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The interaction of general anaesthetics with recombinant $GABA_A$ and glycine receptors expressed in *Xenopus laevis* oocytes: a comparative study

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1 The effects of five structurally dissimilar general anaesthetics were examined in voltage-clamp recordings of agonist-evoked currents mediated by recombinant γ-aminobutyric acid $(GABA)_A$ receptors composed of human $\alpha_1\beta_1$ and γ_{2L} subunits expressed in *Xenopus laevis* oocytes. A quantitative comparison of the effects of these agents was made upon recombinant glycine receptors expressed as a homo-oligomer of human α_1 subunits, or as a hetero-oligomer of human α_1 and rat β subunits.

2 Complementary RNA-injected oocytes expressing $GABA_A$ receptors responded to bath applied GABA with an EC_{50} of 158 ± 34 μM. Oocytes expressing α_1 and $\alpha_1\beta$ glycine receptors subsequent to cDNA injection displayed EC_{50} values of 76 ± 2 μM and 66 ± 2 μM, respectively, in response to bath applied glycine.

3 Picrotoxin antagonized responses mediated by homo-oligomeric α_1 glycine receptors with an IC_{50} of 4.2 ± 0.8 μM. Hetero-oligomeric $\alpha_1\beta$ glycine receptors were at least 100-fold less sensitive to blockade by picrotoxin.

4 With the appropriate agonist EC_{10}, propofol enhanced GABA and glycine-evoked currents to approximately the maximal response produced by a saturating concentration of either agonist (i.e. I_{max}). The calculated EC_{50} values were 2.3 ± 0.2 μM, 16 ± 3 μM and 27 ± 2 μM, for $GABA_A$ $\alpha_1\beta_1\gamma_{2L}$, glycine α_1 and $\alpha_1\beta$ receptors, respectively. At relatively high concentrations, propofol was observed to activate directly both $GABA_A$ and glycine receptors.

5 Pentobarbitone potentiated GABA-evoked currents to $117 \pm 8.5\%$ of I_{max} with an EC_{50} of 65 ± 3 μM. The barbiturate also produced a substantial enhancement of the glycine-evoked currents, I_{max} and EC_{50} values being $71 \pm 2\%$ and 845 ± 66 μM and $51 \pm 10\%$ and 757 ± 30 μM for homomeric α_1 and heteromeric $\alpha_1\beta$ glycine receptors respectively. At high concentrations, pentobarbitone directly activated $GABA_A$, but not glycine, receptors.

6 The potentiation by propofol or pentobarbitone of currents mediated by α_1 homo-oligomeric glycine receptors was in both cases associated with a parallel sinistral shift of the glycine concentration-effect curve. The effects of binary combinations of pentobarbitone and propofol at maximally effective concentrations were mutually occlusive suggesting a common site, or mechanism, of action.

7 GABA-evoked currents were maximally potentiated by etomidate to $79 \pm 2\%$ of I_{max} (EC_{50} of 8.1 ± 0.9 μM). By contrast, glycine-induced currents mediated by α_1 and $\alpha_1\beta$ glycine receptor isoforms were enhanced only to $29 \pm 4\%$ and $28 \pm 3\%$ of I_{max}. Limited solubility precluded the calculation of EC_{50} values for the effect of etomidate at glycine receptors. None of the receptor isoforms examined were directly activated by etomidate.

8 The neurosteroid 5α-pregnan-3α-ol-20-one potentiated GABA-evoked currents to $69 \pm 4\%$ of I_{max}, with an EC_{50} value of 89 ± 6 nM. In contrast, both α_1 homo-oligomeric and $\alpha_1\beta$ heter-oligomeric glycine receptors were insensitive to the action of this steroid. A direct agonist action of the steroid was discernible at $GABA_A$, but not glycine, receptors.

9 Trichloroethanol, the active metabolite of the general anaesthetic chloral hydrate, enhanced glycine-evoked currents to $77 \pm 10\%$ and $94 \pm 4\%$ of I_{max} on α_1 and $\alpha_1\beta$ glycine receptors, with EC_{50} values of 3.5 ± 0.1 mM and 5.9 ± 0.3 mM respectively. On $GABA_A$ receptors, trichloroethanol had a lower maximum enhancement ($52 \pm 5\%$ of I_{max}), but a slightly higher potency (EC_{50} 1.0 ± 0.1 mM). Trichloroethanol activated neither $GABA_A$, nor glycine, receptors.

10 The data demonstrate a variety of intravenous general anaesthetic agents, at clinically relevant concentrations, to augment preferentially GABA-evoked currents mediated by the $\alpha_1\beta_1\gamma_{2L}$ receptor subunit combination as compared to their effects on both α_1 and $\alpha_1\beta$ glycine receptors. However, the presence on glycine receptors of lower affinity modulatory binding sites for pentobarbitone, propofol and trichloroethanol may aid in the identification of the molecular determinants of the CNS actions of these anaesthetics.

Keywords: GABA; glycine; $GABA_A$ receptor; glycine receptor; pentobarbitone; propofol; etomidate; 5α-pregnan-3α-ol-20-one; trichloroethanol; intravenous general anaesthetics

Introduction

The molecular mechanism(s) which underlie the rapid and dramatic behavioural actions of general anaesthetics are unknown. The anaesthetic state represents the culmination of action of compounds which may vary from chemically inert gases, to complex organic molecules that exhibit exquisite structure-activity requirements. Given this structural diversity, a unitary mechanism for general anaesthesia would appear

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untenable. However, surprisingly, a number of studies have now demonstrated that the majority of intravenous anaesthetics, at clinically relevant concentrations, share the common feature of potentiating the actions of γ-aminobutyric acid (GABA) at the $GABA_A$ receptor (Tanelian *et al*., 1993; Franks & Lieb, 1994; Harris *et al*., 1995). Although such observations do not exclude alternative ion channels or receptors as anaesthetic targets, the enhancement of neuronal inhibition through an action at the major inhibitory receptor in the mammalian central nervous system has a simplistic and logical appeal. However, in the brain stem and spinal cord, the majority of inhibitory neural pathways utilize the neurotransmitter glycine, which mediates neuronal depression by activating anion selective strychnine-sensitive glycine receptors. A number of recent studies have suggested that at least one component of the anaesthetic state, the suppression of nocifensive reflexes, may occur at the level of the brain stem and spinal cord, rather than at a higher locus (Antognini & Schwarz, 1993; Rampil *et al*., 1993; Rampil, 1994; Collins *et al*., 1995). Furthermore, in addition to potentiating $GABA_A$ receptor-mediated responses, the inhalational anaesthetics halothane, isoflurane and enflurane (Wakamori *et al*., 1991; Harrison *et al*., 1993; Downie *et al*., 1996), and the intravenous anaesthetic propofol (Hales & Lambert, 1991; Mascia *et al*., 1996), act as positive allosteric modulators of the strychnine-sensitive glycine receptor.

$GABA_A$ and glycine receptors are members of a Cys-loop superfamily of transmitter-gated ion channels (Kuhse *et al*., 1995; Ortells & Lunt, 1995; Barnard, 1996). Both receptors probably exist as a pentameric arrangement of subunits drawn from five families for the $GABA_A$ receptor (α, β, γ, δ and ε) and two families (α and β) for the glycine receptor (Kuhse *et al*., 1995; Smith & Olsen, 1995; Whiting *et al*., 1995). Furthermore, a number of isoforms of these subunits (e.g. $GABA_A$; α_{1-6}, β_{1-3}, γ_{1-3}; glycine; α_{1-4}) have been identified and shown to impart distinct pharmacological and biophysical properties upon the receptor (Rundstrom *et al*., 1994; Kuhse *et al*., 1995; Smith & Olsen, 1995; Whiting *et al*., 1995). These subunits exhibit distinctive expression patterns within the central nervous system (Malosio *et al*., 1991; Wisden *et al*., 1992). Hence, the functional properties of the inhibitory receptors that they form are unlikely to be homogeneous, but may be brain region, or indeed neurone, specific.

In this study, we have utilized the *Xenopus laevis* oocyte expression model to compare the actions of five structurally diverse intravenous anaesthetics (propofol, pentobarbarbitone, 5α-pregnan-3α-ol-20-one, etomidate and trichloroethanol) at recombinant $GABA_A$ (α_1 β_1 γ_{2L}) and glycine (α_1 and $\alpha_1\beta$) receptors, in an attempt to assess the relative importance of these receptors in producing general anaesthesia. The results demonstrate that although some compounds are relatively selective for the $GABA_A$ receptor, it is conceivable that modulation of glycine receptor activity could contribute to the behavioural actions of propofol and trichloroethanol. A preliminary account of a part of this work has appeared in abstract form (Pistis *et al*., 1996).

Methods

Preparation of transcripts and oocyte injection

cDNAs encoding the human α_1, β_1 and γ_{2L} $GABA_A$ receptor subunits were linearized at the *HpaI*, *BamHI* and *NotI* sites in the pCDM8 vector respectively. cRNA transcripts were prepared according to standard protocols (Hope *et al*., 1993). The integrity of the transcripts was examined by denaturing gel electrophoresis prior to injection. The cDNAs coding for human α_1 and rat β glycine receptor subunits were provided in the pCIS vector. The cRNA transcripts (for $GABA_A$ receptors) or cDNAs (for glycine receptors) were injected (30–50 nl of 1 mg ml^{-1} per subunit for GABA subunits, or 20 nl of 3.8–5.0 μg ml^{-1} for the glycine α_1 subunit and 20 nl of 12.5 μg ml^{-1} for the glycine β subunit) into *Xenopus laevis* oocytes (Stage V–VI). The latter had previously been defolliculated by treatment with 2 mg ml^{-1} collagenase 'A' (Boehringer-Mannheim) for 3 h at room temperature (20–23°C) in Barth's saline with Ca^{2+} salts omitted. The cDNA was injected intranuclearly by utilizing the 'blind method' described by Colman (1984). Injected oocytes were individually maintained at 19–20°C for up to 12 days in 96 well plates containing 200 μl of standard Barth's solution (composition in mM: NaCl 88, KCl 1, $NaHCO_3$ 2.4, HEPES 15, $Ca(NO_3)_2$ 0.5, $CaCl_2$ 0.5 and $MgSO_4$ 1.0; adjusted to pH 7.6 with NaOH). The solution was supplemented with 0.1 mg ml^{-1} gentamicin.

