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Potentiation, activation and blockade of $GABA_A$ receptors of clonal murine hypothalamic GT1-7 neurones by propofol

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1 The actions of GABA and the intravenous general anaesthetic propofol (2,6-diisopropylphenol) on $GABA_A$ receptors of self-replicating GT1-7 hypothalamic neurones were investigated by the patch clamp technique.

2 GABA (1 μM - 1 mM) dose-dependently activated inward currents with an EC_{50} of 27 μM, recorded from whole cells voltage-clamped at -60 mV. GABA (100 μM)-activated currents reversed at the Cl^- equilibrium potential.

3 Propofol (0.1 - 100 μM) dose-dependently potentiated GABA (100 μM)-evoked currents with an EC_{50} of 5 μM.

4 In the absence of GABA, propofol (10 μM - 1 mM) activated small inward currents with a reversal potential similar to the Cl^- equilibrium potential. The peak current amplitudes activated by propofol were only 31% of those activated by GABA in the same cells.

5 Like GABA (100 μM)-activated currents, propofol (100 μM)-activated currents were inhibited by the $GABA_A$ receptor antagonist, bicuculline (10 μM) and were abolished by Zn^{2+} (100 μM).

6 Propofol (10, 30 and 100 μM) dose-dependently activated currents in the absence of GABA. However, the peak amplitude of currents activated by propofol declined with concentrations >100 μM. The cessation of application of a high dose of propofol (1 mM) was associated with a current 'surge'.

7 The surge current, seen after application of propofol (1 mM), had a reversal potential similar to the Cl^- equilibrium potential. The ratio between peak current amplitude in the presence of propofol (1 mM) and surge current amplitude after propofol application, were not dependent on holding potential. Thus, it is unlikely that the surge current represents reversal of a voltage-dependent block of $GABA_A$ receptors by propofol.

8 The amplitude of the surge current exceeded the amplitude of the initial propofol (1 mM)-evoked current following brief applications, but declined after prolonged applications of the drug.

9 The observed modulatory actions of propofol may be due to separate potentiation, activation and inhibitory sites for this anaesthetic agent on GT1-7 cell $GABA_A$ receptors.

Keywords: Propofol; general anaesthetics; $GABA_A$ receptors; GT1-7 cells; hypothalamic neurones; patch clamp

Introduction

γ-Aminobutyric acid type A ($GABA_A$) receptors are widely distributed throughout the brain and periphery. Mammalian bicuculline-sensitive $GABA_A$ receptors are modulated by several therapeutic agents with anticonvulsant, anxiolytic, and anaesthetic properties, such as benzodiazepines, anaesthetic barbiturates, anaesthetic steroids, volatile agents, chlormethiazole, etomidate, propanidid, and propofol (see Franks & Lieb, 1994; Hales & Olsen, 1994). $GABA_A$ receptor-chloride-ion channels are thought to comprise five subunits (Olsen & Tobin, 1990). There have been multiple subunits cloned, including α1-6, β1-3, γ1-3 and δ (Burt & Kamatchi, 1991). These subunits are differentially expressed throughout the nervous system (Wisden *et al.*, 1992; Laurie *et al.*, 1992) and can combine to form bicuculline-sensitive $GABA_A$ receptors with varying biophysical and pharmacological properties (Pritchett *et al.*, 1989; Draguhn *et al.*, 1990; Smart *et al.*, 1991; Wafford *et al.*, 1991; Angelotti & Macdonald, 1993; Verdoorn, 1994).

$GABA_A$ receptor subunits are not only differentially distributed between brain regions, (Wisden *et al.*, 1992; Laurie *et al.*, 1992), but are also known to vary within neurones at different developmental stages (Shivers *et al.*, 1989; Smart & Constanti, 1990). This regional and developmental heterogeneity may explain reports of variable $GABA_A$ receptor modulation by some agents (Pritchett & Seeburg, 1990; Smart & Constanti, 1990; Wafford *et al.*, 1991). Clonal cell lines provide a less heterogeneous system for the study of receptor expression and properties. However, although many cell lines express $GABA_A$ receptor mRNAs, few have functional receptors (Hales *et al.*, 1992; Noble *et al.*, 1993; Hales & Tyndale, 1994; Tyndale *et al.*, 1994).

In this study the modulation by the widely used general anaesthetic, propofol, of functional $GABA_A$ receptors in self-replicating GnRH-secreting hypothalamic GT1-7 neurones (Mellon *et al.*, 1990), was investigated. $GABA_A$ receptors of GT1-7 cells have previously been characterized by use of molecular and electrophysiological techniques (Hales *et al.*, 1992; 1994). The clonal nature of these cells, and their stable expression of $GABA_A$ receptors, has allowed a detailed investigation of the modulatory actions of propofol using the whole cell configuration of the patch clamp technique. A preliminary account of a part of this work has appeared in abstract form (Adodra & Hales, 1994).

Methods

Cell culture

Self-replicating hypothalamic GT1-7 neurones were maintained in culture as described previously (Hales *et al.*, 1992; 1994). Cells were grown in Dulbecco's Modified Eagles Medium (DMEM) supplemented with calf serum 5% (v/v), horse serum 5%, penicillin (50 iu ml^{-1}) and streptomycin (0.05 mg ml^{-1}), at 37°C in 95% air/5% CO_2 and 100% relative

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humidity (reagents from Gibco). Cells were used for electrophysiological recordings 3–14 days after subculturing into 35 mm culture dishes (Costar). Experiments were performed at room temperature (22–25°C).

