Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 468 (2003) 75–82
www.elsevier.com/locate/ejphar

Effects of propofol on $GABA_A$ channel conductance in rat-cultured hippocampal neurons

Mansoureh Eghbali[a,b], Peter W. Gage[a], Bryndis Birnir[a,c,*]

[a] *Membrane Biology Program, John Curtin School of Medical Research, Australian National University, Canberra, Australia*
[b] *Department of Anaesthesiology, UCLA School of Medicine, Los Angeles, CA, USA*
[c] *Molecular and Cellular Physiology, Department of Physiological Sciences, Lund University, Lund, Sweden*

Received 17 October 2002; received in revised form 18 March 2003; accepted 21 March 2003

Abstract

Channels were activated, in ripped-off patches from rat-cultured hippocampal neurons, by propofol alone, propofol plus 0.5 μM GABA (γ-aminobutyric acid) or GABA alone. The propofol-activated currents were chloride-selective, showed outward-rectification and were enhanced by 1 μM diazepam. The maximum propofol-activated channel conductance increased with propofol concentration from less than 15 pS (10 μM) to about 60 pS (500 μM) but decreased to 40 pS in 1 mM propofol. Fitting the data from 10 to 500 μM propofol with a Hill-type equation gave a maximum conductance of 64 pS, an EC_{50} value of 32 μM and a Hill coefficient of 1.1. Addition of 0.5 μM GABA shifted the propofol EC_{50} value to 10 μM and increased the maximum channel conductance to about 100 pS. The Hill coefficient was 0.8. The maximum channel conductance did not increase further when 1 μM diazepam was added together with a saturating propofol concentration and GABA. The results are compared to effects other drugs have on $GABA_A$ channels conductance.

Keywords: $GABA_A$ receptor; GABA (γ-aminobutyric acid); Extrasynaptic receptor; Inhibition; Anaesthetic; Benzodiazepine

1. Introduction

The ubiquitous $GABA_A$ receptor is the main inhibitory receptor in the central nervous system. It is a hetero-oligomeric protein and is thought to consist of five subunits organized around a central Cl^--selective channel. The binding of the natural ligand GABA (γ-aminobutyric acid) normally opens the channel (Barnard et al., 1998). Because of its role in neuronal function and its wide distribution in the brain, the $GABA_A$ receptor has been the target for the development of many clinically important drugs such as some general anaesthetics, anxiolytics and antiepileptic drugs. Propofol, an intravenous general anaesthetic, has become widely used due to its favourable clinical properties (Deegan, 1992). Its main site of action is the $GABA_A$ receptor (Tanelian et al., 1993; Reynolds and Maitra, 1996; Bruner and Reynolds, 1998; Belelli et al., 1999; Luo and Sugiyama, 2000). Although it is known that propofol both directly activates $GABA_A$ receptors and enhances GABA-activated whole-cell currents (Hara et al., 1993; Orser et al., 1994; Sanna et al., 1995; Belelli et al., 1996; Krasowski et al., 1998; Lam and Reynolds, 1998), less is known about the effect of propofol on single-channel currents (Hales and Lambert, 1991; Orser et al., 1994). In this study, we examined activation of channels by a range of propofol concentrations (0.5 μM–1 mM) in the presence or absence of 0.5 μM GABA. The results show that propofol not only activates but sets the conductance of the $GABA_A$ channels in rat-cultured hippocampal neurons. In the presence of sub-micromolar GABA, propofol increases the conductance of the channels even further. The results are compared to the effects GABA, diazepam and pentobarbital have on the channel conductance.

2. Materials and methods

Neurons were dissociated from hippocampal slices from newborn rats and maintained in culture (Curmi et al., 1993). Experiments were done at room temperature (20–24 °C). Channels were activated either by switching the solution flowing through the bath to a solution containing propofol or

* Corresponding author. Molecular and Cellular Physiology, Department of Physiological Sciences, Lund University, Tornavägen 10, BMC F11, 221 84 Lund, Sweden. Tel.: +46-46-222-0675; fax: +46-46-222-7763.
E-mail address: bryndis.birnir@mphy.lu.se (B. Birnir).

0014-2999/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0014-2999(03)01641-8

by flowing a solution containing propofol through a narrow tube superfusing the patch. The extracellular solution contained (mM): NaCl 135, KCl 3, $CaCl_2$ 2, $MgCl_2$ 2, TES (*N*-Tris(hydroxymethyl) methyl-2-amino ethane sulphonic acid) 10, pH 7.4. The intracellular solution contained (mM): NaCl or choline Cl 141, KCl 0.3, $CaCl_2$ 0.5, $MgCl_2$ 2, TES 10, pH 7.4. In some experiments, the intracellular solution contained 4 mM ATP (adenosine triphosphate) but this had no detectable effect. In experiments on outside-out patches, the intracellular solution also contained 5 mM EGTA. In some experiments in which the reversal potential of currents was examined, 116 mM NaCl was replaced with 116 mM Na gluconate. γ-aminobutyric acid (GABA, Sigma) and bicuculline methiodide (Sigma) were dissolved in the extracellular solution. Diazepam (Hoffman-La Roche) and propofol (RBI) were first dissolved in dimethyl sulfoxide (DMSO), as described by Eghbali et al. (1997) and then added to the extra-or the intracellular solution. Propofol is lipid soluble and passes through cell membranes (Deegan, 1992) to reach its site of action if applied to the intracellular surface of the plasma membrane.