Electrophysiological recordings

Oocytes were used for experimentation 2–12 days after cRNA or cDNA injection. The methodology was essentially as previously described (Hill-Venning *et al*., 1997). Briefly, electrical recordings were made from oocytes voltage-clamped at −60 mV using an Axoclamp 2A, or a GeneClamp 500 amplifier (Axon Instruments, USA) in the two-electrode voltage-clamp mode. The oocytes were held in a chamber (0.5 ml) and continuously superfused (7–10 ml min^{-1}) with frog Ringer solution (composition in mM: NaCl 120, KCl 2, $CaCl_2$ 1.8, HEPES 5; adjusted to pH 7.4 with NaOH). The voltage-sensing and current-passing electrodes were filled with 3 M KCl and had resistances of 1–2 MΩ when measured in frog Ringer solution. Agonist-induced responses were low pass filtered at a corner frequency of 100 Hz and recorded onto digital audio tape via a Biologic DTR 1204 DAT recorder and simultaneously displayed on a chart recorder. The peak amplitude of the agonist-evoked current was measured manually. All drugs were applied by the superfusion system.

For each oocyte, a maximal concentration of GABA (3 mM) or of glycine (1 mM) was applied and the resultant peak current amplitude determined. This concentration of GABA or glycine was reapplied at 20 min intervals until the current amplitude was consistent to within ±2% over three challenges with GABA or glycine. Once stabilized, the current amplitude remained constant throughout the experimental period. In all cases, care was taken to ensure that the amplitude of the GABA- or glycine-activated current had stabilized before experiments were commenced.

To investigate the enhancement of agonist-evoked responses by putative positive allosteric modulators, a concentration of GABA or glycine producing a peak current approximately 10% of the maximum obtainable (EC_{10}) was determined for each oocyte. The modulator was pre-applied for 30 to 60 s before co-application with the appropriate concentration of GABA or glycine. Potential direct agonist actions of the compounds were also investigated in the absence of GABA or glycine, and when evident, were expressed relative to the current induced by a saturating concentration of the agonist. Concentration-response relationships for either the agonist-modulating or agonist-mimetic actions of the anaesthetics were iteratively fitted, where appropriate, by use of Fig P Version 6c, with the four parameter logistic equation:

$$\frac{I}{I_{max}} = \frac{[A]^{n_H}}{[A]^{n_H} + [EC_{50}]^{n_H}}$$

where for GABA or glycine modulation, I is the amplitude of the agonist-evoked current in the presence of the anaesthetic at concentration [A], I_{max} is the amplitude of the response in the presence of a maximally effective concentration of the anaesthetic, EC_{50} is the concentration of anaesthetic producing half-maximal enhancement and n_H is the Hill coefficient. Concentration-effect relationships for the direct agonist action of the anaesthetics were similarly fitted, where I represents the amplitude of the current evoked by anaesthetic concentration [A], I_{max} is the response in the presence of a maximally effective concentration of anaesthetic and EC_{50} is the concentration of anaesthetic producing a half-maximal response. In instances

where the concentration response relationship for agonist modulation was clearly bell-shaped, curve fitting was restricted to the ascending limb and apparent maximum.

The reduced potency of picrotoxin as an antagonist of hetero-oligomeric $\alpha_1\beta$ glycine receptors versus α_1 homo-oligomers (Pribilla *et al*., 1992; Handford *et al*., 1996) was used to verify the functional expression of the β subunit. An IC_{50} value for the α_1 homo-oligomeric receptor was derived in a manner similar to that described above.

The specific involvement of both $GABA_A$ and glycine receptors in the direct effects of the anaesthetic agents was assessed by examining whether the evoked currents were susceptible to block by the $GABA_A$ and glycine receptor antagonists picrotoxin (30 μM) and RU5135 (3α-hydroxy-16-imino-5β-17-aza-androstan-11-one; 1 μM) and the glycine receptor antagonist strychnine (1 μM). For $GABA_A$ receptors, the direct effects of the anaesthetics were additionally investigated for their sensitivity to potentiation by flunitrazepam (0.3 μM). For glycine receptors, the direct effects of the anaesthetics were examined for their sensitivity to potentiation by 10 μM zinc (Bloomenthal *et al*., 1994; Laube *et al*., 1995). Experiments were conducted at ambient temperature (18–22°C). Quantitative data are presented as the mean ± s.e.mean. The s.e.mean values associated with the EC_{50} and Hill coefficient are those derived from the fitted curve.

Drugs used

γ-Aminobutyric acid (GABA), flunitrazepam, glycine, sodium pentobarbitone, 5α-pregnan-3α-ol-20-one (5α3α), picrotoxin and trichloroethanol, were all obtained from Sigma. R-(+)-etomidate hydrochloride was from Janssen. 2,6-Diisopropylphenol (propofol) was supplied by Aldrich or formulated 1% w/v in Intralipid from Zeneca as Diprivan. RU5135 was a gift of Roussel Uclaf. Stock solutions of all drugs were prepared daily. For $GABA_A$ receptor experiments, propofol (100 mM in ethanol), flunitrazepam (10 mM in ethanol) and 5α3α (10 mM in DMSO) were diluted into frog Ringer solution with a maximal final vehicle concentration of 0.1% v/v, which alone had no effect upon GABA-activated currents. However, these concentrations of ethanol and DMSO enhanced the control response evoked by glycine. Hence, for the glycine receptor experiments, propofol was prepared as a 1 mM stock by diluting Diprivan into frog Ringer and 5α3α as a 10 mM stock into a 2-hydroxypropyl-β-cyclodextrin (RBI) 20% w/v solution in distilled water. The stock solutions were subsequently diluted into frog Ringer solution with a maximal final vehicle concentration of 0.1% v/v, which alone had no effect upon glycine-activated currents. RU5135 (10 mM in DMSO) was applied in frog Ringer solution at a concentration (1 μM) associated with a negative vehicle control. All other drugs were prepared as concentrates in frog Ringer solution.

Results

At a holding potential of −60 mV, oocytes preinjected with cRNA encoding human α_1, β_1, γ_{2L} $GABA_A$ receptor subunits, or with cDNA encoding human α_1 and rat β glycine receptor subunits responded to bath applied GABA and glycine, respectively, with an inward current response. GABA or glycine-evoked currents (Figure 1a) were concentration dependent, with calculated EC_{50} values of 158 ± 34 μM for $\alpha_1\beta_1\gamma_{2L}$ $GABA_A$ receptors and 76 ± 2 μM and 66 ± 2 μM for α_1 and $\alpha_1\beta$ glycine receptors, respectively. Strychnine (1 μM) abolished glycine-evoked currents recorded from oocytes expressing α_1 (not shown) or $\alpha_1\beta$ (Figure 1b) glycine receptors.

For oocytes expressing homo-oligomeric α_1 glycine receptors, picrotoxin (0.1–100 μM) inhibited, in a concentration dependent manner (IC_{50} = 4.2 ± 0.8 μM), the current evoked by glycine at EC_{50} (Figure 2a). In the presence picrotoxin at IC_{50}, such antagonism was associated with a parallel dextral displacement of the glycine concentration-response curve (Figure 2b; see also Lynch *et al*., 1995). As previously shown (Pribilla *et al*., 1992; Handford *et al*., 1996), the coexpression of the glycine β subunit conferred resistance to this inhibitory effect, such that 100 μM picrotoxin reduced the glycine-evoked current to only 88 ± 5% (n = 4) of control (Figure 2). This large (at least 100 fold) difference in the inhibitory potency of picrotoxin was employed throughout this study to confirm the functional expression of the β subunit in oocytes injected with the α_1 and β glycine receptor subunit cDNAs (Figure 2c and d).

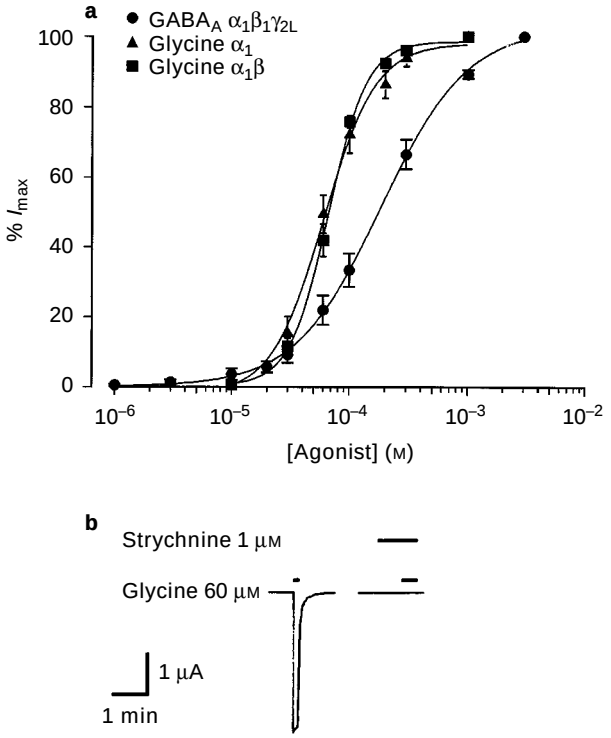

Figure 1 The properties of GABA and glycine-induced currents recorded under voltage-clamp from *Xenopus laevis* oocytes expressing $GABA_A$ ($\alpha_1\beta_1\gamma_{2L}$) and glycine ($\alpha_1$ and $\alpha_1\beta$) receptors. (a) Graphical depiction of the relationship between the concentration of agonist (logarithmic scale) and the peak amplitude of the agonist-evoked current (on a linear scale and expressed relative to the current induced by a maximally effective concentration of the agonist) for oocytes expressing $\alpha_1\beta_1\gamma_{2L}$ $GABA_A$ receptors, human homo-oligomeric α_1 and mammalian (human α_1 and rat β) hetero-oligomeric $\alpha_1\beta$ glycine receptors. Each data point represents the mean and vertical lines associated s.e.mean of data obtained from 4–5 oocytes. (b) Example of a trace showing strychnine (1 μM) to abolish glycine-induced currents recorded from oocytes expressing $\alpha_1\beta$ glycine receptors. All data were obtained from oocytes voltage-clamped at −60 mV.

