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Actions of long chain alcohols on $GABA_A$ and glutamate receptors: relation to *in vivo* effects

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1 The effects of n-alcohols on $GABA_A$ and glutamate receptor systems were examined, and *in vitro* effectiveness was compared with *in vivo* effects in mice and tadpoles. We expressed $GABA_A$, NMDA, AMPA, or kainate receptors in *Xenopus* oocytes and examined the actions of n-alcohols on receptor function using two-electrode voltage clamp recording.

2 The function of $GABA_A$ receptors composed of $\alpha_1\beta_1$ or $\alpha_1\beta_1\gamma_{2L}$ subunits was potentiated by all of the n-alcohols studied (butanol-dodecanol).

3 In contrast to $GABA_A$ receptors, glutamate receptors expressed from mouse cortical mRNA or from cRNAs encoding AMPA (GluR3)- or kainate (GluR6)-selective subunits were much less sensitive to longer chain alcohols. In general, octanol and decanol were either without effect or high concentrations were required to produce inhibition.

4 In contrast to the lack of behavioural effects by long chain alcohols reported previously, decanol produced loss of righting reflex in short- and long-sleep mice, indicating that the *in vivo* effects of decanol may be due in part to actions at $GABA_A$ receptors. Furthermore, butanol, hexanol, octanol, and decanol produce similar potentiation of $GABA_A$ receptor function at concentrations required to cause loss of righting reflex in tadpoles, an *in vivo* model where alcohol distribution is not a compromising factor.

5 Thus, the *in vivo* effects of long chain alcohols are not likely to be due to their actions on NMDA, AMPA, or kainate receptors, but may be due instead to potentiation of $GABA_A$ receptor function.

Keywords: $GABA_A$; NMDA; AMPA; kainate; n-alcohols; *Xenopus* oocytes; loss of righting reflex

Introduction

Both α-aminobutyric $acid_A$ ($GABA_A$) and glutamate receptors are affected by ethanol, suggesting their involvement in the behavioural actions of alcohol intoxication (Huidobro-Toro *et al.*, 1987; Dildy-Mayfield & Harris, 1995b). In examining the actions of a series of n-alcohols on different receptors, it is apparent that alcohols other than ethanol may also have selective sites of action. For example, long chain alcohols were reported to enhance $GABA_A$ receptor function (Nakahiro *et al.*, 1991; Kurata *et al.*, 1993) while having no effect on N-methyl-D-aspartate (NMDA) receptor function (Peoples & Weight, 1995). This lack of effect of long chain alcohols has been termed 'cut-off'. Long chain alcohols also do not affect ATP- (Li *et al.*, 1994) or 5-hydroxytryptamine$_3$-gated channels (Fan & Weight, 1994). Furthermore, short and long chain alcohols may not always share a common site of action at a given receptor (Wood *et al.*, 1991). These studies indicate that within a series of n-alcohols, the potency for altering receptor function does not always correlate with increasing chain length or hydrophobicity, suggesting receptor-specific determinants of alcohol action.

The 'cut-off' in the actions of n-alcohols on NMDA receptors was recently reported to correlate with the behavioural 'cut-off' observed in rodents (Peoples & Weight, 1995). However, the behavioural data (McCreery & Hunt, 1978; Lyon *et al.*, 1981) used for defining the role of NMDA receptors in the *in vivo* action of alcohols may have been misinterpreted due to possibly incomplete distribution of the long chain alcohols to the CNS, as discussed by Lyon *et al.* (1981). To address this possibility, we re-examined the behavioural actions of decanol, an alcohol found to be inactive in an earlier study (McCreery & Hunt, 1978), including measurement of brain decanol levels which have not been previously determined. We then compared the potencies of n-alcohols on defined $GABA_A$ and glutamate receptor subunits expressed in *Xenopus* oocytes in order to define more precisely the roles of these receptors in the behavioural actions of alcohols.

Methods

The n-alcohols were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). For the poorly water soluble alcohols (octanol-dodecanol), stocks were prepared in dimethylsulphoxide (DMSO), and diluted and sonicated in modified Barth's saline (MBS) to a final DMSO concentration not exceeding 0.05%. The alcohols were pre-applied for 30 s to allow for complete equilibration in the bath; for actions on brain kainate responses, octanol alone was exposed for up to 6 h to determine if the lack of effect after only 30 s of pre-exposure was simply due to slow equilibration (see Results). For studies of glutamate receptors and longer chain alcohols, the highest alcohol concentrations attainable, limited by water solubility, were tested.

Mouse cortical mRNA was isolated according to Dildy-Mayfield & Harris (1992). For details on isolation of *Xenopus* oocytes, DNA or RNA microinjection of brain mRNA, glutamate receptors (GluRs), or $GABA_A$ receptors, and two electrode voltage clamp of oocytes, see Dildy-Mayfield & Harris (1992, 1995a) and Mihic *et al.* (1994a,b). GluR6-expressing oocytes were exposed to concanavalin A (1 mg ml^{-1}) to prevent desensitization and to obtain maximal kainate-induced currents (Egebjerg *et al.*, 1991). NMDA responses were recorded in the presence of 10 μM glycine in Mg^{2+}-free MBS.