Conventional patch-clamp techniques were used when establishing a gigaseal and forming patches (Hamill et al., 1981). Pipettes (10–20 MΩ) were made from borosilicate glass, coated with Sylgard (Dow Corning) and fire-polished. Currents were recorded using an Axopatch 1C current-to-voltage converter (Axon instruments), filtered at 5 kHz, digitised at 44 kHz using a pulse code modulator (Sony PCM 501). The currents were played back from the videotape through the Sony PCM and digitised at a frequency of 10 kHz. The currents were then digitally filtered (Gaussian filter) at 5 kHz (or occasionally 2 kHz) and analysed (CHANNEL2, M. Smith, ANU). The amplitude of currents was measured either from all-points current-amplitude probability histograms or from direct measurements of the amplitude of individual currents filtered at 5 kHz. We use the terms "single-channel current" or "single-channel conductance" for the maximum current or conductance levels observed that showed direct transitions to or from the zero current or conductance levels more frequently recorded than would be expected from the opening and closing of two or more independent channels. The average open probability of channels was measured from opening and closing transitions detected by setting thresholds levels just above the baseline noise.

The data in Figs. 2 and 4 were fitted with a form of the Hill equation given by

$$\gamma = \gamma_{max}[\text{propofol}]^n/((EC_{50})^n + [\text{propofol}]^n). \quad (1)$$

3. Results

3.1. Characteristics of propofol-activated channels

Typical single-channel currents activated by 100 μM propofol are shown in Fig. 1 (inside-out patch, − Vp = 80 mV). Before exposure of the patch to propofol, there was no channel activity (Fig. 1Aa) but 3.4 pA (43 pS) currents appeared after applying 100 μM propofol in the bath solution to the patch (Fig. 1Aa and b). The current traces in Fig. 1B are continuous and demonstrate the effect of patch potential on the amplitude and open probability of currents activated by 100 μM propofol. When the patch was held at Vp = 0 mV (the reversal potential), there was no channel activity apparent. Channels with a conductance of about 50 pS were recorded when the patch was depolarised by 50 mV. When the patch was hyperpolarised by 50 mV, the conductance of the channels immediately decreased to 18 pS and then all activity ceased until the patch was depolarised again. In another three patches, the open probability of the channels was only 0.09 ± 0.02 at hyperpolarised 40 mV but increased to 0.45 ± 0.1 when the patches were depolarised by 40 mV.

The currents showed outward rectification ($n = 6$) (Fig. 1B and C), were enhanced by 1 μM diazepam ($n = 3$, Fig. 1Ca) and were carried by chloride ions ($n = 3$, Fig. 1Cb). When in symmetrical chloride solutions, propofol-activated single-channel currents reversed at a pipette potential close to 0 mV (Fig. 1Cb, inside-out patch). When the chloride concentration in the bath was lowered to 30 mM, the zero-current potential shifted to − 39 mV (− Vp), as expected for chloride-selective channels ($n = 3$).

3.2. The propofol concentration sets the single-channel conductance

The maximum conductance of the propofol-activated channels was related to the concentration of propofol applied. The effect of propofol concentration on single-channel conductance is shown in Fig. 2 for an inside-out patch (A, − Vp = 40 mV), an outside-out patch (B, Vp = 40 mV) and a cell-attached patch (C, − Vp = 40 mV). Propofol was applied in the bath solution. In Fig. 2A, before exposure to propofol, there was no channel activity (Fig. 2Aa), but when the patch was exposed to 20 μM propofol, single-channel currents with amplitude of 0.7 pA (17 pS, Fig. 2Aa and b) appeared. When the propofol concentration was increased to 500 μM, the maximum single-channel current amplitude increased to 2.2 pA (55 pS, Fig. 2Aa and c). The maximum single-channel currents were smaller in amplitude in 1 mM propofol as shown in Fig. 2B. Initially, there were no channels active in the patch (Fig. 2Ba) but after being exposed to 1 mM propofol, 1.2 pA single-channel currents (30.5 pS) appeared (Fig. 2Bb). Similar results were recorded in another two patches. Fig. 2C shows cell-attached patch single-channel currents that were evoked when 100 μM propofol was perfused into the bath. Before propofol exposure, there were no channels active in the patch but 52 s after propofol exposure 1.7 pA (Fig. 2Ca and b; 42.5 pS) single-channel currents were recorded. When propofol was washed out of the bath, channel activity ceased (Fig. 2Ca). For propofol to reach its site of action to activate single-channel