Propofol

The general anaesthetic propofol (0.03–10 μM) enhanced currents evoked by GABA at EC_{10} recorded from oocytes expressing $\alpha_1\beta_1\gamma_{2L}$ $GABA_A$ receptors in a concentration-dependent manner. Maximal potentiation was produced by 10 μM propofol (to 95 ± 2% of that evoked by a saturating concentration of GABA (I_{max})) and the calculated EC_{50} was 2.3 ± 0.2 μM (Figure 3 and Table 1). For concentrations of propofol >30 μM, the magnitude of the potentiation decreased (52 ± 13% of I_{max}; n = 4; 100 μM propofol).

At concentrations greater than those required to enhance GABA-evoked currents, propofol (10–300 μM) induced a concentration-dependent inward current in the absence of GABA (Figure 3). This effect was maximal with 300 μM propofol (37 ± 7% of the GABA I_{max}) and the calculated EC_{50} (46 ± 1 μM) was approximately 20 fold greater than that determined for the enhancement of GABA-evoked responses (Table 1). The propofol-induced current was blocked by 30 μM picrotoxin and potentiated by 0.3 μM flunitrazepam (data not

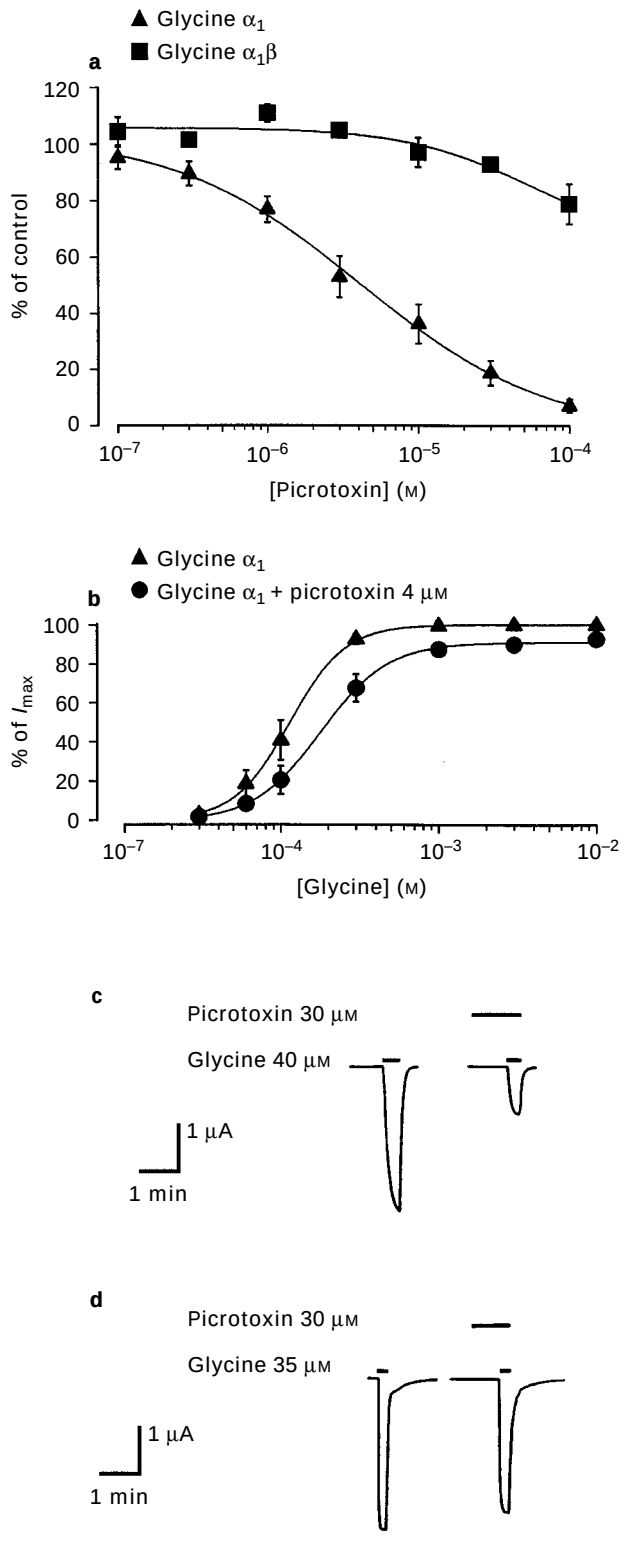

Figure 2 Picrotoxin displays a differential potency as an antagonist of α_1 and $\alpha_1\beta$ glycine receptors. (a) Graph depicting the concentration-dependent suppression by picrotoxin of responses elicited by glycine, at EC_{50}, acting upon α_1 and $\alpha_1\beta$ glycine receptors expressed in *Xenopus laevis* oocytes. Peak response amplitude (as a percentage of control, linear scale) was plotted against the concentration of picrotoxin (logarithmic scale) in the medium. Note the clear separation of the effects of picrotoxin at the two receptor isoforms. Data for α_1 and $\alpha_1\beta$ glycine receptors were obtained from experiments performed on 7 and 4 different oocytes, respectively. (b) Graph illustrating antagonism of responses mediated by α_1 glycine receptors by picrotoxin at IC_{50} (4 μM). Responses elicited by glycine were plotted as a percentage of the response to a saturating concentration of the agonist in the presence and absence of picrotoxin. The data were obtained from experiments on 3 oocytes. In both (a) and (b), data points are the mean and vertical lines show s.e.mean. (c and d) Examples of traces illustrating the differential potency of picrotoxin (30 μM) as an antagonist at (c) α_1 and (d) $\alpha_1\beta$ glycine receptors. In this and all subsequent figures, periods of drug application are indicated by the horizontal bars above each trace. All data were obtained under voltage-clamp at a holding potential of -60 mV.

shown), observations which implicate the $GABA_A$ receptor in this effect. Upon washout of high concentrations ($\geqslant 300$ μM) of the anaesthetic, we occasionally observed the development of a transient 'rebound' current similar to that described below for pentobarbitone.

As previously shown for glycine receptors expressed by mouse spinal neurones in culture (Hales & Lambert, 1991), propofol enhanced currents evoked by glycine at EC_{10} recorded from oocytes expressing α_1 or $\alpha_1\beta$ recombinant receptors. The EC_{50} for this effect was approximately 7–11 fold greater (16 ± 3 μM for α_1; 27 ± 2 μM for $\alpha_1\beta$) than that for $GABA_A$ receptor modulation (Figure 3; Table 1). The maximum potentiation of the glycine-evoked current was produced by 100 μM (α_1 receptors $=85\pm5\%$ of I_{max}) and 300 μM ($\alpha_1\beta$ receptors $=98\pm6\%$ of I_{max}) propofol. Inspection of Figure 3 reveals glycine receptor subunit composition to have little or no influence upon the modulatory effect of propofol.

The modulatory effect of propofol upon the α_1 glycine receptor was further investigated by determining the effect of the anaesthetic upon the approximately linear region of the concentration-response relationship bracketing the glycine EC_{50}. Propofol produced a concentration-dependent and apparently parallel sinistral shift in the glycine concentration effect relationship such that the EC_{50} for glycine was reduced approximately 2.7 fold ($n=2$) and 4.7 fold ($n=3$) in the presence of 30 and 100 μM propofol, respectively (Figure 4a).

Propofol, in the absence of glycine, induced an inward current for oocytes expressing α_1 or $\alpha_1\beta$ glycine receptors. Such currents were evident in the presence of 100 μM propofol and at 1 mM, the anaesthetic induced responses amounted to $12\pm6\%$ of I_{max} for α_1 and $8\pm2\%$ of I_{max} for $\alpha_1\beta$ glycine receptors (Figure 3). Such currents were blocked by the glycine receptor antagonists strychnine (1 μM) and RU5135 (1 μM). In common with glycine-evoked currents (Bloomenthal *et al*., 1994; Laube *et al*., 1995), the currents evoked by propofol were potentiated by zinc (10 μM; see Figure 5).

Pentobarbitone

Pentobarbitone (1–300 μM) elicited a concentration-dependent enhancement of the current evoked by GABA for oocytes expressing $\alpha_1\beta_1\gamma_{2L}$ $GABA_A$ receptors (Figure 6). The current evoked by GABA at EC_{10} was maximally potentiated by 300 μM pentobarbitone (to $117\pm9\%$ of I_{max}; $n=3$) with a calculated EC_{50} of 65 ± 3 μM. At concentrations greater than 300 μM, the magnitude of the pentobarbitone-induced potentiation was reduced ($93\pm8\%$ of I_{max}; $n=3$; 600 μM pentobarbitone).

At concentrations greater than those required for a substantial enhancement of the GABA-evoked response, pentobarbitone (100 μM–2 mM), in the absence of GABA, induced a concentration-dependent inward current response. At relatively high concentrations ($\geqslant 1$ mM), the anaesthetic elicited a complex response consisting of a transient peak current, succeeded by a decline to a plateau and followed by the redevelopment of the current response upon washout of the barbiturate. Such 'rebound currents' may result from a chloride channel blocking action of high concentrations of the barbiturate (Akaike *et al*., 1987; Peters *et al*., 1988). The maximal current was evoked by 2 mM pentobarbitone and amounted to $29\pm4\%$ ($n=3$) of I_{max}. The calculated EC_{50} for the direct effect of pentobarbitone was 1.1 ± 0.02 mM ($n=3$). Such barbiturate-induced currents were blocked by 30 μM picrotoxin and potentiated by 0.3 μM flunitrazepam (data not shown) and thus, are likely to be mediated by the $GABA_A$ receptor.

Pentobarbitone potentiated glycine-evoked currents recorded from oocytes expressing α_1 and $\alpha_1\beta$ glycine receptors, although higher concentrations of the anaesthetic were required compared to those which produced $GABA_A$ receptor modulation (Figure 6). The EC_{50} values for α_1 and $\alpha_1\beta$ glycine receptors were 845 ± 66 μM and 757 ± 30 μM, respectively, i.e. approximately 12–13 fold greater than those determined for the $GABA_A$ receptor. The maximum enhancement of the glycine-induced currents, produced by 3 mM pentobarbitone, was less than that observed for GABA, being $71\pm2\%$ of I_{max} ($n=4$) for α_1, and $51\pm10\%$ of I_{max} ($n=4$) for $\alpha_1\beta$. Hence, the subunit composition of the glycine receptor (α_1 or $\alpha_1\beta$) had little influence on the modulatory actions of this barbiturate.