Bath levels of hexanol-dodecanol were quantified by gas chromatography in a 5890A Hewlett Packard Gas Chromatograph. Alcohols were prepared in MBS, and 0.1 ml samples were taken before and after bath perfusion. Each sample was

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extracted with 0.1 ml of hexane containing an n-alcohol one carbon fewer than the alcohol in the sample. For example, decanol determinations were made with nonanol in hexane as the internal standard. After vortexing for 30 s, the organic phase was collected and samples injected into the injection port (250°C) which was connected to a DB-1 fused silica column (0.53 mm × 30 m). The column temperature was increased as the carbon chain length increased; for example, the initial column temperature was 120°C for hexanol and 200°C for dodecanol. Thus, depending on the n-alcohol, the oven (column) temperature was 120°–200°C for 0.5 min, increasing 5–10°C min^{-1} to a final temperature of 140°–220°C. The nitrogen flow was increased from 7 to 20 psi for determinations of hexanol-dodecanol. The flame ionization detector temperature was 300°C. Although there was no loss of hexanol during oocyte perfusion, there was an approximate 30% loss of heptanol and 50–70% loss of octanol-dodecanol. Polyethylene tubing was used for bath perfusion of all alcohols. The alcohol concentrations in the figures represent corrected bath concentrations.

Long-sleep (LS) and short-sleep (SS) mice obtained from the Institute for Behavioural Genetics (Boulder, CO, U.S.A.) were anaesthetized with pentobarbitone (60 mg kg^{-1}) and chloral hydrate (125 mg kg^{-1}) and indwelling cannulae inserted into the jugular veins. After a two day recovery period, the mice were infused with an emulsion containing corn oil (100 mg ml^{-1}), glycerol (2.25 mg ml^{-1}), and egg lecithin (12 mg ml^{-1}) in water. The concentration and infusion rate of decanol were critical for producing loss of righting reflex without mortality. A decanol concentration of 53.6 mg ml^{-1} and infusion rate of 23.3 μl min^{-1} were optimal for LS mice whereas SS mice required a decanol concentration of 107.2 mg ml^{-1} and infusion rate of 23.3 μl min^{-1}. The sleep time was measured from the time of loss of righting response until the mouse could right itself three times within 1 min after being placed on its back in a plastic trough. At this time, blood (80 μl) samples were obtained from the retro-orbital sinus and the brain was removed for subsequent quantitation of decanol concentration by gas chromatography. Samples were stored at −70°C until analysis. Blood samples (80 μl) were extracted with 160 μl of nonanol (internal standard) in hexane, vortexed, and centrifuged at 1,000 g for 10 min; the upper organic layer was used for injection. For brain samples, the brain was weighed and homogenized with nonanol in hexane. The homogenate (200 μl) was centrifuged at 12,000 g for 10 min and the upper organic layer used for injection. Chromatographic conditions were as described above.

Results

GABA responses in oocytes expressing human $\alpha_1\beta_1$ or $\alpha_1\beta_1\gamma_{2L}$ receptor subunits were potentiated by butanol, hexanol, octanol, decanol, and dodecanol (Figure 1a,b). The GABA concentrations used were 1 and 5 μM for $\alpha_1\beta_1$ and $\alpha_1\beta_1\gamma_{2L}$ receptors, respectively, which represent approximately EC_{10} concentrations for each. The degree of alcohol and anaesthetic potentiation of GABA responses decreases as the GABA concentration is increased (Mihic *et al.*, 1994a,b), and thus low GABA concentrations were used for the alcohol studies. Both constructs responded similarly to these alcohols with large potentiation observed in the presence of even low concentrations of octanol and decanol. The potentiation produced by anaesthetic concentrations of ethanol on these constructs in oocytes has been reported previously not to depend on the presence of specific $GABA_A$ receptor subunits (Mihic *et al.*, 1994b). The alcohol potency for potentiation of $GABA_A$ receptors was dodecanol = decanol > octanol > hexanol > butanol > ethanol. The ED_{50}s in tadpoles for loss of righting reflex at room temperature are 10.8, 0.57, 0.057, 0.037, 0.0126, 0.0081, and 0.0047 mM for butanol, hexanol, octanol, nonanol, decanol, undecanol, and dodecanol, respectively; tridecanol and tetradecanol were nonanaesthetics (Alifimoff *et al.*, 1981). Alcohol concentrations producing 30% potentiation of GABA responses in oocytes expressing $\alpha_1\beta_1\gamma_{2L}$ receptors were 5, 0.2, 0.014, 0.0045, 0.0046, and 0.0054 mM for butanol, hexanol, octanol, decanol, undecanol and dodecanol.

Kainate responses in oocytes expressing mouse cortical mRNA were inhibited by butanol, hexanol, and heptanol; however, very high concentrations of octanol (0.2 mM–1 mM, ~3.5–15 × tadpole ED_{50}) produced only ~20% inhibition while 0.1 mM octanol (~2 × tadpole ED_{50}) and 0.1–0.3 mM nonanol (~2–8 × tadpole ED_{50}) were without effect (Figure 2a). We previously reported the inhibition of kainate responses by methanol to pentanol in oocytes expressing hippocampal mRNA (Dildy-Mayfield & Harris, 1992). The overall alcohol potency for inhibition of brain kainate-mediated responses was heptanol = hexanol > pentanol > butanol > ethanol > methanol.

