British Journal of Pharmacology (1996) 119, 1331-1336

Enhancement of homomeric glycine receptor function by long-chain alcohols and anaesthetics

[1]Maria Paola Mascia, †Tina K. Machu & *R. Adron Harris

Department of Pharmacology, University of Colorado Health Science Center, Denver, CO 80262; *VA Medical Center Denver, CO 80220 and †Department of Pharmacology, Texas Tech University Health Sciences Center, Lubbock, TX 79430, U.S.A.

1 The effects of n-alcohols (ethanol to dodecanol) and anaesthetics on strychnine-sensitive glycine receptors were studied in *Xenopus* oocytes expressing homomeric α1 or α2 glycine receptor subunits, with the two electrode voltage-clamp recording technique.

2 The glycine-induced chloride conductance of homomeric α glycine receptors was potentiated by all the alcohols tested when an EC_2 concentration of glycine was used. Homomeric α1 and α2 receptors were potentiated similarly by the n-alcohols, except that low concentrations of ethanol produced greater potentiation with α1, as previously reported.

3 Increasing the n-alcohol carbon number has been shown to increase the potency of the alcohols up to decanol at concentrations corresponding to EC_{50}s for producing loss of righting reflex in tadpoles. However, dodecanol was no more potent than decanol, and only modest potentiation (30-60%) was obtained with dodecanol, in contrast to marked (150-200%) potentiation with the other alcohols. Thus, a 'cut-off' occurred at about dodecanol.

4 Propofol, alphaxalone, pentobarbitone, halothane and enflurane, reversibly potentiated the function of homomeric α1 glycine receptors at concentrations which represent approximately twice the EC_{50} for production of anaesthesia in mammals, but ketamine and etomidate were ineffective.

5 Two novel cyclobutane compounds were tested; the anaesthetic compound (1-chloro-1,2,2-trifluorocyclobutane) from 0.5 to 5 mM potentiated the action of glycine in a concentration-dependent manner; however, the non-anaesthetic analogue (1,2-dichloro-hexfluorocyclobutane) had no effect on glycine receptor function at concentrations (25 to 80 μM) predicted to be anaesthetic, based on the lipid solubility of this compound.

6 These results suggest that the α subunits of strychnine-sensitive glycine receptors contain sites of action for n-alcohols, propofol, alphaxalone, pentobarbitone and volatile anaesthetics, but not for ketamine and etomidate. Potentiation of glycine receptor function may contribute to the anaesthetic action of n-alcohols and volatile agents.

Keywords: Anaesthetics; n-alcohols; strychnine-sensitive glycine receptors; α subunits

Introduction

Glycine and γ-aminobutyric acid (GABA) are the most important inhibitory neurotransmitters in the nervous system of vertebrates, as well as many invertebrates. GABA is considered the major inhibitory neurotransmitter in many brain regions, and glycine plays a major role in brain stem and spinal cord. However, glycine receptors are also widely distributed in supraspinal regions, including subcortical structures (Betz, 1991). Both glycine and GABA inhibit neuronal firing by activating receptors linked to an integral chloride channel. These ligand-gated ion channels represent the strychnine-sensitive glycine receptor and the $GABA_A$ subtype of GABA receptor. This type of glycine receptor consists of two different subunits (α and β) that assemble in a pentameric structure with a proposed *in vivo* stoichiometry of 3α and 2β (Betz *et al.*, 1991; Bechade *et al.*, 1994). This pentameric stoichiometry is comparable to that of the nicotinic acetylcholine receptor and to other members of the ligand-gated ion channel receptor superfamily. The α subunits, which contain the binding site for glycine agonists and antagonists (e.g., strychnine) (Vandenberg *et al.*, 1992), exists in 4 subtypes (α 1-4) that differ in developmental and regional expression (Malosio *et al.*, 1991). Moreover, when expressed in *Xenopus* oocytes or cultured mammalian cells, these subunits are able to assemble into functional homomeric glycine receptors whose activation is blocked by the antagonist, strychnine (Taleb & Betz, 1994). The β subunit is not required for ligand binding (Pribilla *et al.*, 1992) and exists as a single subtype that is widely distributed throughout the CNS during all developmental stages (Malosio *et al.*, 1991).

The effects of alcohols and anaesthetics on ion channels gated by GABA and glutamate agonists have been the subject of numerous studies (Sanna & Harris, 1993; Harris *et al.*, 1995; Mihic & Harris, 1996). However, there are relatively few reports of the effects of these drugs on strychnine-sensitive glycine receptors. Recent studies have indicated a positive effect of ethanol on the glycine-receptor complex. Ethanol potentiated glycine receptor function in embryonic spinal neurones of mice and chick (Celentano *et al.*, 1988; Aguayo & Pancetti, 1994), and in synaptoneurosomes prepared from whole rat brain (Engblom *et al.*, 1991). Moreover, we recently demonstrated that ethanol enhances the function of recombinant homomeric glycine receptor expressed in *Xenopus* oocytes (Mascia *et al.*, 1996).

Electrophysiological studies, performed on neurones from the rat nucleus tractus solitarius, show that high concentrations of halothane or enflurane potentiate glycine receptor function (Wakamori *et al.*, 1991). In addition, isoflurane enhanced the glycine response of homomeric α2 glycine receptors expressed in HEK 293 cells (Harrison *et al.*, 1993) and halothane potentiated the glycine receptor function of homomeric α1 and α2 glycine receptor subunit expressed in *Xenopus* oocytes (Machu & Harris, 1994). Moreover, a study performed on embryonic spinal neurones found a considerable potentiation of glycine receptor function by propofol, but not by pentobarbitone (Hales & Lambert, 1991). Similarily, we found

[1] Author for correspondence at: Department of Pharmacology, C236, University of Colorado Health Sciences Center, 4200 E. Ninth Ave., Denver, CO 80262 U.S.A.

that pharmacologically relevant concentrations of propofol potentiated glycine-induced Cl^- currents in homomeric glycine receptors expressed in *Xenopus* oocytes (Mascia *et al*, 1996).

