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Inhibition by propofol (2,6 di-isopropylphenol) of the N-methyl-D-aspartate subtype of glutamate receptor in cultured hippocampal neurones

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1 The effects of propofol (2,6 di-isopropylphenol) on responses to the selective glutamate receptor agonists, N-methyl-D-aspartate (NMDA) and kainate, were investigated in cultured hippocampal neurones of the mouse. Whole cell and single channel currents were recorded by patch-clamp techniques. Drugs were applied with a multi-barrel perfusion system.

2 Propofol produced a reversible, dose-dependent inhibition of whole cell currents activated by NMDA. The concentration of propofol which induced 50% of the maximal inhibition (IC_{50}) was approximately 160 μM. The maximal inhibition was incomplete leaving a residual current of about 33% of the control response. This inhibitory action of propofol was neither voltage- nor use-dependent.

3 Analysis of the dose-response relation for whole cell NMDA-activated currents indicated that propofol caused no significant change in the apparent affinity of the receptor for NMDA .

4 Outside-out patch recordings of single channel currents evoked by NMDA (10 μM) revealed that propofol (100 μM) reversibly decreased the probability of channel opening but did not influence the average duration of channel opening or single channel conductance.

5 Whole-cell currents evoked by kainate (50 μM) were insensitive to propofol (1 μM-1 mM).

6 These results indicate that propofol inhibits the NMDA subtype of glutamate receptor, possibly through an allosteric modulation of channel gating rather than by blocking the open channel. Depression of NMDA-mediated excitatory neurotransmission may contribute to the anaesthetic, amnesic and anti-convulsant properties of propofol.

Keywords: Propofol (2,6 di-isopropylphenol); NMDA; kainate; hippocampal neurones; patch-clamp; anaesthesia

Introduction

Propofol (2,6 di-isopropylphenol) is an alkylphenol used as an intravenous general anaesthetic and hypnotic (James & Glen, 1980). Since its introduction into clinical practice in the 1980's, propofol has been used extensively because of its favourable pharmacokinetic properties and limited number of side-effects (for review see Skues & Prys-Roberts, 1989; Borgeat *et al.*, 1994). Despite numerous investigations of the behavioural properties of propofol, there is relatively little known about its site(s) and molecular mechanism(s) of action. Recent studies indicate that propofol potentiates γ-aminobutyric-acid (GABA)-mediated inhibitory synaptic transmission and directly activates the $GABA_A$ receptor (Hales & Lambert, 1992; Hara *et al.*, 1993; Orser *et al.*, 1994). In addition, several reports suggest that propofol may also inhibit glutamate receptor-mediated excitatory synaptic transmission (Lodge & Anis, 1984; Bianchi & Galzigna, 1991).

Glutamate, the major excitatory neurotransmitter in the central nervous system activates at least two major types of ionotropic receptors which can be classified as: N-methyl-D-aspartate (NMDA) and non-NMDA or α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)/kainate receptors. Electrophysiological recordings from the cat spinal cord have previously shown that propofol attenuates glutamate-mediated polysynaptic reflexes, as does the noncompetitive NMDA receptor antagonist, ketamine (Lodge & Anis, 1984). Furthermore, propofol inhibits glutamate-stimulated entry of Ca^{2+} into rat synaptosomes (Bianchi & Galzigna, 1991). In contrast to these findings, propofol has been reported to have little effect on glutamate, kainate, or NMDA-induced depolarizations recorded from the surface of rat olfactory cortical slices (Collins, 1988). In the present study we examined the direct effect of propofol on currents activated by NMDA and kainate in cultured hippocampal neurones of the mouse. Part of this work has been published in abstract form (Orser *et al.*, 1993; Bertlik *et al.*, 1994).

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Methods

Dissociation and culture of hippocampal neurones

Cultures of embryonic hippocampal neurones were prepared from Swiss White mice as previously described (MacDonald *et al.*, 1989). Briefly, foetal hippocampi were obtained from mice killed by cervical dislocation and neurones were dissociated by enzymatic digestion and mechanical trituration. Cells were plated on collagen-coated dishes and maintained in culture for 10-21 days.

Whole cell current recordings

Prior to recording, cells were thoroughly rinsed with a standard extracellular recording solution containing (in mM): NaCl 140, $CaCl_2$ 1.3, KCl 5.4, *N*-2-hydroxy-ethylpiperazine-*N'*-2-ethanesulphonic acid (HEPES 25), glucose 33, tetrodotoxin 300 nM, glycine 3 μM, buffered to a pH of 7.4 with NaOH and adjusted to 297-300 mOsm. Strychnine (1 μM) was added to the extracellular solution to prevent the enhancement of glycine-mediated currents by propofol (Hales & Lambert, 1992).

Patch electrodes were constructed from thin walled borosilicate glass (1.5 mm diameter, World Precision Instruments

Inc.) with a two-stage vertical puller (Narishige PP-83). The electrodes were filled with a solution containing (in mM): CsF 140, $CaCl_2$ 1, $MgCl_2$ 2, HEPES 10 and EGTA 11. In some experiments, CsF was replaced with Cs_2SO_4. For most experiments the pipette solution also included an 'ATP support solution' containing (mM): adenosine-triphosphate (Mg^{2+} salt) 4, phosphocreatine 20 and creatine phosphokinase 50 u ml^{-1}, to prevent run down of NMDA and kainate-activated currents (MacDonald *et al.*, 1989; Wang *et al.*, 1991). Cells were voltage-clamped with an Axopatch 1B amplifier (Axon Instruments Inc.).

We have previously shown that propofol elicits a Cl^--mediated response by directly activating the $GABA_A$ receptor in cultured hippocampal neurones (Orser *et al.*, 1994). Therefore, cells were voltage-clamped at −65 mV, close to the reversal potential for responses activated by GABA or propofol, in order to minimize the contribution of such currents. Drugs and agonists were applied to the cells by use of a multi-barrel perfusion system which ensured the rapid exchange of extracellular solutions (Johnson & Ascher, 1987). All experiments were conducted at room temperature (20°−25°C).

