 μM; 6.5 μM free). Scatchard analysis revealed that propofol significantly increased the K_D without affecting the B_{max} for [^{3}H]-BTX-B binding.

3 Kinetic studies of [^{3}H]-BTX-B binding in the presence of various concentrations (25-200 μM) of propofol showed no significant changes in the association rate of [^{3}H]-BTX-B. However, propofol at 200 μM significantly increased the rate of dissociation of [^{3}H]-BTX-B, consistent with an indirect allosteric competitive mechanism of inhibition.

4 [^{3}H]-saxitoxin binding to receptor site 1 and [^{3}H]-brevetoxin-3 binding to receptor site 5 of the Na^+ channel were not inhibited by propofol (10-200 μM).

5 Propofol (10-100 μM) exhibited concentration-dependent inhibition of veratridine-evoked Na^+ influx either in the absence or presence of scorpion toxin with IC_{50} values of 46 μM (8.8 μM free) and 44 μM (8.5 μM free), respectively.

6 These results suggest that propofol inhibits voltage-dependent Na^+ channels due to a preferential interaction with the inactivated state of the channel. Blockade of Na^+ channels by propofol, which is known to inhibit glutamate release from synaptosomes, may contribute to its anaesthetic, anticonvulsant and neuroprotective properties.

Keywords: Batrachotoxin; brevetoxin; general anaesthesia; Na^+ influx; propofol; saxitoxin; scorpion toxin; synaptosomes; veratridine; voltage-dependent Na^+ channel

Introduction

Voltage-dependent sodium channels (Na^+ channels) of excitable membranes are involved in the initiation and rapid transmission of action potentials. Multiple neurotoxin receptor sites that modulate several aspects of channel function have been characterized on Na^+ channels (Ritchie & Rogart, 1977; Catterall & Gainer, 1985; Poli *et al.*, 1986; Lombet *et al.*, 1987; Catterall, 1995). Tetrodotoxin and saxitoxin bind at site 1 and inhibit ion conductance through the channel. The lipid-soluble toxins batrachotoxin, veratridine, aconitine and grayanotoxin bind at site 2 and cause persistent activation of the channel by blocking inactivation and shifting the voltage-dependence of activation to more negative membrane potentials; they also alter single channel conductance and ion selectivity. Small molecular weight polypeptide toxins such as the α-scorpion toxins and sea anemone toxins bind at site 3 and slow channel inactivation; they also potentiate the action of neurotoxins that act at site 2 through an allosteric interaction. The β-scorpion toxins shift the voltage-dependence of Na^+ channel activation to more negative membrane potentials by binding at site 4. The hydrophobic polyether brevetoxins and ciguatoxin bind at site 5 and inhibit inactivation and shift activation to more negative membrane potentials. Distinct sites have also been identified on Na^+ channels for pyrethroids, which shift channel activation to more negative membrane potentials and block inactivation.

Propofol (2,6 di-isopropylphenol) is a highly lipophilic compound that is widely used for sedation and the induction and maintenance of general anaesthesia. Propofol, as well as many other general anaesthetics, has potent potentiation and agonist effects at the $GABA_A$ receptor (see Tanelian *et al.*, 1993). Electrophysiological studies have also implicated Na^+ channels as a molecular site of action for several general anaesthetics (Frenkel *et al.*, 1993; Rehberg *et al.*, 1996), including propofol (Frenkel & Urban, 1991), although the pharmacological relevance of these effects has been questioned (Franks & Lieb, 1994). Recently, we found that propofol at clinically relevant concentrations inhibits veratridine-evoked increases in $^{22}Na^+$ flux, intracellular free Na^+ levels and Na^+ channel-dependent glutamate release in rat cerebrocortical synaptosomes, evidence which supports a role for Na^+ channel blockade in the action of propofol (Ratnakumari & Hemmings, 1996). Synaptosomes are pinched-off nerve terminals that retain a number of the functional properties of intact nerve endings (Krueger *et al.*, 1980; Tamkun & Catterall, 1981; Nicholls, 1993), including neurotoxin binding to Na^+ channels (Ray *et al.*, 1978; Catterall *et al.*, 1979; 1981; Grima *et al.*, 1986; Pauwells *et al.*, 1986; Poli *et al.*, 1986). In the present study, we analysed the effects of propofol on [^{3}H]-saxitoxin, [^{3}H]-batrachotoxinin-A 20-α-benzoate ([^{3}H]-BTX-B) and [^{3}H]-brevetoxin-3 binding to Na^+ channel neurotoxin receptor sites 1, 2 and 5, respectively, in rat cerebrocortical synaptosomes. The results provide biochemical evidence for an interaction between propofol and central nervous system (CNS) Na^+ channels, and further support a role for Na^+ channels as a possible target for general anaesthetic action.

[1] Author for correspondence at: Department of Anesthesiology, Box 50, LC-203A, Cornell University Medical College, 525 East 68th Street, New York, NY 10021, U.S.A.

Methods

Preparation of synaptosomes from rat cerebral cortex

Synaptosomes were prepared by a modification of the procedure of Dunkley *et al.* (1986). Adult male (150–175 g) Sprague-Dawley rats were anaesthetized with 80% CO_2/20% O_2 and killed by decapitation. Brains were immediately removed and rinsed in ice-cold 0.32 M sucrose. The cortical grey matter was homogenized in ten volumes of 0.32 M sucrose by a motor-driven polytetrafluoroethylene-glass (Potter-Elvehjem) homogenizer at 900 r.p.m. for 10 up-and-down strokes. The homogenate was centrifuged at $1000 \times g$ for 2 min. The supernatant fraction was collected and centrifuged at $15000 \times g$ for 12 min. The resulting pellet was resuspended in 8 ml of 0.32 M sucrose. Aliquots (2 ml) of this fraction were loaded onto discontinuous gradients consisting of three 2.5 ml layers of filtered (0.45 μm) Percoll density gradient medium (23%, 10% and 3%) in 0.32 M sucrose, 0.25 mM dithiothreitol, and 1 mM ethylenediaminetetraacetic acid, pH 7.4. The gradients were centrifuged at $25,000 \times g$ for 6.5 min. The synaptosomal fraction was collected from the 23%/10% Percoll interface and diluted about 5 fold either in low Na^+ buffer (130 mM choline chloride, 5.4 mM KCl, 5 mM NaCl, 0.8 mM $MgSO_4$, 5.5 mM D-glucose, 50 mM HEPES-Tris, pH 7.4) for $^{22}Na^+$ influx studies or in Na^+-free buffer (130 mM choline chloride, 5.4 mM KCl, 0.8 mM $MgSO_4$, 5.5 mM D-glucose, 50 mM HEPES-Tris (pH 7.4)) for neurotoxin binding studies; both buffers were equilibrated with 95% O_2/5% CO_2. The synaptosomes were centrifuged at $23,000 \times g$ for 10 min and resuspended in the appropriate buffer. The protein concentration of the synaptosomal preparation was determined by the method of Bradford (1976) with bovine serum albumin (BSA) as a standard.