Recent reports emphasize the importance of spinal cord and brain stem circuits in the production of general anaesthesia (Antognini & Schwartz, 1993; Rampil *et al.*, 1993) and raise the possibility that glycine receptors may be an important site of action for these drugs. To define better the actions of alcohols and anaesthetics on glycine receptors, we tested a series of n-alcohols, including long-chain alcohols that produce anaesthesia *in vivo* and show a 'cut-off' for effects on ligand-gated ion channels (Dildy-Mayfield *et al.*, 1996). We also tested several clinically useful anaesthetics as well as two novel compounds which are structurally related, but only one of which produces anaesthesia (Koblin *et al.*, 1994; Mihic *et al.*, 1994). In order to examine the actions of different alcohols and anaesthetics in both α1 and α 2 glycine receptors, we used the *Xenopus* oocyte expression system.

Methods

Oocyte preparation, microinjection and electrophysiological recording

Preparation of the oocytes and microinjection of the cDNA was performed as described elsewhere (Lin *et al.*, 1992). Isolated oocytes were placed in modified Barth's saline (MBS) containing (in mM): NaCl 88, KCl 1, HEPES 10, $MgSO_4$ 0.82, $NaHCO_3$ 2.4, $CaCl_2$ 0.91, and $Ca(NO_3)_2$ 0.33 adjusted to pH 7.5. Glycine receptor subunit cDNAs (α1 or α2, 0.4 ng 30 nl^{-1}) were injected into the animal poles of oocytes by the 'blind' method of Colman (Colman *et al.*, 1984). The injected oocytes were cultured at 15–19°C in sterile MBS supplemented with 10 mg l^{-1} streptomycin, 10,000 units l^{-1} of penicillin, 50 mg l^{-1} of gentamicin, 90 mg l^{-1} theophylline and 220 mg l^{-1} pyruvate.

Oocytes were used for recording on days 1–4 after injection. Oocytes were placed in a rectangular chamber (approximately 100 μl volume) and perfused (2 ml min^{-1}) with MBS with or without drugs, via a roller pump (Cole-Parmer Instrument Co., Chicago, IL, U.S.A.) through 18-gauge polyethylene tubing (Clay Adams Co., Parsippany, NJ, U.S.A.) that delivered the drugs solutions to the recording chamber. The animal poles of oocytes were impaled with two glass electrodes (0.5–10 MΩ) filled with 3M KCl and voltage-clamped at −50 mV with an Axoclamp 2A amplifier (Burlingame, CA, U.S.A.). A strip-chart recorder (Cole-Parmer Instrument Co.) continuously plotted the clamping currents. Glycine was dissolved in MBS and applied for 20 s. Octanol to dodecanol were first dissolved in dimethylsulphoxide (DMSO), then diluted in MBS to a final DMSO concentration not exceeding 0.05%, followed by sonication to facilitate the equilibration with MBS. Oocytes were perfused with ethanol or long chain alcohols for 2 min, to allow for complete equilibration in the bath, before a 20 s, coapplication with glycine. Anaesthetics were perfused for 5 min before being coapplied with glycine for 20 s. A 5 min washout period was allowed between drug applications when the concentrations of drugs used were low (below 5 μM, 200 mM, 10 mM, 350 μM, 26 μM, 9 μM and 6 μM respectively for propofol, ethanol, butanol, exanol, octanol, decanol and dodecanol), and a 15 min washout was allowed for higher concentrations. Propofol and alphaxalone were dissolved in DMSO. The final DMSO concentration in MBS was 0.02%. This concentration of DMSO did not affect the glycine responses. Ketamine, etomidate and pentobarbitone were diluted in MBS from stock aqueous solutions. The solutions of volatile compounds were freshly prepared immediately before use. The loss of concentration from vial to bath was approximately 50–60% for all volatile anaesthetics tested and 50–70% for long chain alcohols from octanol to dodecanol (Dildy-Mayfield *et al.*, 1996). Concentrations given in the figures represent the final bath concentrations.

Materials

Adult female *Xenopus laevis* frogs were obtained from Xenopus I (Ann Arbor, MI, U.S.A.); glycine was obtained by Bio-Rad Laboratories (Hercules, CA, U.S.A.); ethanol was purchased from Aaper Alcohol and Chemical Co. (Shelbyville, KY, U.S.A.); butanol, hexanol, octanol, decanol and dodecanol were purchased from Sigma Chemical Co. (St Louis, MO, U.S.A.); propofol was obtained from Aldrich Chemical Co (Milwaukee, Wi, U.S.A.); halothane was bought from Halocarbons Laboratories (River Edge, NJ, U.S.A.) and enflurane from Anaquest Co. (Madison, WI, U.S.A.); etomidate was obtained from Janssen Pharmaceutica (Beerse, Belgium); alphaxalone was donated by Glaxo (Greenford, UK); ketamine was purchased from Sigma Chemical CO. (St Louis, MO, U.S.A.); F3 (1-chloro-1,2,2trifluorocyclobutane) and F6 (1,2-dichloro-hexafluorocyclobutane) were obtained from PCR Inc. (Gainesville, FL, U.S.A.). All other reagents used were of reagent grade. Human α1, α2 glycine receptor subtypes cDNAs (Grenningloh *et al.*, 1990) were subcloned into the mammalian expression vector pCIS 2 (Sontheimer *et al.*, 1989).