Because longer chain alcohols might have difficulty reaching

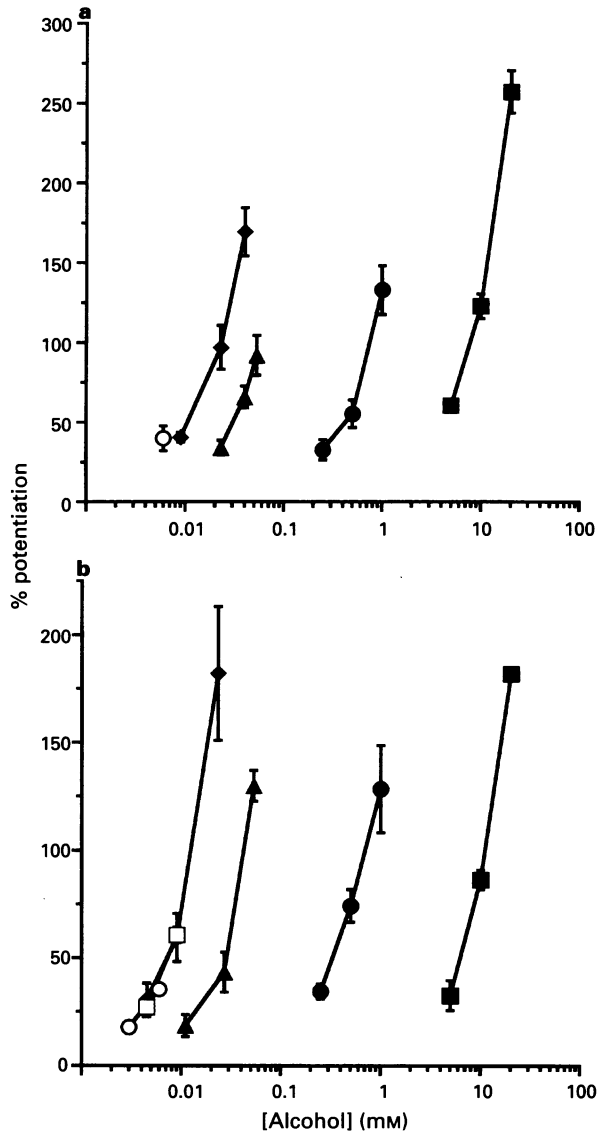

Figure 1 Short and long chain alcohols potentiate $GABA_A$ receptors composed of $\alpha_1\beta_1$ or $\alpha_1\beta_1\gamma_{2L}$ subunits. (a) Percentage potentiation of the effect of GABA (1 μM) by butanol (C4; ■), hexanol (C6; ●), octanol (C8; ▲), decanol (C10; ◆), and dodecanol (C12; ○) in oocytes expressing $\alpha_1\beta_1$ subunits. Data represent the mean ± s.e. of $n = 4–9$ oocytes. (b) Percentage potentiation of the effect of GABA (5 μM) by butanol (C4; ■), hexanol (C6; ●), octanol (C8; ▲), decanol (C10; ◆), undecanol (C11; □), and dodecanol (C12; ○) on oocytes expressing $\alpha_1\beta_1\gamma_{2L}$ subunits. Data represent the mean ± s.e. of $n = 3–5$ oocytes.

their sites of action (Raines & Miller, 1994), we tested the effects of longer pre-exposure times of octanol on kainate responses from brain mRNA expressing oocytes. Octanol (0.05 mM, which is approximately equivalent to the tadpole ED_{50}) applied for up to 30 min had no effect on kainate-induced currents in agreement with its lack of effect after only 30 s of perfusion (data not shown). Furthermore, for oocytes incubated in 0.5 mM octanol ($\sim 8 \times$ tadpole ED_{50}) for 6 h, mean currents in response to 400 μM kainate were not significantly different from control oocytes incubated only in vehicle (0.05% DMSO) (data not shown).

Compared to kainate-mediated currents, NMDA-stimulated currents in oocytes expressing mouse cortical mRNA were more sensitive to inhibition by the alcohols (Figure 2b). The potency for alcohol inhibition of NMDA responses were nonanol = octanol > hexanol > butanol > ethanol. In contrast to kainate responses in oocytes expressing brain mRNA, NMDA responses were inhibited by octanol and nonanol, although high concentrations (0.1 mM and greater) were required to inhibit NMDA responses compared to the low concentrations (0.01 – 0.05 mM) which potentiated $GABA_A$ responses. Unlike $GABA_A$ receptors, NMDA receptors were relatively insensitive to the actions of decanol (Figure 2b).