Equilibrium binding assays

[^{3}H]-batrachotoxinin-A 20-α-benzoate binding [^{3}H]-batrachotoxinin-A 20-α-benzoate ([^{3}H]-BTX-B) binding was determined as described by Postma and Catterall (1984). Synaptosomes were incubated for 60 min at 37°C in Na^+-free buffer plus 10 nM [^{3}H]-BTX-B, 1 μM tetrodotoxin, 80 μg ml^{-1} scorpion venom, 1 mg ml^{-1} BSA, with or without the indicated concentrations of propofol. Tetrodotoxin was included in the assay to inhibit residual Na^+ flux through Na^+ channels activated by scorpion venom and BTX-B (Catterall *et al.*, 1981). Binding reactions were initiated by rapid mixing by synaptosomes (200 μg protein in 100 μl) with 150 μl of the above reaction mixture, and were terminated after 60 min by the addition of 3 ml of ice-cold washing buffer (163 mM choline chloride, 0.8 mM $MgSO_4$, 1.8 mM $CaCl_2$, 5 mM HEPES-Tris, pH 7.4). The synaptosomes were collected on glass fibre filters (Brandel, Gaitherburg, MD) for vacuum filtration and washed three times with 3 ml of washing buffer. Bound [^{3}H]-BTX-B was determined by liquid scintillation spectrometry in Bio-Safe NA scintillation cocktail (Research Products International Corp., Mount Prospect, IL). Non-specific binding was determined in the presence of 0.3 mM veratridine, which binds at the same site as BTX-B. Nonspecific binding was 10–20% of total binding at 10 nM [^{3}H]-BTX-B.

[^{3}H]-brevetoxin-3 binding [^{3}H]-brevetoxin-3 ([^{3}H]-PbTx-3) binding was performed by the procedure of Edwards *et al.* (1992). Synaptosomes were suspended in Na^+-free buffer as for [^{3}H]-BTX-B binding with the addition of 25 nM [^{3}H]-PbTx-3 and 0.01% (v/v) Pluronic F-127, a nonionic detergent required to solubilize the high concentrations of unlabelled PbTx-3 used to determine nonspecific binding (Poli *et al.*, 1986). Synaptosomes (100 μg protein in 100 μl) were added to the above reaction mixture of 100 μl. After rapid mixing, synaptosomes were incubated at 4°C for 1 h, following which the reaction was stopped by the addition of 3 ml of ice-cold washing buffer. The synaptosomes were collected on glass fibre filters by vacuum filtration and washed twice with 3 ml of washing buffer. Bound [^{3}H]-PbTx-3 was determined by liquid scintillation spectrometry in Bio-Safe NA scintillation cocktail. Nonspecific binding was measured in the presence of 10 μM unlabelled PbTx-3, and was 10–15% of total binding at 25 nM [^{3}H]-PbTx-3.

[^{3}H]-saxitoxin binding [^{3}H]-saxitoxin ([^{3}H]-STX) binding was determined as described by Catterall *et al.* (1979). Synaptosomes (100 μg in 100 μl) were added to a reaction mixture (100 μl) consisting of Na^+-free buffer as for [^{3}H]-BTX-B binding with the addition of 3 nM [^{3}H]-STX. Samples were rapidly mixed and incubated at 37°C for 30 min. The binding reactions were stopped by the addition of 3 ml of ice-cold washing buffer, and the synaptosomes were collected on glass fibre filters by vacuum filtration and washed twice over 10–15 s. Bound [^{3}H]-STX was determined by liquid scintillation spectrometry by Bio-Safe NA scintillation cocktail. Nonspecific binding was determined in the presence of 1 μM tetrodotoxin which binds at the same site as STX. Nonspecific binding was 10–15% of total binding at 3 nM [^{3}H]-STX.

Kinetic binding assays

The rate of dissociation of [^{3}H]-BTX-B from the Na^+ channel receptor complex was measured by incubating synaptosomes for 60 min with 10 nM [^{3}H]-BTX-B, 80 μg ml^{-1} scorpion venom (*Leiurus quinquestriatus*) and 1 μM tetrodotoxin at 37°C as in the equilibrium binding assays. Measurement of dissociation was initiated by adding 0.3 μM veratridine ± propofol (25, 50, 100 or 200 μM) at zero time. Reactions were terminated (at 15, 30, 45 or 60 min) by vacuum filtration and washing, followed by determination of bound [^{3}H]-BTX-B.

The dissociation rate constant (k_{-1}) was calculated from the equation $\ln(SB_t/SB_o) = -k_{-1}.t$ (Hill *et al.*, 1989), where SB_t = specific binding of [^{3}H]-BTX-B at time t, and SB_o = specific binding of [^{3}H]-BTX-B at time zero. A plot of $\ln(SB_t/SB_o)$ *versus* t, in the absence and presence of propofol, was linear with a slope of k_{-1}.

The rate of association of [^{3}H]-BTX-B was measured by incubating synaptosomes as in the equilibrium binding assays with 80 μg ml^{-1} scorpion venom (*Leiurus quinquestriatus*) for 15 min at 37°C in the absence or presence of propofol (25, 50, 100 or 200 μM). [^{3}H]-BTX-B (10 nM) was then added and synaptosomes were incubated for 5, 10, 20 or 30 min at 37°C. Assays were carried out in parallel in the presence of 0.3 mM veratridine to determine nonspecific binding at each time point. Incubations were terminated by vacuum filtration and washing followed by determination of bound [^{3}H]-BTX-B.

The association rate constant (k_{+1}) of [^{3}H]-BTX-B binding was calculated from the equation $\ln(SB_{eq}/SB_{eq} - SB_t) = ([L]k_{+1} + k_{-1})t$ (Hill *et al.*, 1989), where SB_{eq} = specific binding of [^{3}H]-BTX-B at equilibrium, SB_t = specific binding of [^{3}H]-BTX-B at time t, [L] = concentration of [^{3}H]-BTX-B, and k_{-1} = dissociation rate constant for [^{3}H]-BTX-B dissociation from the Na^+ channel receptor complex at the ambient drug concentration. A plot of $\ln(SB_{eq}/SB_{eq} - SB_t)$ *versus* t was linear, with slope = $[L]k_{+1} + k_{-1}$, from which k_{+1} could be calculated. Only the data obtained in the absence or presence of 200 μM propofol are presented in the figure for clarity.

Measurement of Na^+ influx

Na^+ influx was measured by a modification of the method of Tamkun & Catterall (1981). Synaptosomes (approximately 600–700 μg protein in 150 μl of low Na^+ buffer) were preincubated at 37°C for 5 min with or without the indicated concentrations of propofol. Following preincubation with propofol, 60 μM veratridine ± 80 μg ml^{-1} scorpion venom was added, and the samples were incubated for 10 min at 37°C. Uptake was initiated by the addition of 1.3 μCi of carrier-free $^{22}NaCl$ in 50 μl of low Na^+ buffer, and was terminated after

5 s by the addition of 3 ml of ice-cold washing buffer and rapid vacuum filtration through Whatman GF/C glass fibre filters. The filters were washed twice with 3 ml of washing buffer. Filter radioactivity was determined by liquid scintillation spectrometry in Bio-Safe NA scintillation cocktail. Nonspecific (Na^+ channel-independent) $^{22}Na^+$ uptake was determined in the presence of 1 μM tetrodotoxin.