Electrophysiology

The whole cell configuration of the patch clamp technique was used to record GABA- and propofol-evoked currents from single GT1-7 cells voltage-clamped at −60 mV (unless otherwise stated). The recording chamber was continuously and rapidly (20 ml min^{-1}) perfused by gravity feed with an extracellular solution of the following composition (in mM): NaCl 140, KCl 2.8, $MgCl_2$ 2.0, $CaCl_2$ 1.0, glucose 6.0 and HEPES-NaOH (pH 7.2). Recording electrodes contained CsCl 140, $MgCl_2$ 2.0, $CaCl_2$ 0.1, ATP (Mg salt) 3, EGTA 1.1, and HEPES-CsOH (pH 7.2). Membrane currents were monitored with an Axopatch-1A (Axon Instruments Inc.) patch clamp amplifier. Currents were low-pass filtered at a cutoff frequency of 2 kHz (Bessel characteristics), digitized with a digital audio processor (Sony PCM-501ES) and recorded onto VCR tapes for subsequent analysis.

In experiments examining activation of GABA$_A$ receptors, GABA or propofol was applied locally by pressure ejection (General Valve Picospritzer II) at a pressure of 70 kPa, from glass micropipettes positioned approximately 50 μm from the cell under investigation. Cells were continuously and rapidly superfused with recording solution to help prevent GABA$_A$ receptor desensitization. In dose-response experiments randomized doses of GABA or propofol were applied via different pipettes with similar resistances positioned in the same location (with the aid of an eye piece graticule). During concentration-response experiments, agonists were applied for prolonged periods (≥1 s) in order to achieve equilibrium concentrations. However, it is likely that dilution occurs at some receptor sites therefore concentrations represent maximum estimates of the true agonist concentrations. A period of at least 4 min wash was allowed between each application to prevent desensitization. All other drugs were bath-applied, as was propofol in experiments investigating its GABA-potentiating effect.

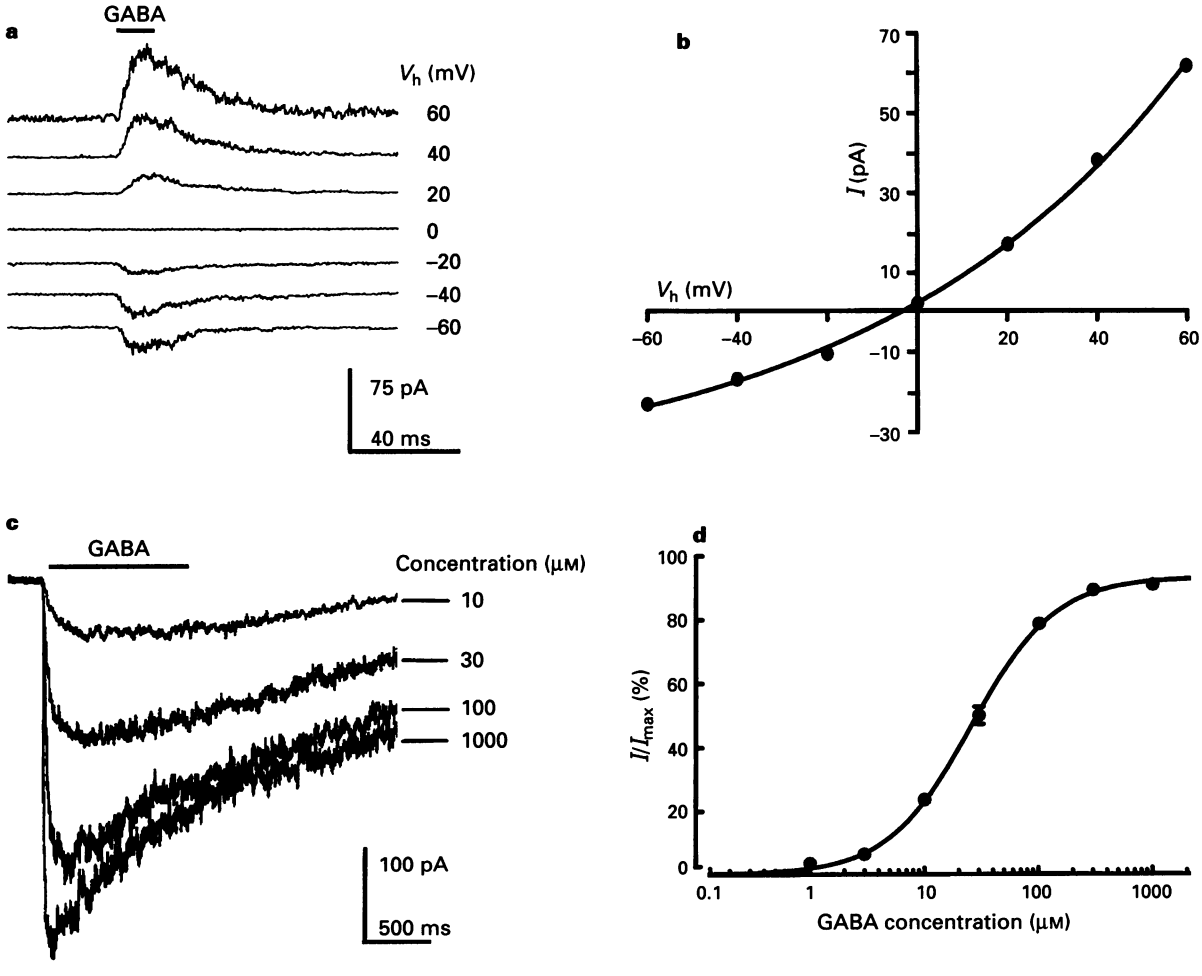

Figure 1 GABA-evoked currents recorded from GT1-7 cells. (a) Traces illustrating whole cell currents evoked by locally applied GABA (100 μM, for 10 ms at 70 kPa) at holding potentials (V_h) from −60 mV to 60 mV. Traces are averages of two currents at each potential. (b) The relationship between holding potential and amplitude of the currents in (a) is shown graphically, and indicates that GABA-induced currents have a reversal potential of approximately 0 mV with 144 mM and 148 mM Cl^- inside and outside the cell, respectively. (c) Dose-dependent activation of GABA$_A$ receptors by GABA. Prolonged local applications (1 s) of GABA (10 μM–1 mM) to a cell voltage-clamped at −60 mV activate Cl^- currents in a dose-dependent manner. (d) A graph of the mean amplitude of the GABA-induced currents is plotted against the concentration of GABA (on a logarithmic scale). Peak current amplitudes were measured and expressed as a percentage of the highest amplitude current recorded from individual cells. The points are mean dose-response data from six cells. Where bigger than the data points, s.e.means are shown. The curve was fitted to a logistics equation (see Methods) and provides an EC_{50} value of 26.6 μM, and a Hill coefficient of 1.6.

