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Trichloroethanol potentiation of γ-aminobutyric acid-activated chloride current in mouse hippocampal neurones

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1 The action of 2,2,2-trichloroethanol on γ-aminobutyric acid (GABA)-activated Cl^- current was studied in mouse hippocampal neurones in tissue culture by use of whole-cell patch-clamp recording.

2 Trichloroethanol increased the amplitude of currents activated by 1 μM GABA or 0.1 μM muscimol. Trichloroethanol, 1–25 mM, potentiated current activated by 1 μM GABA in a concentration-dependent manner with an EC_{50} of 3.0 ± 1.4 mM and a maximal response (E_{max}) of 576 ± 72% of control.

3 Trichloroethanol potentiated currents activated by GABA concentrations $< 10\,\mu M$, but did not increase the amplitude of currents activated by concentrations of GABA $\geq 10\,\mu M$. Despite marked potentiation of currents activated by low concentrations of GABA, trichloroethanol did not significantly alter the EC_{50}, slope, or E_{max} of the GABA concentration-response curve.

4 Trichloroethanol, 5 mM, potentiated GABA-activated current in neurones in which ethanol, 10–500 mM, did not. The effect of trichloroethanol was not altered by the putative ethanol antagonist, Ro 15-4513. Trichloroethanol did not potentiate currents activated by pentobarbitone.

5 In the absence of exogenous GABA, trichloroethanol at concentrations ≥ 2.5 mM activated a current that appeared to be carried by Cl^- as its reversal potential changed with changes in the Cl^- gradient and as it was inhibited by the $GABA_A$ antagonists, bicuculline methiodide and picrotoxin.

6 Since trichloroethanol is thought to be the active metabolite of chloral hydrate and other chloral derivative anaesthetics, potentiation of the GABA-activated current in central nervous system neurones by trichloroethanol may contribute to the sedative/hypnotic effects of these agents.

Keywords: $GABA_A$ receptor; trichloroethanol; anaesthetic; hippocampal neurones; chloride current; membrane ion current; alcohol; receptor modulation; neurotransmitter receptor

Introduction

A number of sedative/hypnotic agents have been found to enhance the action of γ-aminobutyric acid (GABA) at the $GABA_A$ receptor subtype in the CNS. Despite great structural diversity, benzodiazepines (Haefely *et al.*, 1975; Choi *et al.*, 1977; MacDonald & Barker, 1978), barbiturates (Nicoll *et al.*, 1975; Ransom & Barker, 1975), anaesthetic steroids (Harrison & Simmonds, 1984; Majewska *et al.*, 1986), ethanol (Nestoros, 1980; Suzdak *et al.*, 1986b; Mehta & Ticku, 1988), and inhalational anaesthetics (Gage & Robertson, 1985; Nakahiro *et al.*, 1989; Jones *et al.*, 1992) have all been reported to enhance $GABA_A$ receptor-mediated cellular responses. As GABA is believed to be the predominant inhibitory neurotransmitter in the brain, enhancement of central GABAergic transmission may mediate or contribute to the CNS depression produced by these agents.

Trichloroethanol is the principal active metabolite of chloral derivative sedative/hypnotic agents, such as chloral hydrate, and is believed to be responsible for the pharmacological effects of these agents (Breimer, 1977; Rall, 1990). Although chloral derivatives have been widely used both clinically and experimentally, the cellular mechanism of action of chloral derivatives or their active metabolite, trichloroethanol, has not been established. Previous studies in the periphery have found that α-chloralose can activate a Cl^- current in frog dorsal root ganglion neurones (Ishizuka *et al.*, 1989), and that trichloroethanol decreases the amplitude and prolongs the duration of acetylcholine-mediated currents at the neuromuscular junction (Pennefather & Quastel, 1980; Sterz *et al.*, 1981). Recently, trichloroethanol has been reported to potentiate $5\text{-}HT_3$ receptor-activated current in nodose ganglion neurones (Lovinger & Zhou, 1993), to increase the amplitude and duration of $GABA_A$-receptor-mediated currents evoked in response to pressure application of GABA in hippocampal neurones in culture and to prolong inhibitory postsynaptic currents between hippocampal neurones in culture or in brain slices (Lovinger *et al.*, 1993). The present study was performed in order to characterize the effect of trichloroethanol on the $GABA_A$ receptor-ion channel complex. Some of the results presented here have been reported previously in preliminary form (Peoples & Weight, 1991).