Chloride channels endogenous to the oocytes may be activated by calcium influx through the NMDA receptor-associated channel (Leonard & Kelso, 1990), and we showed that this chloride current becomes a more prominent component of the NMDA response as the extracellular calcium concentration is increased (Dildy-Mayfield & Harris, 1992). However, in the presence of the physiological extracellular calcium concentration used in the present study, there is minimal contribution of the oocyte's calcium-stimulated chloride current to the NMDA response. Furthermore, we reported that 100 – 200 mM ethanol (Sanna *et al.*, 1994; Dildy-Mayfield & Harris,

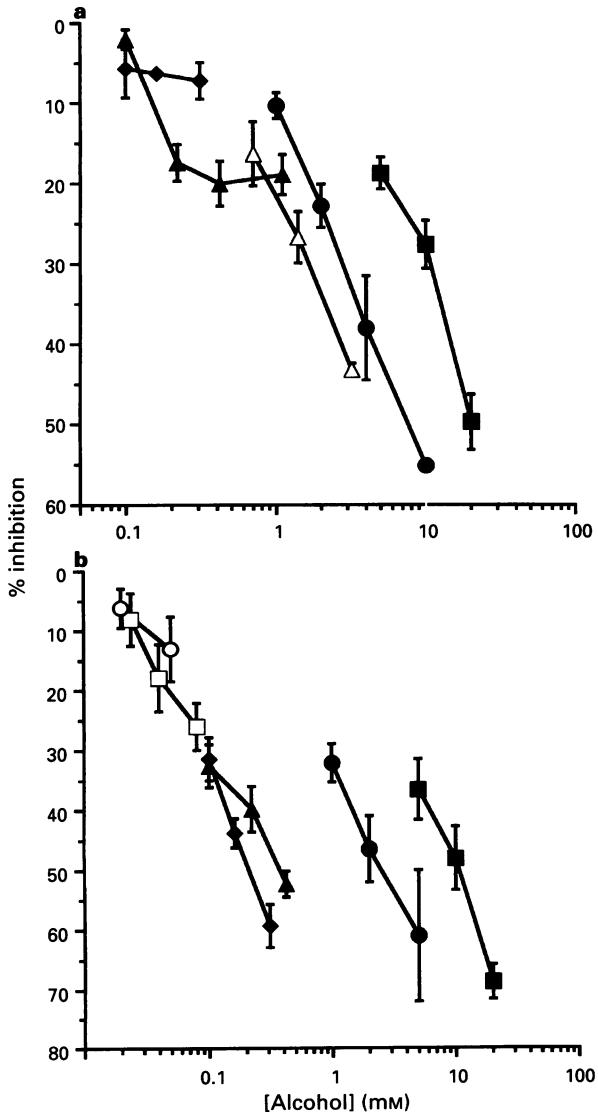

Figure 2 Inhibition of kainate and NMDA responses is greater for short chain than long chain alcohols. (a) Percentage inhibition of the effect of kainate (400 μM) by butanol (C4; ■), hexanol (C6; ●), heptanol (C7; △), octanol (C8; ▲), and nonanol (C9; ◆) in oocytes expressing mouse cortical mRNA. Data represent the mean ± s.e. of $n = 4-12$ oocytes. (b) Percentage inhibition of the effect of NMDA (200 μM + 10 μM glycine) by butanol (C4; ■), hexanol (C6; ●), octanol (C8; ▲), nonanol (C9; ◆), decanol (C10; □), and undecanol (C11; ○) in oocytes expressing mouse cortical mRNA. Data represent the mean ± s.e. of $n = 4-11$ oocytes.