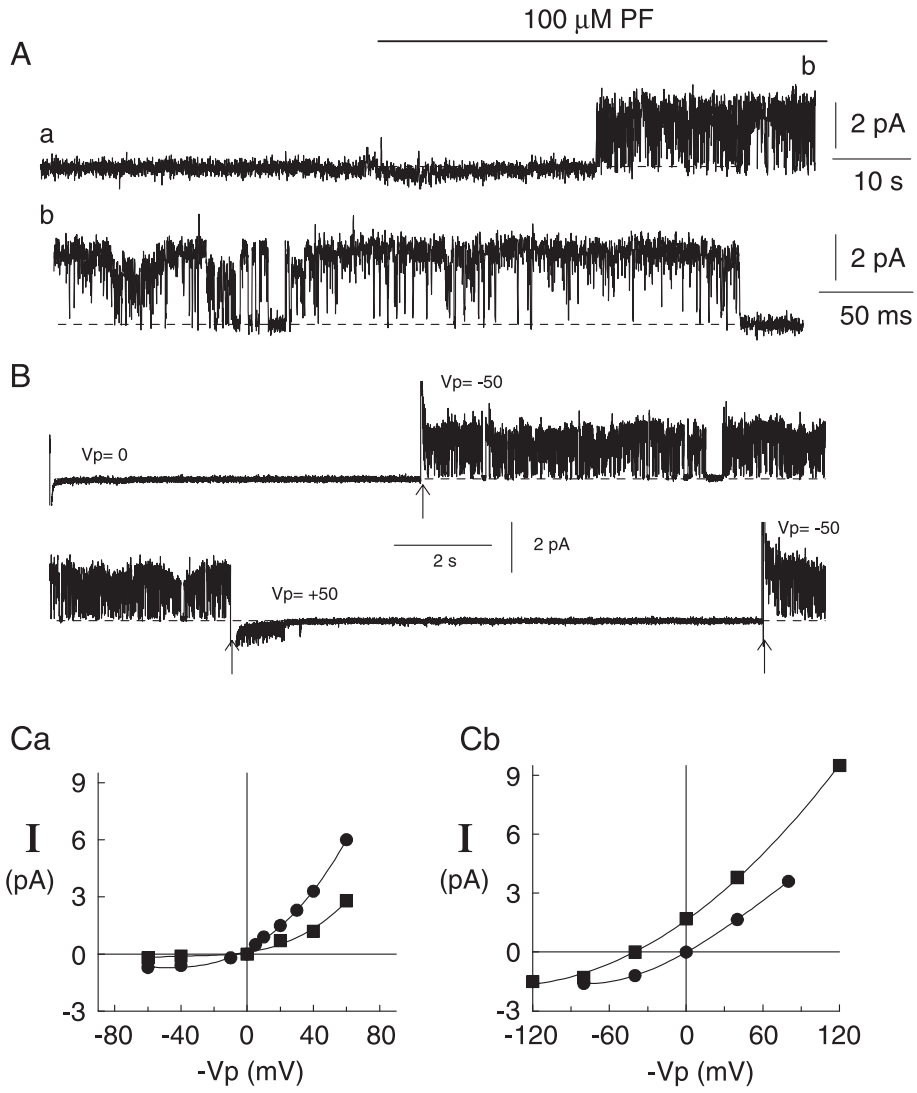

Fig. 1. Characteristics of channels activated by 100 μM propofol. (A) Single-channel currents from an inside-out patch (− Vp = 80 mV). (a) Before application of propofol, there were no channels active in the patch. When 100-μM propofol was added in the bath solution 3.4 pA (43 pS) currents were activated. b. The propofol-activated currents at a faster time scale. The currents were filtered at 5 kHz and the broken line represents the baseline current level. (B) The effect of membrane potential on the amplitude and open probability of channels activated by 100 μM propofol in an inside-out patch. The two 16-s traces are continuous, the pipette potential is given above the trace and arrows indicate when the potential was changed. The broken line represents the baseline current at Vp = 0 mV. (Ca) Current–voltage relationship. Currents recorded in 500 μM propofol alone (squares) and 500 μM propofol plus 1 μM diazepam (circles) in an inside-out patch. Diazepam was applied in the bath solution. (Cb) Chloride selectivity. Single-channel current amplitude in an inside-out patch is plotted against the pipette potential. The currents were first recorded in symmetrical Cl^- solutions (circles, 146 mM) and then when the bath Cl^- concentration had been lowered to 30 mM (isoosmolality was maintained with gluconate, squares). The reversal of the currents was shifted to − Vp = − 39 mV.

currents, it must have first crossed the cell membrane into the cell and then entered the membrane in the patch. That propofol diffuses readily across membranes and rapidly attains equilibrium is not surprising as it is very lipophilic (Deegan, 1992). When all channel activity had ceased, 100 μM propofol was again applied in the bath and activated the full conductance channel in 58 s (data not shown). Propofol was applied to seven other cell-attached patches and in six of those single-channel currents were evoked.

Single-channel conductance as a function of propofol concentration recorded in 25 patches is shown in Fig. 2D. The data from inside-out and outside-out patches are pooled because there did not appear to be any difference between them. Current amplitudes recorded in patches depolarised by 40 or 60 mV were used to calculate single-channel conductance since the current–voltage relationship in this potential range was nearly linear (see Fig. 1C). The average conductance increased as the propofol concentration was raised. It was 13 ± 4 pS in 10 μM propofol but had increased to 62 ± 7 pS in 500 μM propofol. In 1 mM propofol, the single-channel conductance decreased to 40 ± 7 pS. The data were fitted with a Hill-type equation (Eq. (1)) over the range of 1–500 μM propofol. The half-saturating concentration for propofol (EC_{50}) was 32 ± 4 μM, the Hill coefficient