Whole cell currents were filtered (2 kHz), recorded with a pen recorder (Gould Inc.) and simultaneously digitized (TL-1 Interface, Axon Instruments Inc.) and stored on a microcomputer using PClamp software (Axon Instruments Inc.). For the analysis of the dose-response curves, data were plotted using *Sigma Plot* (Jandel Scientific) and the curves fitted to a modified version of the Michaelis-Menten equation by a least-squares method. The effective concentration of NMDA that produced 50% of the maximal response (EC_{50}) and the Hill coefficient (n_H) were determined according to the equation: $I = I_{max}\, C^n/(C^n + EC_{50}^n)$ where I is the observed current, I_{max} is the maximal response and C is the concentration of agonist. The concentration of propofol that produced 50% of the maximal inhibition (IC_{50}) was determined from the concentration-inhibition curve. Data points normalized to the maximal response were fit to the equation: $I = (I_{max}(C^n/(C^n + IC_{50}^n))) + c$, where c is the fraction of the response that was insensitive to the block by propofol. Data are shown as the mean ± s.e.mean unless otherwise indicated.

Single channel recordings

Electrodes to be used for single channel recording (6−10 MΩ) were coated with Sylgard (Dow-Corning Corp.) and filled with a solution containing (in mM): CsF or Cs_2SO_4 140, HEPES 10, EGTA 11, $CaCl_2$ 1, adjusted to a pH of 7.4 with CsOH. Outside-out patches were positioned in the outflow from one of the perfusion barrels. Single channel currents activated by NMDA (10 μM) were recorded in the presence or absence of propofol (100 μM).

Signals were filtered at 2 kHz (−3 dB) with a low pass filter (4 pole Bessel filter, Axopatch 200, Axon Instruments Inc.), digitized, and recorded on a VHS tape for off-line analysis. Current records were sampled at 100 μs per point and analysed using Fetchan (PClamp, Axon Instruments Inc.). The amplitude of single channel openings was determined by two methods, both of which produced similar results. Firstly, sample data were displayed and the amplitudes determined by manually placing a cursor at the open and closed current level. The amplitudes of multiple events were averaged to obtain the mean value. Alternatively, the distribution of all digitized currents was plotted as a histogram. The peaks of the histograms were fitted by a non-linear least-squares curve fitting method (Levenberg-Marquardt fitting algorithm, PClamp, Axon Instruments Inc.). The 50% threshold crossing method was used to measure the amplitude and duration of events. Openings or closings with a duration of less than 300 μs were ignored and no compensation was applied for undetected events (Twyman & MacDonald, 1992). Data were binned into event duration histograms and the estimates of the time constants were obtained by fitting a bi-exponential function to the histograms by a non-linear least-squares method. The binning parameters for all open duration histograms were identical for records obtained under the various recording conditions. The probability of channel opening (P_o) was calculated as previously described (Oh & Benos, 1993). It was assumed that in records containing multiple levels, patches contained as many channels as levels observed and that each channel behaved independently. A large variation in the frequency of channel opening and P_o was observed between patches, presumably because of differences in the number of channels in each patch. In order to compare the effect of propofol on open state probability, values for each patch were normalized to those obtained under control conditions. A detailed analysis of burst kinetics and the effects of various concentrations of propofol is beyond the scope of the current presentation.

Drugs and other chemicals

Propofol was prepared from Diprivan (I.C.I. Pharma. Mississauga, Canada). Each ml of Diprivan contains: 2,6 di-isopropylphenol 10 mg, soyabean oil 100 mg, egg lecithin 12 mg and glycerol 22.5 mg. Propofol stock (10^{-2} M) was prepared on the day of the experiment. Vehicle solution was prepared from Intralipid 10% (KabiVitrum Canada Inc. Toronto, Canada). Each ml of Intralipid contains: soyabean oil 100 mg, egg lecithin 12 mg and glycerin 22.5 mg, pH adjusted to 7.2 with NaOH. Unless specified otherwise, all other compounds were purchased from Sigma Chemical Co (St. Louis MO, U.S.A.).

Results

Effects of propofol on NMDA-activated whole cell currents

In cells voltage-clamped at −65 mV, applications of NMDA (50 μM) activated inward currents which peaked and then declined to an apparent steady-state. Co-applications of propofol (10−1000 μM) produced a reversible and concentration-dependent inhibition of the current as indicated in Figure 1a.

Propofol consistently reduced the amplitude of the steady-state current to a greater extent than the peak response: propofol, at concentrations of 100 μM and 600 μM, decreased the amplitude of the peak response by 11.6 ± 2.7% ($n = 6$) and 13.9 ± 5.9% ($n = 5$), whereas the steady-state currents were decreased by 27.6 ± 5.9% and 48.4 ± 5.0%, respectively. In order to determine if this apparent discrepancy was due to the slow onset of propofol block, relative to the rapid rate of activation of the NMDA response, the time course of propofol inhibition was further examined. Propofol applications (10 μM−6 mM) were superimposed upon a 100 s application of NMDA as illustrated in Figure 2a. These experiments demonstrated that the onset of, and recovery from, inhibition by propofol were slower than the rate of activation of the receptor by NMDA. The time constant of the onset of the block (τ_{on}) was estimated by fitting the decay phase of the steady-state response using a single exponential function.