Data analysis

Propofol and GABA-evoked whole cell currents were replayed onto a chart recorder (Gould Brush 2400) for manual analysis, or digitized (Digidata 1200 interface, Axon Instruments Inc.) at a frequency of 10 kHz onto the hard drive of an IBM PC and analysed with pCLAMP software (Axon Instruments Inc.). Illustrations of raw data were produced either by reproduction of chart recordings or by retrieval of digitized information from the IBM PC onto a laser printer. GABA and propofol concentration-response curves were compiled and then best fitted using the logistic equation:

$$I = I_{max}/(1 + (EC_{50}/C)^{n_H})$$

Where I is the agonist activated current amplitude (as % of maximum current observed), I_{max} the maximum current (as % maximum observed), EC_{50} the concentration of agonist eliciting a half maximal response, C the agonist concentration and n_H the Hill coefficient. When fitting propofol potentiation dose-response curves the equation was modified to:

$$I = 100 + I_{max}/(1 + (EC_{50}/C)^{n_H})$$

Where I is the amplitude of the GABA-activated current (expressed as % control), I_{max} is the maximum propofol-potentiated current (as % control), EC_{50} is the concentration of propofol eliciting a half maximal potentiation, C the propofol concentration and n_H the Hill coefficient.

All quantitiative results are expressed as the arithmetic mean ± s.e.mean.

Drugs used

Drugs used in this study were: γ-aminobutyric acid (GABA), bicuculline methiodide (both from Sigma) and 2,6-diisopropylphenol (propofol, from Aldrich). Stock solutions of propofol were made up at 100 mM in ethanol. In potentiation