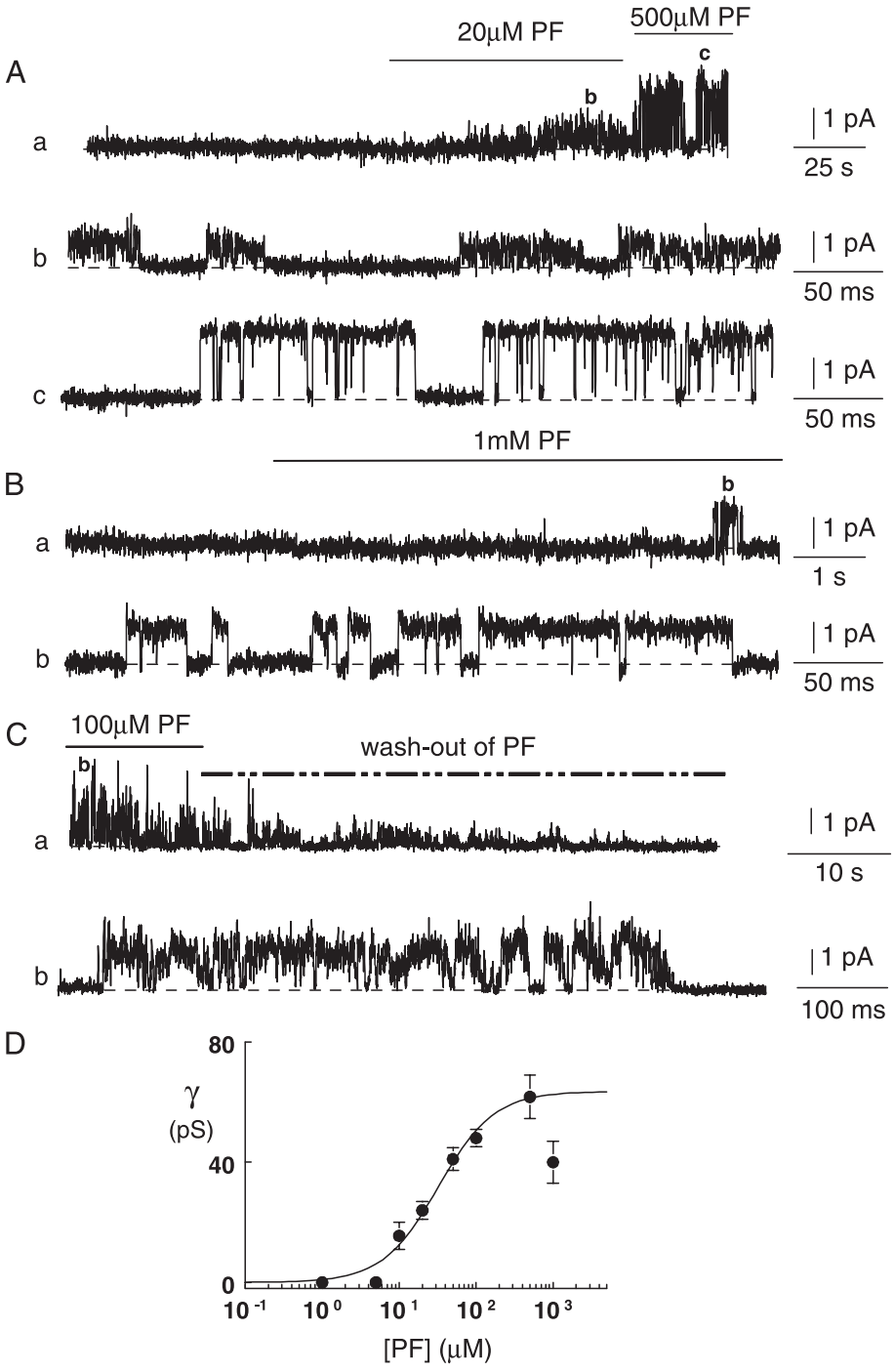

Fig. 2. The propofol concentration determines single-channel current amplitude. (A) Single-channel currents from an inside-out patch (− Vp = 40 mV). Currents, 0.7 pA (17 pS), were activated by 20 μM propofol (a and b) and increased to 2.2 pA (55 pS) when the propofol concentration was raised to 500 μM (a and c). (B) Single-channel currents in an outside-out patch (Vp = 40 mV). Propofol (1 mM) activated 1.2 pA (30 pS) currents (a and b). (C) Single-channel currents in a cell-attached patch (− Vp = 40 mV). 1.7 pA (45.2 pS) currents were activated by 100 μM propofol (a and b). Initially more than one channel was active in the patch (a). When propofol was washed out, the amplitude of the single-channel currents became smaller until they disappeared (a). Currents were filtered at 5 kHz in A and B and 2 kHz in C. The propofol was applied in the bath solution. The solid lines above the current traces represent the time drugs were applied for, broken lines the level of the baseline currents and the broken/dotted line in C, the period when propofol was washed out. The placement of the lower case letters in Aa, Ba and Ca indicate from where the expanded traces in b and c were taken. The same terminology is applied throughout he paper. (D) Propofol dose–conductance relationship. The data were obtained from 21 inside-out and four outside-out patches at depolarised potentials of 40 or 60 mV. Data points represent the average maximum conductance for three or more patches ± S.E.M. if larger than the symbol. The curve is a fit by Eq. (1) (see Materials and methods) to the data apart from the conductance evoked by 1 mM propofol that was not included.

1.1 ± 0.1 and the maximal channel conductance was 64 ± 3 pS ($r^2 = 0.99$).

3.3. Currents activated by sub-micromolar concentration of GABA plus propofol

To examine the effect of propofol on low-conductance (< 30 pS) GABA-activated channels, different concentrations of propofol were added to patches already exposed to 0.5 μM GABA in the pipette solution. Similar to the currents activated by propofol alone, the currents activated by GABA alone showed outward-rectification (results not shown), as reported previously (Eghbali et al., 1997). The effects of propofol in three inside-out patches are shown in Fig. 3. In the patch shown in Fig. 3A (− Vp = 40 mV), the single-channel current amplitude activated by 0.5 μM GABA was 1.4 pA (35 pS, Fig. 3Aa and b). It increased to 3.2 pA (80 pS, Fig. 3Aa and c) in the presence of 100 μM propofol.