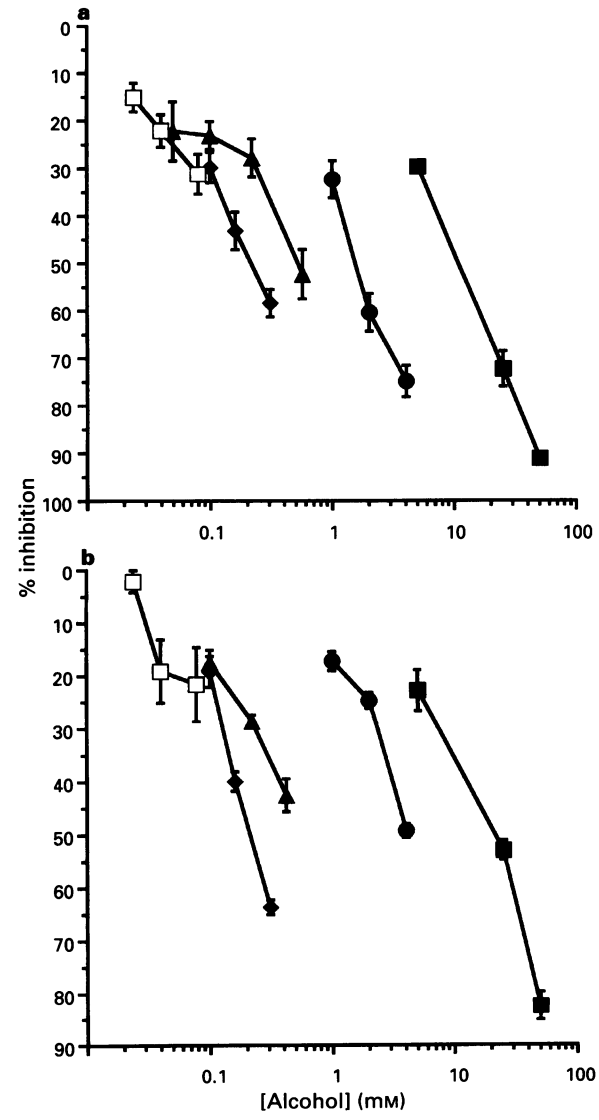

Figure 3 Inhibition of cloned AMPA and kainate-selective receptors is greater for short chain than long chain alcohols. (a) Percentage inhibition of the effect of kainate (400 μM) by butanol (C4; ■), hexanol (C6; ●), octanol (C8; ▲), nonanol (C9; ◆), and decanol (C10; □) in oocytes expressing GluR3 (AMPA) receptors. Data represent the mean ± s.e. of $n = 4-10$ oocytes. (b) Percentage inhibition of the effect of kainate (0.1 μM) by butanol (C4; ■), hexanol (C6; ●), octanol (C8; ▲), nonanol (C9; ◆), and decanol (C10; □) in oocytes expressing GluR6 (kainate) receptors. Data represent the mean ± s.e of $n = 4-10$ oocytes.

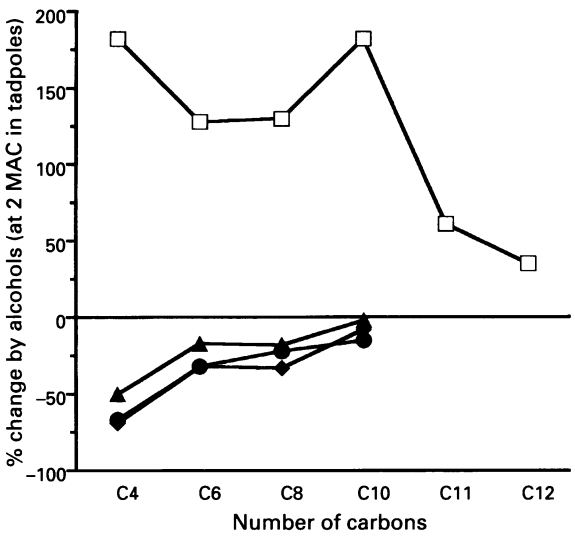

Figure 4 Comparison of changes in $GABA_A$ and glutamate receptor function by alcohol concentrations which produce loss of righting reflex in tadpoles. The percentage change of GABA ($\alpha_1\beta_1\gamma_{2L}$; □) and glutamate (GluR3, ●; GluR6, ▲; NMDA, ◆) receptor function produced by the alcohols butanol-dodecanol (using twice the EC_{50} for producing loss of righting reflex in tadpoles from Alifimoff *et al.*, 1989) was plotted as a function of the carbon chain length.

1995a) and volatile anaesthetics (Lin *et al.*, 1993) have no direct effects on calcium-stimulated chloride channels in oocytes. Our work agrees with that of Ilyn & Parker (1992) who showed that even a very high ethanol concentration (320 mM) produced only a 15% inhibition of the chloride current in oocytes. Thus, effects of alcohols on NMDA responses are likely to be due to direct actions on the NMDA receptor complex and not to indirect interactions with chloride channels.

We next tested the effects of alcohols on specific GluRs expressed in oocytes (Figure 3a,b). Kainate is an agonist at the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor, GluR3, and the kainate-selective receptor, GluR6, although the sensitivity to kainate varied markedly; for example, the kainate EC_{50} is ~1–2 μM for GluR6 receptors compared to ~100 μM for GluR3 receptors (Egebjerg *et al.*, 1991; Dildy-Mayfield & Harris, 1995a). The alcohol sensitivity of these two different subtypes was similar in the presence of a maximal concentration of kainate for GluR3 and a minimal concentration of kainate for GluR6 receptors (Figure 3a,b). However, decreased sensitivity to the alcohols in the presence of a maximal kainate concentration (10 μM) for GluR6 response was observed (data not shown); and, thus, for these studies with GluR6 receptors, a low kainate concentration (0.1 μM) was used in the presence of the different alcohols in order to observe maximal alcohol effects which is similar to the conditions used for $GABA_A$ receptors. Thus, concentrations of agonists were chosen which would allow maximal alcohol effects to be measured, thus ensuring that all receptors were compared under the most favourable conditions. The decreased alcohol sensitivity in the presence of a maximal kainate concentration agrees with our previous work with ethanol which demonstrated differing sensitivities depending on kainate concentration for GluR6 receptors (Dildy-Mayfield & Harris, 1995a). The potency profiles for alcohol inhibition of GluR3 and GluR6 receptors were similar under these conditions: nonanol > octanol > hexanol > butanol > ethanol. Decanol (0.02–0.08 mM) produced only weak inhibition (Figure 3a,b).