Currents evoked by NMDA gradually recovered to control amplitude following termination of the application of propofol. The recovery phase was also fitted with a single exponential function and the time constants for recovery from block (τ_{off}) were determined. The concentration-dependencies of τ_{on} and τ_{off} are illustrated in Figure 2b. Note that both the on and off rates depend upon the concentrations of propofol and that the rates do not appear to saturate at the concentrations examined. Moreover, a 100 fold increase in the concentration of propofol decreased τ_{on} 3.3 fold. The concentration of propofol at which the rates of onset and offset were approximately equivalent was estimated to be 140 μM. This concentration is consistent with the IC_{50} value calculated from the concentration-inhibition curve ($IC_{50} = 160$ μM, see Figure 3).

The concentration-dependence of the inhibition of steady-state currents by propofol is summarized in Figure 3. Analysis of the dose-response relation indicated that the half-maximal inhibitory concentration of propofol and the Hill coefficient were 160 μM and 0.77, respectively. The block by propofol was incomplete with an extrapolated maximal block of about 67%. The effects of Intralipid, which is used as an emulsifying agent in Diprivan, was examined at concentrations equivalent to those associated with 10 μM – 1 mM propofol. This substance did not significantly inhibit or potentiate NMDA-evoked responses (not shown).

An alternative explanation for the apparent difference in sensitivity of the peak and steady-state responses to propofol (see Figure 1) is that propofol might have induced a use-dependent block of the receptor, similar to that observed with uncompetitive antagonists (MacDonald *et al.*, 1987). Uncompetitive blockers, such as PCP, ketamine or MK-801, demonstrate a much higher affinity for the open state relative to the closed state of the NMDA receptor. Therefore, the onset of, and recovery from, the blockade by ketamine is enhanced by prolonged or repeated activation of the receptor. Moreover, exposure to ketamine in the absence of agonist produces a minimal reduction in the peak response to an initial application of agonist (MacDonald *et al.*, 1987; 1989). In contrast, propofol reduced the peak and steady-state responses to NMDA even when it was pre-applied in the absence of agonist. In addition, recovery from the block occurred in the absence of agonist. (Figure 4). These observations suggest that propofol can gain access to its binding site regardless of the conformation of the receptor.

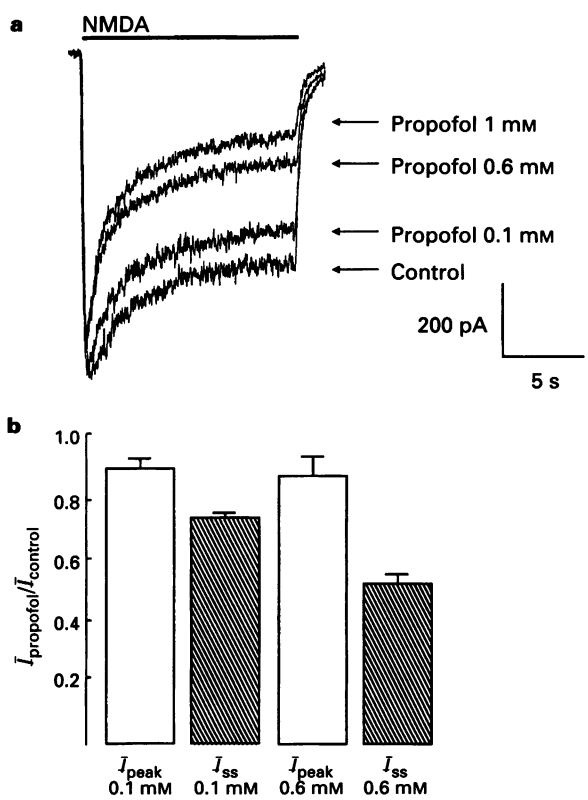

Figure 1 Propofol inhibition of NMDA-activated currents recorded from hippocampal neurones. (a) Representative traces of currents evoked by NMDA (50 μM), in the absence or presence of propofol are shown. Traces are superimposed upon each other and the period of perfusion is indicated by the horiziontal bar at the top of the traces. Propofol caused a concentration-dependent reduction in the amplitude of these currents. Note that the steady-state component is reduced to a greater extent than the peak response. (b) The fractional reduction of peak (I_{peak}) and steady-state currents (I_{ss}), recorded in the presence of propofol 0.1 mM ($n=6$) and 0.6 mM ($n=5$) is illustrated. Neurones were voltage-clamped at a holding potential of −65 mV.

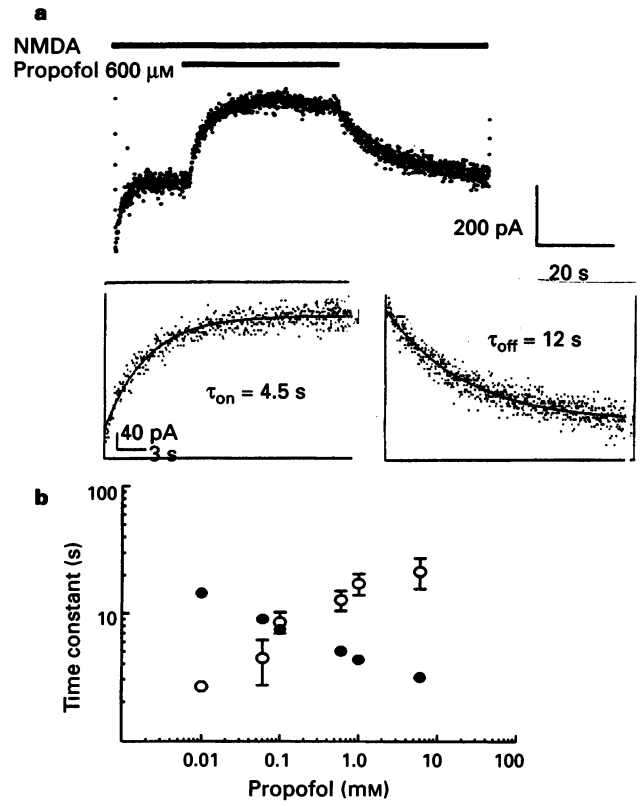

Figure 2 Propofol inhibition of the steady-state NMDA response. (a) The τ_{on} and τ_{off} of propofol block was determined using the illustrated protocol. Propofol (10 μM – 10 mM) was applied for 40 s during a 100 s application of NMDA (50 μM). The solid lines drawn through the data points in the insets represent the theoretical fit to a single exponential function. (b) The concentration-dependencies of τ_{on} and τ_{off} are shown. The time constants for onset (●) and recovery (○) were averaged from four neurones and data presented as mean ± s.e.mean.