Results from another patch are shown in Fig. 3B. Representative current traces are shown and the all-points histograms are from 20 s current records. Before exposure to propofol, the maximum conductance of single-channels activated by 0.5 μM GABA was 20 pS (− Vp = 40 mV, Fig. 3Ba). Following exposure of the patch to 1 μM propofol, the single-channel conductance increased to 33 pS (Fig. 3Bb) and to 55 pS in the presence of 10 μM propofol as can been in the all-points histograms shown to the right of the current traces (Fig. 3Bc). Single-channel conductance increased further following exposure to 100 μM propofol, and there were two channels present in the patch, each with a conductance of 72 pS represented by the two large peaks in the corresponding all-points histogram (Fig. 3Bd). Results from the third inside-out patch are shown in Fig. 3C. In the presence of 0.5 μM GABA alone, there were no channels active in the patch (results not shown) but upon addition of 500 μM propofol, channels were activated. The maximum single-channel conductance activated by the 500 μM propofol in the presence of 0.5 μM GABA was 90 pS (− Vp = 60 mV, Fig. 3Ca and b). Following exposure of the patch to 10 μM propofol in the continuing presence of the GABA, the maximum conductance decreased to 60 pS (Fig. 3Ca and c).

The relationship between propofol concentration and channel conductance in the presence of 0.5 μM GABA is illustrated in Fig. 4. The concentration-conductance curve for channels activated by propofol plus GABA was bell-shaped. As in the absence of GABA, the single-channel current amplitude was depressed in 1 mM propofol (Fig. 4).

The data were obtained from 16 inside-out patches (− Vp = 40 or 60 mV) and were fitted with a Hill-type

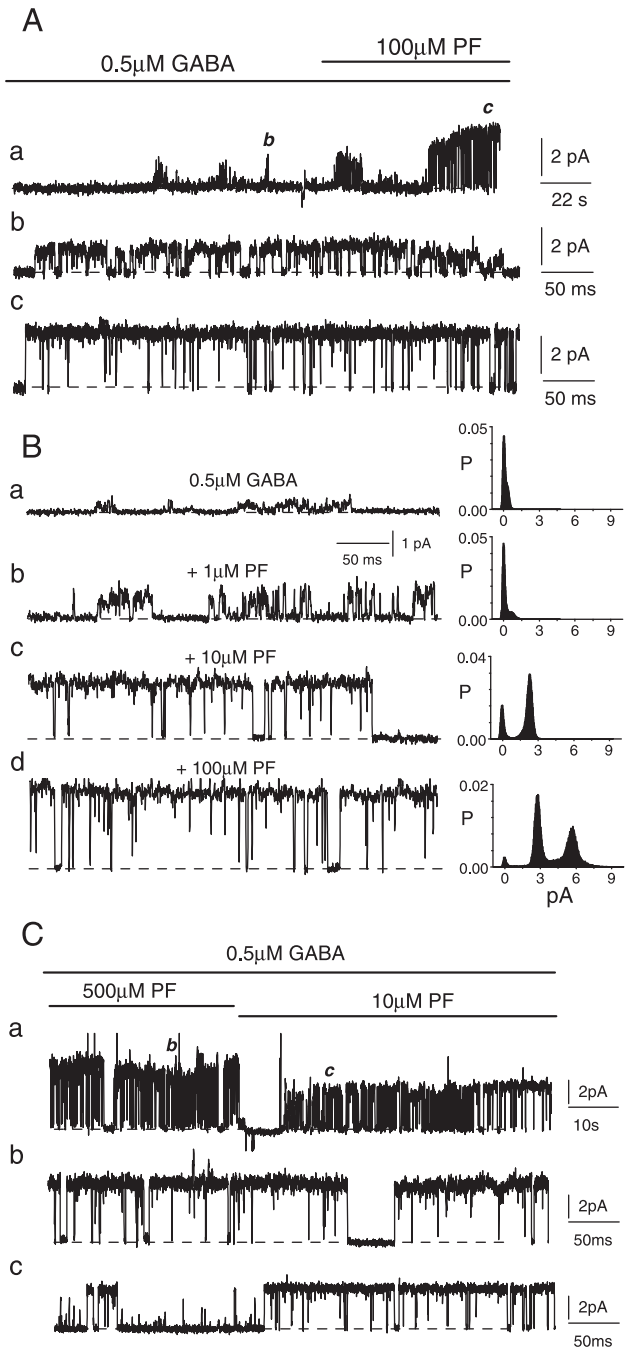

Fig. 3. The effect of a range of propofol concentrations on conductance in the presence of 0.5 μM GABA. (A) Single-channel currents from an inside-out patch (− Vp = 40 mV). 1.4 pA (35 pS) currents were activated by 0.5 μM GABA alone (a and b) but increased to 3.2 pA (80 pS) in the presence of 100 μM propofol plus 0.5 μM GABA (a and c). (B) Single-channel currents from an inside-out patch (− Vp = 40 mV). The all-points histograms are from 20 s of current records and representative currents are shown. The single-channel current amplitudes in 0.5 μM GABA alone (a) or together with 1 (b), 10 (c) or 100 (d) μM propofol were 0.8, 1.3, 2.2, 2.8 pA or 20, 33, 55 and 72 pS, respectively. (C) Single-channel currents from an inside-out patch. The pipette potential was − 60 mV apart from a brief period when the patch potential was changed to 60 mV that can be seen in (a) where the currents are reversed. In the presence of 0.5 μM GABA alone, no channels were activated during the recording period. 5.4 pA (90 pS) channels were activated in the presence of 500 μM propofol plus 0.5 μM GABA (a and b) and decreased to 3.6 pA (60 pS) in amplitude when the propofol concentration was lowered to 10 μM (a and c) in the presence of 0.5 μM GABA. The broken lines indicate the level of the baseline current. GABA was present in the pipette solution whereas propofol was applied in the bath solution.