As for NMDA, GluR3 channels are calcium-permeable which induces activation of chloride channels under conditions of elevated calcium (Dildy-Mayfield & Harris, 1995a). However, under normal buffer conditions, the kainate current is due primarily to sodium influx (Dildy-Mayfield & Harris, 1995a). EGTA injection did not affect the kainate current in normal buffer, although it reduced the current in a high calcium buffer (Dildy-Mayfield & Harris, 1995a), indicating that there is no detectable contribution of the chloride current in the kainate-stimulated response under normal conditions, as used in this study. As discussed above, effects of alcohols on glutamate receptor activation under the recording conditions presented here are not likely to be confounded by secondary alcohol actions on chloride channels in the oocytes.

Previous *in vivo* studies of longer chain alcohols did not measure brain alcohol levels and the lack of behavioural effects of longer chain alcohols may have been due to incomplete distribution to the CNS (McCreery & Hunt, 1978; Lyon *et al.*, 1981). We tested the effects of decanol injections and observed loss of righting reflex in SS and LS mice. Although different concentrations of decanol were used for LS and SS mice (see Methods), the blood and brain levels at awakening were not different, indicating that the different concentrations required for LS and SS mice can be explained by pharmacokinetic rather than pharmacodynamic mechanisms. The sleep times and brain levels of decanol at awakening were not significantly differently for LS and SS mice, and thus the data were combined. The mean sleep time was 5.7 ± 8 min and the brain decanol level at awakening was 60.1 ± 2.7 μg g^{-1} ($n = 12$). No effect of the vehicle used for decanol infusion (see Methods) was observed (data not shown). If brain tissue is 80% water and the membrane/buffer partition coefficient of decanol is 1910, then the calculated aqueous concentration of decanol in brain at regaining righting reflex is ~1 μM (see McCreery & Hunt, 1978, for values and calculation). This concentration is similar to the low micromolar concentrations (e.g., 5 μM) which potentiate $GABA_A$ receptor function in oocytes.

Because decanol produced loss of righting reflex in mice, it appears that the previous behavioural measures of the lack of long chain alcohol effects in rodents were not accurate. A study of alcohols on loss of righting reflex in tadpoles, however, showed that the alcohol potency increased with chain length up to dodecanol (Alifimoff *et al.*, 1989) which agrees with our *in vivo* results with decanol in mice. Furthermore, the low micromolar ED_{50} for decanol anaesthesia in tadpoles is consistent with the 1 μM aqueous concentration of decanol at regaining righting reflex in mice, suggesting that the tadpole studies are appropriate *in vivo* models for the action of n-alcohols. We plotted the percentage change of glutamate and $GABA_A$ receptor function produced by the alcohols (using twice the concentration that produces loss of righting reflex in tadpoles) as a function of the carbon chain length (Figure 4). For the $GABA_A$ receptor ($\alpha_1\beta_1\gamma_{2L}$ constructs), butanol-decanol produced similar, large potentiation, of GABA responses with the degree of potentiation decreasing (although still significant) for undecanol and dodecanol. In contrast, for glutamate receptors the degree of inhibition declines after butanol with decanol producing no or very weak inhibition. We considered the possibility that the decreased potentiation of $GABA_A$ responses by undecanol and dodecanol could be due to a problem with delivery of alcohol to its site of action. However, when oocytes were preincubated with tadpole ED_{50} concentrations of octanol, decanol or dodecanol for 30 min, we still saw less of an effect with dodecanol than with the other two alcohols (data not shown). There was a greater effect of all three alcohols after the 30 min preincubation compared to the 30 s preincubation, suggesting that pharmacokinetic factors may play a role in the magnitude of the potentiation observed.

Discussion

This is the first paper to compare the effects of alcohols on defined subtypes of $GABA_A$ and glutamate receptor clones and the first *in vivo* study showing loss of righting reflex by decanol in mice. In contrast to previous papers, we measured levels of the n-alcohols in our *in vitro* and *in vivo* studies. We used the same cell type, *Xenopus* oocytes, to examine alcohol action on

each type of receptor to compare directly alcohol sensitivity among classes of receptors in an identical expression system. Our results suggest that $GABA_A$ receptors are preferentially sensitive to the longer chain alcohols in contrast to brain NMDA and kainate responses and AMPA- and kainate-selective glutamate receptors which are relatively insensitive. Although brain kainate and NMDA responses showed somewhat different alcohol potencies and alcohol 'cut-offs', AMPA and kainate receptor clones showed similar alcohol effects as brain NMDA responses, suggesting that the glutamate class of receptors are relatively similar and distinct from the $GABA_A$ class of receptors. The potent actions of longer chain alcohols on $GABA_A$ receptors agree with *in vivo* studies of tadpoles showing increased potency for producing loss of righting reflex as the alcohol carbon chain length was increased up to dodecanol (Alifimoff *et al.*, 1989) and also agrees with our *in vivo* measurements of loss of righting reflex produced by decanol in mice.