In common with propofol, pentobarbitone produced a concentration-dependent leftward shift of the glycine concentration-effect relationship. In the presence of 1 and 3 mM pentobarbitone, the glycine EC_{50} was reduced 2 fold and 2.4 fold, respectively (Figure 4b). Whether propofol and pento-

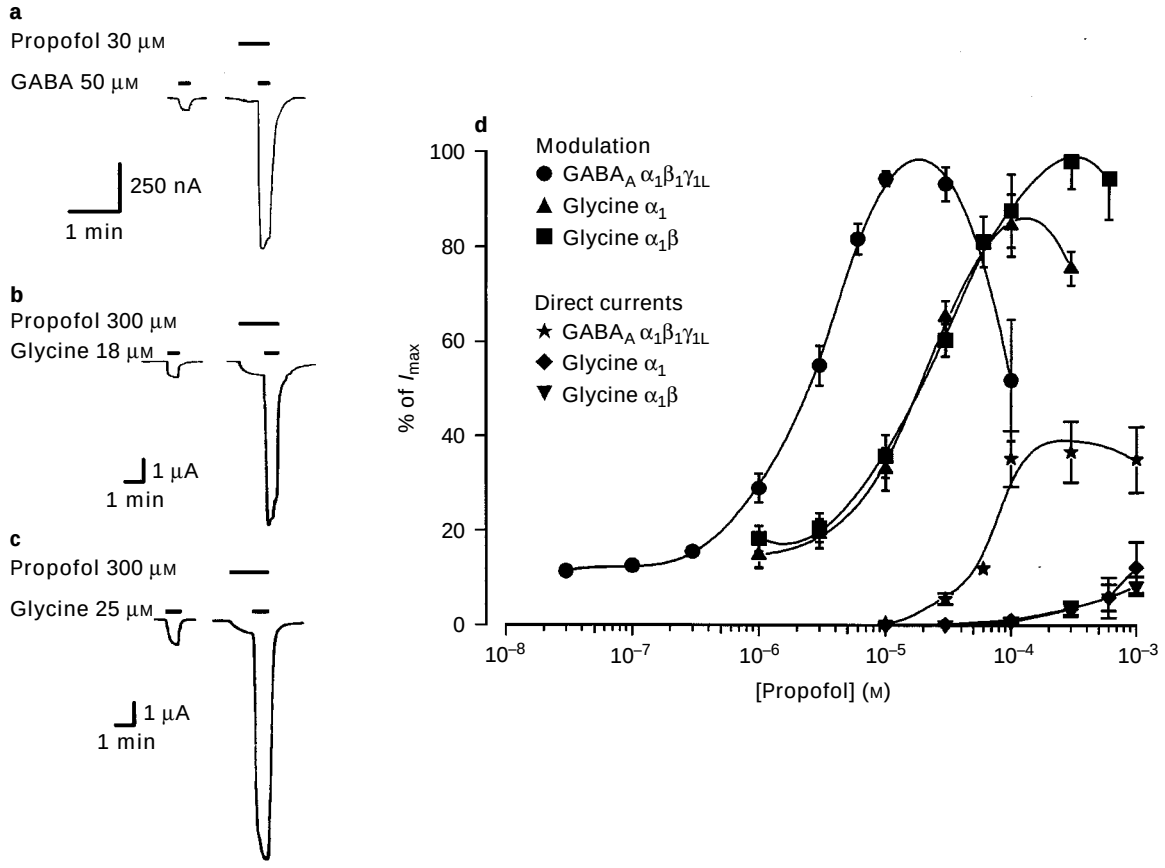

Figure 3 Propofol acted as a positive allosteric modulator of $GABA_A$ and glycine receptors. (a) Bath applied propofol (30 μM) greatly potentiated the current evoked by 50 μM GABA (approximate EC_{10} in this example) recorded from an oocyte expressing the human $\alpha_1\beta_1\gamma_{2L}$ $GABA_A$ receptor subunit combination. (b) A ten fold greater concentration of propofol (300 μM) produced an equivalent enhancement of the current evoked by an EC_{10} concentration of glycine recorded from oocytes expressing glycine receptors composed of α_1 (b) and $\alpha_1\beta$ (c) subunits. (d) Graph illustrating the relationship between the concentration of bath applied propofol (logarithmic scale) and the peak amplitude of the agonist-evoked current (on a linear scale and expressed relative to the maximum current induced by a saturating concentration of agonist). The data show the potentiation of GABA (EC_{10}) acting at the $\alpha_1\beta_1\gamma_{2L}$ $GABA_A$ receptor and of glycine (EC_{10}) acting at homo-oligomeric α_1 and hetero-oligomeric $\alpha_1\beta$ glycine receptors. Additionally, the peak direct current elicited by propofol alone at $GABA_A$ and glycine receptor isoforms is plotted. All records were obtained from oocytes voltage-clamped at -60 mV. Each data point represents the mean and associated s.e.mean (vertical lines) of data obtained from 4–5 oocytes. Note that the EC_{50} values for propofol (and other anaesthetics) quoted in the text and in Table 1 were calculated from curve fits restricted to the ascending limb of the concentration-response relationship. Curves illustrated in this and subsequent figures were fitted by eye and have no theoretical significance (see Methods).

Table 1 A comparison of the modulatory actions of a variety of structurally diverse anaesthetics across recombinant human $\alpha_1\beta_1\gamma_{2L}$ $GABA_A$ receptors, α_1 homo-oligomeric and $\alpha_1\beta$ hetero-oligomeric glycine receptors

	$GABA_A$ receptor $\alpha_1\beta_1\gamma_{2L}$			*Glycine receptor* α_1			$\alpha_1\beta$		
Compound	ED_{50}	I_{max}	n	EC_{50}	I_{max}	n	EC_{50}	I_{max}	n
Pentobarbitone	65 ± 3 μM	$117\pm9\%$	3	845 ± 66 μM	$71\pm2\%$	4	757 ± 3 μM	$51\pm10\%$	4
Propofol	2.3 ± 0.2 μM	$95\pm2\%$	4	16 ± 3 μM	$85\pm5\%$	3	27 ± 2 μM	$98\pm6\%$	3
Etomidate	8.1 ± 0.9 μM	$79\pm2\%$	3	ND	$29\pm4\%$	3	ND	$28\pm3\%$	3
Trichloroethanol	1.0 ± 0.08 mM	$52\pm5\%$	3	3.5 ± 0.1 mM	$77\pm10\%$	3	5.9 ± 0.3 mM	$94\pm4\%$	3
5α3α	89 ± 6 nM	$69\pm4\%$	3	ND	ND	3	ND	ND	3

The I_{max} is expressed as a percentage of the maximum response to either GABA or glycine. ND= not determined due to the small magnitude of the effect. All data were obtained from oocytes voltage-clamped at -60 mV.

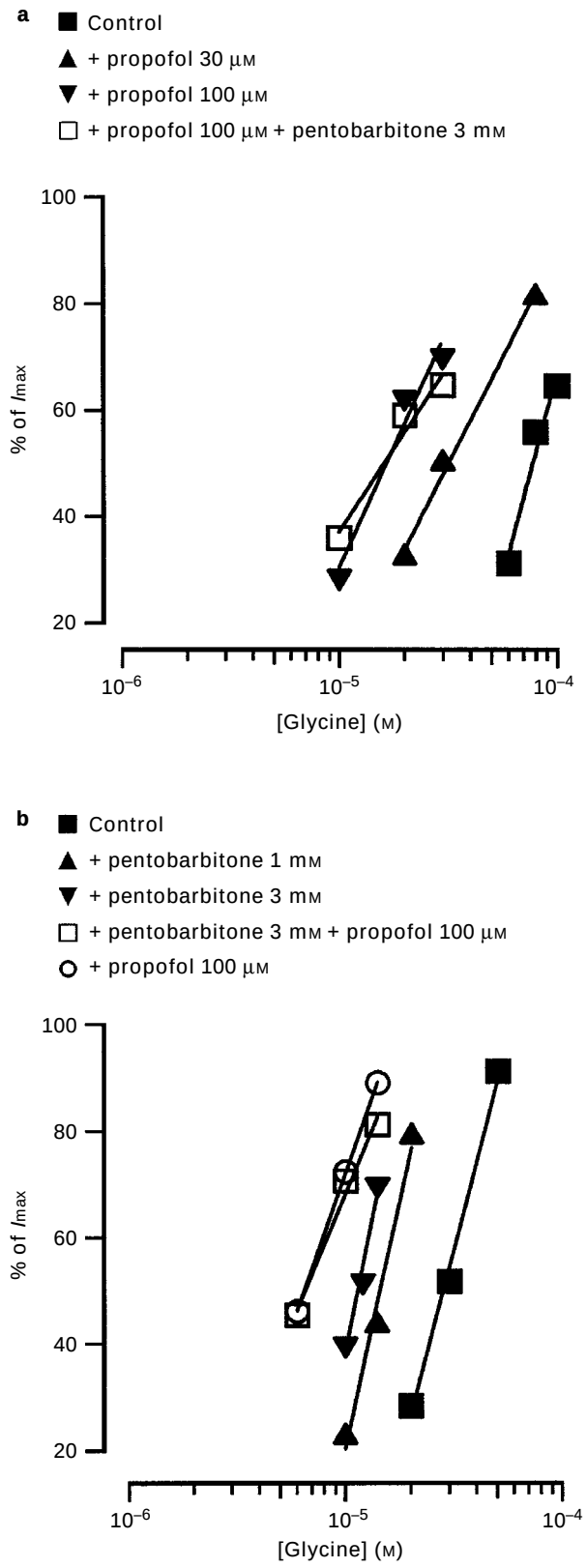

Figure 4 The actions of propofol and pentobarbitone on the glycine concentration-response relationship. (a) Graph depicting the relationship between the amplitude of the glycine evoked current (expressed as a percentage of the maximal response to glycine, ordinate) and the concentration of bath applied glycine (logarithmic scale, abcissa scale). Currents were recorded from oocytes expressing the α_1 glycine receptor in the absence of any modulator, in the presence of propofol (30 μM or 100 μM) and in the combined presence of propofol 100 μM and pentobarbitone 3 mM. Under each condition, 3 concentrations of glycine bracketing the EC_{50} were examined. Note that propofol produced a concentration-dependent and parallel sinistral shift of the glycine concentration-effect curve over the range 30–100 μM. The co-application of pentobarbitone 3 mM with propofol (100 μM) produced no further shift. (b) A plot similar to that described above for glycine alone, and in the presence of pentobarbitone (1 mM or 3 mM). The combined application of propofol 100 μM and pentobarbitone 3 mM produced a further parallel sinistral shift of the glycine concentration-response relationship, but this was identical to that produced by 100 μM propofol alone in this oocyte. The data obtained in (a) and (b) were obtained from separate oocytes and are representative of those obtained in two further experiments.

barbitone interact with a common binding site on the glycine receptor is not known. To investigate this question, the effects of binary combinations of propofol and pentobarbitone upon the glycine EC_{50} were determined. As noted above, a maximally effective concentration of propofol (100 μM; Figure 3) produced a 4.7 fold decrease of the glycine EC_{50} (Figure 4a). In the presence of 100 μM propofol, the addition of pentobarbitone (3 mM) had no further influence upon the glycine EC_{50} which remained displaced from control by approximately 4.6 fold (Figure 4a). In the converse experiment, employing a maximally effective concentration of pentobarbitone (3 mM), the introduction of propofol (100 μM) was associated with a further reduction in the glycine EC_{50} (from 2.4 fold to 4.6 fold-relative to control; Figure 4b). However, it should be emphasised that the magnitude of the total shift is identical to that produced by propofol (100 μM) alone. One interpretation of these data is that the two anaesthetics act via a common saturable site, or mechanism, at the glycine receptor.