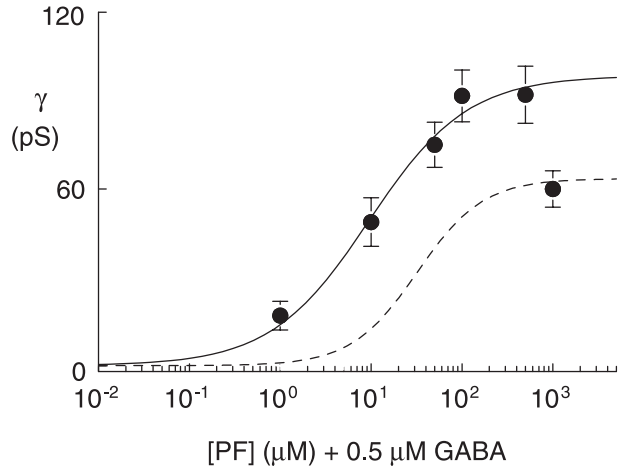

Fig. 4. Relationship between channel conductance and propofol concentration in the presence of 0.5 μM GABA. The data were obtained from 16 inside-out patches at depolarised potentials of 40 or 60 mV. Data points represent the average maximum conductance for three or more patches ± S.E.M. if larger than the symbol. The solid curve is a fit of Eq. (1) (see Materials and methods) to the data over the concentration range of 1–500 μM propofol. The broken line shows the relationship between 1 and 500 μM propofol in the absence of GABA (from Fig. 2D) for comparison.

equation (Eq. (1)) over the range of 1–500 μM propofol. The half-saturating concentration (EC_{50}) was 10 ± 3 μM, the Hill coefficient 0.8 ± 0.2 and the maximal channel conductance was 98 ± 8 pS ($r^2 = 0.98$). The dependence of channel conductance on propofol concentration in the absence of GABA is shown for comparison (broken line).

In five experiments, 1 μM diazepam was added to patches in which channels had been activated with high (100 or 500 μM, $n = 3$) or low (10 or 50 μM, $n = 2$) concentrations of propofol in the presence of 0.5 μM GABA. Only with the low concentrations of propofol (plus GABA) was channel conductance increased by diazepam; from 50 to 70 pS and from 80 to 119 pS in the presence of 10 and 50 μM propofol, respectively. For comparison, channels activated by 0.5 μM GABA plus 1 μM diazepam had an average conductance of 68 ± 6 pS (three outside-out patches, Vp = 40 mV).

The open probability of the channels in the presence of propofol plus GABA was greater than in the presence of either drug alone. In the presence of 0.5 μM GABA alone, it was 0.05 ± 0.02 (− Vp = 40 mV; $n = 3$ inside-out patches) but upon addition of propofol, the open probability increased to 0.87 ± 0.01. A similar observation was made by Hales and Lambert (1991). The open probability of chloride channels directly activated by 100 μM propofol alone was 0.49 ± 0.12 (− Vp = 40; $n = 7$ inside-out patches).

4. Discussion

Propofol is generally thought to depress excitatory activity of neurons by enhancing the inhibitory GABAergic system. The results show that propofol both activates and modulates $GABA_A$ channel conductance in rat-cultured hippocampal neurons.

4.1. Direct activation of channels by propofol

The graded effect of propofol on channel conductance has not been described before although direct activation of channels by propofol has been reported previously. Hales and Lambert (1991) activated 29 pS channels with 30 μM propofol in outside-out patches in bovine chromaffin cells. In their study, the lowest concentration of propofol that could activate channels was 10 μM, whereas Orser et al. (1994) using 2 μM propofol, activated channels of about 30 pS in outside-out patches from cultured murine hippocampal neurones. Hales and Lambert (1991) did not activate whole-cell currents when propofol was applied to the inside of bovine chromaffin cells. This is perhaps not surprising if propofol enters its binding side from the extracellular side of the membrane. Propofol passing the cell membrane might immediately be diluted in the much larger volume of the bath solution and never reach the required concentration to activate channels. This is in contrast to the cell-attached and inside-out patches used in this study, where the volume of the solution in the pipette is much smaller than the volume of the bath solution, and propofol can, therefore, rapidly attain equilibrium concentration across the membrane. In whole-cell recordings from neurones or cells expressing reconstituted $GABA_A$ receptors, the EC_{50} values for direct activation by propofol range from 1 to 129 μM (Hara et al., 1993; Orser et al., 1994; Krasowski et al., 1998; Belelli et al., 1996). In the present study, the EC_{50} value (32 ± 4 μM) for direct activation by propofol of single channels is within the EC_{50} value range reported from whole-cell current measurements.

The slow rate of activation of the channels that is observed can stem from at least two sources: in inside-out and cell-attached patches, it may reflect the time it takes for propofol to reach the equilibrium concentration across the membrane. This can, however, not be the case for the outside-out patches and indicates a slow rate of activation of the receptors, similar to what we have reported for GABA-activated channels in initially silent, outside-out patches (see Birnir et al., 2001). It is possible that this property of the receptors is related to their non-synaptic location.