Other workers (Wafford *et al.*, 1991; Harris *et al.*, 1995) demonstrated that low concentrations of ethanol (~20 mM) could only enhance the effect of GABA in receptors containing the γ_{2L} subunit. However, Mihic *et al.* (1994b) found that higher ethanol concentrations (>100 mM) could potentiate GABAergic currents even in receptors not containing the γ_{2L} subunit, suggesting that there were separate low- and high-dose effects of ethanol on $GABA_A$ receptors. Since all of the alcohols used in the current study had similar effects on $\alpha\beta$ and $\alpha\beta\gamma$ receptors, it is concluded that these alcohol effects correspond to the high dose ethanol effect reported by Mihic *et al.* (1994b). Kurata *et al.* (1993) also observed that the potentiation of $GABA_A$ responses by octanol did not require the γ subunit. In the oocyte expression system, glutamate (Dildy-Mayfield & Harris, 1992; 1995a) and $GABA_A$ (Mihic *et al.*, 1994a,b) receptors are sensitive to intoxicating and anaesthetic concentrations of ethanol. The present report shows that these receptors also share similar sensitivity to butanol whereas at hexanol the sensitivity of glutamate receptors begins to decline.

Our results in oocytes generally agree with previous reports of alcohol action on $GABA_A$ and glutamate receptors in mammalian cells. Octanol potentiated $GABA_A$ currents in HEK 293 cells expressing $\alpha_1\beta_2$ or $\alpha_1\beta_2\gamma_{2S}$ subunits (Kurata *et al.*, 1993), although longer chain alcohols were not tested. Peoples & Weight (1995) found that nonanol and decanol also potentiated GABA responses in hippocampal neurones. In agreement with these studies, our data from oocytes expressing the $GABA_A$ receptor subunits $\alpha_1\beta_1$ or $\alpha_1\beta_1\gamma_{2L}$ show that these receptors are also potentiated by octanol and decanol and extends the characterization to include undecanol and dodecanol. Shorter chain alcohols have been tested extensively on various NMDA-mediated responses in neurones, and the alcohol potency increased with increasing chain length (Teichberg *et al.*, 1984; Lovinger *et al.*, 1989, 1990; Fink & Gothert, 1990; Gonzales *et al.*, 1991); however, only the n-alcohols methanol-hexanol were studied previously. Our results and those of Peoples & Weight (1995) now extend these observations and show the relative insensitivity of NMDA responses to long chain alcohols. However, we found that NMDA receptors were inhibited by octanol and nonanol, although at higher concentrations than those which affected $GABA_A$ responses. Peoples & Weight (1995) found only ~20% inhibition of mouse hippocampal NMDA-activated currents by 1 mM octanol and no effect of up to 0.5 mM nonanol on these currents whereas we observed approximately 50–60% inhibition by 0.3–0.4 mM octanol and nonanol on NMDA-activated currents in oocytes expressing mouse cortical mRNA. It is possible that the different brain areas studied or the use of oocytes vs. neurones accounts for the different sensitivity of these NMDA receptors to octanol and nonanol, but it should be noted that in contrast to the results of Peoples & Weight (1995), octanol (0.02 mM) was reported to inhibit the mean open time for the NMDA channel in hippocampal neurones (McLarnon *et al.*, 1991). Furthermore, Peoples & Weight (1995) did not measure bath levels of the alcohols, and thus the concentrations of octanol and nonanol which failed to alter NMDA responses may be lower than they reported. Despite these discrepancies, the general conclusion is that NMDA responses are less sensitive to octanol and nonanol than $GABA_A$ responses and are insensitive to decanol, unlike $GABA_A$ responses.

The effects of n-alcohols on AMPA/kainate responses have received little attention. We previously described the effects of methanol to pentanol on AMPA/kainate responses in oocytes expressing hippocampal brain mRNA (Dildy-Mayfield & Harris, 1992) and Teichberg *et al.* (1984) examined the effects of these alcohols on kainate responses in striatal neurones, although effects of longer chain alcohols on brain or cloned AMPA/kainate responses have not been previously tested. Our present results show that, like NMDA, AMPA/kainate receptors expressed from brain mRNA or from cloned AMPA or kainate receptor subunits are also insensitive to longer chain alcohols.

Because similar alcohol effects were obtained using specific AMPA and kainate receptors and brain mRNA expression of AMPA/kainate receptors in oocytes, this suggests that multiple subtypes and assemblies of AMPA/kainate receptors are insensitive to long chain alcohols. Also, the similar alcohol actions on NMDA receptors expressed in hippocampal neurones (Peoples & Weight, 1995) and in oocytes expressing cortical mRNA suggest that different NMDA receptor constructs are relatively insensitive to long chain alcohols. Furthermore, specific $GABA_A$ receptor subunits expressed in oocytes (present paper) or HEK 293 cells (Kurata *et al.*, 1993) show similar alcohol sensitivity of hippocampal $GABA_A$ receptors (Peoples & Weight, 1995). Thus, within a class of receptors, neither the specific receptor subunit nor the cell type in which the subunits (whether endogenous or exogenous) are expressed appear to affect markedly the alcohol sensitivity.