Materials

[11-^{3}H]-saxitoxin (28 Ci mmol^{-1}) was purchased from Amersham (Arlington Heights, IL). [^{3}H]-batrachotoxin-A 20-α-benzoate (34 Ci mmol^{-1}) and [^{22}NaCl] (1 mCi ml^{-1}) were obtained from DuPont-New England Nuclear (Boston, MA). [42-^{3}H]-brevetoxin-3 (14.25 Ci mmol^{-1}) and *Ptychodiscus brevis* toxin-3 (PbTx-3) were from Chiral Corp. (Miami, FL). Percoll density gradient medium was from Pharmacia/LKB (Uppsala, Sweden). Propofol was from Aldrich Chemicals Inc. (Milwaukee, WI). Tetrodotoxin, veratridine, aconitine, scorpion venom (*Leiurus quinquestriatus*), and dimethylsulphoxide were from Sigma Chemical Co. (St. Louis, MO). Pluronic F-127 was from Molecular Probes, Inc. (Eugene, OR). All other chemicals were from commercial sources and were of analytical grade. Dimethylsulphoxide at a final concentration of 0.05% (v/v) was used as a vehicle for propofol and veratridine; control experiments showed that the vehicle alone had no effect on the activities measured (data not shown).

Data analysis

Statistical differences between control and experimental values were determined by analysis of variance (ANOVA) with the Fisher *post hoc* test. The equilibrium dissociation constant (K_D) and the maximum number of binding sites (B_{max}) were calculated for [^{3}H]-BTX-B from Scatchard plots by use of the Enzfit kinetic program (Elsevier-Biosoft, Cambridge, U.K.). Concentration-effect data were analysed by a graded dose-response programme that carried out linear regression analysis on data between 20% and 80% of the maximal response (Pharm/PCS Pharmacologic Calculation System, Version 4.2, Springer-Verlag, New York, NY). Curve fitting was performed by using commercially available software (CA-Cricket Graph III, Islandia, NY).

Experiments were carried out in accordance with the NIH guide for the care and use of laboratory animals as approved by the Cornell University Medical College Institutional Animal Care and Use Committee.

Results

Effect of propofol on [^{3}H]-BTX-B binding

Equilibrium [^{3}H]-BTX-B binding to intact synaptosomes was determined in the presence of scorpion venom (*Leirus quinquestriatus*), which allosterically enhances binding by increasing Na^+ channel affinity for [^{3}H]-BTX-B (Catterall *et al.*, 1981). Scorpion venom enhanced specific [^{3}H]-BTX-B binding about 16 fold from 24±3 fmol mg^{-1} to 380±53 fmol mg^{-1} without affecting nonspecific binding. The effect of propofol on specific [^{3}H]-BTX-B binding in synaptosomes is shown in Figure 1. Propofol inhibited specific [^{3}H]-BTX-B binding in a concentration-dependent manner (IC_{50}=26 μM; n=3), and had no effect on nonspecific binding (data not shown). Scatchard analysis of [^{3}H]-BTX-B binding in the absence of propofol (Figure 2) revealed binding to a single class of high-affinity binding sites with a K_D of 110±12 nM (mean± s.e.mean; n=3), and a maximal binding capacity (B_{max} of 2.6±0.2 pmol mg^{-1} protein. These values are in good agreement with those obtained previously (Catterall *et al.*, 1981; Grima *et al.*, 1986; Velly *et al.*, 1987). Propofol (25 μM) increased the K_D for [^{3}H]-BTX-B to 260±62 nM (Figure 2) without significantly affecting B_{max} (2.8±0.2 pmol mg^{-1} protein). This effect is consistent with a competitive mechanism for propofol inhibition of [^{3}H]-BTX-B binding. The Hill coefficient for [^{3}H]-BTX-B binding in the absence or presence of propofol was 1.0 (data not shown).

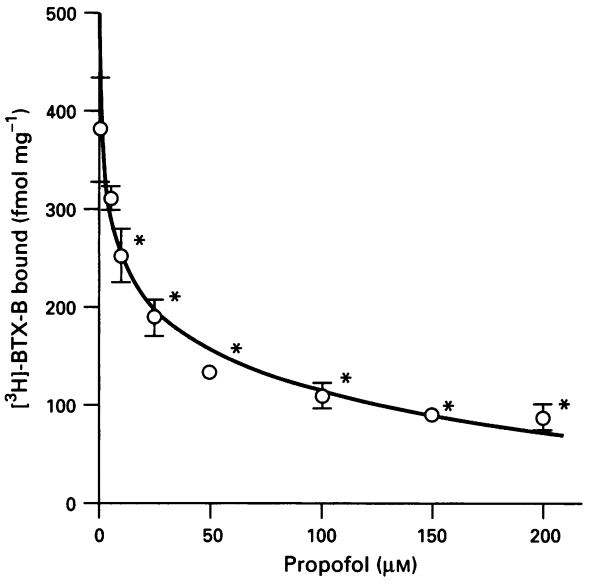

Figure 1 Effect of propofol on specific [^{3}H]-BTX-B binding to synaptosomes. Synaptosomes (200 μg) were incubated with 10 nM [^{3}H]-BTX-B in the presence of 80 μg ml^{-1} scorpion toxin, 1 μM tetrodotoxin and the indicated concentrations of propofol at 37°C for 1 h. Each point is the mean of 3 independent experiments performed in triplicate; vertical lines show s.e.mean. *P<0.05 *versus* control (no propofol) by ANOVA followed by Fisher's *post hoc* test.

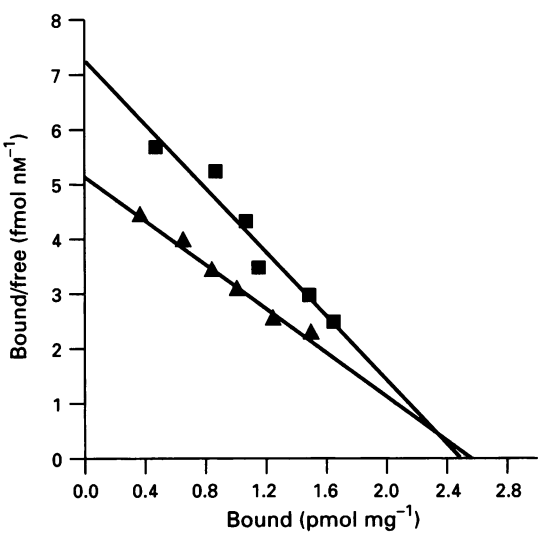

Figure 2 Scatchard analysis of the effect of propofol on [^{3}H]-BTX-B binding to synaptosomes. Synaptosomes were incubated with various concentrations of [^{3}H]-BTX-B in the presence (▲) or absence (■) of 25 μM propofol. The results represent data from a single representative experiment in which duplicate determinations were made. Values for B_{max} were 2.7 pmol mg^{-1} and 3.0 pmol mg^{-1} in absence or presence of propofol, respectively. The K_D values were 103 μM and 207 μM in the absence or presence of propofol, respectively. Two additional independent experiments gave similar results.