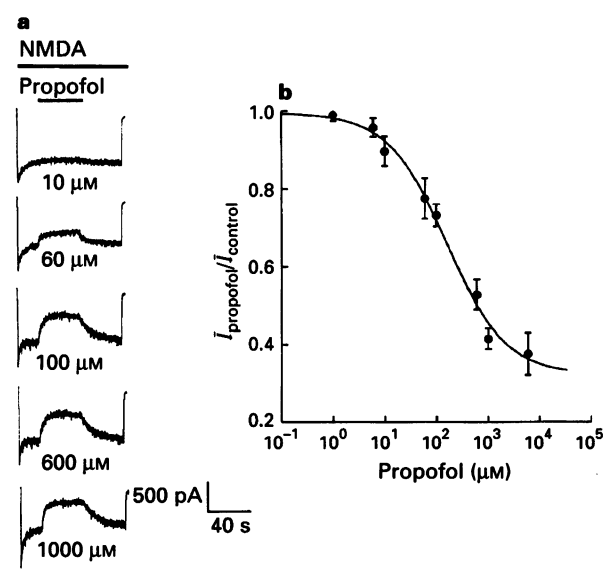

Figure 3 The dose-dependence of the inhibition of NMDA-activated currents by propofol. (a) During prolonged applications of NMDA, various concentrations of propofol were superimposed. The threshold concentration for channel inhibition was approximately 10 μM and 6 mM propofol produced a block of 63% ($n=6$). The solid line was derived by fitting to the modified Hill equation. The concentration of propofol that induced 50% of the maximum block (IC_{50}) and Hill coefficient were 160 μM and 0.77, respectively. The extrapolated maximum block was approximately 67%.

The possible voltage-dependence of the block by propofol was examined by recording NMDA-evoked currents at various holding potentials in the absence or presence of propofol (600 μM). Analysis of the current-voltage ($I-V$) relation for steady-state currents evoked by NMDA, revealed that propofol reduced the amplitude of the responses equally at both hyperpolarized and depolarized membrane potential (Figure 5a). The slope of the $I-V$ curve was decreased and the reversal potential was unchanged by the presence of propofol indicating that this anaesthetic reduced whole cell conductance but did not alter the ion selectivity of NMDA-activated channels.

Propofol may have reduced NMDA-activated responses by decreasing the binding of the agonist to the receptor. In order to determine if propofol reduced the affinity of the receptor for NMDA, the dose-response curves for NMDA-activated currents were examined in the presence or absence of propofol. Control responses were first obtained by applying various concentrations of NMDA (0.6–1000 μM). Cells were then perfused for several minutes with propofol (30 or 100 μM) and NMDA was subsequently re-applied in the continued presence of propofol. Individual dose-response curves were constructed from the control responses for each cell and the EC_{50} values were determined from the fitted curves. All currents were normalized with respect to the control EC_{50} value for each cell (Figure 6). Propofol reduced the peak responses to applications of NMDA, as indicated by the depression of the dose-response relations. Propofol did not, however, substantially modulate the apparent affinity (K_d) of the receptor for NMDA suggesting a non-competitive mechanism of action. The maximal response to NMDA was decreased, in the presence of propofol 30 μM or 100 μM by 20.3±2.5% ($n=3$) and 35.7±11.3% ($n=5$), respectively. The reduction of the peak response following exposure to propofol 100 μM (35.7%) was slightly greater than the depression of the steady-state currents observed with the co-application paradigm (27%, see Figure 1). The reduction of the peak response may have been over estimated due to a rundown of current which was not completely prevented by the high-energy support solution.

Propofol effects on NMDA receptor single channel openings

In patches held at −70 mV, applications of NMDA (10 μM) evoked channel openings with a mean current amplitude of 2.6±0.1 pA ($n=5$), which is indicative of a single channel conductance of 37 pS. Application of 100 μM propofol did not change the amplitude (2.6±0.1 pA, $n=5$) or the slope conductance as estimated from the $I-V$ relation (Figure 7a). In the presence or absence of propofol, channel openings were

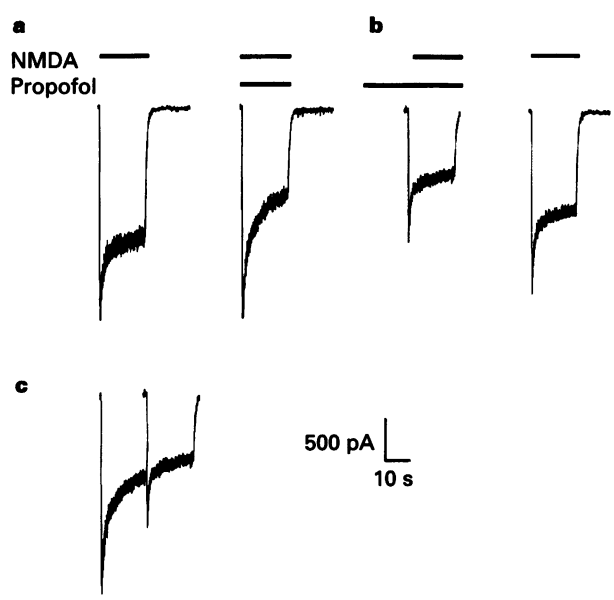

Figure 4 The inhibition of currents evoked by NMDA does not demonstrate the features typical of a use-dependent block. (a) Under control conditions, a 20 s application of NMDA (50 μM) activated a current which rapidly reached a peaked then declined. The second trace demonstrates the response to a co-application of propofol (600 μM). The peak current was decreased by 4% whereas the nondesensitized component, measured at the end of the application, was decreased by 37%. (b) Pre-application of 600 μM propofol reduced the peak and quasi steady-state response by 46% and 49%, respectively. A subsequent application of NMDA alone generated a peak response which recovered to 80% of the control value. (c) Currents recorded during the co-application (a, second trace) or following pre-exposure to propofol (b, first trace) are shown superimposed upon each other. These data suggest that the rate of current decay is similar regardless of the method of application of propofol.