Statistical analyses

Statistical analyses were performed on normalized data using the two-way ANOVA and Fishers' post hoc test, using the SOLO programme (BMPD statistical software, Los Angeles, CA, U.S.A.) running on an IBM compatible computer.

Results

Glycine responses in *Xenopus* oocytes expressing homomeric α1 or α2 glycine receptors were potentiated by ethanol, butanol, hexanol, octanol, decanol and dodecanol (Figure 1). The potencies of these alcohols in enhancing glycine receptor function increased as the carbon chain length was increased. However, this effect was not strictly linear with respect to alcohol chain length. We found a smaller increase in potency between octanol and hexanol than between hexanol and butanol and a very small increase in potency between octanol and decanol. Dodecanol produced a much smaller enhancement than the other alcohols (Figure 1), and tridecanol was almost completely inactive (not shown). The potencies were similar in α1 and α 2 glycine receptors with the exception of the lower concentrations of ethanol, which showed a significantly greater effect on α1 than on α2 receptors (Mascia *et al.*, 1996). Because we previously found that glycine was more potent at α1 ($EC_{50} = 155 \pm 19$) than α2 ($EC_{50} = 257 \pm 14$) receptors (Mascia *et al.*, 1996), we performed all experiments using the same effective concentration of glycine. This was a concentration that produced a 2% of the maximal current observed (60 to 150 nA); i.e. EC_2, and was determined for each oocyte used. Figure 1c shows a sample of inward currents induced by application of an EC_2 concentration of glycine on oocytes expressing α2 glycine receptor subunits. The glycine-induced responses were potentiated by 26 μM octanol. The reversal potential of glycine-induced currents was close to −20 mV. No desensitization was found when low concentrations of glycine were applied, however the desensitization rates became much greater as the glycine concentrations were raised.

To determine if long-chain alcohols enhanced the maximal effect of glycine, we performed glycine concentration- response curves in the absence and presence of low (100 μM) and high (500 μM) concentrations of octanol in oocytes expressing homomeric α2 glycine receptor subunits (Figure 2). Both 100 and 500 μM octanol significantly shifted the glycine concentration-response curve to the left in a parallel manner ($P < 0.04$ and $P < 0.0001$ by ANOVA). Octanol 100 and 500 μM also decreased the glycine EC_{50} from 149 ± 16 (control) to 117 ± 18 (100 μM) and 80 ± 13 (500 μM). The octanol po-

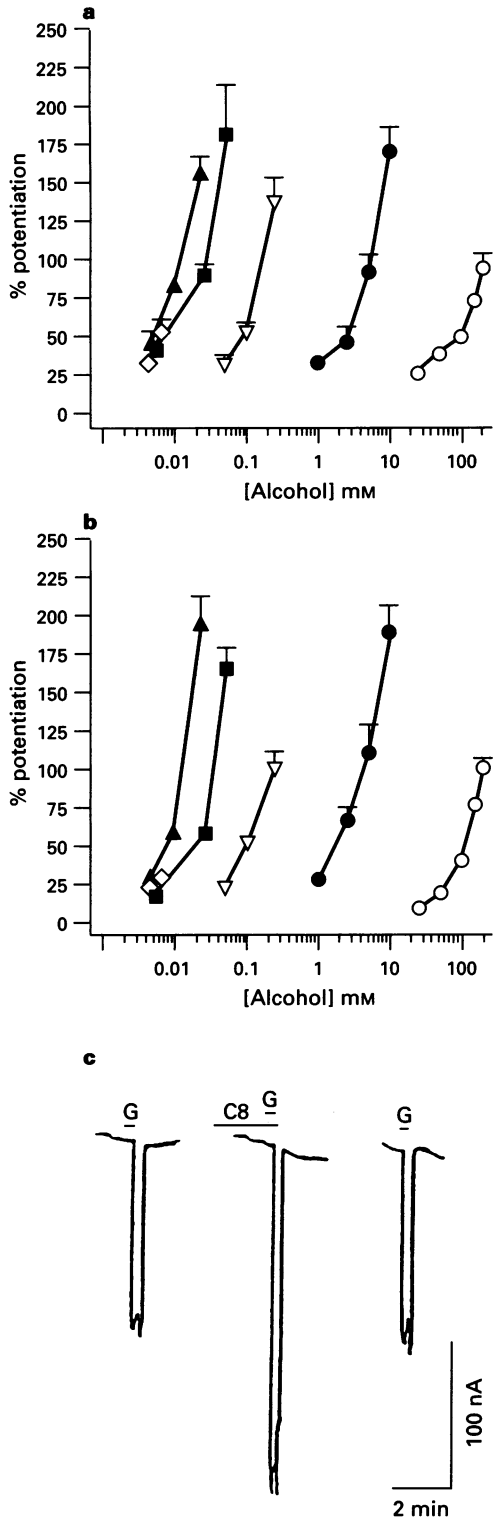

Figure 1 Short and long chain alcohols potentiate homomeric α1 or α2 glycine receptors expressed in *Xenopus* oocytes. Percentage potentiation by ethanol (○), butanol (●), hexanol (▽), octanol (■), decanol (▲) and dodecanol (◇), of currents evoked by an EC_2 concentration of glycine in α1 homomeric glycine receptor subunit (a) or α2 homomeric glycine receptor subunit (b). The EC_2 concentration of glycine, which roughly corresponds to 10 μM glycine for α1 and 14 μM glycine for α2, was determined for each oocyte. Alcohols were preapplied for 2 min before being coapplied with glycine for 20 s. The amplitude of the control glycine response (0% potentiation) was between 60 and 150 nA. Values represent the mean ± s.e.mean of 5 to 8 different oocytes. (c) Sample tracings of glycine induced Cl^- currents in oocytes expressing α2 glycine receptor subunit. The tracings represent the effect of 26 μM octanol (C8) on Cl^- currents evoked by an EC_2 concentration of glycine. Octanol was preapplied for 2 min before being coapplied with glycine for 20 s. The interval between glycine applications was 5 min.