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Methods

Cultures of hippocampal neurones grown on glial feeder layers were prepared from 15–17 day foetal mice essentially as described by Forsythe & Westbrook (1988). Neurones were maintained in medium containing 95% MEM, 5% heat-inactivated equine serum, and a serum supplement (final concentrations in $\mu g\,ml^{-1}$: corticosterone 4, insulin 10, progesterone 1, putrescine 320, selenium 1, transferrin 200 and triiodothyronine 2); this medium was given half-changes weekly. Neurones were cultured for 1–4 weeks prior to use in experiments.

Patch-clamp recording of whole-cell currents was performed in hippocampal neurones at 25°C using a List EPC-7 or an Axopatch-1D patch-clamp amplifier. Electrodes with tip resistances of 2–5 MΩ were used; series resistances of 3–10 MΩ were compensated by 40–80%. Neurones were superfused at 1–2 $ml\,min^{-1}$ in an extracellular medium containing (in mM): NaCl 150, KCl 5, $CaCl_2$ 2.5, $MgCl_2$ 2, HEPES 10, glucose 10, tetrodotoxin 0.0002–0.001, pH was adjusted to 7.4 with NaOH and osmolality to 340

mosmol kg^{-1} with sucrose. Unless stated otherwise, the patch-pipette contained (in mM): CsCl 140, $MgCl_2$ 2, Mg_4ATP 2, BAPTA 10, HEPES 10, pH was adjusted to 7.4 with CsOH and osmolality to 310 mosmol kg^{-1} with sucrose. Drug solutions were prepared in extracellular medium and in most experiments were applied to neurones by gravity flow using a linear multi-barrel pipette array (diameter of each pipette $\sim$200 μm) placed within 100 μm of the cell body. Cells were constantly bathed in extracellular medium flowing from one barrel (flow rate $\sim$3 μl s^{-1}), and drug solutions were applied by opening a valve connected to another barrel and moving the barrel array so that the desired solution superfused the cell. In some experiments, drug solutions were applied by gravity flow from a single large-bore pipette (diameter $\sim$100 μm) placed within 100 μm of the cell body. Concentrations of agonists producing receptor desensitization (e.g., GABA concentrations $>$1 μM) were applied at intervals of 3–5 min to allow for full recovery from desensitization; low concentrations of agonists were applied at intervals of at least 90 s. Data were displayed on a digital oscilloscope (Nicolet 1090-IIIA) and recorded on a chart recorder (Gould 2400S); in most cases data were also filtered at 2 kHz, digitized at 1–5 kHz and stored on a microcomputer.

Statistical analysis of concentration-dependent data was performed using the nonlinear curve-fitting programme ALLFIT (DeLean *et al.*, 1978). Values reported from concentration-response analysis are those obtained by fitting the data to the logistic equation

$$y = ((E_{max}-E_{min})/(1 + (x/EC_{50})^{-n})) + E_{min},$$

where x and y are concentration and response, respectively, E_{min} is the minimal response, E_{max} is the maximal response, EC_{50} is the half-maximal concentration, and n is the slope factor. Time constants of decay were calculated by fitting data to a single exponential function using the programme NFIT. Statistical comparisons were performed using Student's *t* tests or analyses of variance (in some cases followed by Student's Newman-Keuls ranges tests), as noted. Average values are reported as the mean ± s.e.