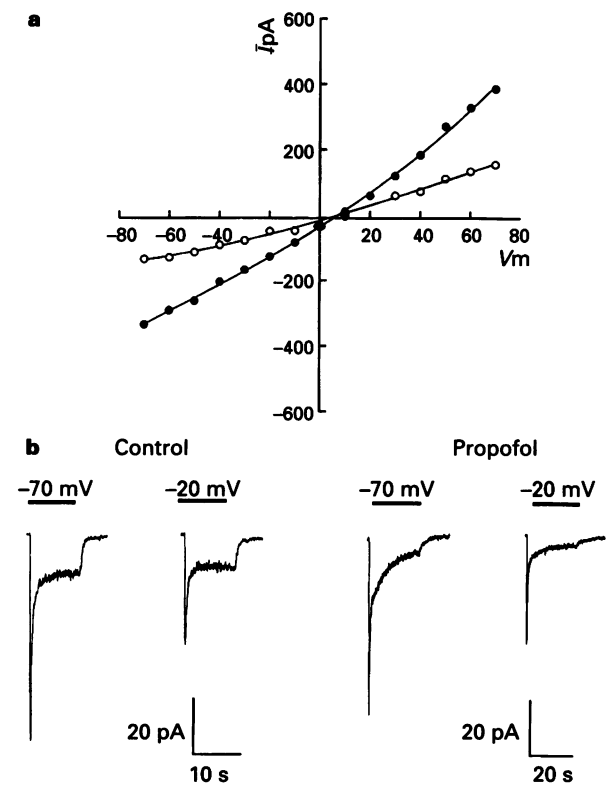

Figure 5 Propofol inhibition of NMDA-evoked currents is independent of membrane potential. (a) Current-voltage relation for responses activated by 100 μM NMDA were recorded in the absence (●) or presence (○) of propofol (600 μM). Cells were voltage clamped at −65 mV and exposed to a prolonged application of agonist. Currents were observed to peak and then decline to an apparent steady-state. Once a stable current level was reached, the membrane potential was adjusted in 10 mV steps ranging from −70 mV to +70 mV for 100 ms and the steady-state current recorded. The cell was then voltage clamped at −65 mV and NMDA washed away. This protocol was repeated in the presence of NMDA plus propofol (600 μM). In addition, the amplitude of currents activated by propofol (600 μM) alone was measured at the various holding potentials and subtracted from the NMDA plus propofol current records. The $I-V$ plot illustrates the amplitude of the steady-state responses. Leak and capacitance currents, measured in the absence of agonist and propofol were also subtracted from the records. NMDA-evoked currents were depressed equally at hyperpolarizing and depolarizing potentials: propofol reduced responses to 40% of control at −70 mV and +70 mV. (b) In a different cell, the voltage-dependence of block by propofol was further investigated. Cells were voltage-clamped at −70 mV or −20 mV and NMDA, or NMDA plus propofol, applied for 10 s. Propofol (1 mM) was seen to reduce the amplitude of the steady-state response to 42% of control, at both holding potentials.

brief with no evidence of 'flickering'. Propofol did however, reduce the probability of single channel opening (P_o) to 45.2±16% of control values. The mean open probability for five patches, measured before and after exposure to 100 μM propofol, was 0.1±0.02 and 0.031±0.007 ($n=5$, $P<0.05$, Student's t test), respectively. The reduction in P_o resulted primarily from a decrease in the frequency of channel opening rather than a reduction in the duration of open events (Table 1). In addition, cumulative open time histograms were constructed and fitted using a bi-exponential function. Propofol had no apparent effect on the distribution of the channel open times nor upon the fast or slow time constants. In three of the five patches, propofol was washed away for several minutes and P_o again determined. Following this washout of drug, P_o increased 2.7, 1.2 and 1.8 fold (mean 1.95±0.4). It is interesting to note that this concentration of propofol decreased P_o by more than 50% whereas the same concentration reduced the whole cell response by only 28%. The reason for this apparent discrepancy is unknown but may relate to changes in the sensitivity of the receptor to propofol block following patch excision. For example, Covarrubias & Steinbach, (1990) demonstrated that channel kinetics are influenced by patch excision. Also, we previously observed that the sensitivity of the NMDA receptor to other compounds that modulate channel gating is influenced by the recording configuration (Wang *et al.*, 1994). Furthermore, the concentrations of propofol required to blocked macroscopic nicotinic acetylcholine receptor (AChR)-mediated currents were higher and not simply related to those that inhibited single channel openings (Dilger *et al.*, 1994).