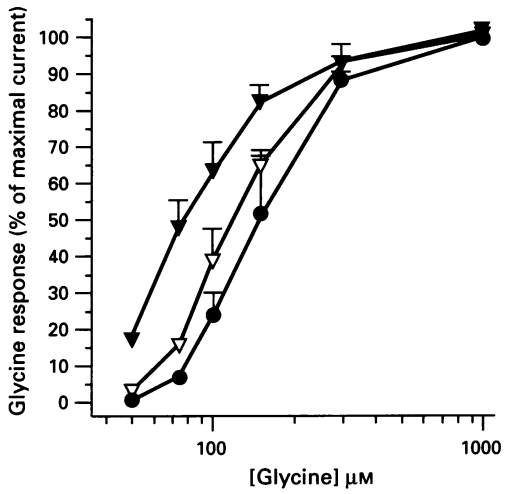

Figure 2 Effect of octanol on the glycine concentration-response of homomeric α2 glycine receptors. The glycine EC_{50} was 149 ± 16 μM in the absence of octanol (●), decreasing to 117 ± 18 and 80 ± 13 μM in the presence of 100 (▽) and 500 (▼) μM octanol respectively. The Hill coefficients were 2.8, 3.2 and 2.8. Data represent the mean ± s.e.mean of 4 to 8 different oocytes. See text for statistics.

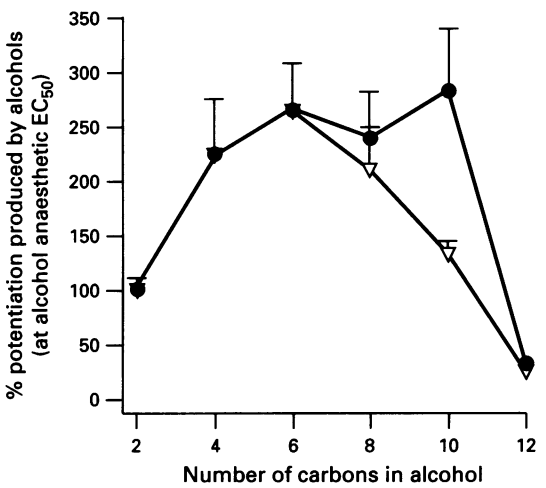

Figure 3 Comparison of potentiation of α1 glycine receptor function by n-alcohols concentrations corresponding to EC_{50}s for producing loss of righting reflex in tadpoles. The percentage potentiation of glycine-induced currents produced by concentrations of alcohols (ethanol-dodecanol) that correspond to their respective EC_{50}s for producing loss of righting reflex in tadpoles (Alifimoff *et al.*, 1989) is plotted as a function of the carbon chain length. Homomeric α1 glycine receptors were used and alcohols were preincubated for 2 min (●) or 20 min (▽) before being coapplied with an EC_2 concentration of glycine for 20 s. Values represent the mean ± s.e.mean of 4 different oocytes.

tentiation was greater with lower concentrations of glycine and minimal or no effects were seen at high glycine concentrations (> 300 μM glycine).

The enhancement of glycine receptor function produced by the alcohols (ethanol-dodecanol), was compared at EC_{50} concentration of these alcohols for producing loss of righting reflex in tadpoles (Alifimoff *et al.*, 1989) (Figure 3). In most experiments, *Xenopus* oocytes expressing the α1 glycine receptor subunits were pre-exposed to these alcohols for 2 min. However, longer chain alcohols may have more difficulty in reaching their site of action than short chain alcohols, so we also performed several experiments pre-exposing oocytes to these alcohols for a longer time (20 min). All alcohols tested potentiated the glycine receptor function when oocytes were