Results

Trichloroethanol potentiation of GABA- and muscimol-activated current

Figure 1 illustrates potentiation of currents activated by GABA or muscimol by trichloroethanol. In a typical neurone, 1 μM GABA activated an inward current that was potentiated over fourfold by 2.5 mM trichloroethanol (Figure 1a). Application of 2.5 mM trichloroethanol in the absence of GABA activated only a very small inward current (20 pA).

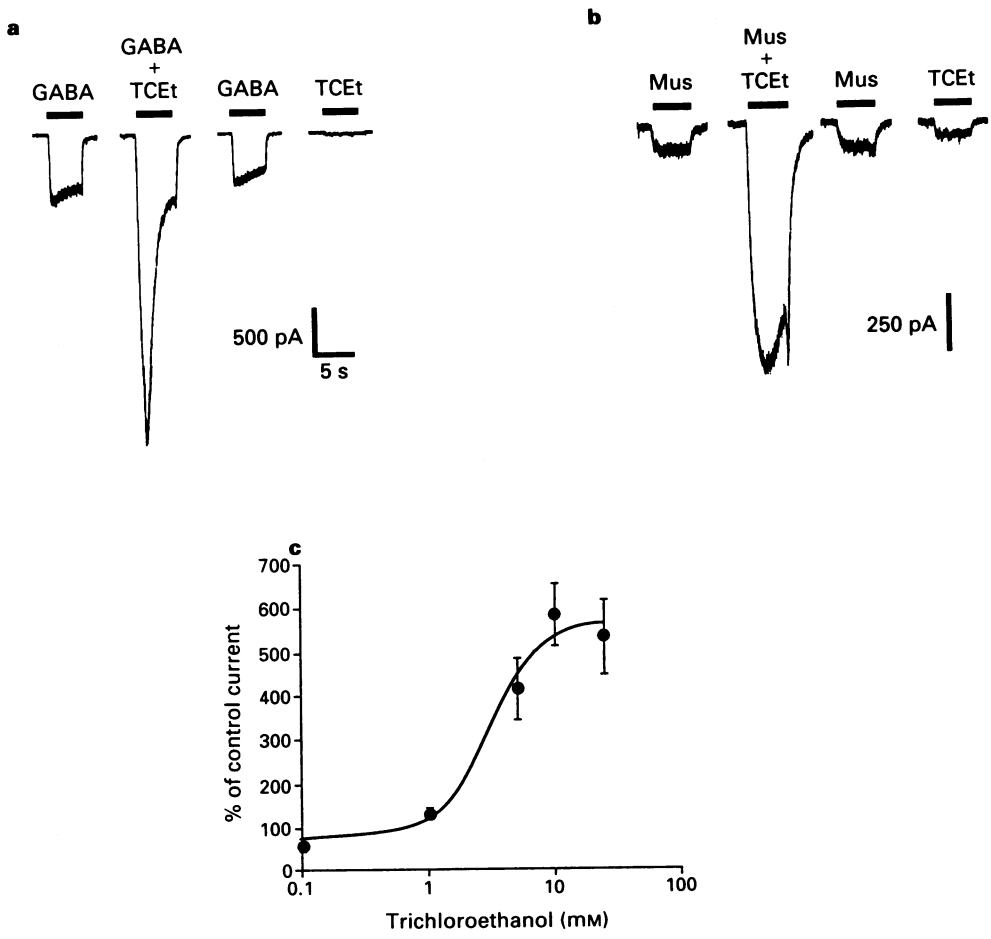

Figure 1 Trichloroethanol potentiation of current activated by GABA agonists. (a) Records of current activated by 1 μM GABA and its potentiation by 2.5 mM trichloroethanol (TCEt). Record at far right shows effect of 2.5 mM trichloroethanol alone (without added GABA). (b) Records of current activated by 0.1 μM muscimol (Mus) and its potentiation by 5 mM trichloroethanol (TCEt). Record at far right shows effect of 5 mM trichloroethanol alone (without added muscimol). Records in (a) and (b) are from different hippocampal neurones. Membrane holding potential was − 50 mV. Solid bar above each record indicates time of agonist and/or drug application, as labelled. (c) Concentration-response curve for potentiation by trichloroethanol of current activated by 1 μM GABA. Each data point is mean ± s.e. of at least 6 neurones voltage-clamped at − 50 mV. The curve shown is the best fit of the data to the logistic equation described in the Methods; the EC_{50} for trichloroethanol was 3.0 ± 1.4 mM, the slope factor was 2.1, and the E_{max} was 576 ± 72% of control.