4.2. Modulation of channels by propofol

In the presence of 0.5 μM GABA, the half-maximal effect of propofol on channel conductance was shifted to a lower concentration (10 μM) and the maximal single-channel conductance was greater (100 pS) than with propofol alone (34 μM, 60 pS). For modulation of GABA-activated currents by propofol in whole-cell experiments on neurons or cells expressing reconstituted receptors, the EC_{50} values are similar and range from about 3 to 30 μM (Hara et al., 1993; Adodra and Hales, 1995; Belelli et al., 1996; Krasowski et al., 1998; Lam and Reynolds, 1998). The channel was

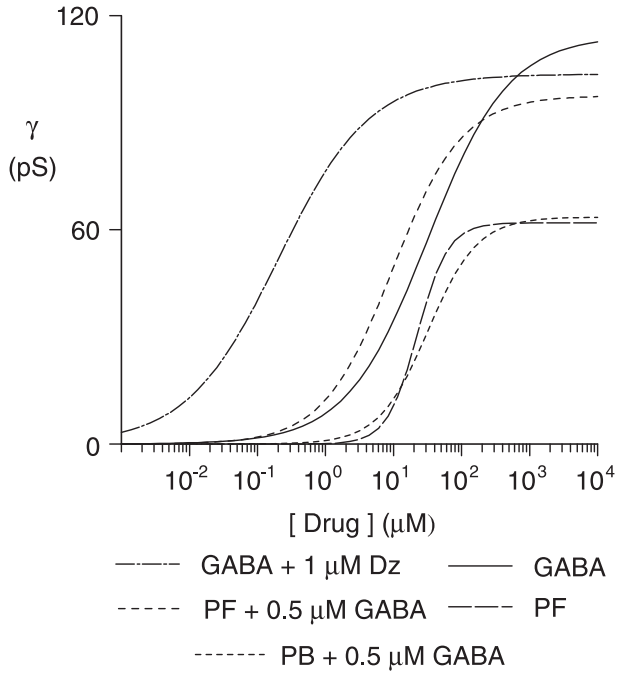

Fig. 5. Effects of drugs on channel conductance. Drugs evoke single-channel conductance that is related to the drug itself and the drug concentration. The maximal single-channel conductance and the drug half-saturation concentration (EC_{50} value) are determined by the combination of drugs present. The curves represent fits to single-channel conductance data by a Hill-type equation. The fits for propofol (PF) were calculated from the data shown in Figs. 2 and 4. The fits for GABA alone and GABA plus 1 μM diazepam (Dz) are from Birnir et al. (2001) and for pentobarbital (PB) from Eghbali et al. (2000).

more open in the presence of both drugs than in either GABA or propofol alone.

4.3. Effects of drugs on conductance

The physiological and pharmacological properties of the $GABA_A$ receptors are dictated by the subunit isoforms that make up the receptors (Barnard et al., 1998). In cultured neurons, a wide range of channel conductances (7 to 110 pS) has been reported, with the most commonly reported conductance being in the range of 20 to 30 pS (see, e.g. Mathers, 1991). The wide range of channel properties reported in cultured neurons may be partly due to different types of $GABA_A$ receptors present, different levels of intracellular modifications or associations with intracellular proteins, but are probably also related to different conductance stage (subconductance stage) of the single-channel. The effect of propofol on the channel conductance is not unique. We have previously shown that the conductance is related to the GABA concentration (Fig. 5; Birnir et al., 2001) and both benzodiazepines and barbiturates can increase the conductance of low conductance $GABA_A$ channels (Fig. 5; Eghbali et al., 1997, 2000; Guyon et al., 1999; Birnir et al., 2000, 2001). Single-channel conductance has also been correlated with agonist concentration in cyclic nucleotide-gated channels (Ruiz and Karpen, 1997) and in AMPA receptors (Rosenmund et al., 1998; Smith and Howe, 2000).

The maximal single-channel conductance in the presence of saturating propofol concentration plus 0.5 μM GABA is similar to the maximal conductance reported for the channels when activated by GABA alone or GABA plus 1 μM diazepam (Fig. 5; Birnir et al., 2001). This suggests that the limiting conductance for these channels, in 146 mM Cl^- experimental solutions, is around 100 to 120 pS. Propofol appears to be more effective in modulating the channels than pentobarbital as saturating pentobarbital concentrations plus 0.5 μM GABA only gave maximal conductance of 60 pS (Fig. 5; Eghbali et al., 2000). The results indicate that the drugs act synergistically to increase the channel conductance.

4.4. Drug effects and physiology

Together, our results show that it is the final combination of drugs that determines the single-channel characteristics and the apparent affinity of the receptors for the agonist (see Fig. 5). These receptors are most likely non-synaptic in origin and can be expected to affect baseline properties of the neuron. They may have a significant role in effects of drugs on neuronal activity where the time scale is long such as, e.g., during anaesthesia. Inhibition is graded and precisely tuned by the concentration of GABA and other drugs present. The end result is a dynamic and efficient inhibitory system. Most of our studies have been done on cultured neurons. It is therefore necessary to examine if similar effects of GABA and other drugs are observed at non-synaptic $GABA_A$ receptors on neurons in brain tissue.

Acknowledgements

We thank the John Curtin School of Medical Research, ANU, Australia, the Swedish Medical Research Council (grant nos. Y0924; K2002-33X-13408-03A), the Crafoord Stiftelse, the Segerfalks Stiftelse, A. O. Swärds and U. Eklunds Stiftelse and the Medical Faculty, Lund University, for financial support.

References

Adodra, S., Hales, T.G., 1995. Potentiation, activation and blockade of $GABA_A$ receptors of clonal murine hypothalamic GT1-7 neurones by propofol. Br. J. Pharmacol. 115, 953–960.