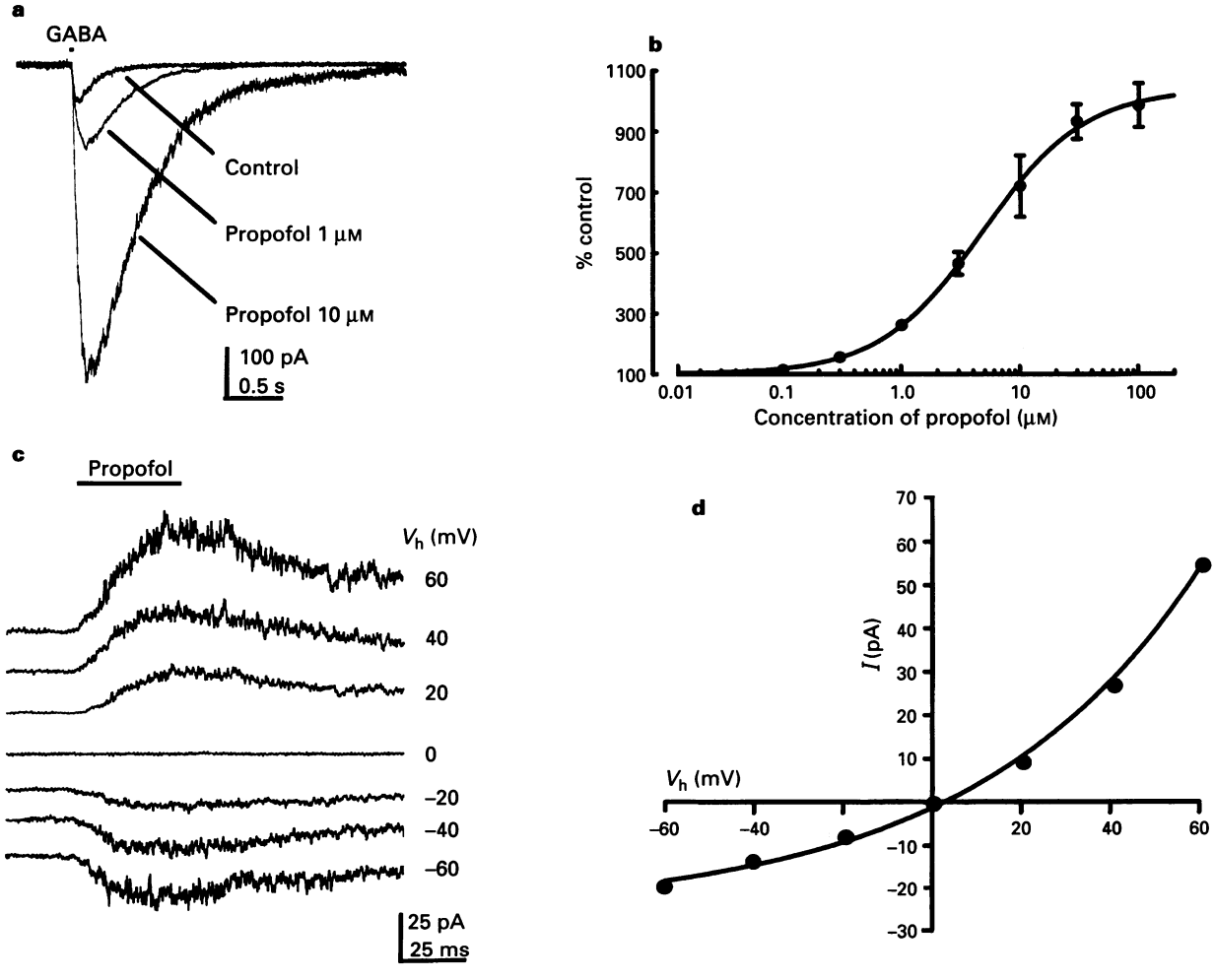

Figure 2 Potentiation of GABA-evoked currents and activation of GABA$_A$ receptors by propofol. (a) Traces illustrating potentiation of GABA-evoked currents by propofol (1–10 μM) recorded from a cell voltage-clamped at −60 mV. GABA (100 μM) was pressure-applied (70 kPa for 10 ms) and propofol was bath-applied at the indicated doses. Propofol-evoked potentiations were reversed on wash out of the drug. Superimposed traces are averages of three currents under each condition. (b) A graph illustrating the cumulative dose-response relationship for propofol-evoked potentiation of GABA-activated currents. Each data point represents the mean potentiation (expressed as a percentage of control GABA response amplitude) induced by propofol in 4–10 separate cells. Propofol concentrations are plotted on a logarithmic scale, and s.e.means are shown. The fitted logistic curve provides an EC_{50} value of 4.8 μM, and a Hill coefficient of 1.0. (c) Pressure applied (45 ms at 70 kPa) propofol (100 μM) directly activated small currents in the absence of GABA, recorded from a cell with 144 mM and 148 mM Cl^- inside and outside the cell, respectively. Currents were recorded from the same cell, with varying holding potentials from −60 mV to 60 mV. Traces represent averages of two currents recorded at each potential. (d) A graph of the relationship between the peak response amplitude and the holding potential, for the currents in (c). The equilibrium potential for propofol-evoked currents in this case was 0 mV, similar to the equilibrium potential for GABA-activated currents (see Figure 1). The mean equilibrium potential for propofol-activated currents was calculated to be 0.2 ± 0.1 mV (n = 4).

experiments the final concentration of ethanol did not exceed 0.1% (v/v). This concentration of ethanol has no effect on GABA-evoked currents recorded from GT1-7 cells (Hales *et al.*, 1992). In experiments examining the direct activation of $GABA_A$ receptors by propofol, concentrations of the drug up to 1 mM were tested, necessitating a maximum final ethanol concentration of 1%. While this concentration of ethanol has small potentiating effects on GABA-evoked currents there are no direct actions of ethanol (1%) in GT1-7 cells (Hales *et al.*, 1992).

Results

Activation of $GABA_A$ receptors by GABA

Self-replicating hypothalamic GT1-7 neurones have been reported to express functional $GABA_A$ receptors (Hales *et al.*, 1992). In confirmation of this previous report all GT1-7 cells tested ($n>100$) responded to locally applied GABA (100 μM) with inward whole cell currents recorded at a holding potential of −60 mV (Figure 1). From the current-voltage relationship it was determined that GABA-activated currents exhibited outward rectification and reversed close to the Cl^- equilibrium potential of ~0 mV with approximately equal concentrations of the ion on both sides of the GT1-7 cell membrane (Hales *et al.*, 1992; Figure 1a and b).

GABA (1 μM–1 mM), pressure applied for 1 s, dose-dependently activated currents recorded from GT1-7 cells voltage-clamped at −60 mV (Figure 1c and d). The threshold for activation was around 1 μM GABA with an EC_{50} of 26.6 μM. Peak currents were activated by 300 μM GABA ($n=6$).

Potentiation of GABA-evoked currents by propofol

GABA-evoked currents recorded from GT1-7 cells are modulated by the anaesthetic barbiturate, pentobarbitone and the anaesthetic steroid, 5α-pregnan-3α-ol-20-one, but are insensitive to the benzodiazepine, diazepam (Hales *et al.*, 1992). In this study, currents activated by brief (10 ms) applications of GABA (100 μM), were dramatically and dose-dependently potentiated by propofol (0.3–100 μM), when the general anaesthetic was bath applied (Figure 2a and b). Propofol-evoked potentiations were fully reversed upon washing out the drug. The threshold concentration of propofol for potentiation of GABA-evoked currents was approximately 300 nM and the EC_{50} was 4.8 μM. Propofol at concentrations >10 μM generally caused transient potentiations of GABA-evoked currents even in the continued presence of the drug (data not shown). This phenomemon could be due to desensitization and/or block of $GABA_A$ receptors by propofol (see Figures 4 and 5). In order to minimize the effect of desensitization and/or block, in such cases the peak amplitude of the current in the presence of propofol was used to calculate the degree of po-