Trichloroethanol, 1–50 mM, increased the amplitude of currents activated by 0.1–2.5 μM GABA in all neurones tested ($n = 72$). Trichloroethanol also enhanced current activated by the $GABA_A$-selective agonist, muscimol (Figure 1b). In this neurone, 0.1 μM muscimol or 5 mM trichloroethanol, alone, activated small, nondesensitizing currents, whereas the same concentrations of muscimol and trichloroethanol in combination activated a current that was over six times greater than the sum of the individual currents. On average, 5 mM trichloroethanol increased the amplitude of current activated by 0.1 μM muscimol by 569 ± 149% (paired t test, $P < 0.05$; $n = 4$). The enhancement of GABA-activated current amplitude by trichloroethanol exhibited a clear concentration-dependence (Figure 1c). The EC_{50} for trichloroethanol enhancement of current activated by 1 μM GABA was 3.0 ± 1.4 mM, the slope factor was 2.1, and the maximal effect was 576 ± 72% of control.

Dependence of trichloroethanol potentiation on GABA concentration

Figure 2a shows records of currents activated by various concentrations of GABA and their modulation by trichloroethanol in a hippocampal neurone. In this neurone, 5 mM trichloroethanol increased the amplitude of currents activated by 0.25 μM or 2.5 μM GABA by 1150% and 61%, respectively. In contrast, 25 μM GABA activated a large, rapidly-desensitizing current in this neurone that was 86% of control in the presence of 5 mM trichloroethanol.

Figure 2b shows the concentration-response curves for GABA-activated ion current in the absence and presence of 5 mM trichloroethanol. GABA-activated current exhibited a clear concentration-dependence between 1 and 100 μM GABA. The average maximal current activated by GABA in these neurones was 2566 ± 133 pA, the EC_{50} for GABA was 15.4 ± 2.2 μM, and the slope factor was 1.4 ± 0.3. In the presence of trichloroethanol, the average maximal current was 2259 ± 164 pA, the EC_{50} for GABA was 12.2 ± 2.8 μM, and the slope factor was 1.0 ± 0.3; these values did not differ significantly from those of the control GABA concentration-response curve (analysis of variance, $P > 0.05$).

Figure 2c illustrates the dependence of trichloroethanol potentiation of GABA-activated current on GABA concentration. Trichloroethanol, 5 mM, augmented the currents activated by 0.1, 0.25, 1 and 2.5 μM GABA (580 ± 196, 639 ± 142, 514 ± 246, and 300 ± 72% of control, respec-