Effect of propofol on association and dissociation kinetics of [^{3}H]-BTX-B binding

The effect of propofol on the association rate of [^{3}H]-BTX-B is shown in Figure 3a. The association rate constant (k_{+1}) of

[^{3}H]-BTX-B binding in the absence of propofol was 0.0023 ± 0.0002 min^{-1} (mean ± s.e.mean $n=3$). Propofol (up to 200 μM) did not influence the rate of association of [^{3}H]-BTX-B ($k_{+1} = 0.0024 \pm 0.0002$ min^{-1}).

The effects of propofol on the dissociation rate of [^{3}H]-BTX-B binding are shown in Figure 3b. The dissociation rate constant (k_{-1}) for [^{3}H]-BTX-B dissociation from the Na^+ channel receptor complex in the presence of the competitive ligand veratridine (0.3 mM) was 0.0058 ± 0.0006 min^{-1} (mean ± s.e.mean; $n=3$). Propofol increased the dissociation rate constant of [^{3}H]-BTX-B binding, but this effect achieved statistical significance only at 200 μM propofol (about 10 times its IC_{50} value) ($k_{-1} = 0.0080 \pm 0.0003$ min^{-1}, $P<0.05$). An effect of propofol on rebinding of dissociated [^{3}H]-BTX-B can be ignored since dissociation was determined in the presence of a saturating concentration of veratridine, a direct competitive inhibitor of [^{3}H]-BTX-B binding (Catterall *et al.*, 1981).

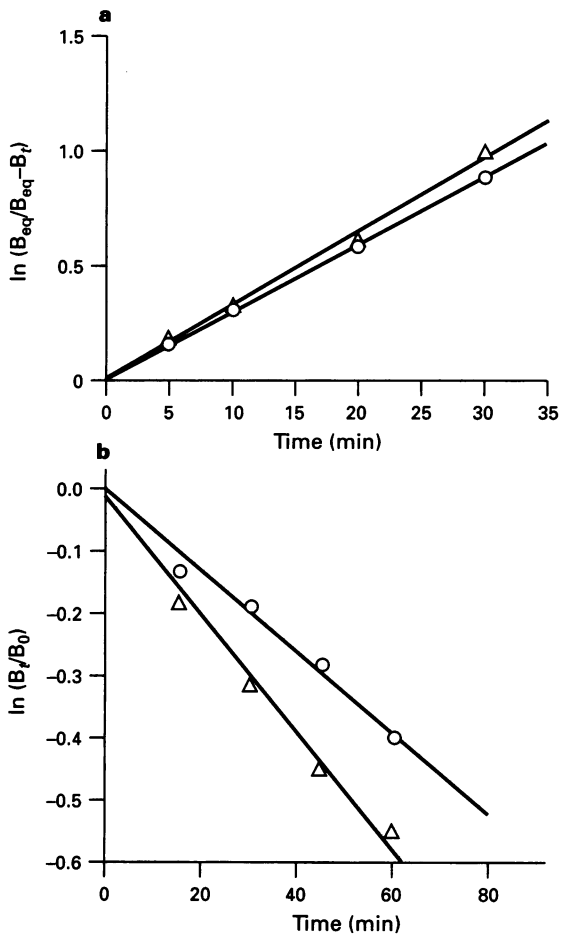

Figure 3 (a) Effect of propofol on the association rate of [^{3}H]-BTX-B with synaptosomes. Synaptosomes were incubated at 37°C in the presence of 80 μg ml^{-1} scorpion toxin, 1 μM tetrodotoxin in the absence (○) or presence (△) of 200 μM propofol. After the addition of 10 nM [^{3}H]-BTX-B, incubation was continued and specifically bound [^{3}H]-BTX-B was determined at the indicated times. The association rate constant (k_{+1}) was determined from the slope by linear regression. (b) Effect of propofol on the dissociation rate of [^{3}H]-BTX-B from synaptosomes. Synaptosomes were incubated for 60 min with 10 nM [^{3}H]-BTX-B in the presence of 80 μg ml^{-1} scorpion toxin and 1 μM tetrodotoxin. At time zero, dissociation was initiated by the addition of 0.3 mM veratridine in the absence (○) or presence (△) of 200 μM propofol. Incubation was continued at 37°C and specifically bound [^{3}H]-BTX-B was determined at the indicated times. The dissociation rate constant (k_{-1}) was determined from the slope by linear regression. In (a) and (b) each value is the mean of 3 independent experiments performed in duplicate.

Effect of propofol on [^{3}H]-STX and [^{3}H]-PbTx-3 binding

The effects of propofol on neurotoxin binding to receptor sites 1 and 5 of the Na^+ channel in intact synaptosomes were measured with [^{3}H]-STX and [^{3}H]-PbTx-3, respectively (Figure 4). The specific binding of [^{3}H]-STX was 100 ± 5 fmol mg^{-1} protein (mean ± s.e.mean; $n=3$). Propofol (up to 200 μM) had no significant effect on [^{3}H]-STX binding. [^{3}H]-PbTx-3 binding in the absence of propofol was 2.10 ± 0.02 pmol mg^{-1} protein (mean ± s.e.mean; $n=3$). Low concentrations of propofol (≤ 50 μM) marginally but significantly increased [^{3}H]-PbTx-3 binding (14–22% over control; $P<0.05$), while higher concentrations (≥ 75 μM) did not affect binding significantly.

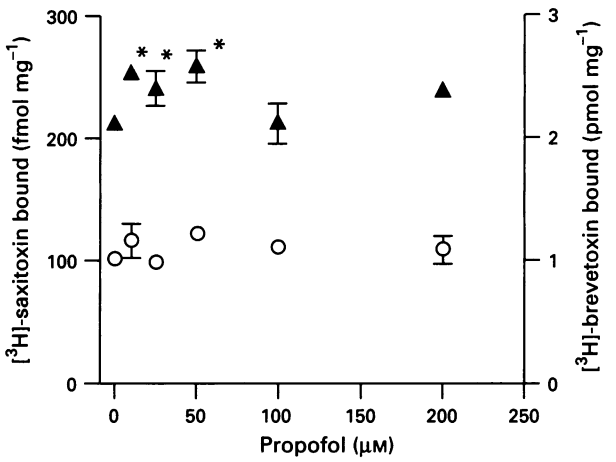

Figure 4 Effect of propofol on specific binding of [^{3}H]-saxitoxin and [^{3}H]-brevetoxin-3 to synaptosomes. Synaptosomes (100 μg) were incubated with either 3 nM [^{3}H]-saxitoxin (○) or 25 nM [^{3}H]-brevetoxin-3 (▲) with the indicated concentrations of propofol. Saxitoxin binding was performed at 37°C for 30 min, while brevetoxin-3 binding incubation was performed at 4°C for 1 h. Each point is the mean of 3 independent experiments performed in duplicate; vertical lines show s.e.mean. *$P<0.05$ *versus* control (no propofol) by ANOVA followed by Fisher's *post hoc* test.