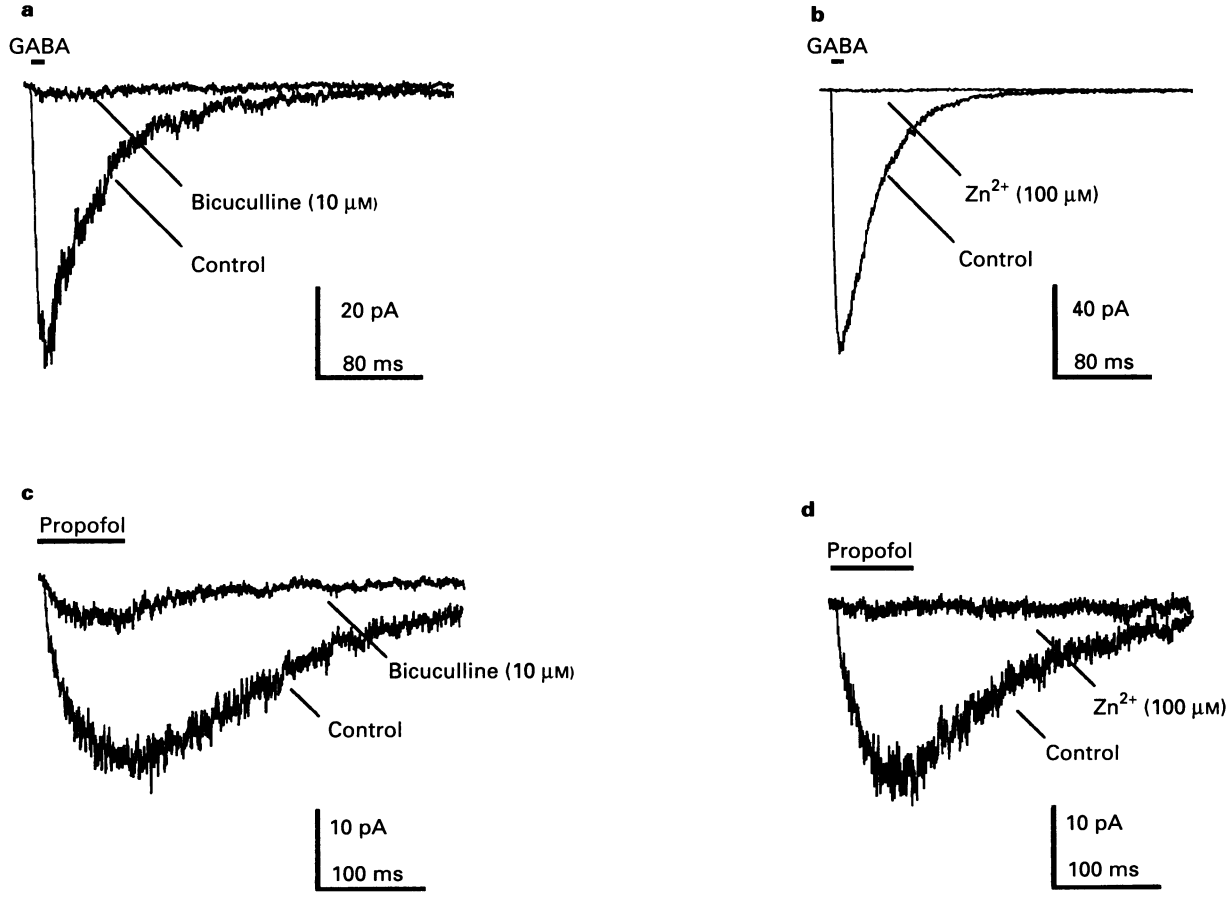

Figure 3 Bicuculline methiodide and Zn^{2+} inhibit both GABA- and propofol-evoked currents. (a) Bicuculline methiodide (10 μM) inhibited currents evoked by GABA (100 μM). (b) Zn^{2+} (100 μM) abolished currents evoked by GABA (100 μM). In (a) and (b), GABA was applied locally (for 6.5 ms at 70 kPa). Bicuculline methiodide and Zn^{2+} were bath applied. (c) Bicuculline methiodide (10 μM) reduced the amplitude of currents evoked by propofol (100 μM), to 31 ± 2.4% of control ($n=6$). (d) Zn^{2+} (100 μM) abolished ($n=6$) currents activated by local application of propofol (100 μM). For both (c) and (d), propofol was applied locally (for 80 ms at 70 kPa). Zn^{2+} and bicuculline methiodide were bath-applied. All currents were recorded at −60 mV, and superimposed traces represent the average of three currents.

tentiation. Maximum potentiation was achieved with 100 μM propofol, which increased GABA-evoked responses to 988 ± 73% (n = 6) of control current amplitude.

Activation of GABA$_A$ receptors by propofol

Propofol has been reported to activate directly GABA$_A$ receptors of adrenomedullary chromaffin cells (Hales & Lambert, 1991) and hippocampal neurones (Hara *et al.*, 1993; Orser *et al.*, 1994) in the absence of GABA. Pressure application of propofol (100 μM, for 45 ms) to GT1-7 cells activated small inward currents at negative potentials which became outward when cells were clamped at potentials above 0 mV (Figure 2c and d). The reversal potential of propofol-evoked currents with approximately equal Cl^- on both sides of the cell membrane, was 0.2 ± 0.1 mV (n = 4), similar to the theoretical reversal potential of ~0 mV. Like GABA-evoked currents recorded from GT1-7 cells, propofol-activated currents also exhibited outward rectification (n = 4).

Currents recorded from GT1-7 cells, activated by brief applications of GABA (100 μM) were inhibited when the GABA$_A$ receptor selective antagonist, bicuculline methiodide (10 μM), was applied via the superfusate (n = 5; Figure 3a). GABA-evoked currents were also abolished by Zn^{2+} (100 μM, n = 6; Figure 3b). Low concentrations of Zn^{2+} are thought to inhibit GABA$_A$ receptors in GT1-7 cells due to the absence of γ subunits (Hales *et al.*, 1992). In support of propofol (100 μM) activating GABA$_A$ receptors in GT1-7 cells lacking γ subunits, propofol-evoked currents were also inhibited by bicuculline methiodide (10 μM, n = 4) and blocked by Zn^{2+} (100 μM, n = 4; Figure 3c and d).

In order to characterize the dose-dependence of the activation of GT1-7 cell GABA$_A$ receptors by propofol, the anaesthetic was pressure-applied for prolonged periods at different concentrations, from micropipettes situated adjacent to cells voltage-clamped at −60 mV. Propofol (10, 30 and 100 μM) dose-dependently activated whole cell currents (Figure 4a and b). However, current amplitudes declined when the propofol concentration exceeded 100 μM. This hindrance of propofol-activated current was associated with a 'surge' current seen on cessation of application of high concentrations of propofol (300 μM and 1 mM; Figures 4 and 5). When peak GABA- and propofol-activated current amplitudes were compared in the same cells the latter were found to be only 31 ± 3% of the former (n = 9).