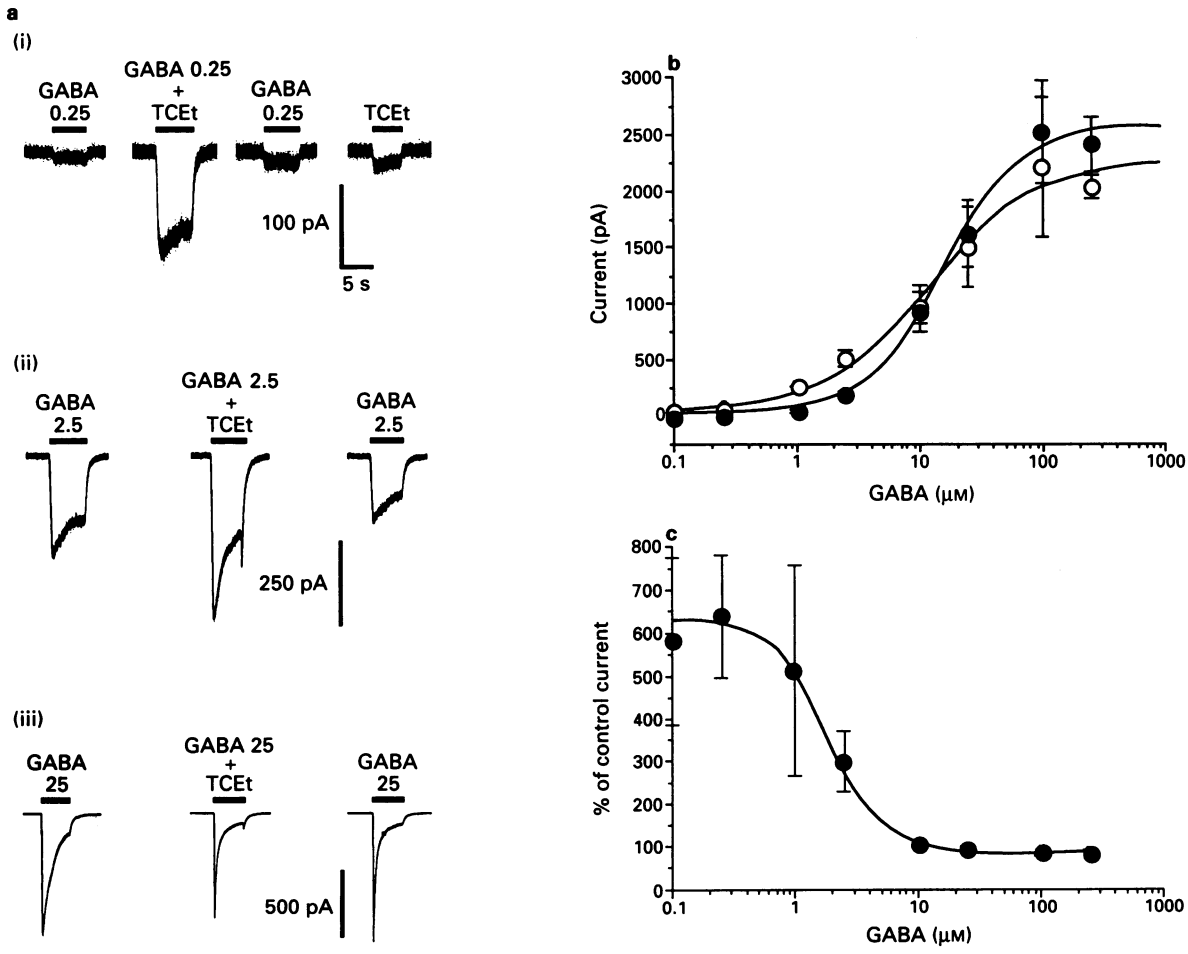

Figure 2 Dependence of trichloroethanol potentiation upon GABA concentration. (a) Records of current activated by 0.25 μM (i), 2.5 μM (ii), and 25 μM (iii) GABA in the absence and presence of 5 mM trichloroethanol (TCEt) in a hippocampal neurone. Record at far right in (i) shows effect of 5 mM trichloroethanol alone (without added GABA). Membrane holding potential was − 10 mV. Solid bar above each record indicates time of agonist and/or drug application, as labelled. (b) Concentration-response curves for GABA-activated current in the absence (●) and presence (○) of 5 mM trichloroethanol. Each data point is the mean ± s.e. of 4–6 neurones. Membrane holding potential was − 10 mV. The curves shown were fitted to the data using the logistic equation described in the Methods. The E_{max} of GABA was 2566 ± 133 pA, the EC_{50} was 15.4 ± 2.2 μM, and the slope factor was 1.4. In the presence of 5 mM trichloroethanol, the E_{max} of GABA was 2259 ± 164 pA, the EC_{50} was 12.2 ± 2.8 μM, and the slope factor was 1.0. These values in the presence of trichloroethanol did not differ significantly from those of the control GABA concentration-response curve (analysis of variance, $P > 0.05$). (c) Plot of potentiation of GABA-activated current by 5 mM trichloroethanol as a function of GABA concentration. Trichloroethanol significantly enhanced currents activated by 0.1, 0.25, 1 and 2.5 μM GABA (repeated measures analysis of variance, $P < 0.05$). The curve shown is the best fit of the data to the logistic equation given in the Methods. Data points are means ± s.e. of 4–6 neurones; error bars not visible are smaller than the size of the symbols.