In contrast to its effect on human $\alpha_1\beta_1\gamma_{2L}$ $GABA_A$ receptors, pentobarbitone (100 μM–6 mM) did not directly activate either form (α_1 or $\alpha_1\beta$) of the glycine receptor.

Etomidate

In agreement with previous observations (Belelli *et al*., 1996; Hill-Venning *et al*., 1997), etomidate enhanced the current evoked by GABA at EC_{10} from oocytes expressing human $\alpha_1\beta_1\gamma_{2L}$ $GABA_A$ receptors. Potentiation of the GABA-evoked response was maximal with 100 μM of this anaesthetic ($79\pm2\%$ of I_{max}) and the EC_{50} was 8.1 ± 0.9 μM (Figure 7). The magnitude of the potentiation was reduced for concentrations of etomidate greater than 100 μM (Figure 7). We have previously demonstrated that the modulatory actions of etomidate are greater and more potent for β_2 and β_3 containing $GABA_A$ receptors (Belelli *et al*., 1997; Hill-Venning *et al*., 1997) and, therefore, the effects of etomidate at α_1 β_2 γ_{2L} receptors are also shown for comparison. In contrast to both pentobarbitone and propofol, etomidate ($\geqslant$600 μM) did not elicit an inward current when applied to oocytes expressing $\alpha_1\beta_1\gamma_{2L}$ $GABA_A$ receptor subunits. These results are consistent with the selectivity of the agonist-like actions of this anaesthetic for β_2- or β_3-containing receptors found previously (Belelli *et al*., 1997; Hill-Venning *et al*., 1997; Lambert *et al*., 1997a,b).

Glycine evoked currents were only modestly potentiated by etomidate over a range of concentrations that greatly enhanced GABA-evoked currents (Figure 7). The potentiation was concentration-dependent, and at the highest concentration tested (300 μM), etomidate enhanced the glycine-evoked response to $29\pm4\%$, (α_1; $n=3$) and $28\pm3\%$ ($\alpha_1\beta$; $n=3$) of I_{max}. Higher concentrations of etomidate were not tested due to limited solubility. The absence of a clear maximal effect of etomidate precluded calculation of the EC_{50}. Finally, for oocytes expressing α_1 or $\alpha_1\beta$ glycine receptors, etomidate did not directly induce an inward current over the concentration range (1–300 μM) tested.

5α-Pregnan-3α-ol-20-one

The potent allosteric modulation of the $GABA_A$ receptor by some naturally occurring neurosteroids is well documented (reviewed in Lambert *et al*., 1995). Consistent with these studies, acting upon oocytes expressing human recombinant

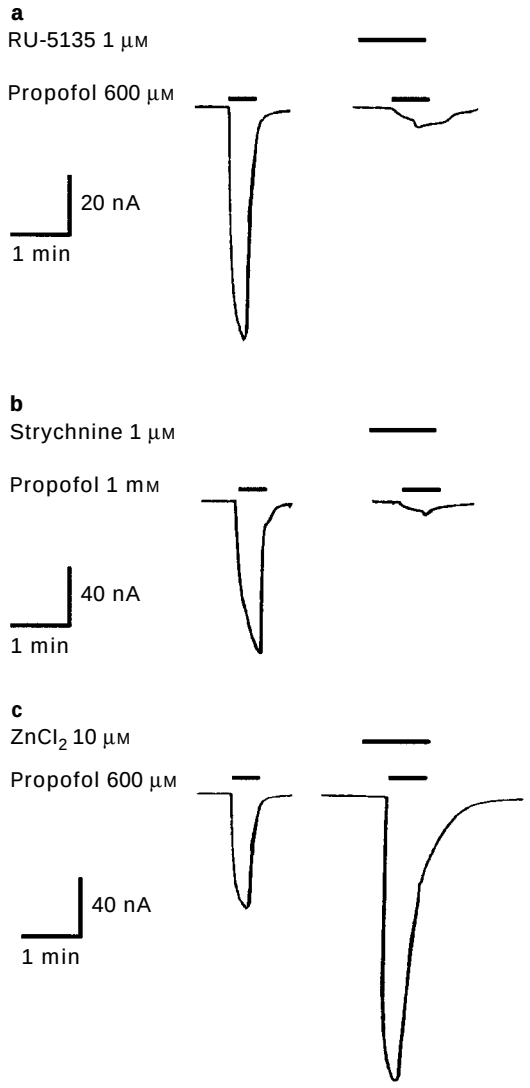

Figure 5 Propofol acted as a glycine-mimetic. In the absence of glycine, the bath application of a relatively high concentration (600 μM – 1 mM) of propofol to oocytes expressing α_1 recombinant receptors induced an inward current response which was antagonized by the co-application of the glycine receptor antagonist RU5135 (1 μM; a) or strychnine (1 μM; b). Propofol induced currents were enhanced by the co-application of zinc (10 μM; c). All records were obtained at a holding potential of -60 mV.

$\alpha_1\beta_1\gamma_{2L}$ $GABA_A$ receptors, the anaesthetic steroid 5α-pregnan-3α-ol-20-one (5α3α; 10 nM – 1 μM) elicited a concentration-dependent enhancement of GABA-evoked currents. Potentiation by the steroid was maximal at 1 μM (69 ± 4% of the I_{max}; $n = 3$) with a calculated EC_{50} of 89 ± 6 nM (Figure 8, Table 1). At concentrations $\geqslant$300 nM, the steroid evoked a small inward current response in the absence of GABA. Such currents never exceeded 1% of I_{max}, but were potentiated by flunitrazepam 0.3 μM and blocked by picrotoxin 30 μM (data not shown) and are thus likely to be $GABA_A$ receptor-mediated.

In contrast to its potent effect on $GABA_A$ receptors, 5α3α (30 nM – 10 μM) did not enhance the glycine-evoked current recorded from oocytes expressing either the recombinant homo-oligomeric α_1 or the hetero-oligomeric $\alpha_1\beta$ receptors (Figure 8). Nor did the steroid produce detectable activation of the receptor.

Trichloroethanol

Trichloroethanol is the principal active metabolite of the anaesthetic chloral hydrate. Previous studies have shown this anaesthetic to potentiate GABA-activated chloride currents recorded from mouse hippocampal neurones (Lovinger *et al*., 1993; Peoples & Weight, 1994).

In the present study, trichloroethanol (100 μM – 3 mM) produced a concentration-dependent enhancement of the GABA-evoked current recorded from oocytes expressing $\alpha_1\beta_1\gamma_{2L}$ $GABA_A$ receptors (Figure 9). The maximum enhancement (to 52 ± 5% of I_{max}; $n = 3$) was produced by 3 mM of the anaesthetic. Higher concentrations were associated with a reduced potentiation, giving rise to a 'bell shaped' concentration-response curve. The calculated EC_{50} for trichloroethanol was 1.0 ± 0.1 mM. At relatively high concentrations ($\geqslant$3 mM), trichloroethanol in the absence of GABA, induced an inward current. However, this current was neither blocked by picrotoxin (30 μM), nor enhanced by flunitrazepam (300 nM), and therefore is unlikely to be mediated by $GABA_A$ receptor activation. Indeed, we have previously observed such currents on uninjected oocytes (Downie *et al*., 1995).

Trichloroethanol (100 μM – 3 mM) produced a concentration-dependent enhancement of glycine-evoked currents recorded from oocytes expressing α_1 or $\alpha_1\beta$ glycine receptor subunits (Figure 9). The calculated EC_{50} values for this effect were 3.5 ± 0.1 mM (α_1) and 5.9 ± 0.3 mM ($\alpha_1\beta$). The maximal enhancement (to 77 ± 10% and 94 ± 4% of I_{max} for α_1 and $\alpha_1\beta$ glycine receptors, respectively) occurred with 30 mM trichloroethanol for both the hetero-oligomeric and homo-oligomeric forms of the receptor. Hence, the subunit composition of the glycine receptor has little influence on the positive allosteric actions of this anaesthetic. Concentrations of trichloroethanol greater than 1 mM produced a direct inward current in the absence of glycine. However, such currents were not influenced by the glycine receptor antagonist strychnine, and, as mentioned above, are observed on uninjected oocytes (Downie *et al*., 1995) and are therefore, unlikely to be mediated by the glycine receptor.

Discussion

The present study has examined the actions of five structurally dissimilar anaesthetic agents upon current responses mediated by recombinant $GABA_A$ and glycine receptor isoforms. For propofol, pentobarbitone and trichloroethanol, substantial modulation of both classes of receptor was evident. In addition, propofol directly activated both $GABA_A$ and glycine receptors. These features are discussed below in the context of the clinically relevant actions of the agents studied.