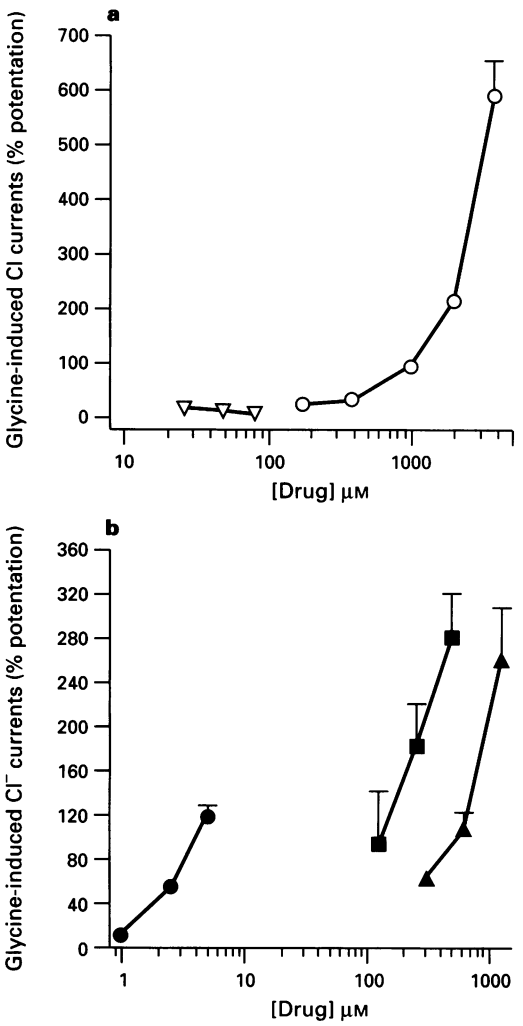

Figure 4 Comparison of effects of an anaesthetic (F3) (○) and non-anesthetic (F6) (▽) cyclobutane derivatives on glycine receptor function (a). The anaesthetic F3, ($(CF_2)_2CF_2CFCl$) potentiated currents evoked by an EC_2 concentration of glycine in oocytes expressing α1 glycine receptors. Lack of effect of the nonanaesthetic F6, ($(CF_2)_2$ $(CFCl)_2$) on glycine receptor function is also shown. The MAC for F3 is 0.8 mM while the predicted MAC for F6 is 18 μM. F3 and F6 were preapplied for 5 min before being coapplied with glycine for 20 s. Values represent the mean ± s.e.mean of 4 to 6 different oocytes. (b) Comparison of effects of volatile anaesthetics and propofol on glycine receptor function. Halothane (■) and enflurane (▲) potentiate, in a concentration-dependent manner, currents evoked by an EC_2 concentration of glycine in oocytes expressing α1 glycine receptor subunits. For comparison the published effect of propofol (●) on α1 glycine receptor (Mascia *et al.*, 1996) is included. Pharmacologically-relevant concentrations of halothane and enflurane which correspond to approximately 0.5, 1 and 2 times the anaesthetic concentrations were preapplied for 5 min before being coapplied with glycine for 20 s. Values represent the mean ± s.e.mean of 6 different oocytes.

pre-exposed either at 2 or 20 min. The longer incubation period did not alter the potentiation produced by butanol, hexanol, or octanol, but decanol was more effective with the longer incubation. A marked 'fall' in potency was found with dodecanol, and this was not affected by the length of the incubation period.

Several anaesthetic compounds were next examined for their ability to potentiate glycine receptor function in *Xenopus* oocytes expressing homomeric α1 glycine receptor subunits. Figure 4a illustrates the effects on homomeric glycine receptor of two novel halogenated compounds: 1-

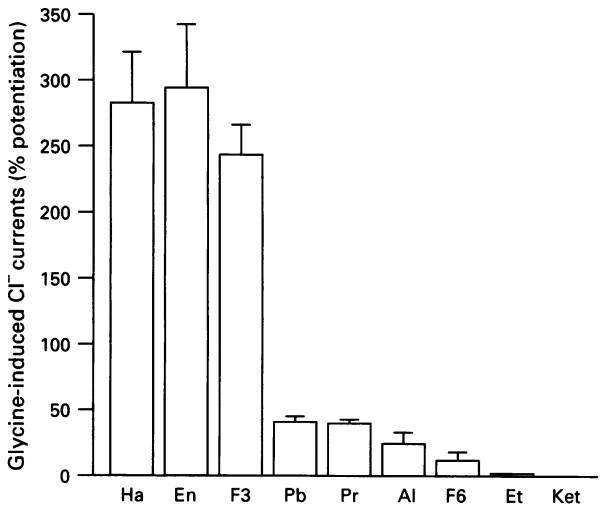

Figure 5 Comparison of the effects of volatile and intravenous anaesthetics (and the non-anaesthetic F6) in enhancing the function of homomeric α1 glycine receptors. The concentrations tested were: 500 μM halothane (Ha), 1240 μM enflurane (En), 1600 μM F3, 100 μM pentobarbitone (Pb), 1.6 μM propofol (Pr), 6 μM alphaxalone (Al), 36 μM F6, 1 μM etomidate (Et) and 365 μM ketamine (Ket). These concentrations represent approximately twice the EC_{50} for production of anaesthesia in mammals. See text for details. Anaesthetics were preapplied for 5 min before being coapplied with an EC_2 concentration of glycine for 20 s. Values represent the mean ± s.e.mean of 5–6 different oocytes.

chloro-1,2,2-trifluorocyclobutane (F3) and 1,2-dichlorohexafluorocyclobutane (F6). Both compounds have a high lipid solubility, and the Meyer-Overton rule predicts that both should act as anaesthetics *in vivo* (Meyer, 1899; Overton, 1901; Koblin *et al.*, 1994). However, only F3 is an anaesthetic and a comparison of the effects of these compounds may be useful in testing candidate sites of anaesthetic action (Mihic *et al.*, 1994). F3 (MAC = 0.8 mM) strongly potentiated chloride currents induced by an EC_2 concentration of glycine. In contrast, F6, despite having a predicted MAC of 18 μM, had no effect on glycine receptor function at concentration up to 80 μM. Anaesthetic and sub-anaesthetic concentrations of the volatile anaesthetics, halothane and enflurane, were also tested on *Xenopus* oocytes expressing homomeric α1 glycine receptor subunits. Their effects on glycine receptor function were compared to those previously reported for the intravenous anaesthetic propofol (Mascia *et al.*, 1996) (Figure 4b). All these compounds potentiated the response induced by an EC_2 concentration of glycine. However, halothane and enflurane were more effective than propofol when these agents were compared at pharmacological relevant concentrations.