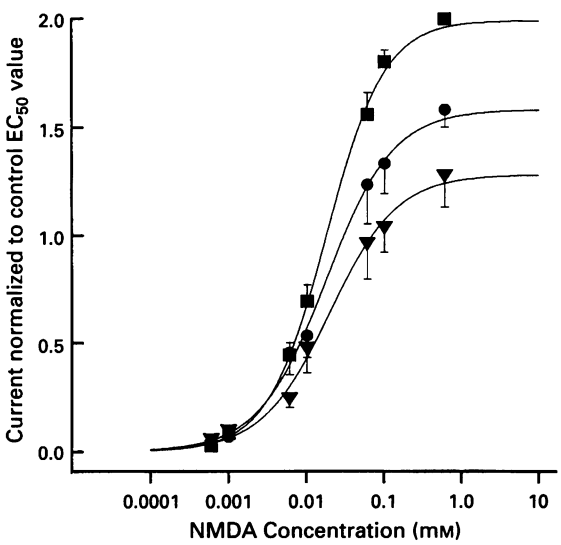

Figure 6 Concentration-response curves for peak NMDA-evoked currents recorded in the presence or absence of propofol. Neurones were voltage clamped at −65 mV and currents were activated by applying NMDA (0.6 μM−1 mM). Cells were exposed to propofol (30 μM or 100 μM) and NMDA was re-applied. Paired dose-response curves were constructed and responses normalized to the concentration of NMDA that evoked the half-maximal response (EC_{50}) under control conditions. The ordinate scale indicates peak responses normalized to the NMDA EC_{50} value for individual neurones and the abscissa scale shows the concentration of NMDA (μM). The apparent affinity of the receptor for NMDA (K_d) and Hill coefficients (n_H) for the various recording conditions were determined by fitting the curves to a standard logistic equation. No significant differences observed in the absence (■) or presence of propofol 100 μM (▼), 30 μM (●), for the K_d: 20.6±3.4 μM ($n=9$), 19.9±2.88 μM ($n=5$), 24.0±10.2 μM ($n=4$), respectively, or n_H values: 1.24±0.06, 1.38±0.16, 1.31±0.31, ($P>0.05$ Student's t test).

Effects of propofol on kainate-activated whole cell currents

The effects of propofol on AMPA/kainate receptors were also investigated. Kainate was used as an agonist rather than AMPA, as AMPA currents exhibit substantial desensitization. Stable currents were evoked by application of 50 μM kainate, a concentration close to the EC_{50} value for currents recorded from cultured hippocampal neurones (Wang *et al.*, 1994). Neither pre-exposure of the cells to propofol nor the co-application of propofol (10 μM−1 mM) had any consistent effect on kainate-evoked responses (Figure 8). In addition, propofol (100 μM), applied for 20 s during a 40 s application of kainate (50 μM) failed to influence significantly the amplitude of the steady state response ($O_{propofol}=92.7\pm2.7\%$ of the control response, $n=4$, $P<0.05$).

Discussion

Our results demonstrate that propofol causes a concentration-dependent and reversible inhibition of NMDA receptors expressed in cultured hippocampal neurones. The onset of, and recovery from propofol block occurred in the absence of agonist, no use-dependency was detected and proprofol did not change the apparent affinity of the receptor NMDA. Furthermore, propofol reduced the frequency, but not the duration or amplitude, of NMDA-activated single channel openings.

These results are in agreement with a preliminary report by Sanna *et al.* (1993) which indicates that propofol inhibits NMDA-evoked responses in *Xenopus* oocytes injected with brain mRNA. These authors observed that propofol, at concentrations of 25 μM and 300 μM, decreased responses by 8% and 34%, respectively. We found that these concentrations of propofol depressed NMDA-evoked currents recorded from hippocampal neurones by 13% and 42%, respectively (Figure 3). In addition, Sanna *et al.* (1993) reported that [^{3}H]-propofol failed to bind to HEK 293 cells transfected with cDNA for the GluR6 glutamate receptor clone and that propofol had little effect on kainate responses. Together with our own results, these data demonstrate that propofol blocks the NMDA subtype of the glutamate receptor but does not influence receptors activated by kainate.

Table 1 Effects of propofol on NMDA-activated single channel openings

		Single channel kinetics			
	pA	*Open time* (ms)	*Close time* (ms)	τ_1 (ms)	τ_2 (ms)
Control NMDA	2.61±0.14	2.85±0.93	16.3±14.5	0.46±0.05	2.95±0.87
NMDA + propofol 100 μM	2.63±0.12	2.90±0.68	40.9±24.2	0.52±0.87	3.24±1.03

Mean±s.d.; $n=5$ patches.