Propofol

Patch- and voltage-clamp studies performed on endogenous and recombinant $GABA_A$ receptors have demonstrated low micromolar concentrations of propofol to enhance allosterically the interaction of the receptor with GABA. At higher concentrations, propofol acts as a GABA-mimetic (Hales & Lambert, 1991; Hara *et al*., 1993, 1994; Orser *et al*., 1994; Sanna *et al*., 1995; Wafford *et al*., 1996, reviewed in Lambert *et al*., 1997a,b). In the present study, low micromolar concentrations of propofol produced a large enhancement of the GABA-evoked response mediated by $\alpha_1\beta_1\gamma_{2L}$ receptors whereas much higher concentrations (approximately 20 fold) were required for a GABA-mimetic effect.

We have previously demonstrated that propofol, at concentrations greater than those required for $GABA_A$ receptor modulation, enhances glycine-evoked currents mediated by the strychnine-sensitive glycine receptor of mouse spinal cord neurones in culture (Hales & Lambert, 1991). Consistent with these observations, propofol produced a large, concentration dependent enhancement of the glycine-evoked current for both α_1 homo-oligomeric and $\alpha_1\beta$ hetero-oligomeric recombinant glycine receptors. Similar observations have been made for oocytes injected with rat brain mRNA (Shepherd *et al*., 1996). The effects of propofol were not dependent upon the subunit composition of the receptor (α_1 or $\alpha_1\beta$) and occurred over a

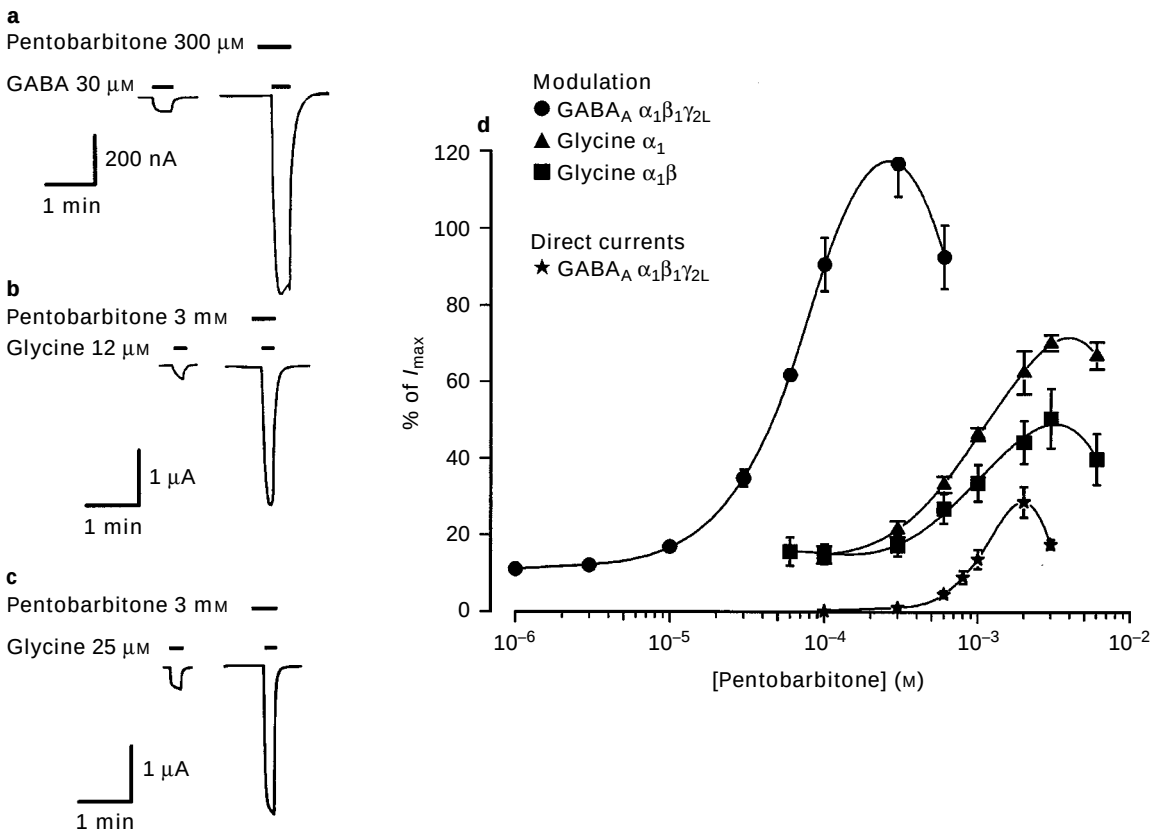

Figure 6 Pentobarbitone was shown to be a positive allosteric modulator of $GABA_A$ and glycine receptors. (a) Bath applied pentobarbitone (300 μM) greatly potentiated the current evoked by 30 μM GABA (approximate EC_{10} in this example) recorded from an oocyte expressing the human $\alpha_1\beta_1\gamma_{2L}$ $GABA_A$ receptor subunit combination. (b and c) A ten fold greater concentration of pentobarbitone (3 mM) produced a substantial enhancement of the current evoked by an EC_{10} concentration of glycine recorded from oocytes expressing (b) α_1 and (c) $\alpha_1\beta$ glycine receptors. (d) Graph illustrating the relationship between the concentration of bath applied pentobarbitone (logarithmic scale) and the peak amplitude of the agonist-evoked current (on a linear scale and expressed relative to the maximum current induced by a saturating concentration of agonist). Data show potentiation of GABA (EC_{10}) at $\alpha_1\beta_1\gamma_{2L}$ receptors and of glycine (EC_{10}) at homo-oligomeric α_1 and hetero-oligomeric $\alpha_1\beta$ glycine receptors. Additionally, the peak direct current elicited by pentobarbitone alone at α_1 β_1 γ_{2L} $GABA_A$ receptors was also plotted. All records were obtained from oocytes voltage-clamped at -60 mV. Each data point represents the mean and associated s.e.mean (vertical lines) of data obtained from 3–4 oocytes.

concentration range approximately 7–12 fold greater than that required for $GABA_A$ receptor modulation. Hence, a modulatory binding site for this anaesthetic, although of lower apparent affinity than for the $GABA_A$ receptor, is present on the α_1 homo-oligomeric glycine receptor. Furthermore, the effect of propofol does not appear to be influenced by the co-expression of the β subunit. It is important to note, in this regard, that functional expression of the β subunit was always confirmed by the differential potency of picrotoxin (Pribilla *et al*., 1992; Handford *et al*., 1996). Interestingly, glycine-evoked currents recorded from rat hippocampal neurones have been shown to be insensitive to micromolar concentrations of propofol (Hara *et al*., 1993). The subunit composition of the glycine receptors native to the hippocampus are not known, although both α_2 and β subunits are well represented (Malosio *et al*., 1991). To date the effect of propofol on $\alpha_2\beta$ glycine receptors has not been studied, although propofol does enhance glycine-evoked currents recorded from oocytes expressing homo-oligomeric α_2 glycine receptors (Mascia *et al*., 1996). At very high concentrations (100 μM–1 mM), propofol induced a small inward current which was inhibited by glycine receptor antagonists and enhanced by zinc. Collectively, these observations suggest that propofol can directly activate the glycine receptor.

Pentobarbitone

Numerous electrophysiological studies have demonstrated that the interaction of GABA with endogenous and recombinant $GABA_A$ receptors is enhanced by pentobarbitone (e.g. Thompson *et al*., 1996). In addition, higher concentrations of this anaesthetic directly activate the receptor (see Lambert *et al*., 1997a,b for reviews). Here, pentobarbitone produced a large concentration dependent enhancement of the GABA-evoked current recorded from oocytes expressing human $\alpha_1\beta_1\gamma_{2L}$ receptors with an EC_{50} of 65 μM. Approximately 15 fold greater concentrations of pentobarbitone ($EC_{50} = 1.1$ mM) were required for the GABA-mimetic effects of this anaesthetic.

The strychnine-sensitive glycine receptor has been shown to be relatively insensitive to barbiturates (Barker & Ransom, 1978; Akaike *et al*., 1985; Hales & Lambert, 1991; Koltchine *et al*., 1996). In agreement, we have demonstrated that although pentobarbitone substantially enhances the glycine-evoked response, such effects are only evident at high concentrations (>300 μM). Indeed, the EC_{50} for this effect is 12–13 fold greater than that determined for $GABA_A$ receptor modulation. Similar to propofol, the incorporation of the β subunit into the receptor ($\alpha_1\beta$ versus α_1) had little influence on the allosteric actions of this barbiturate.

Pentobarbitone acting at the $GABA_A$ receptor produces a parallel sinistral shift of the GABA concentration response relationship, suggesting an increase in the apparent affinity of the receptor for the agonist (Owen *et al*., 1986; Parker *et al*., 1986; Belelli *et al*., 1996). Similarly, in this study, pentobarbitone produced a leftward shift of glycine-response relationship and a reduction of the glycine EC_{50}, which may similarly reflect an increase in affinity. Furthermore, the results of experiments with binary combinations of propofol and pentobarbitone