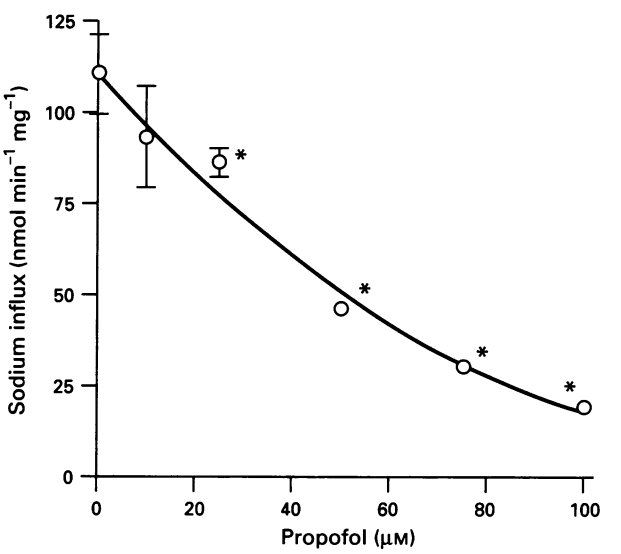

Figure 5 Effect of propofol on $^{22}Na^+$ influx into synaptosomes. Synaptosomes were preincubated in the presence of the indicated concentrations of propofol, and Na^+ channel-dependent Na^+ influx was evoked with 60 μM veratridine plus 80 μg ml^{-1} scorpion venom. Nonspecific uptake in the presence of 1 μM tetrodotoxin was subtracted to yield specific uptake (shown as mean ± s.e.mean (vertical lines); $n=3-6$). The IC_{50} value for inhibition of Na^+ influx was 44 μM. *$P<0.05$ *versus* control (no propofol) by ANOVA followed by Fisher's *post hoc* test.

Effect of propofol on $^{22}Na^+$ influx

The alkaloid toxin veratridine was used to activate Na^+ flux through Na^+ channels in synaptosomes. Veratridine (60 μM) increased Na^+ uptake 4 fold from 26 ± 5 nmol min^{-1} mg^{-1} (basal) to 98 ± 1 nmol min^{-1} mg^{-1} (mean $\pm$ s.e.mean; $n = 3-6$). Scorpion venom was included in some experiments to potentiate veratridine-stimulated $^{22}Na^+$ flux Scorpion venom further stimulated veratridine-evoked $^{22}Na^+$ influx to 140 ± 11 nmol min^{-1} mg^{-1}. Propofol concentration-dependently inhibited veratridine-evoked $^{22}Na^+$ influx in the absence ($IC_{50} = 46$ μM; data not shown) or presence ($IC_{50} = 44$ μM; Figure 5) of scorpion toxin with comparable efficacy and potency.

Discussion

Recent electrophysiological studies have shown that CNS voltage-dependent Na^+ channels are sensitive to inhibition by clinically relevant concentrations of both intravenous and volatile general anaesthetics (Frenkel *et al.*, 1993; Rehberg *et al.*, 1996). Propofol was found to reduce Na^+ channel open-time ($ED_{50} = 20$ μM) by electrophysiological recording of single Na^+ channels from human cerebral cortex (Frenkel & Urban, 1991). Inhibition of Na^+ channels by propofol has been implicated in its effects on veratridine-evoked [^{3}H]-noradrenaline release from bovine cultured adrenal medullary chromaffin cells (Minami *et al.*, 1996) and veratridine-evoked glutamate release from cerebrocortical synaptosomes (Ratnakumari & Hemmings, 1996). Blockade of presynaptic Na^+ channels may also underlie inhibition of neurotransmitter release by volatile anaesthetics in synaptosomes (Schlame & Hemmings, 1995). The results presented here indicate that propofol selectively inhibits neurotoxin binding to Na^+ channels and Na^+ channel function at clinically relevant concentrations.

Propofol inhibited both [^{3}H]-BTX-B binding and veratridine-evoked Na^+ influx in rat cortical synaptosomes. The potency for inhibition of [^{3}H]-BTX-B binding by propofol ($IC_{50} = 26$ μM) was comparable to its potency for inhibition of veratridine-evoked Na^+ influx ($IC_{50} = 44$ μM), and also for inhibition of veratridine-evoked increases in intracellular Na^+ concentration ($IC_{50} = 21$ μM) (Ratnakumari & Hemmings, 1996). Scatchard analysis revealed a competitive mechanism for inhibition of [^{3}H]-BTX-B binding by propofol. Kinetic analysis indicated that propofol did not affect the association rate of [^{3}H]-BTX-B with the Na^+ channel, but increased its dissociation rate. An increase in dissociation rate without a change in association rate indicates that propofol interacts with a site distinct from that of [^{3}H]-BTX-B binding to inhibit [^{3}H]-BTX-B binding allosterically.

Binding of the neurotoxins [^{3}H]-STX to site 1 or of [^{3}H]-PbTx-3 to receptor site 5 of the Na^+ channel was not inhibited by concentrations of propofol (up to 200 μM) that inhibited [^{3}H]-BTX-B binding and $^{22}Na^+$ influx. Minami *et al.* (1996) also failed to observe significant effects of propofol on [^{3}H]-STX binding in bovine adrenal chromaffin cells. A slight stimulation of [^{3}H]-PbTx-3 binding was observed at lower concentrations of propofol. The mechanism for this small biphasic effect is unclear. Together, these data demonstrate that propofol exhibits a specific interaction with synaptosomal Na^+ channels by selective inhibition of neurotoxin binding to receptor site 2. Scorpion venom (which binds at site 3) was included in the analysis of [^{3}H]-BTX-B binding to increase [^{3}H]-BTX-B binding to measurable levels by an allosteric interaction with site 2 of the Na^+ channel (Catterall *et al.*, 1981). Inhibition by propofol of [^{3}H]-BTX-B binding is not likely to be due to an indirect effect on scorpion toxin binding at site 3, since propofol inhibited veratridine-evoked $^{22}Na^+$ influx in either the presence or absence of scorpion toxin with similar efficacy and potency. Since both [^{3}H]-BTX-B and veratridine interact with neurotoxin receptor site 2 of the Na^+ channel (Catterall *et al.*, 1981), the inhibitory action of propofol in both assays probably involves a selective allosteric interaction with this site.