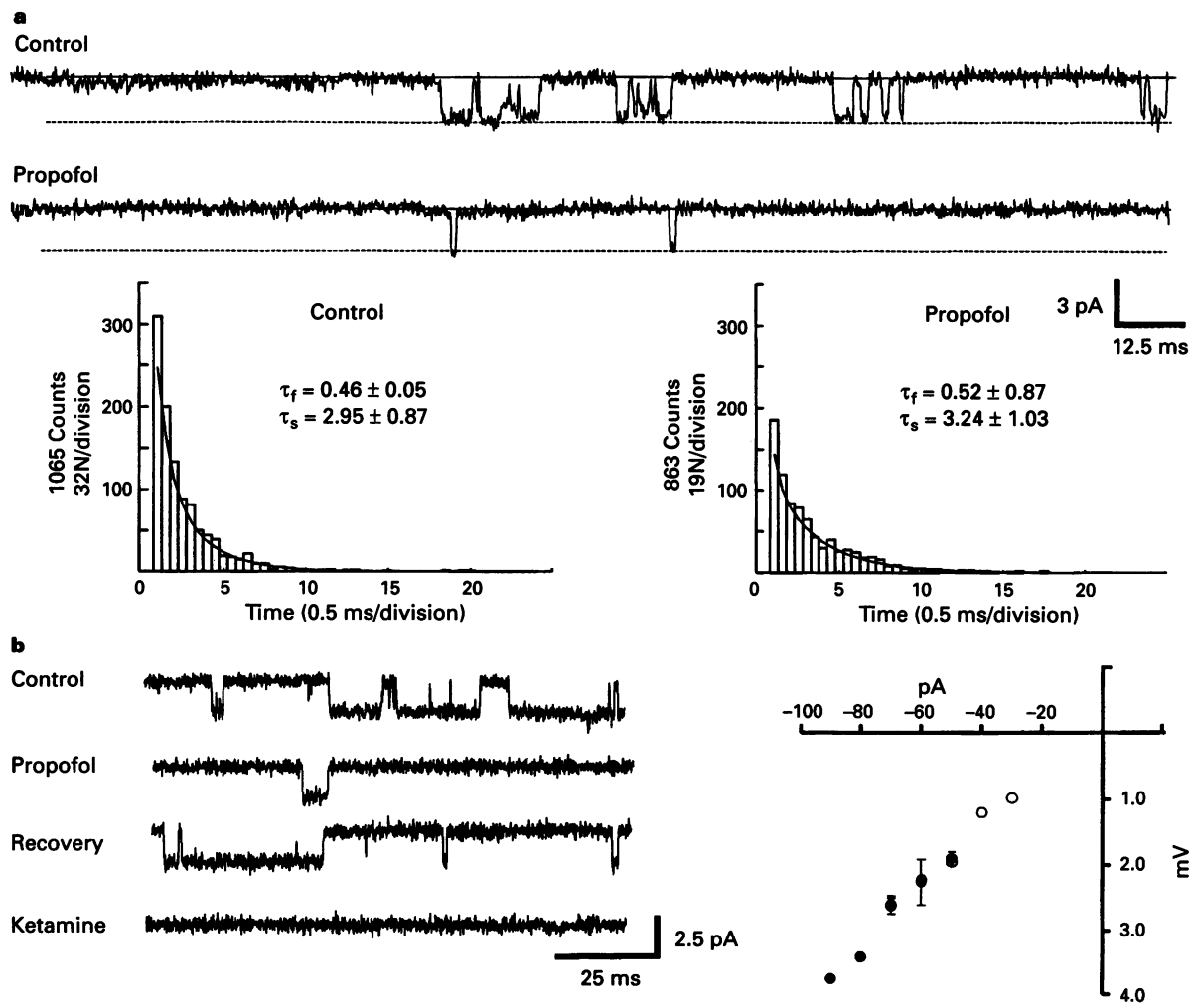

Figure 7 Propofol reduces the frequency of NMDA channel opening. (a) An example of single channel currents from an outside-out patch. The pipette solution contained Cs_2SO_4 and the patch was voltage clamped at −70 mV. Traces represent current low passed filtered at 2 kHz (−3 dB) and digitized at 0.1 ms/point. Inward current is represented by a downward deflection and the closed and open states are indicated by the solid and dashed lines respectively. The addition of 100 μM propofol to the perfusate containing 10 μM NMDA, caused a reversible decrease in frequency of channel opening. The open duration histograms for the control and propofol currents are indicated below the tracings. The time constants for the illustrated control and propofol curves were $\tau_f = 0.75$ ms, $\tau_s = 2.43$ ms, and $\tau_f = 0.43$, $\tau_s = 2.94$, respectively. Propofol had no apparent effect on the fast (τ_f), or slow (τ_s) time constants. (b) Currents from a different patch, voltage-clamped at −50 mV, recorded in the absence and presence of propofol are shown. Ketamine (20 μM), added to the bath solution abolished residual open events providing further confirmation of the identity of the channels. The $I-V$ plot for single channel currents activated by 10 μM NMDA revealed a slope-conductance of 47 pS and 45 pS for currents recorded in the absence (●) and presence (○) of 100 μM propofol. The mean current amplitudes ± s.e.mean are shown ($n = 5$ patches).

The kinetics of the actions of propofol are complex as indicated by Figure 2 and do not support a simple receptor occupancy model. Notably, the off rates depend on the concentration of propofol and exceed the on rates at low concentrations. It is therefore likely that the off rates reflect the slow clearance of propofol from the membrane domain rather than dissociation of drug from the receptor. Propofol is a highly lipophylic compound with an octanol/water partition coefficient of 4,300 and p$Ka = 11$ (Tonner *et al.*, 1992; Veintemilla *et al.*, 1992). Unlike a charged blocker that can access a binding site via the channel pore, propofol probably diffuses into the membrane and binds to a hydrophobic region of the protein or a site located at the lipid-protein interface. Interestingly, propofol modulation of the $GABA_A$ receptor demonstrates similar slow kinetics (rate of association: 3×10^5 M^{-1} s^{-1}) suggesting that binding of propofol to a hydrophobic site modulates the gating of both excitatory and inhibitory amino acid receptors (Orser *et al.*, 1994).

Propofol (100 μM) inhibited the frequency but not the duration of NMDA channel openings. In contrast, propofol (100 μM) decreased the mean open duration of the nicotinic AChR by more than 50% (Wachtel & Wegrzynowicz, 1992; Dilger *et al.*, 1994). This reduction in channel open time suggests that the blockade of the open state of the nicotinic AChR contributes, at least in part, to the inhibitory actions of propofol on this receptor (Dilger *et al.*, 1994). Propofol may have failed to influence the duration of NMDA channel opening due to the slow kinetics of the block. However, for several reasons our data are inconsistent with an open channel blocking mechanism. Consider the following kinetic scheme:

Scheme 1

$$\text{Closed} \underset{\alpha}{\overset{B}{\rightleftharpoons}} \text{Open} \underset{k_{-B}}{\overset{k_{+B}[C]}{\rightleftharpoons}} \text{Blocked}$$

where B and α are the opening and closing rates of the channel, k_{+B} and K_{-B}, are the rates of association and dissociation of the blocker, respectively, and [C] is the concentration of blocker (Adams, 1976). According to scheme 1, the mean single channel open time (τ_{open}) is inversely related to the sum of transition rates leading from the open state where $\tau_{open} = 1/$