Barnard, E.A., Skolnick, P., Olsen, R.W., Mohler, H., Sieghart, W., Biggio, G., Braestrup, C., Bateson, A.N., Langer, S.Z., 1998. International Union of Pharmacology: XV. Subtypes of γ-aminobutyric acid A receptors: classification on the basis of subunit structure and receptor function. Pharmacol. Rev. 50, 291–313.

Belelli, D., Callachan, H., Hill-Venning, C., Peters, J.A., Lambert, J.J., 1996. Interaction of positive allosteric modulators with human and Drosophila recombinant GABA receptors expressed in Xenopus laevis oocytes. Br. J. Pharmacol. 118, 563–576.

Belelli, D., Pistis, M., Peters, J.A., Lambert, J.L., 1999. General anaesthetic

action at transmitter-gated inhibitory amino acid receptors. TIPS 20, 496–502.

Birnir, B., Eghbali, M., Everitt, A.B., Gage, P.W., 2000. Bicuculline, pentobarbital and diazepam modulate spontaneous $GABA_A$ channels in rat hippocampal neurons. Br. J. Pharmacol. 131, 695–704.

Birnir, B., Eghbali, M., Cox, G.B., Gage, P.W., 2001. GABA concentration sets the conductance of delayed $GABA_A$ channels in outside-out patches from rat hippocampal neurons. J. Membr. Biol. 181, 171–183.

Bruner, K.R., Reynolds, J.N., 1998. Propofol modulation of [^{3}H]flunitrazepam binding to $GABA_A$ receptors in guinea pig cerebral cortex. Brain Res. 806, 122–125.

Curmi, J.P., Premkumar, L.S., Birnir, B., Gage, P.W., 1993. The influence of membrane potential on chloride channels activated by GABA in rat cultured hippocampal neurones. J. Membr. Biol. 136, 273–280.

Deegan, R.J., 1992. Propofol: a review of the pharmacology and applications of an intravenous anesthetic agent. Am. J. Med. Sci. 304, 45–49.

Eghbali, M., Curmi, J.P., Birnir, B., Gage, P.W., 1997. Hippocampal $GABA_A$ channel conductance increased by diazepam. Nature 388, 71–75.

Eghbali, M., Gage, P.W., Birnir, B., 2000. Pentobarbital modulates γ-aminobutyric acid-activated single-channel conductance in rat cultured hippocampal neurons. Mol. Pharmacol. 58, 463–469.

Guyon, A., Laurent, S., Paupardin-Tritsch, D., Rossier, J., Eugene, D., 1999. Incremental conductance levels of $GABA_A$ receptors in dopaminergic neurons of the rat substantia nigra pars compacta. J. Physiol. 516, 719–737.

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.

Hamill, O.P., Marty, A., Neher, E., Sakmann, B., Sigworth, F.J., 1981. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflugers Arch. 391, 85–100.

Hara, M., Kai, Y., Ikemoto, Y., 1993. Propofol activates $GABA_A$ receptor-chloride ionophore complex in dissociated hippocampal pyramidal neurons of the rat. Anesthesiology 79, 781–803.

Krasowski, M.D., Koltchine, V.V., Rick, C.E., Ye, Q., Finn, S.E., Harrison, N.L., 1998. Propofol and other intravenous anesthetics have sites of action on the γ-aminobutyric acid type A receptor distinct from that for isoflurane. Mol. Pharmacol. 53, 530–538.

Lam, D.W., Reynolds, J.N., 1998. Modulatory and direct effects of propofol on recombinant $GABA_A$ receptors expressed in *Xenopus* oocytes: influence of α- and γ_2-subunits. Brain Res. 16, 179–187.

Luo, A., Sugiyama, K., 2000. Propofol combined with diazepam synergistically potentiates the GABA-activated chloride current in rat sensory neurons. Chin. Med. J. 113, 840–843.

Mathers, D.A., 1991. Activation and inactivation of the $GABA_A$ receptor: insights from comparison of native and recombinant subunit assemblies. Can. J. Physiol. Pharmacol. 69, 1057–1063.

Orser, B.A., Wang, L.Y., Pennefather, P.S., MacDonald, J.F., 1994. Propofol modulates activation and desensitization of $GABA_A$ receptors in cultured murine hippocampal neurons. J. Neurosci. 14, 7747–7760.

Reynolds, J.N., Maitra, R., 1996. Propofol and flunitrazepam act synergistically to potentiate $GABA_A$ receptor activation in human recombinant receptors. Eur. J. Pharmacol. 314, 151–156.

Rosenmund, C., Stern-Bach, Y., Stevens, C.F., 1998. The tetrameric structure of a glutamate receptor channel. Science 280, 1596–1599.

Ruiz, M.L., Karpen, J.W., 1997. Single cyclic nucleotide-gated channels locked in different ligand-bound states. Nature 389, 389–392.

Sanna, E., Mascia, M.P., Klein, R.L., Whiting, P.J., Biggio, G., Harris, R.A., 1995. Actions of the general anesthetic propofol on recombinant human $GABA_A$ receptors: influence of receptor subunits. J. Pharmacol. Exp. Ther. 274, 353–360.

Smith, T.M., Howe, J.R., 2000. Concentration-dependent substate behaviour of native AMPA receptors. Nat. Neurosci. 3, 992–997.

Tanelian, D.L., Kosek, P., Mody, I., MacIver, M.B., 1993. The role of the $GABA_A$ receptor/chloride channel complex in anesthesia. Anesthesiology 78, 757–776.