Studies have shown that other ionotropic receptors including ATP and 5-hydroxytryptamine$_3$ (5-HT_3) have 'cut-offs' for relatively short chain alcohols. For example, ethanol and propanol but not butanol or pentanol inhibited ATP-gated channels (Li *et al.*, 1994), and propanol to pentanol but not hexanol or octanol potentiated 5-HT_3 receptor function (Fan & Weight, 1994). In contrast, nicotinic acetylcholine receptors are sensitive to long chain alcohols including undecanol (Murrell *et al.*, 1991), dodecanol, and tridecanol (McKenzie *et al.*, 1995). Some of these studies have discussed the concept of the alcohols binding to a hydrophobic pocket of fixed dimensions on the protein as originally proposed by Franks & Lieb (1985). A small hydrophobic pocket would allow short chain alcohols to bind and therefore cause changes in receptor function whereas long chain alcohols would be unable to fit and therefore be unable to alter receptor function. According to this model, ATP, 5-HT_3, and glutamate receptors would have relatively small alcohol binding sites, thus accounting for the inability of longer chain alcohols to alter their receptor function. Because we observed that glutamate and $GABA_A$ receptors did not exhibit a low or sudden alcohol 'cut-off' as for the ATP receptor, it is unlikely that this model is correct for the receptors studied here. For the nicotinic acetylcholine receptor, short and long chain alcohols have been reported to have distinct sites of action (Wood *et al.*, 1991). Furthermore, the anaesthetic binding sites on neuronal nicotinic acetylcholine receptors have been described as amphiphilic, with polar and apolar portions (McKenzie *et al.*, 1995). According to this model, the binding site for n-alcohols on the $GABA_A$ receptor complex would accommodate alcohols up to decanol, whereas the extension of the carbon backbone beyond decanol would force part of the n-alcohol into a more aqueous environment. This could explain the similar responses produced by decanol, undecanol, and dodecanol at $GABA_A$ receptors. Thus, the anaesthetic binding site may not necessarily exclude alcohols above decanol but rather its size would preclude increased effectiveness of these alcohols. The lack of a sudden alcohol 'cut-off' at $GABA_A$ and glutamate receptors supports this model which could explain the similar responses despite in-

creasing chain length. However, the carbon endpoint at which the apolar binding site is fully occupied would be different for these two classes of receptors.

We disagree with the interpretation reached by Peoples & Weight (1995) using loss of righting reflex in mice (Lyon *et al.*, 1981) and ataxia in rats (McCreery & Hunt, 1978) as behavioural correlates for their *in vitro* alcohol effects on NMDA responses. Peoples & Weight (1995) suggested that the *in vitro* 'cut-off' in alcohol action on NMDA responses at approximately heptanol or octanol corresponded with the *in vitro* 'cut-off' for loss of righting reflex in mice and ataxia in rats which occurred at approximately heptanol. However, the *in vivo* studies by Lyon *et al.* (1981) and McCreery & Hunt (1978) did not measure brain levels of the alcohols tested; and, thus, the lack of behavioural activity of heptanol and longer chain alcohols may have been simply due to incomplete distribution to the brain of these alcohols as discussed by Lyon *et al.* (1981). We present evidence that this explains their results because our *in vivo* work showed that injections of decanol produced loss of righting reflex in mice. We found that the route and rate of decanol injection were important factors in producing loss of righting reflex, and the inability or ability of decanol to produce loss of righting reflex correlated with brain decanol levels. In contrast, the studies by McCreery & Hunt (1978) and Lyon *et al.* (1981) simply used i.p. injections of the alcohols and did not measure brain alcohol levels. Therefore, the behavioural measures showing alcohol 'cut-offs' at heptanol and above were apparently incorrect, making the relationship of *in vitro* NMDA alcohol sensitivity and *in vivo* alcohol actions proposed by Peoples & Weight (1995) also incorrect. Rather the potency for loss of righting reflex in tadpoles which increases with increasing carbon chain length up to dodecanol (Alifimoff *et al.*, 1989) appears to be a more accurate *in vivo* measure of the potency for different alcohols. In this system, the confounding variable of poor or incomplete alcohol distribution is avoided. Based on the alcohol EC_{50}s for loss of righting reflex in tadpoles and our *in vivo* results with decanol, we propose that the *in vivo* alcohol 'cut-off' for producing loss of righting reflex is greater than decanol, in good agreement with the *in vitro* action of alcohols on $GABA_A$ but not glutamate receptors.

Of the ionotropic receptors studied to date, $GABA_A$ and nicotinic are the only receptors which have been reported to be sensitive to long chain alcohols up to dodecanol, suggesting that the binding site for alcohols on these receptor complexes is larger compared to the other ionotropic receptors or that long chain alcohols have a distinct site of action at these receptors which is missing in glutamate and other ionotropic receptors discussed above. Potentiation of $GABA_A$ receptor function with increasing alcohol chain length is not likely to be explained simply on the basis of increasing membrane disorder because glutamate receptor function does not show a corresponding change in function with increasing chain length. Furthermore, the hydrophobicity of a compound is not necessarily a predictor of potency at $GABA_A$ receptors (Mihic *et al.*, 1994a) or of anaesthetic potency *in vivo* (Koblin *et al.*, 1994). These results suggest that $GABA_A$ receptors represent a specific protein site for the behavioural actions of alcohols.

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