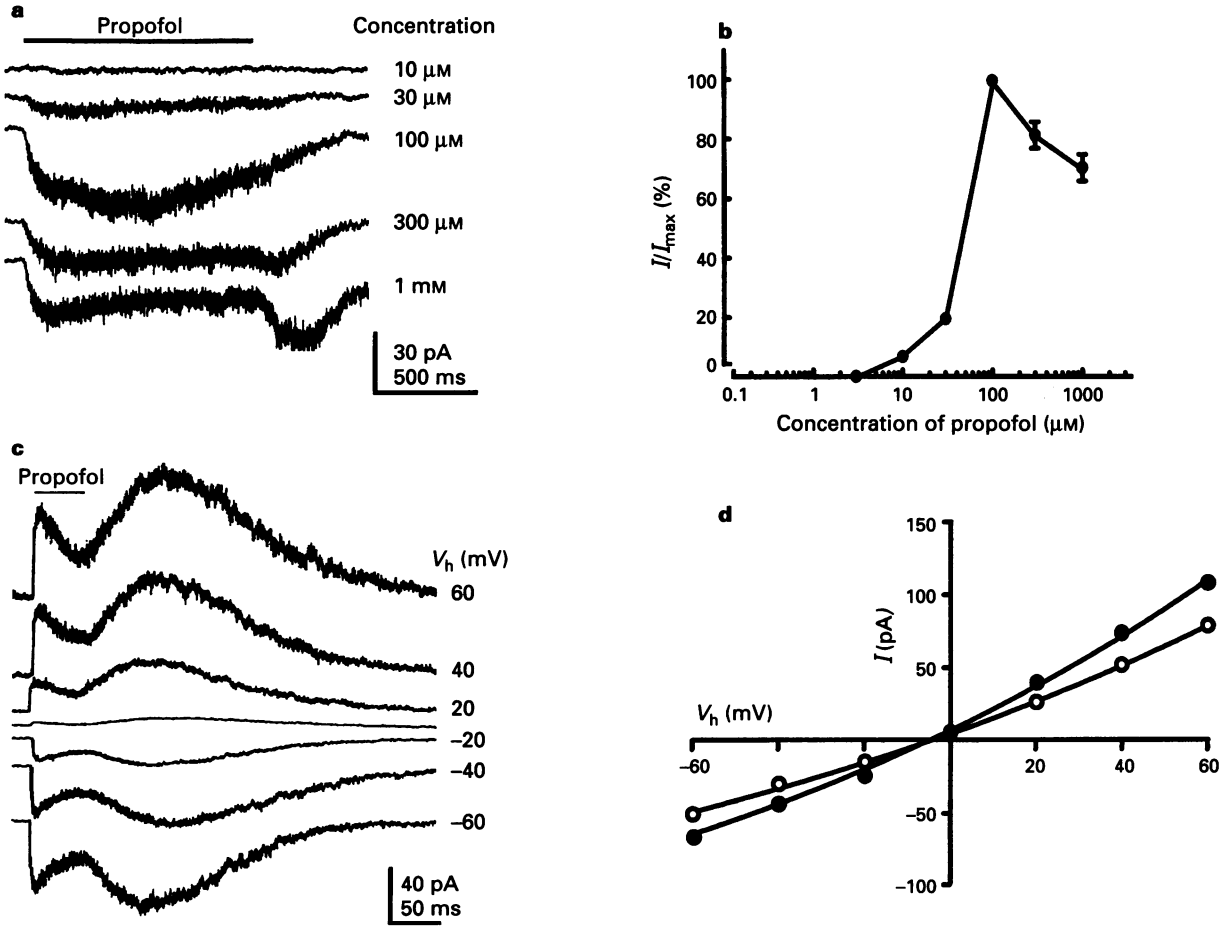

Figure 4 Dose-dependent activation and block of GABA$_A$ receptors by propofol. (a) Traces illustrating dose-dependent activation of GT1-7 cell GABA$_A$ receptors by propofol (10 μM − 1 mM). Cessation of application of propofol concentrations of greater than 100 μM (300 μM and 1 mM) caused a 'surge' current. (b) Log concentration-response curve for currents activated by propofol (3 μM − 1 mM). The threshold for activation of propofol was approximately 10 μM. Current amplitudes are expressed as a percentage of the highest amplitude response evoked on each cell. Each point represents mean data from six cells. A logistics curve could not be fitted to the data points due to the reduction in current amplitude caused by propofol concentrations greater than 100 μM. However, it is estimated from the graph that the half-maximal current would occur with approximately 50 μM propofol. (c) Propofol (1 mM)-activated currents recorded from a GT1-7 cell clamped at potentials between −60 and 60 mV. (d) A graph of the current-voltage relationship for the currents illustrated in (c): (○) represent peak current amplitudes in the presence of propofol; (●) peak amplitudes of surge currents appearing on cessation of propofol application. Both peak and surge currents reversed at approximately the Cl^- equilibration potential of ~0 mV (with 144 mM and 148 mM Cl^- inside and outside the cell, respectively). Additionally, the ratios between the peak and surge currents did not appear to vary with voltage.