The pattern of inhibition by propofol of neurotoxin binding to Na^+ channels is similar to that obtained for therapeutic concentrations of local anesthetics, class I anticonvulsants, and class I antiarrhythmic drugs (Catterall, 1981; Willow & Catterall, 1982; Creveling *et al.*, 1983; Postma & Catterall, 1984; Gusovsky *et al.*, 1990). These compounds are allosteric competitive inhibitors of [^{3}H]-BTX-B binding to neurotoxin receptor site 2, and have no significant effects on neurotoxin binding at other receptor sites (Catterall, 1981). Evidence from electrophysiological studies revealed that these drugs inhibit Na^+ channels in a voltage- and frequency-dependent manner by preferential interaction with the inactivated state of the channel, as described by the modulated receptor hypothesis (Catterall, 1987). This results in selective effects of specific antiarrhythmics and anticonvulsants and low concentrations of local anaesthetics on abnormally firing or depolarized cells while normally functioning cells are left relatively unaffected. The similar pharmacological properties of propofol (this study; Frenkel & Urban, 1991) suggest that an analogous mechanism underlies its Na^+ channel blocking effects. Selective antagonism of repetitively active neurones may contribute to both the anticonvulsant (De Riu *et al.*, 1992) and general anaesthetic properties of propofol. Inhibition of Na^+ channels in depolarized neurones would be expected to exert a neuroprotective effect (Urenjak & Obrenovitch, 1996), although results from studies of the neuroprotective properties of propofol have been inconsistent (Kochs *et al.*, 1992; Ridenour *et al.*, 1992; Amorim *et al.*, 1995; Arcadi *et al.*, 1996).

Allosteric inhibition by propofol and other drugs of the interaction between activating neurotoxins and Na^+ channel receptor site 2 is best explained by selective high affinity drug binding to the inactivated state of the Na^+ channel. The inactivated conformation of the Na^+ channel appears to possess a hydrophobic drug binding site(s) that is distinct from, but allosterically coupled to, neurotoxin receptor site 2. This binding site(s) is able to interact with a number of chemically diverse drugs including local anaesthetics, antiarrythmics, anticonvulsants (Catterall, 1987), neuroprotective agents (Urenjak & Obrenovitch, 1996) and propofol (this study). Our data suggest that propofol binds to a site(s) on the Na^+ channel that is exposed or formed in the inactivated conformation of the channel. Further experiments will be required to determine whether this site(s) overlaps that for other drugs with similar neurotoxin binding interactions, and whether other general anaesthetics have similar effects.

A highly lipophilic compound such as propofol can be expected to have multiple actions on excitable membranes. It is therefore necessary to correlate the concentrations of propofol that affect Na^+ channels *in vitro* with concentrations that are clinically effective. The plasma EC_{50} of propofol in man during continuous infusion for suppression of response to skin incision in the absence of other drugs is 85 μM (Smith *et al.*, 1994). The actual brain concentration of propofol is considerably higher, with a brain/plasma concentration ratio in rats of 7.8–8.5 (Shyr *et al.*, 1995), although it is difficult to estimate the effect site concentration of propofol *in vivo* due to protein and lipid binding. The mean IC_{50} values of propofol for inhibition of Na^+ influx (44 μM) or [^{3}H]-BTX-B binding (26 μM) obtained in the present study are comparable to the clinically effective plasma concentration of propofol, and suggest that significant effects on Na^+ channel function would occur with clinical use. Taking into account its extensive protein binding (Cockshott *et al.*, 1992), the inhibitory effects of propofol on Na^+ channels should occur at much lower free concentrations.

Propofol has also been shown to affect several other ion channels and neurotransmitter receptors. Numerous studies have demonstrated potent effects at γ-aminobutyric acid $(GABA)_A$ receptors (Tanelian *et al.*, 1993). For example, propofol potentiates surface depolarizations evoked by GABA in rat olfactory cortex slices (Collins, 1988) and the actions of $GABA_A$ receptors of rodent neurones and bovine chromaffin cells (Hales & Lambert, 1991). Potentiation of glycine receptors by propofol has also been demonstrated in mouse

spinal neurones (Hales & Lambert, 1991). Propofol inhibits whole cell currents activated by N-methyl-D-aspartate (NMDA) without affecting kainate-evoked currents in cultured mouse hippocampal neurones (Orser *et al.*, 1995). Propofol also inhibits T-type and L-type voltage-dependent Ca^{2+} channels in chick dorsal root ganglion neurones (Olcese *et al.*, 1994), as well as KCl-evoked, and presumably Ca^{2+} channel-mediated, [^{3}H]-noradrenaline release from human neuroblastoma cells (Lambert *et al.*, 1996). However, propofol does not affect KCl-evoked glutamate release from rat cortical slices (Bikler *et al.*, 1995) or rat cortical synaptosomes (Ratnakumari & Hemmings, 1996), which indicates that propofol does not block the Ca^{2+} channel type coupled to cortical glutamate release. The observations that propofol inhibits 4-aminopyridine- and veratridine-evoked (IC_{50} values of 39 μM and 30 μM, respectively), but not KCl-evoked, glutamate release from synaptosomes (Ratnakumari & Hemmings, 1996) and veratridine-evoked, but not KCl-evoked, [^{3}H]-noradrenaline release from bovine adrenal chromaffin cells (Minami *et al.*, 1996), suggest that Na^+ channels are more sensitive to inhibition by propofol than are the Ca^+ channels coupled to neurotransmitter release. It should also be noted that propofol inhibits [^{3}H]-PN 200-110 binding to L-type Ca^{2+} channels in rat cerebrocortical membranes with an IC_{50} of 97μM (Hirota & Lambert, 1996), which is 4 times higher than its IC_{50} (26 μM) for inhibition of [^{3}H]-BTX-B binding to intact synaptosomes isolated from rat cerebral cortex (Figure 1).

In conclusion, propofol inhibits CNS Na^+ channels as determined by a number of independent methods, including electrophysiological (Frenkel & Urban, 1991), Na^+ flux (this study), and neurotoxin binding (this study) measurements. Propofol appears to interact selectively with the inactivated state of the Na^+ channel as indicated by its specific effects on neurotoxin binding. This hypothesis is also consistent with the observed difference between the concentration of propofol that exerts a significant effect on equilibrium binding of [^{3}H]-BTX-B to synaptosomes (10 μM – wherein propofol has to interact with channels that are in the resting or inactive state) and that which affects the dissociation rate of [^{3}H]-BTX-B from the receptor complex (200 μM – wherein propofol has to interact with activated channels, i.e., channels already bound to [^{3}H]-BTX-B). Inhibition of Na^+ channels has been implicated in the inhibitory effects of propofol on neurotransmitter release from neuroendocrine cells (Minami *et al.*, 1996) and synaptosomes (Ratnakumari & Hemmings, 1996). The voltage-dependent Na^+ channel is a sensitive and functionally relevant site of propofol action that may contribute to its anaesthetic, anticonvulsant, neuroprotective and haemodynamic effects.