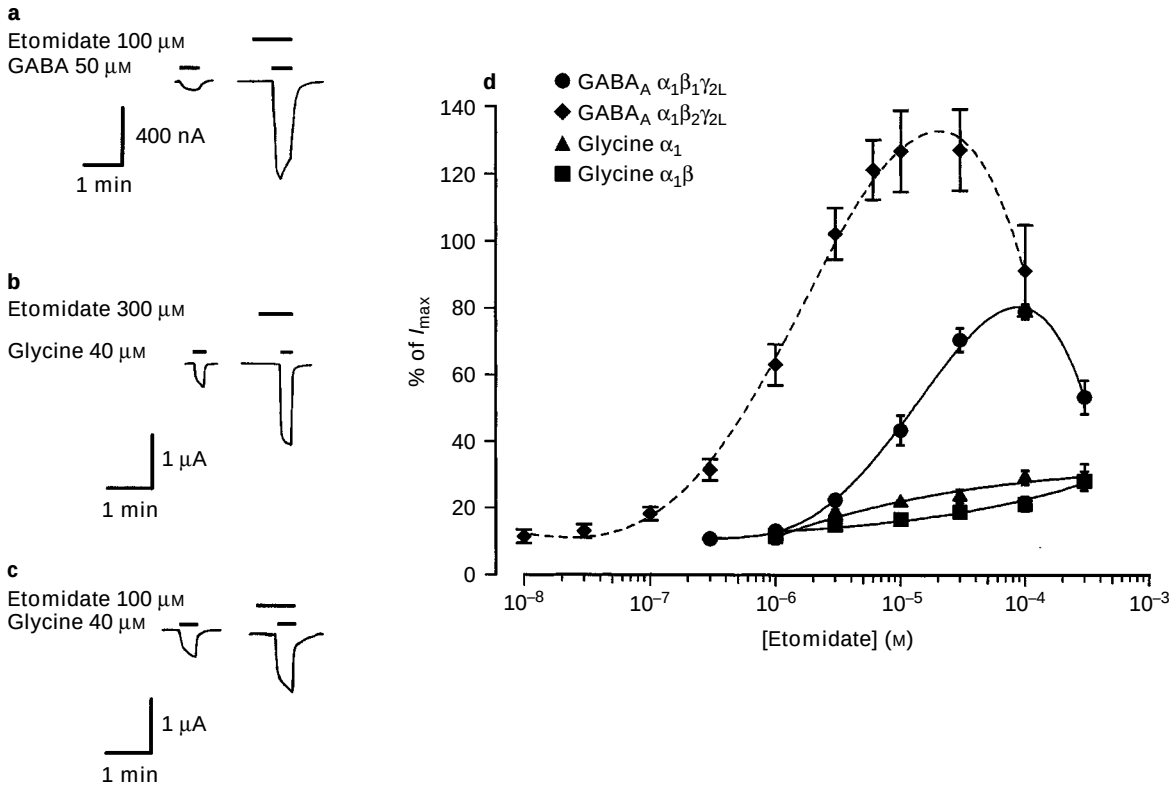

Figure 7 Etomidate was found to be a potent positive allosteric modulator of $GABA_A$ receptors but exerted only a weak effect at glycine receptors. (a) Bath applied etomidate (100 μM) greatly potentiated the current evoked by 50 μM GABA (approximate EC_{10} in this example) recorded from an oocyte expressing the human $\alpha_1\beta_1\gamma_{2L}$ $GABA_A$ receptor subunit combination. (b and c) At identical or higher concentrations (100–300 μM), etomidate produced a much reduced enhancement of the current evoked by an EC_{10} concentration of glycine recorded from oocytes expressing (b) α_1 homo-oligomeric or (c) $\alpha_1\beta$ hetero-oligomeric glycine receptors. (d) Graph illustrating the relationship between the concentration of bath applied etomidate (logarithmic scale) and the peak amplitude of the agonist-evoked current (on a linear scale and expressed relative to the maximum current induced by a saturating concentration of agonist). Data show potentiation of GABA (EC_{10}) at $\alpha_1\beta_1\gamma_{2L}$ receptors and of glycine (EC_{10}) at homo-oligomeric α_1 and hetero-oligomeric $\alpha_1\beta$ glycine receptors. For comparative purposes, the potentiation of GABA (at EC_{10}) acting at $\alpha_1\beta_2\gamma_{2L}$ $GABA_A$ receptors was reproduced from the data of Hill-Venning *et al.* (1997). All records were obtained from oocytes voltage-clamped at −60 mV. Each data point represents the mean and associated s.e.mean (vertical lines) of data obtained from 3–4 oocytes.

suggest that the anaesthetics compete for a common site on the glycine receptor.

Etomidate

In common with propofol and pentobarbitone, etomidate possesses both GABA-modulatory and GABA-mimetic actions (Robertson, 1989; Belelli *et al.*, 1996; Hill-Venning *et al.*, 1997). Uniquely, the interaction of etomidate with the $GABA_A$ receptor is influenced by the β isoform present within the receptor complex, with both the GABA-modulatory and GABA-mimetic actions being favoured by β_2- or β_3-subunit containing receptors versus those incorporating a β_1 subunit (Hill-Venning *et al.*, 1997; Belelli *et al.*, 1997; Sanna *et al.*, 1997). Consistent with these observations, in the present study, both the GABA modulatory and the GABA-mimetic effects of etomidate are favoured by α_1 β_2 γ_{2L} compared with α_1 β_1 γ_{2L} receptors. Recent studies have demonstrated that this selectivity resides with a single amino acid (asparagine in β_2 and β_3, serine in β_1) which is located within the ion channel forming region (M2) (Belelli *et al.*, 1997). In comparison to propofol and pentobarbitone, high concentrations of etomidate produced only a modest potentiation of glycine-evoked currents mediated by either α_1 or α_1 β glycine receptors.

5α-Pregnan-3α-ol-20-one

The naturally occurring neurosteroid 5α-pregnan-3α-ol-20-one is a potent general anaesthetic and $GABA_A$ receptor modulator (Lambert *et al.*, 1995). In this study, the neurosteroid potently enhanced the $GABA_A$ receptor (α_1 β_1 γ_{2L}) response (EC_{50} = 89 nM), but much higher concentrations had no effect on the glycine (α_1 or $\alpha_1\beta$) receptor. Similarly, the glycine receptor of chick spinal neurones is insensitive to this anaesthetic (Wu *et al.*, 1990). However, the water soluble steroidal anaesthetic minaxolone does act as a positive allosteric modulator of both $GABA_A$ and glycine receptors, but is approximately 20 fold less potent at the latter (Shepherd *et al.*, 1996). By contrast, the steroids 20α-hydrocortisone, α-cortol and hydrocortisone have been shown to enhance selectively the glycine receptor mediated depolarization of rat optic nerve, but to have no effect on $GABA_A$ receptor mediated responses (Prince & Simmonds, 1992). Hence, the steroid structure activity relationship for these related inhibitory receptors is distinct.

Trichloroethanol

Trichloroethanol is the principal active metabolite of the anaesthetic chloral hydrate. In the present study, this anaesthetic potentiated GABA-evoked currents with an EC_{50} of 1 mM. Similar concentrations of trichloroethanol are reported to enhance GABA-gated chloride currents in rodent hippocampal neurones (Lovinger *et al.*, 1993; Peoples & Weight, 1994) and mammalian fibroblasts stably expressing recombinant $GABA_A$ receptors (Krasowski *et al.*, 1997). In rat hippocampal slices, this effect is seen as a prolongation of the inhibitory postsynaptic current (Lovinger *et al.*, 1993). Concentrations of tri-

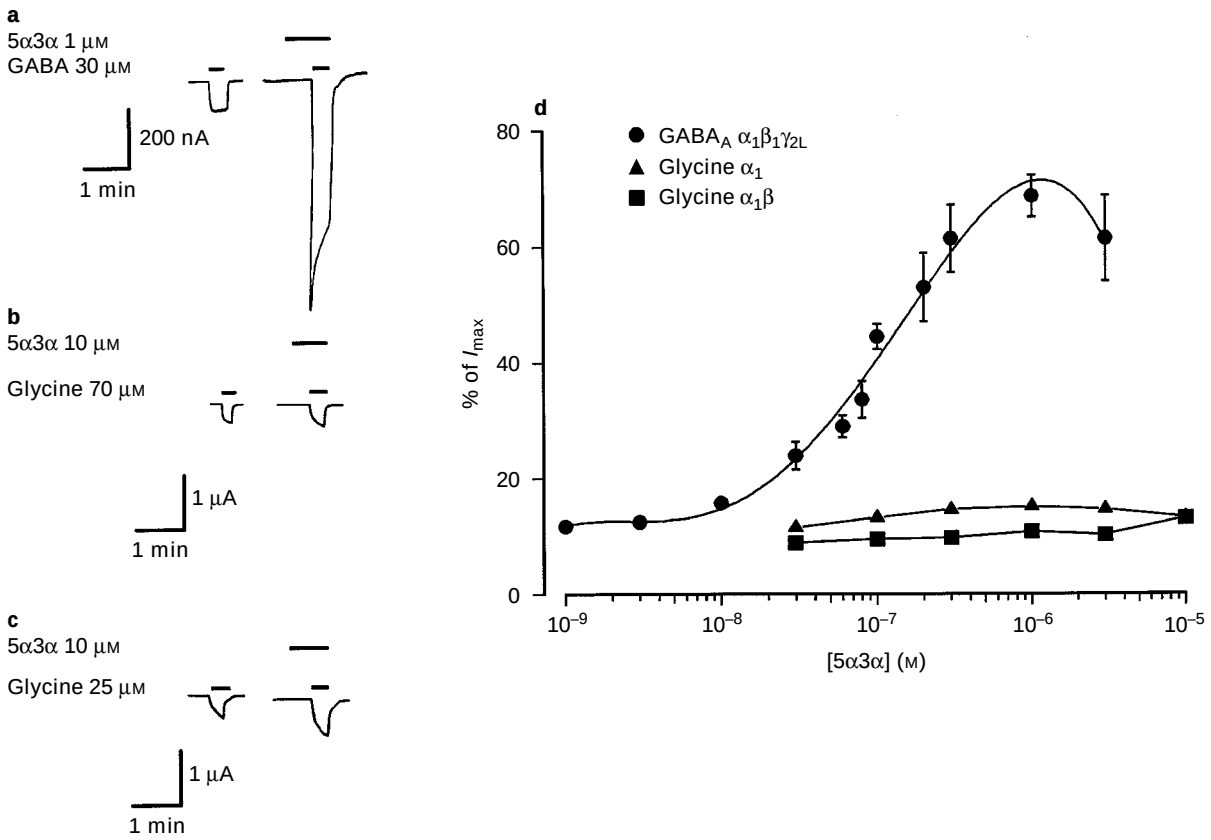

Figure 8 5α-pregnan-3α-ol-20-one (5α3α) was shown to be a selective positive allosteric modulator of the $GABA_A$ receptor. (a) Bath applied 5α3α (1 μM) greatly potentiated the current evoked by 30 μM GABA (approximate EC_{10} in this example) recorded from an oocyte expressing the human $\alpha_1\beta_1\gamma_{2L}$ $GABA_A$ receptor subunit combination. (b and c). By contrast, a ten fold higher concentration of the neurosteroid had little influence on the current evoked by an EC_{10} concentration of glycine recorded from oocytes expressing (b) α_1 homo-oligomeric and (c) $\alpha_1\beta$ hetero-oligomeric glycine receptors. (d) The graph illustrates the relationship between the concentration of bath applied 5α3α (logarithmic scale) and the peak amplitude of the agonist-evoked current (on a linear scale and expressed relative to the maximum current induced by a saturating concentration of agonist). Data show the potentiation of GABA (EC_{10}) at the $\alpha_1\beta_1\gamma_{2L}$ receptors and of glycine (EC_{10}) at homo-oligomeric α_1 and hetero-oligomeric $\alpha_1\beta$ glycine receptors. All records were obtained from oocytes voltage-clamped at −60 mV. Each data point represents the mean and, where greater than the size of the symbol, the associated s.e.mean (vertical lines) of data obtained from 3–4 oocytes.

chloroethanol greater than those required for GABA-modulatory activity on rodent hippocampal neurones are GABA-mimetic (Lovinger *et al*., 1993; Peoples & Weight, 1994). Here, although high concentrations of trichloroethanol did induce a direct inward current this was not mediated by the $GABA_A$ receptor. It is possible that the GABA-mimetic actions of this anaesthetic are subunit-dependent and are not supported by the $\alpha_1\beta_1\gamma_{2L}$ receptors expressed in this study. Trichloroethanol also enhanced glycine-evoked currents over a similar concentration range to those effective at the $GABA_A$ receptor. The EC_{50} for this effect was little influenced by the presence of the β subunit (α_1 = 3.5 mM, $\alpha_1\beta$ = 5.9 mM). Interestingly, the maximal effect was greater for the glycine receptor than for the $GABA_A$ receptor. However, such differences must be interpreted with caution given the probable influence of receptor desensitization upon the peak amplitude of agonist evoked responses recorded from oocytes.