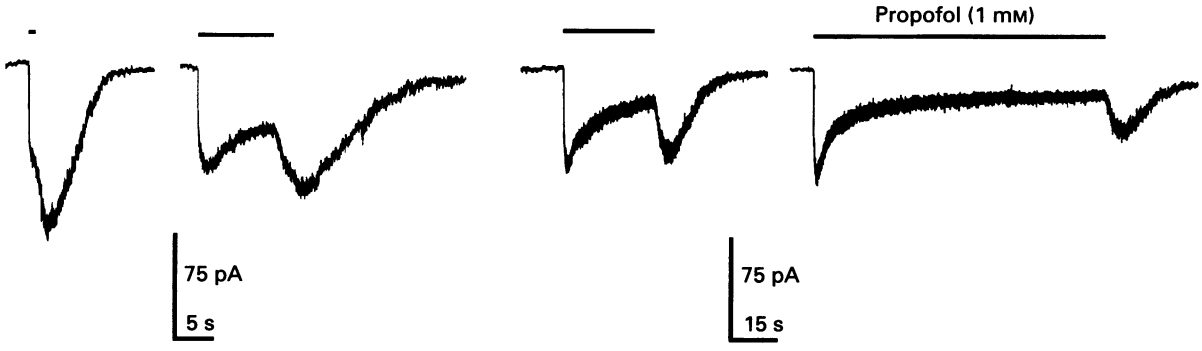

Figure 5 The relationship between duration of propofol application and surge current amplitude. Currents recorded from the same cell activated by propofol (1 mM) applied for 1, 10, 30 and 100 s. The current amplitude declined in the continued presence of propofol, but rebounded on cessation of the application. The amplitude of the surge current seen following 1 and 10 s propofol applications was greater than that of the peak current in the presence of the drug. However, the amplitude of the surge current declined with the duration of propofol application.

Propofol block of $GABA_A$ receptors

The post-propofol (1 mM) surge current could be caused by rapid recovery from: (i) blockade of $GABA_A$ receptor-Cl^--ion channels; (ii) desensitization; or (iii) allosteric inhibition of $GABA_A$ receptor-Cl^- channel gating. To test whether the surge current was caused by reversal of propofol-induced voltage-dependent block of $GABA_A$ receptor-Cl^--ion channels, the relationship between the holding potential and surge current amplitude was investigated (Figure 4c and d). Both the transient current in the presence of propofol (1 mM) and the surge current following application of the drug reversed at 0 mV (Figure 4c and d). The ratio between the two currents showed no dependence on holding potential and their current-voltage relationships resembled that seen for the lower concentration of propofol (100 μM). Taken together, these data suggest that neither the propofol-evoked hindrance, nor the reversal of this phenomenon (seen as a current surge) show voltage-dependence.

The possible role of desensitization in the hindrance of the propofol (1 mM)-evoked current and subsequent surge current was investigated by examining the surge current amplitude after drug applications of different durations (Figure 5). This protocol revealed that the surge current amplitude declined as the length of propofol application was increased (Figure 5). This is unlikely to occur if the surge current represents rapid recovery from desensitization since there would be more recovery (and hence a bigger surge) following protocols which caused greater desensitization. It is likely that the decline in the surge amplitude with prolongation of propofol application represents the relatively slow onset of desensitization (Figure 5).

Discussion

The results of this study support and extend previous reports of functional $GABA_A$ receptors on self-replicating GnRH-secreting hypothalamic GT1-7 neurones (Hales *et al.*, 1992; 1994; Bosma, 1993). This clonal cell line was further exploited here to investigate the actions of the widely used intravenous general anaesthetic, propofol, on $GABA_A$ receptors.

Low concentrations of propofol potentiate GABA-evoked currents recorded from bovine chromaffin cells, rodent and murine hippocampal neurones (Hales & Lambert, 1991; Hara *et al.*, 1994; Orser *et al.*, 1994), and GT1-7 cells. In addition, the general anaesthetic directly activates $GABA_A$ receptors at higher concentrations, relevant to total intravenous anaesthesia (Hales & Lambert, 1991; Hara *et al.*, 1993; Orser *et al.*, 1994). Numerous other compounds with general anaesthetic properties potentiate the actions of GABA and at higher doses directly activate $GABA_A$ receptors. These agents include anaesthetic barbiturates (Macdonald *et al.*, 1986), anaesthetic steroids (Cottrell *et al.*, 1987; Harrison *et al.*, 1987), etomidate (Robertson, 1989), chlormethiazole (Hales & Lambert, 1992) and volatile agents (Jones *et al.*, 1992; Yang *et al.*, 1992). The anticonvulsant and anxiolytic benzodiazepines are also potent potentiators of GABA-evoked responses, but are unable to activate directly $GABA_A$ receptors in the absence of GABA. It has been suggested that this difference in the action of general anaesthetics and the benzodiazepines on the $GABA_A$ receptor may underlie their different therapeutic properties (Schulz & Macdonald, 1981).

GT1-7 cell $GABA_A$ receptors are insensitive to the anxiolytic benzodiazepine, diazepam and membranes from these cells do not bind [^{3}H]-flunitrazepam (Hales *et al.*, 1992). It is thought that this lack of benzodiazepine modulation is caused by the absence of a γ subunit from the functional receptors. It is known from recombinant $GABA_A$ receptor studies, that the γ subunit is a prerequisite for benzodiazepine modulation of the $GABA_A$ receptor and that the selectivity of benzodiazepines in this respect is influenced by the identity of the α subunit (Pritchett *et al.*, 1989; Ymer *et al.*, 1990; Puia *et al.*, 1991). The long form of the alternatively spliced γ2 subunit has also been implicated in the actions of relatively low concentrations of ethanol (20 mM) on $GABA_A$ receptors (Wafford *et al.*, 1991). Ethanol, at this concentration, has no effect on GABA-evoked currents recorded from GT1-7 cells (Hales *et al.*, 1992). To add to the evidence for a lack of γ subunits in GT1-7 cell $GABA_A$ receptors, GABA-evoked currents recorded from these cells are blocked potently by Zn^{2+} (Hales *et al.*, 1991); Zn^{2+} is less potent as an inhibitor of GABA responses recorded from cells expressing γ subunits (Draguhn *et al.*, 1990; Smart *et al.*, 1991).