The potentiation of homomeric α1 glycine receptor function by F3, F6, halothane, enflurane, propofol, alphaxalone, pentobarbitone, etomidate and ketamine, was compared at concentrations representing approximately 2 times the EC_{50} for producing anaesthesia in mammals. For halothane, enflurane, pentobarbitone, F3 and propofol, anaesthetic concentrations are from Franks & Lieb (1994), Mihic *et al.* (1994) and Harris *et al.* (1995). For alphaxalone and etomidate, the concentrations found in serum (corrected for protein binding) during anaesthesia, are from Sear & Prys-Roberts (1979), Frangen (1994) and Lin *et al.* (1992). The concentration of ketamine, was equivalent to that found in brain during anaesthesia (Lin *et al.*, 1992). Halothane, enflurane and F3 produced the strongest potentiation of glycine receptor function. Alphaxalone, pentobarbitone and propofol weakly stimulated the function of homomeric α1 glycine receptor, while etomidate, F6 and ketamine were completely ineffective (Figure 5).

Discussion

In the present paper, we investigated the effects of n-alcohols and structurally different anaesthetics on the function of human homomeric glycine receptors expressed in *Xenopus* oocytes. Our results clearly show that short and long chain alcohols enhance the function of the glycine receptors. Moreover, we also demonstrate that anaesthetics produce distinct actions on glycine receptor function. Halothane, enflurane, F3, propofol, alphaxalone and pentobarbitone potentiated glycine receptor function, while etomidate, ketamine and the non-anaesthetic cyclobutane derivative (F6) were completely ineffective. The differential sensitivity of the glycine response to these agents argues against membrane lipids as a site of action because the inactive (or weakly active) compounds such as tridecanol, dodecanol, pentobarbitone and alphaxalone are lipid-soluble and known to disorder membrane bilayers (Franks & Lieb, 1994).

Previous reports showed ethanol enhancement of the strychnine-sensitive glycine receptor, including electrophysiological studies performed on spinal neurones of chick (Celentano *et al.*, 1988) and mice (Aguayo & Pancetti, 1994). Moreover behavioural reports suggest the importance of glycine receptors in the central depressant effects induced by ethanol as measured by a loss of righting reflex (Williams *et al.*, 1995). In the present study we extend our previous results with ethanol potentiation of glycine receptor function in *Xenopus* oocytes (Machu & Harris, 1994; Mascia *et al.*, 1996) to the long-chain alcohols.

The n-alcohols tested in this study enhanced the responses of homomeric α glycine receptors with a potency that increased with the carbon chain length up to dodecanol. A possible explanation for this 'cut-off' effect could be, as proposed by Franks & Lieb (1985), the existence of hydrophobic binding pockets, located in receptor proteins and acting as n-alcohol binding sites. According to this model, this pocket cannot completely accommodate alcohols larger than decanol. Thus, dodecanol cannot fit all the carbons into the binding site, and part of the molecular would be forced into a polar environment, with a consequent decrease of the binding affinity. Our 'cut-off' for the n-alcohols is very similar to that found for recombinant human $GABA_A$ receptors ($\alpha1\beta1$ and $\alpha1\beta1\gamma2L$ subunits) expressed in *Xenopus* oocytes (Dildy-Mayfield *et al.*, 1996). Moreover, our result agree with *in vivo* studies performed in tadpoles, which demonstrate that n-alcohol potency increases as the carbon backbone increases, although for tadpoles dodecanol is more effective than would be predicted from the oocyte data (Alifimoff *et al.*, 1989). The effects of decanol were also studied in mice by Dildy-Mayfield *et al.* (1996). In this paper, the concentration of decanol in mouse brain at the time of regaining righting reflex was calculated to be about 1 μM and it is of interest to note that 5 μM decanol potentiated glycine and $GABA_A$, but not glutamate (NMDA, AMPA or kainate) receptors (Dildy-Mayfield *et al.*, 1996). These results, taken together, suggest that glycine and GABA, but not glutamate receptors are important in mediating the effects of higher chain alcohols such as decanol.

Recent reports strongly suggest that spinal cord and brain stem are important targets for the action of general anaesthetics. The spinal cord, for example, has an important role in inhibition of motor responses to noxious stimuli, the most common parameter used to measure the presence of anaesthesia (Collins *et al.*, 1995). Moreover, the importance of these CNS areas in anaesthesia is emphasized by two different *in vivo* studies. Antognini & Schwartz (1993) used a goat model to asses the role of brain stem and spinal cord in producing anaesthesia. When only forebrain was perfused with isoflurane, the concentration required to suppress motor responses to noxious stimuli was about 3 times greater than that required when spinal cord and brain stem also received isoflurane, Furthermore, Rampil *et al.* (1993) demonstrated that the MAC for isoflurane was not altered in rats which underwent precollicular decerebration. Because of the importance of glycine receptors in spinal cord function, these studies raise the question of whether anaesthetics increase this form of synaptic inhibition. Indeed, several electrophysiological studies showed that clinical concentrations of halothane and enflurane potentiate the glycine-induced Cl^- currents in dissociated neurones from the nucleus tractus solitarius of the rat (Wakamori *et al.*, 1991). In addition, Harrison *et al.* (1993) reported that isoflurane positively modulates the function of $\alpha2$ human glycine receptors transiently expressed in HEK 293 cells. Moreover, halothane potentiated glycine receptor function in oocytes expressing $\alpha1$ or $\alpha2$ subunits (Machu & Harris, 1994). In agreement with these reports, we found that sub-anaesthetic and anaesthetic concentrations of halothane and enflurane potentiated glycine receptor function in homomeric glycine receptors expressed in *Xenopus* oocytes. A clear correlation between compounds able to induce anaesthesia *in vivo*, and potentiation of the glycine receptor function was found when we tested two cyclobutane derivatives: the anaesthetic F3 and the non-anaesthetic F6. The anaesthetic F3 potentiated the function of $\alpha1$ glycine receptors but the non-anaesthetic F6 had no effect on glycine-evoked currents. These results are similar to those reported by Mihic *et al.* (1994) for $\alpha1\beta2$ and $\alpha1\beta2\gamma2s$ recombinant $GABA_A$ receptors expressed in *Xenopus* oocytes. Several other anaesthetics including alphaxalone, pentobarbitone, etomidate and ketamine are known to potentiate $GABA_A$ receptor function at concentrations that have no effect on responses to glycine (Simmonds, 1983). In our study, etomidate and ketamine were completely ineffective on glycine receptor function, while alphaxalone and pentobarbitone produced a modest potentiation of homomeric $\alpha1$ glycine receptor function. Our results are in agreement with previous findings, that alphaxalone (Hill-Venning *et al.*, 1992) and pentobarbitone (Hales & Lambert, 1991) had no effect on glycine receptors in cultured neurones.