Relevance to anaesthesia

Are the actions of general anaesthetics on $GABA_A$ and glycine receptors relevant to the production of the anaesthetic state? Although an unequivocal answer to this question is difficult, a rather strong case can be made for the involvement of GABA, based on the concentration-dependency and stereoselectivity of anaesthetic action *in vitro* and *in vivo*. The concentration of propofol during total intravenous anaesthesia in man is 6–9 μg ml^{-1} (Shafer, 1993) which, allowing for extensive protein binding (97.8%), equates to an aqueous concentration of 0.75 to 1.1 μM. However, this value may be an underestimate, since in rats the concentration determined in the brain and spinal cord during anaesthesia is approximately nine fold greater than that of plasma (Shyr *et al*., 1995). For etomidate, a plasma concentration of 0.5 μg ml^{-1} is required to maintain anaesthesia in patients premedicated with diazepam and fentanyl (Doenicke *et al*., 1982) which, correcting for protein binding (76.5% in man), translates into a free aqueous concentration of only 0.7 μM (Meuldermanns & Heykants, 1976). However, this value might also be an underestimate, because in rats the concentration of etomidate in the brain exceeds that in plasma (Heykants *et al*., 1975) and, furthermore, premedication may have reduced the anaesthetic requirement in man. In the absence of precise knowledge, we assume low micromolar concentrations of propofol and etomidate to be clinically relevant. Much higher aqueous concentrations of pentobarbitone (approximately 50 μM; Franks & Lieb, 1994) and trichloroethanol (0.8–1.6 mM; Butler, 1948) are required to suppress movement in response to a painful stimulus in the rat or the wink response in the dog, respectively. Unfortunately, the concentration of 5α-pregnan-3α-ol-20-one in the brain during anaesthesia is not known.

A comparison of the above estimates of potency *in vivo* (where available) with the present *in vitro* data reveals that the anaesthetics studied are likely to enhance $GABA_A$ receptor activity at clinically relevant concentrations. For propofol (Orser *et al*., 1994) pentobarbitone (De Koninck & Mody, 1994) and trichloroethanol (Lovinger *et al*., 1993; Peoples & Weight, 1994), this effect is probably manifest as an increase in

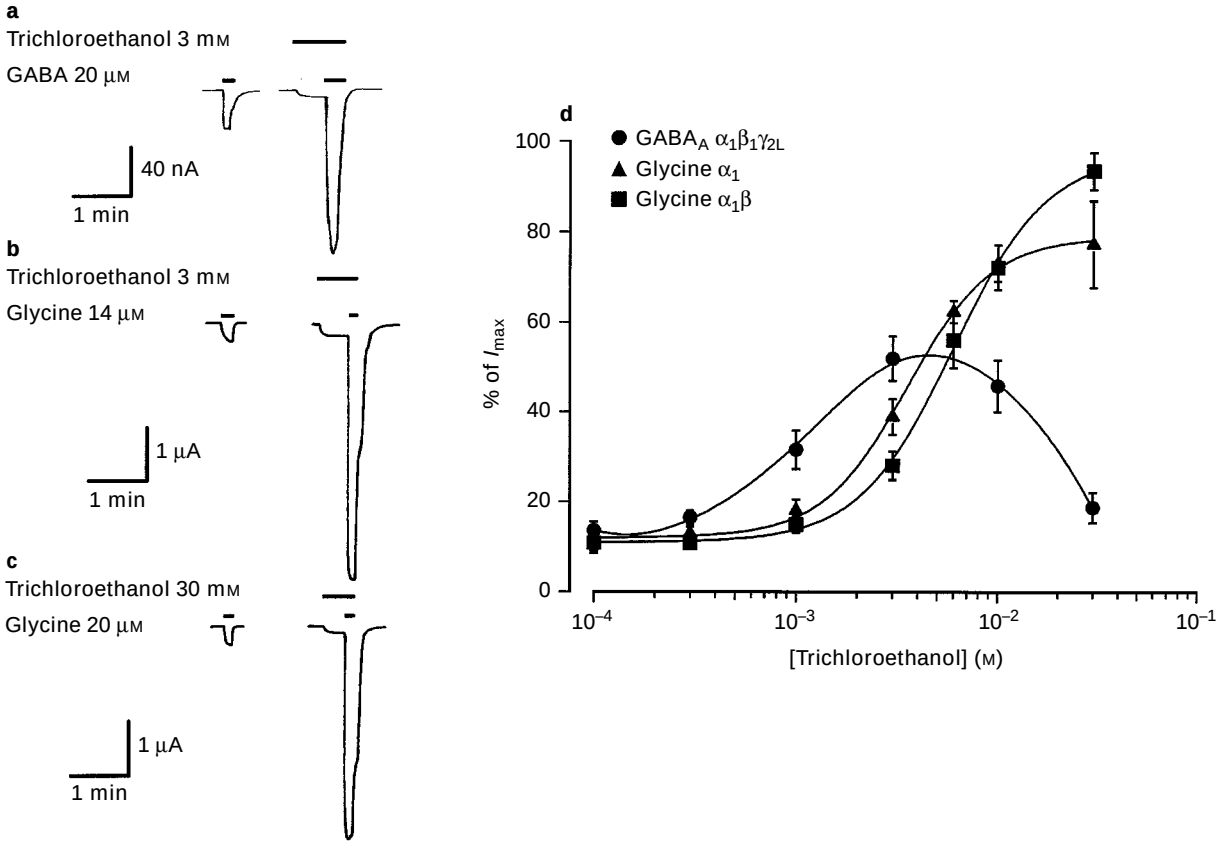

Figure 9 Trichloroethanol was shown to be a positive allosteric modulator of $GABA_A$ and glycine receptors. (a) Bath applied trichloroethanol (3 mM) greatly potentiated the current evoked by 20 μM GABA (approximate EC_{10} in this example) recorded from an oocyte expressing the human $\alpha_1\beta_1\gamma_{2L}$ $GABA_A$ receptor subunit combination (b and c). Trichloroethanol (30 mM) also produces a large enhancement of the current evoked by an EC_{10} concentration of glycine recorded from oocytes expressing (b) α_1 homo-oligomeric and (c) $\alpha_1\beta$ hetero-oligomeric glycine receptors. (d) The graph illustrates the relationship between the concentration of bath applied trichloroethanol (logarithmic scale) and the peak amplitude of the agonist-evoked current (on a linear scale and expressed relative to the maximum current induced by a saturating concentration of agonist). Data show the potentiation of GABA (EC_{10}) at the $\alpha_1\beta_1\gamma_{2L}$ receptors and of glycine (EC_{10}) at homo-oligomeric α_1 and hetero-oligomeric $\alpha_1\beta$ glycine receptors. Each data point represents the mean and associated s.e.mean (vertical lines) of data obtained from 3–4 oocytes.

the duration of the inhibitory post synaptic potential at GABA-ergic synapses (Mody *et al*., 1994). However, GABA-mimetic effects are unlikely to be elicited by the concentrations of anaesthetics encountered clinically. Several additional observations support the contention that modulation of $GABA_A$ receptor activity is a phenomenon central to anaesthetic action. Pentobarbitone, etomidate, and 5α-pregnan-3α-ol-20-one steroids exist as enantiomers with differing anaesthetic potencies. In each case, enantioselectivity in anaesthetic potency (i.e. as seen with the eutomers **S**-(−)-pentobarbitone, **R**-(+)-etomidate and (+)-5α-pregnan-3α-ol-20-one) is paralleled by appropriate stereoselectivity of action at the $GABA_A$ receptor (Christensen & Lee, 1973; Huang & Barker, 1980; Olsen *et al*., 1986; Wittmer *et al*., 1996; Doenicke & Ostwald, 1997; Lambert *et al*., 1997b).

If it is assumed that the aforementioned estimates of anaesthetic potency *in vivo* are not grossly inaccurate, the present data suggest that, in therapeutic use, only propofol and trichloroethanol are likely to exert any significant activity at glycine receptors. Hence, the behavioural effects of these anaesthetics may result from a dual action at these inhibitory receptors. Unusually, over a similar concentration range, trichloroethanol also potentiates the actions of 5-hydroxytryptamine (5-HT) at the excitatory 5-HT_3 receptors (Downie *et al*., 1995). It would be of interest to investigate the actions of this anaesthetic at other members of the ligand-gated ion channel family.

In summary, the present study suggests that the intravenous anaesthetic agents investigated act preferentially at the $GABA_A$ receptor, especially within the clinically relevant range of concentrations. However, pentobarbitone, propofol and trichloroethanol clearly possess the capacity to modulate glycine receptor activity, albeit with lower apparent affinity than at the $GABA_A$ receptor isoform examined. Such commonality of action may be indicative of structurally similarities between these two classes of inhibitory amino acid receptor and might eventually assist in the elucidation of the basis of anaesthetic sensitivity and selectivity at the molecular level.

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