Note added in proof

Determination of free propofol concentration

The concentrations of propofol mentioned throughout the text include both bound and free (total) fractions of propofol. The concentration of free propofol present in parallel samples duplicating each experimental condition was determined by equilibrium dialysis using a Spectrum Equilibrium Dialyzer (Spectrum Medical Industries, Los Angeles, CA, U.S.A.) equipped with Spectrapore 2 dialysis membrane (molecular weight cut-off = 12 000). The sample chamber contained 200 μl of a solution containing all components of each assay condition (binding assay or Na^+ flux) at the appropriate concentrations, while the dialysate chamber was identical except for the exclusion of protein and lipid components (synaptosomes and BSA). Various concentrations of propofol were added to each sample chamber and allowed to dialyse for 4 h at 37°C. Aliquots of the sample and dialysate chambers were analysed for propofol by HPLC as described by Pavan *et al.* (1992). The ratio of free (concentration in dialysate) to total (concentration in sample) propofol concentration, determined over a range of propofol concentrations (25–200 μM), was found to be constant for each experimental condition and was used to calculate the free propofol concentration corresponding to the total propofol concentration added. The total–free ratios of propofol were 4:1 in the [^{3}H]BTX-B binding assay (0.2 mg synaptosomal protein and 1 mg ml^{-1} BSA in a 0.25 ml reaction volume) 1.4:1 in the assays of [^{3}H]saxitoxin and [^{3}H]brevetoxin-3 binding (0.1 mg synaptosomal protein in a 0.2 ml reaction volume) and 5.2:1 in the assays of $^{22}Na^+$ flux (0.65 mg synaptosomal protein in a 0.2 ml reaction volume). Accordingly, the nominal IC_{50} values for inhibition of [^{3}H]BTX-B binding (26 μM) or of Na^+ influx, in the absence (46 μM) or presence (44 μM) of scorpion toxin, correspond to free propofol concentrations of 6.5 μM, 8.8 μM and 8.5 μM, respectively. These values for the free IC_{50} of propofol, which is pharmacologically relevant to drug efficacy, are similar to those achieved clinically.

This work was supported by a Cornell Scholar Award in Biomedical Sciences and NIH grant GM 52441 to H.C.H. We thank Daniel N Duch, Ph.D. for helpful comments on this work, and Ms. Anna Adamo for technical help.

References

AMORIM, P., CHAMBERS, G., COTTRELL, J. & KASS, I.S. (1995). Propofol reduces neuronal transmission damage and attenuates the changes in calcium, potassium, and sodium during hyperthermic anoxia in the rat hippocampal slice. *Anesthesiology*, **83**, 1254–1265.

ARCADI, F.A., RAPISARDA, A., DE LUCA, R., TRIMARCHI, G.R. & COSTA, G. (1996). Effect of 2,6-diisopropylphenol on the delayed hippocampal cell loss following transient forebrain ischemia in the gerbil. *Life Sci.*, **58**, 961–970.

BICKLER, P.E., BUCK, L.T. & FEINER, J.R. (1995). Volatile and intravenous anesthetics decrease glutamate release from cortical brain slices during anoxia. *Anesthesiology*, **83**, 1233–1240.

BRADFORD, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, **72**, 248–254.

CATTERALL, W.A. (1981). Inhibition of voltage-sensitive sodium channels in neuroblastoma cells by antiarrhythmic drugs. *Mol. Pharmacol.*, **20**, 356–362.

CATTERALL, W.A. (1987). Common modes of drug action on Na^+ channels: local anesthetics, antiarrhythmics and anticonvulsants. *Trends Pharmacol. Sci.*, **8**, 57–65.

CATTERALL, W.A. (1995). Structure and function of voltage-gated ion channels. *Ann. Rev. Biochem.*, **64**, 493–531.

CATTERALL, W.A. & GAINER, M. (1985). Interaction of brevetoxin A with a new receptor site on the sodium channel. *Toxicon*, **23**, 497–504.

CATTERALL, W.A., MORROW, C.A., DALY, J.W. & BROWN, G.B. (1981). Binding of batrachotoxin A 20-α-benzoate to a receptor site associated with sodium channels in synaptic nerve ending particles. *J. Biol. Chem.*, **256**, 8922–8927.

CATTERALL, W.A., MORROW, C.S. & HARTSHORNE, R.P. (1979). Neurotoxin binding to receptor sites associated with voltage-sensitive sodium channels in intact, lysed, and detergent solubilized brain membranes. *J. Biol. Chem.*, **254**, 11379–11387.

COCKSHOTT, I.D., DOUGLAS, E.J., PLUMMER, G.F. & SIMONS, P.J. (1992). The pharmacokinetics of propofol in laboratory animals. *Xenobiotica*, **22**, 369–375.

COLLINS, G.G.S. (1988). Effects of the anaesthetic 2,6-diisopropylphenol on synaptic transmission in the rat olfactory cortex slice. *Br. J. Pharmacol.*, **95**, 939–949.

CREVELING, C.R., MCNEAL, E.T., DALY, J.W. & BROWN, G.B. (1983). Batrachotoxin-induced depolarization and [^{3}H]Batrachotoxinin-A 20α-benzoate in a vesicular preparation from guinea pig cerebral cortex. Inhibition by local anesthetics. *Mol. Pharmacol.*, **23**, 350–358.

DE RIU, P.L., PETRUZZI, V., TESTA, C., MULAS, M., MELIS, F., CARIA, M.A. & MAMELI, O. (1992). Propofol anticonvulsant activity in experimental epileptic status. *Br. J. Pharmacol.*, **69**, 177–181.

DUNKLEY, P.R., JARRIE, P.E., HEATH, J.W., KIDD, G.J. & ROSTAS, J.A.P. (1986). A rapid method for isolation of synaptosomes on Percoll gradients. *Brain Res.*, **372**, 115–129.

EDWARDS, R.A., TRAINER, V.L. & BADEN, D.G. (1992). Brevetoxins binds to multiple classes of sites in rat brain synaptosomes. *Mol. Brain Res.*, **14,** 64–70.

FRANKS, N.P. & LIEB, W.R. (1994). Molecular and cellular mechanisms of general anaesthesia. *Nature*, **367,** 607–614.

FRENKEL, C., DUCH, D.S. & URBAN, B.W. (1993). Effects of i.v. anaesthetics on human brain sodium channels. *Br. J. Anaesth.*, **71,** 15–24.

FRENKEL, C. & URBAN, B.W. (1991). Human brain sodium channels as one of the molecular target sites for the new intravenous anaesthetic propofol (2,6-diisopropylphenol). *Eur. J. Pharmacol.*, **208,** 75–79.

GRIMA, M., SCHWARTZ, J., SPACH, M.O. & VELLY, J. (1986). Antianginal arylalkylamines and sodium channels: [^{3}H]-batrachotoxinin-A 20-α-benzoate and [^{3}H]-tetracaine binding. *Br. J. Pharmacol.*, **89,** 641–646.

GUSOVSKY, F., NISHIZAWA, Y., PADGETT, W., MCNEAL, E.T., RICE, K., KIM, C-H., CREVELING, C.R. & DALY, J.W. (1990). Voltage-dependent sodium channels in synaptoneurosomes: studies with $^{22}Na^+$ influx and [^{3}H]saxitoxin and [^{3}H]batrachotoxinin-A 20-α-benzoate binding. Effects of proparacaine isothiocyanate. *Brain Res.*, **518,** 101–106.

HALES, T.G. & LAMBERT, J.J. (1991). The actions of propofol on inhibitory amino acid receptors of bovine adrenomedullary chromaffin cells and rodent central neurones. *Br. J. Pharmacol.*, **104,** 619–628.

HILL, R.J., DUFF, H.J. & SHELDON, R.S. (1989). Class I antiarrhythmic drug receptor: Biochemical evidence for state-dependent interaction with quinidine and lidocaine. *Mol. Pharmacol.*, **36,** 150–159.