Use of homomeric receptors allows some conclusions that may be applicable to heteromeric receptors, such as $\alpha+\beta$ glycine receptors, or the more complex $GABA_A$ receptors. First, it is clear that both $\alpha1$ and $\alpha2$ subunits have the sites of action for n-alcohols, up to dodecanol, as well as the sites for volatile anesthetics. However, they lack sites for several intravenous anesthetics (propofol is an exception, but it might be considered a bulky alcohol). Thus, the sites required for alcohols and volatile anesthetics are in regions conserved between $\alpha1$ and $\alpha2$ glycine receptor subunits that may also be homologous to regions in $GABA_A$ subunits. In contrast, the sites for some intravenous anaesthetics are present on $GABA_A$, but not glycine, subunits. Construction of chimaeric receptors combining portions of glycine and $GABA_A$ subunits may be useful in elucidating the molecular sites of alcohol and anaesthetic action on these receptors.

In conclusion, we demonstrate that short and long chain alcohols and several anaesthetics positively modulate homomeric α glycine receptors expressed in *Xenopus* oocytes. This indicates a potential role for the strychnine-sensitive glycine receptor in the depressant actions of these compounds in the central nervous system.

Note added in proof

Findings similar to some of those reported in this paper were recently published by D.L. Downie *et al.* in *Br. J. Pharmacol.*, **118**, 493–502 (1996).

We thank Drs John Mihic, Neil Harrison, Ted Eger and Joan Kending for many helpful discussions and Drs Heinrich Betz and Peter Schofield for kindly providing the $\alpha1$ and $\alpha2$ glycine receptor clones. Supported by funds from the Department of Veterans Affairs (R.A.H.) and NIH grants GM47818 (R.A.H.), AA06399 (R.A.H.), and AA10561 (T.K.M.).

References

AGUAYO, L.G. & PANCETTI, F.C. (1994). Ethanol modulation of the γ-aminobutyric acid$_A$- and glycine-activated Cl^- currents in cultured mouse neurons. *J. Pharmacol. Exp. Ther.*, **270,** 61–69.

ALIFIMOFF, J.K., FIRESTONE, L.L. & MILLER, K.W. (1989). Anaesthetic potencies of primary alkanols: implications for the molecular dimension of the anaesthetic site. *Br. J. Pharmacol.*, **96,** 9–16.

ANTOGNINI, J.F. & SCHWARTZ, K. (1993). Exaggerated anesthetic requirements in the preferentially anesthetized brain. *Anesthesiology*, **79,** 1244–1249.

BECHADE, C., SUR C. & TRILLER A. (1994). The inhibitory neuronal glycine receptor. *BioEssays*, **16,** 735–744.

BETZ, H. (1991). Glycine receptors: heterogeneous and widespread in the mammalian brain. *Trends Neurosci.*, **14,** 458–461.

CELENTANO, J.J., GIBBS, T.T. & FARB, D.H. (1988). Ethanol potentiates GABA and glycine induced currents in chick spinal cord neurons. *Brain Res.*, **455,** 377–380.

COLLINS, J.G., KENDING, J.J. & MASON, P. (1995). Anesthetic actions within the spinal cord: contributions to the state of general anesthesia. *Trends Neurosci.*, **18,** 549–553.

COLMAN, A. Expression of exogenous DNA in *Xenopus* oocytes. (1984). In *Transcription and Translation: a Practical Approach*. ed Hames, B.D. & Higgins, S.J. pp. 49–59. Washington D.C: Oxford Press.

DILDY-MAYFIELD, J.E., MIHIC, S.J., LIU, Y., DEITRICH, R.A. & HARRIS, R.A. (1996). Actions of long chain alcohols on GABA$_A$ but not glutamate receptors correlate with their *in vivo* effects. *Br. J. Pharmacol.*, **118,** 378–384.

ENGBLOM, A.C. & AKERMAN, K.E.O. (1991). Effect of ethanol on γ-aminobutyric acid and glycine receptor-coupled Cl^- fluxes in rat brain synaptoneurosomes. *J. Neurochem.*, **57,** 384–390.

FRANGEN, R.J. (1994). Clinical pharmacology and applications of intravenous anesthetic induction agents. In *The Pharmacological Basis of Anesthesiology*. ed Bowdle, T.A., Horita, A., Kharash, E.D. pp. 319–336. New York: Churchill Livingstone.

FRANKS, N.P. & LIEB, W.R. (1985). Mapping of general anaesthetic target sites provides a molecular basis for cut off effects. *Nature*, **316,** 349–351.

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

GRENNINGLOH, G., SCHMIEDEN, V., SCHOFIELD, P.R., SEEBURG, P.H., SIDDIQUE, T., MOHANDAS, T.K., BECKER, C-M. & BETZ, H. (1990). Alpha subunit variants of the human glycine receptor: primary structures, functional expression and chromosomal localization of the corresponding genes. *EMBO J.*, **9,** 771–776.