Unlike benzodiazepines and ethanol, low propofol concentrations cause profound potentiation of GABA-activated currents recorded from GT1-7 cells. The anaesthetic steroid, 5α-pregnan-3α-ol-20-one and the anaesthetic barbiturate, pentobarbitone, also potentiated GABA-activated currents recorded from these cells (Hales *et al.*, 1992). These data suggest that the γ subunit is not required for potentiation of GABA-evoked currents by these general anaesthetics. Studies of recombinant receptors lacking γ subunits have also demonstrated that this subunit is not required for the GABA potentiating actions of anaesthetic barbiturates and steroids (Puia *et al.*, 1990; Horne *et al.*, 1993). In fact, of all the mammalian receptor subunit combinations tested to date, only homomeric ρ GABA receptors localized in the retina have been reported to mediate GABA-evoked currents which are not potentiated by anaesthetic barbiturates and steroids (Shimada *et al.*, 1992; Woodward *et al.*, 1992; Hales & Olsen, 1994). However, these receptors are also unusual in that their activation by GABA is not blocked by the selective $GABA_A$ receptor antagonist, bicuculline (Shimada *et al.*, 1992).

Unlike low concentrations of propofol which potentiate GABA responses, in the absence of GABA high concentrations of the drug were required to cause discernible direct activation of Cl^- currents. Consistent with previous reports of propofol activation of $GABA_A$ receptors (Hales & Lambert, 1992; Hara *et al.*, 1993; Orser *et al.*, 1994), propofol-evoked currents recorded from GT1-7 cells were blocked by bicuculline methiodide. Propofol-activated currents were also blocked by Zn^{2+} suggesting that these, like GABA-evoked currents, are mediated by activation of $GABA_A$ receptors lacking γ subunits in this cell line. Propofol concentrations required for activation of GT1-7 $GABA_A$ receptors were higher than those reported for $GABA_A$ receptor activation in bovine chromaffin cells (Hales & Lambert, 1991) and rodent hippocampal neurones (Hara *et al.*, 1993).

The large separation between the threshold for potentiation and activation of $GABA_A$ receptors in GT1-7 cells may be a feature of their subunit combination(s). It is possible that the absence of the γ subunit contributes to this difference in the potency of propofol as a potentiator and an activator. Potentiation and activation by propofol may require different subunits and hence some receptor combinations may be more sensitive to one action of the anaesthetic than the other. Interestingly, Orser and colleagues (1994) reported a high threshold of propofol activation of $GABA_A$ receptors in embryonic murine hippocampal neurones. The dose-response relationship for this action of propofol in their preparation looks strikingly similar to that reported here. It may be significant that GABA-responses recorded from embryonic neurones tend to be more sensitive to Zn^{2+} (Smart & Constanti, 1990) and this has been explained by a lack of γ subunits (Draguhn *et al.*, 1990; Smart *et al.*, 1991). Hence the absence of a γ subunit from the $GABA_A$ receptor may make propofol less potent and/or efficacious as an activator in GT1-7 cells and embryonic neuronal preparations. Rigorous pharmacological comparisons of various subunit combinations of recombinant $GABA_A$ receptors will be required to investigate this and other possibilities further.

There is little known about the actions of propofol on $GABA_A$ receptors of different subunit combinations. Both propofol and pentobarbitone directly activate homomeric human $\beta1$ $GABA_A$ receptors expressed in *Xenopus* oocytes (Sanna *et al.*, 1995). The same study demonstrated that a dose of propofol which directly activated recombinant $\alpha1\beta1\gamma2$ receptors eliciting large currents, activated barely detectable currents when applied to oocytes expressing $\alpha1\beta1$ receptors. Recent studies of $\alpha2\beta1$ and $\alpha2\beta1\gamma2$ receptors expressed in human embryonic kidney cells also suggest that smaller currents are activated by propofol relative to GABA in cells lacking the γ subunit (Jones *et al.*, 1995). These observations support the hypothesis that the presence of the γ subunit increases the potency and/or efficacy of propofol as an activator of $GABA_A$ receptors.

In addition to potentiation and activation, propofol also inhibits current through GT1-7 cell $GABA_A$ receptor Cl^- ion channels at high concentrations. The inhibitory action of propofol is exposed by the surge current associated with the termination of application of high concentrations of the drug. A similar phenomenon has been seen in experiments on chromaffin cells (Peters *et al.*, 1989) and dorsal root ganglion neurones (Robertson, 1989) with pentobarbitone and etomidate. More recently it has also been observed in experiments involving high concentrations of propofol in embryonic murine hippocampal neurones (Orser *et al.*, 1994).

In all these cases the blocking action of these drugs is manifested as a surge current following their application at relatively high concentrations. The present study suggests that the blocking effect of propofol is independent of voltage. $GABA_A$ receptor block by pentobarbitone also appears to be independent of voltage (Robertson, 1989). Taken together, these observations suggest that $GABA_A$ receptor inhibition by these anaesthetics is not due to a channel block similar to that seen at the N-methyl-D-aspartate receptor with charged blocking compounds such as the dissociative anaesthetic, ketamine (Halliwell *et al.*, 1989).

The onset of block and the appearance of the surge current are probably too rapid to represent the onset and recovery from propofol-evoked desensitization (Orser *et al.*, 1994). The suggestion that this phenomenon is not related to desensitization is supported by the observation of smaller surge currents following longer, desensitizing applications of propofol. The most likely explanation would seem to be a low-affinity propofol site on the $GABA_A$ receptor through which the agent allosterically inhibits channel gating. This site is presumably only accessed at high concentrations and rapidly left during wash, resulting in the appearance of a surge current caused by propofol still bound at its agonist site.

Thus propofol may have three modulatory sites on the GT1-7 cell $GABA_A$ receptor for potentiation, activation and inhibition. These sites may all have different subunit specificities, but do not appear to require the γ subunit. The prevalence of agonist and inhibitory sites on different receptor subtypes may influence the apparent efficacy of propofol as a $GABA_A$ receptor activator and perhaps also as a general anaesthetic.

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