HIROTA, K. & LAMBERT, D.G. (1996). I.V. anesthetic agents inhibit dihydropyridine binding to L-type voltage-sensitive Ca^{2+} channels in rat cerebrocortical membranes. *Br. J. Anaesth.*, **77,** 248–253.

KOCHS, E., HOFFMAN, W.E., WERNER, C., THOMAS, C., ALBRECHT, R. & ESCH, J.S. (1992). The effects of propofol on brain electrical activity neurologic outcome, and neuronal damage following incomplete ischemia in rats. *Anesthesiology*, **76,** 245–252.

KRUEGER, B.K., BLAUSTEIN, M.P. & RATZLAFF, R.W. (1980). Sodium channels in presynaptic nerve terminals. Regulation by neurotoxins. *J. Gen. Physiol.*, **76,** 287–313.

LAMBERT, D.G., WILLETS, J.M., ATCHESON, R., FROST, C., SMART, D., ROWBOTHAM, D.J. & SMITH, G. (1996). Effects of propofol and thiopentone on potassium- and carbachol-evoked [^{3}H]noradrenaline release and increased $[Ca^{2+}]_i$ from SH-SY5Y human neuroblastoma cells. *Biochem. Pharmacol.*, **51,** 1613–1621.

LOMBET, A., BIDARD, J-N. & LAZDUNSKI, M. (1987). Ciguatoxin and brevetoxins share a common receptor site on the neuronal voltage-dependent Na^+ channel. *FEBS Lett.*, **219,** 355–359.

MINAMI, K., YANAGIHARA, N., SEGAWA, K., TSUTSUI, M., SHIGEMATSU, A. & IZUMI, F. (1996). Inhibitory effects of propofol on catecholamine secretion and uptake in cultured bovine adrenal medullary cells. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **353,** 572–578.

NICHOLLS, D.G. (1993). The glutamatergic nerve terminal. *Eur. J. Biochem.*, **212,** 613–631.

OLCESE, R., USAI, C., MAESTRONE, E. & NOBILE, M. (1994). The general anesthetic propofol inhibits transmembrane calcium current in chick sensory neurons. *Anesth. Analg.*, **78,** 955–960.

ORSER, B.A., BERTLIK, M., WANG, L-Y. & MACDONALD, J.F. (1995). Inhibition by propofol (2,6-di-isopropylphenol) of the N-methyl-D-aspartate subtype of glutamate receptors in cultured hippocampal neurones. *Br. J. Pharmacol.*, **116,** 1761–1768.

PAUWELS, P.J., LEYSEN, J.E. & LADURON, P.M. (1986). [^{3}H]Batrachotoxinin A 20-α-benzoate binding to sodium channels in rat brain: Characterization and pharmacological significance. *Eur. J. Pharmacol.*, **124,** 291–298.

PAVAN, I., BUGLIONE, E., MASSICCIO, M., GREGORETTI, C., BURBI, L. & BERARDINO, M. (1992). Monitoring propofol serum levels by rapid and sensitive reversed-phase high-performance liquid chromatography during prolonged sedation in ICU patients. *J. Chromatogr. Sci.*, **30,** 164–166.

POLI, M.A., MENDE, T.J. & BADEN D.G. (1986). Brevetoxins, unique activators of voltage-sensitive sodium channels, bind to specific sites in rat brain synaptosomes. *Mol. Pharmacol.*, **30,** 129–135.

POSTMA, S.W. & CATTERALL, W.A. (1984). Inhibition of binding of [^{3}H]Batrachotoxinin A 20-α-benzoate to sodium channels by local anesthetics. *Mol. Pharmacol.*, **25,** 219–227.

RATNAKUMARI, L. & HEMMINGS, H.C. (1996). Effects of intravenous anesthetic propofol on voltage-dependent sodium channels in rat brain synaptosomes. *J. Neurochem.*, **66** (suppl 1), S51B.

RAY, R., MORROW, C.S. & CATTERALL, W.A. (1978). Binding of scorpion toxin to receptor sites associated with voltage-sensitive sodium channels in synaptic nerve ending particles. *J. Biol. Chem.*, **253,** 7307–7313.

REHBERG, B., XIAO, Y-H. & DUCH, D.S. (1996). Central nervous system sodium channels are significantly suppressed at clinical concentrations of volatile anesthetics. *Anesthesiology*, **84,** 1223–1233.

RIDENOUR, T.R., WARNER, D.S., TODD, M.M. & GIONET, T.X. (1992). Comparative effects of propofol and halothane on outcome from temporary middle cerebral artery occlusion in the rat. *Anesthesiology*, **76,** 807–812.

RITCHIE, J.M. & ROGART, R.B. (1977). The binding of saxitoxin and tetrodotoxin to excitable tissue. *Rev. Physiol. Biochem. Pharmacol.*, **79,** 1–51.

SCHLAME, M. & HEMMINGS, H.C. (1995). Inhibition by volatile anesthetics of endogenous glutamate release from synaptosomes by a presynaptic mechanism. *Anesthesiology*, **82,** 1406–1416.

SHYR, M-H., TSAI, T-H., TAN, P.P.C., CHEN, C-F. & CHEN, S.H.H. (1995). Concentration and regional distribution of propofol in brain and spinalcord during propofol anesthesia in the rat. *Neurosci. Lett.*, **184,** 212–215.

SMITH, C., MCEWAN, A.I., JHAVERI, R., WILKINSON, M., GOODMAN, D., SMITH, R., CANADA, A.T. & GLASS, P.S.A. (1994). The interaction of fentanyl on the CP_{50} of propofol for loss of consciousness and skin incision. *Anesthesiology*, **81,** 820–828.

TAMKUN, M.M. & CATTERALL, W.A. (1981). Ion flux studies of voltage sensitive sodium channels in synaptic nerve-ending particles. *Mol. Pharmacol.*, **19,** 78–86.

TANELIAN, D.L., KOSEK, P., MODY, I. & MACLVER, M.B. (1993). The role of the $GABA_A$ receptor/chloride channel complex in anesthesia. *Anesthesiology*, **78,** 757–776.

URENJAK, J. & OBRENOVITCH, T.P. (1996). Pharmacological modulation of voltage-gated Na^+ channels: A rational and effective strategy against ischemic brain damage. *Pharmacol. Rev.*, **48,** 21–67.

VELLY, J., GRIMA, M., MARCINIACK, G., SPACH, M.O. & SCHWARTZ, J. (1987). Effects of some antianginal and vasodilating drugs on sodium influx and on the binding of ^{3}H-batrachotoxinin-A 20-α-benzoate and ^{3}H-tetracaine. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **335,** 176–182.

WILLOW, M. & CATTERALL, W.A. (1982). Inhibition of binding of [^{3}H]Batrachotoxinin-A 20-α-benzoate to sodium channels by the anticonvulsant drugs diphenylhydantoin and carbamazepine. *Mol. Pharmacol.*, **22,** 627–635.

(Received June 21, 1996
Revised August 26, 1996
Accepted September 9, 1996)