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

HARRIS, R.A., MIHIC, S.J., DILDY-MAYFIELD, J.E. & MACHU, T.K. (1995). Actions of anesthetics on ligand-gated ion channels: role of receptor subunit composition. *FASEB J.*, **9,** 1454–1462.

HARRISON, N.L., KUGLER, J.L., JONES, M.V., GREENBLATT, E.P. & PRITCHETT, D.B. (1993). Positive modulation of human γ-aminobutyric acid type A and glycine receptors by the inhalation anesthetic isoflurane. *Mol. Pharmacol.*, **44,** 628–632.

HILL-VENNING, C., PETERS, J.A. & LAMBERT, J.J. (1992). The interaction of steroids with inhibitory and excitatory amino acid receptors. *Clin. Neuropharmacol.*, **15,** 683A–684A.

KOBLIN, D.D., CHORTKOFF, B.S., LASTER, M.J., EGER II, E.I., HALSEY, M.J. & IONESCU, P. (1994). Polyhalogenated and perfluorinated compounds that disobey the Meyer-Overton hypothesis. *Anesth. Analg.*, **79,** 1043–1048.

LIN, L-H., CHEN, L.L., ZIRROLLI, J.A. & HARRIS, R.A. (1992). General anesthetics potentiate GABA actions on GABA$_A$ receptors expressed by *Xenopus* oocytes: lack of involvement of intracellular calcium. *J. Pharmacol. Exp. Ther.*, **263,** 569–578.

MACHU, T.K. & HARRIS, R.A. (1994). Alcohols and anesthetics potentiate glycine receptor responses. *Alcohol Clin. Exp. Res.*, **18,** 517.

MALOSIO, M-L., MARQUEZE-POUEY, B., KUHSE, J. & BETZ, H. (1991). Widespread expression of glycine receptor subunit mRNAs in the adult and developing rat brain. *EMBO J.*, **10,** 2401–2409.

MASCIA, M.P., MIHIC, S.J., VALENZUELA, C.F., SCHOFIELD, P.R. & HARRIS, P.A. (1996). A single amino acid determines differences in ethanol actions on strychnine-sensitive glycine receptors. *Mol. Pharmacol.*, **50,** 402–406.

MEYER, H.H. (1899). Theorie der alkoholnarkose. *Arch. Exp. Pathol. Pharmakol.*, **42,** 109–118.

MIHIC, S.J. & HARRIS, R.A. (1996). Alcohol actions at the GABA$_A$ receptor/chloride channel complex. In *Pharmacological Effects of Ethanol on the Nervous System*. ed Deitrich, R.A. & Erwin, V.G. pp. 51–71. New York: CRC Press.

MIHIC, S.J., MCQUILKIN, S.J., EGER, II, E.I., IONESCU, P. & HARRIS, R.A. (1994). Potentiation of α-aminobutyric acid type A receptor-mediated chloride currents by novel halogenated compounds correlates with their abilities to induce general anesthesia. *Mol. Pharmacol.*, **46,** 851–857.

OVERTON, E. (1901). *Studien uber die narkose zugleich ein beitrag zur allgemeinen pharmacologie*. Jena, Switzerland: Gustav Fischer.

PRIBILLA, I., TAKAGI, T., LANGOSCH, D., BORMANN, J. & BETZ, H. (1992). The atypical M2 segment of the β subunit confers picrotoxinin resistance to inhibitory glycine receptor channels *EMBO J.*, **11,** 4305–4311.

RAMPIL, I.J., MASON, P. & SINGH, H. (1993). Anesthetic potency (MAC) is independent of forebrain structures in the rat. *Anesthesiology*, **78,** 707–712.

SANNA, E. & HARRIS, R.A. (1993). Neuronal ion channels. *Recent Dev. Alcohol.*, **11,** 169–186.

SEAR, J.W. & PRYS-ROBERTS, C. (1979). Plasma concentrations of alphaxalone during continuous infusion of althesin. *Br. J. Anaesth.*, **51,** 861–865.

SIMMONDS, M.A. (1983). Depolarizing responses to glycine, β-alanine and muscimol in isolated optic nerve and cuneate nucleus. *Br. J. Pharmacol.*, **79,** 799–806.

SONTHEIMER, H., BECKER, C-M., PRITCHETT, D.B., SCHOFIELD, P.R., GRENNINGLOH, G., KETTENMANN, H., BETZ, H. & SEEBURG, P.H. (1989). Functional chloride channels by mammalian cell expression of rat glycine receptor subunit. *Neuron.*, **2,** 1491–1497.

TALEB, O. & BETZ, H. (1994). Expression of the human glycine receptor α1 subunit in *Xenopus* oocytes: apparent affinities of agonists increase at high receptor density. *EMBO. J.*, **13,** 1318–1324.

VANDENBERG, R.J., HANDFORD, C.A. & SCHOFIELD, P.R. (1992). Distinct agonist- and antagonist-binding sites on the glycine receptor. *Neuron*, **9,** 491–496.

WAKAMORI, M., IKEMOTO, Y. & AKAIKE, N. (1991). Effects of two volatile anesthetics and a volatile convulsant on the excitatory and inhibitory amino acid responses in dissociated CNS neurones of the rat. *J. Neurophysiol.*, **66,** 2014–2021.

WILLIAMS, K.L., FERKO, A.P., BARBIERI, E.J. & DI GREGORIO, G.J. (1995). Glycline enhances the central depressant properties of ethanol in mice. *Pharmacol. Biochem. Behav.*, **50,** 199–205.

(Received May 16, 1996
Revised August 14, 1996
Accepted August 22, 1996)