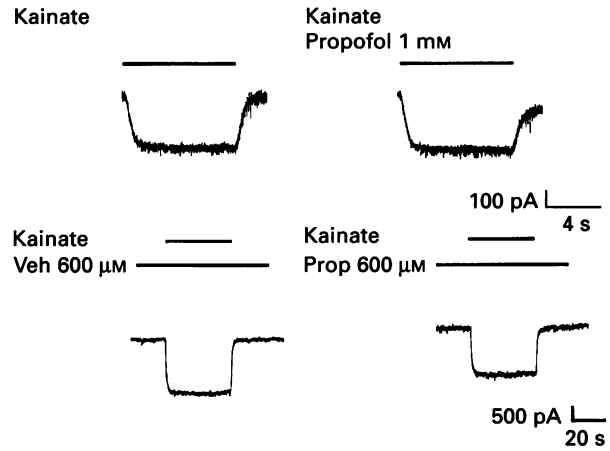

Figure 8 Propofol does not inhibit kainate-activated current. Co-application of propofol (1 mM) did not significantly decrease the amplitude of current activated by kainate (50 μM). Similarly, pre-exposure to propofol (600 μM) for 20 s did not substantially reduce the responses to kainate (50 μM).

($\alpha + k_{+B}$ [C]) (Colquhoun & Hawkes, 1983). Furthermore, the time course of inhibition of the *macroscopic* current (τ_{on}) is determined by $1/P_o$ ($\alpha + k_{+B}$ [C]), where P_o is the probability that the channels are in the open state.

In the absence of propofol, the mean duration of NMDA single channel opening is equal to 2.85 ms (Table 1) and the rate of channel closure ($\alpha = 1/\tau_{open}$) is approximately 0.35 ms^{-1}. The association rate constant for propofol blockade of the NMDA receptor is not known. If we assume, however, that the Po is equal to 1, then k_{+B} can be estimated from the rate of decay of the macroscopic current. Under these conditions, k_{+B} is estimated to be less than 1.34 M^{-1} ms^{-1}, (see Figure 2b, $\tau_{on} = 7.43$ s for 100 μM propofol). Therefore, in the presence of 100 μM propofol, we would not anticipate a *detectable* change in channel open time: $\tau_{open} \cong 1/(0.35\ ms^{-1} + 1.34\ M^{-1}\ ms^{-1} \times (1.0 \times 10^{-4}\ M))$. In contrast to our results, the association rate constant for propofol binding to the nicotinic AChR is estimated to be $6.0 \times 10^3\ M^{-1}\ ms^{-1}$ while the rate of channel closure equals 0.31 ms^{-1}. Accordingly, propofol (100 μM) reduces the mean open duration of the nicotinic AChR by approximately 61% (see Figure 2a, Dilger *et al.*, 1994).

It is likely however, that the probability of NMDA channel opening is considerably less than 1 (Huettner & Bean, 1988). In this case, the forward and reverse rate constants of propofol inhibition (estimated from τ_{on}) would be underestimated and the rate of blockade of the *macroscopic* current would depend on the opening and closing rates of the channel. Our results suggest that the inhibition by propofol is relatively independent of channel gating as it occurs in the absence of agonist. Furthermore, NMDA-activated responses decay at a similar rate, whether propofol is applied with or before the application of the agonist (see Figure 4c).

For several additional reasons, our data are inconsistent with an open channel blocking mechanism. Scheme 1 does not account for the decrease in the frequency of NMDA channel opening induced by propofol (Neher & Steinbach, 1978). Furthermore, the inhibition by propofol is insensitive to membrane potential, unlike the voltage-dependent block induced by uncompetitive NMDA receptor antagonists (MacDonald & Nowak, 1990). We therefore favour a model whereby propofol allosterically influences the gating of the NMDA channel, possibly by prolonging the dwell time of the receptor in a closed or desensitized state. These actions would be analogous to the effects of a number of neurotoxins on the activation and inactivation of Na^+ channels (Catterall, 1980). These neurotoxins are also highly lipid soluble, prompting the suggestion that their site of action is located within the hydrophobic core of the protein or at the lipid-protein interface (Hille, 1992).

Clinically effective concentrations of propofol, measured in human serum during induction of anaesthesia, range from 8–50 μM (Cockshott, 1985; Shafer *et al.*, 1988). Recovery from anaesthesia occurs when the serum concentration is less than 6 μM (Kanto, 1988). Our results indicate that *anaesthetic* concentrations of propofol reduce NMDA-evoked currents from murine hippocampal neurones by approximately 10–20% (Figure 3). Thus it is unlikely, that subanaesthetic concentrations of propofol (those used for nonhypnotic therapeutic applications) would be sufficient to inhibit significantly the NMDA receptor (Borgeat *et al.*, 1994). In contrast, anaesthetic concentrations of propofol probably depress NMDA channel activity and may reduce the duration of excitatory postsynaptic currents (Lester *et al.*, 1990).

NMDA receptor-mediated Ca^{2+} entry is thought play an important role in the triggering of long-term potentiation (a cellular model of learning and memory) and activity-dependent synaptic reorganization. Interestingly, propofol induces profound amnesia and impairs processes associated with the storage, consolidation and retrieval of new formation (Pang *et al.*, 1993; Polster *et al.*, 1993). Receptor block might also contribute to some of the undesirable side-effects of the propofol. For example, the post-operative dreams, mood disturbances, and hallucinations observed following propofol anaesthesia, are more commonly associated with the dissociative anaesthetic ketamine (Young, 1988; Suresh, 1991; Oxorn *et al.*, 1994). Propofol also blocks several non-ligand gated channels and exerts opposing actions on excitatory and inhibitory neurotransmitter receptors (Frenkel & Urban, 1991; Hales & Lambert, 1992; Magnelli *et al.*, 1992; Veintemilla *et al.*, 1992; Wachtel & Wegrzynowicz, 1992, Baum 1993; Hara *et al.*, 1993; Dilger *et al.*, 1994; Olcese *et al.*, 1994; Orser *et al.*, 1994). Modulation of these receptors might also contribute to the clinical properties of propofol.

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