tively; repeated measures analysis of variance, $P<0.05$), but did not affect the currents activated by 10, 25, 100 and 250 μM GABA (107 ± 10, 94 ± 6, 87 ± 6, 86 ± 6% of control, respectively; repeated measures analysis of variance, $P>0.05$). The lack of a potentiating effect of trichloroethanol on currents activated by GABA at concentrations of 10 μM or greater was also observed when a maximally effective concentration of trichloroethanol, 25 mM, was tested. Trichloroethanol, 25 mM, significantly reduced the amplitude of current activated by 10 μM GABA (2311 ± 366 vs 1344 ± 342 pA in the absence and the presence of trichloroethanol, respectively; paired t test, $P<0.05$; $n=4$) and 25 μM GABA (3518 ± 599 vs 1800 ± 670 pA in the absence and the presence of trichloroethanol, respectively; paired t test, $P<0.05$; $n=4$). In addition, trichloroethanol did not significantly alter the rate of decay of current activated by 10 μM GABA (time constants: 1335 ± 185 vs 1093 ± 225 ms in the absence and presence of 5 mM trichloroethanol, respectively; paired t test, $P>0.1$; $n=5$) and 25 μM GABA (time constants: 787 ± 185 vs 736 ± 70 ms in the absence and presence of 5 mM trichloroethanol, respectively; paired t test, $P>0.5$; $n=6$).

Comparison of ethanol and trichloroethanol effects on GABA-activated current

Ethanol has been reported to enhance responses mediated by $GABA_A$ receptors in the CNS (Nestoros, 1980; Suzdak *et al.*, 1986b; Mehta & Ticku, 1988), although there have also been reports that ethanol does not affect GABA responses in some preparations (Gage & Robertson, 1985; Barker *et al.*, 1987; Harrison *et al.*, 1987; White *et al.*, 1990). We compared the effects of ethanol and trichloroethanol on GABA-activated current in the same neurones. In order for the comparison to be valid, we chose to use an anaesthetic concentration of trichloroethanol (see Discussion) and concentrations of ethanol below, in, and above the anaesthetic range (60–100 mM; Little, 1991). Figure 3a shows records of ion current evoked by GABA in the absence and presence of trichloroethanol or ethanol in a hippocampal neurone. In this cell, the amplitude of current activated by 1 μM GABA was enhanced by 5 mM trichloroethanol (274% of control), but was not affected by 100 or 500 mM ethanol (116 and 111% of control, respectively). Figure 3b illustrates that 10–500 mM ethanol did not affect the average amplitude of current activated by 1 μM GABA (analysis of variance and Student's Newman-Keuls ranges test, $P>0.05$; $n=6$). In contrast, in the same neurones, the average current activated by 1 μM GABA in the presence of 5 mM trichloroethanol was 310 ± 76% of control (analysis of variance and Student's Newman-Keuls ranges test, $P<0.01$; $n=6$).

Effect of the putative ethanol antagonist, Ro 15-4513

The augmentation by ethanol of responses mediated by $GABA_A$ receptors has been reported to be antagonized by the imidazodiazepine partial-inverse agonist Ro 15-4513 (ethyl 8-azido-5,6-dihydro-5-methyl-6-oxo-4H-imidazo-[1,5-a][1,4] benzodiazepine-3-carboxylate) (Suzdak *et al.*, 1986a; Harris *et al.*, 1988). Figure 4 illustrates that the enhancement

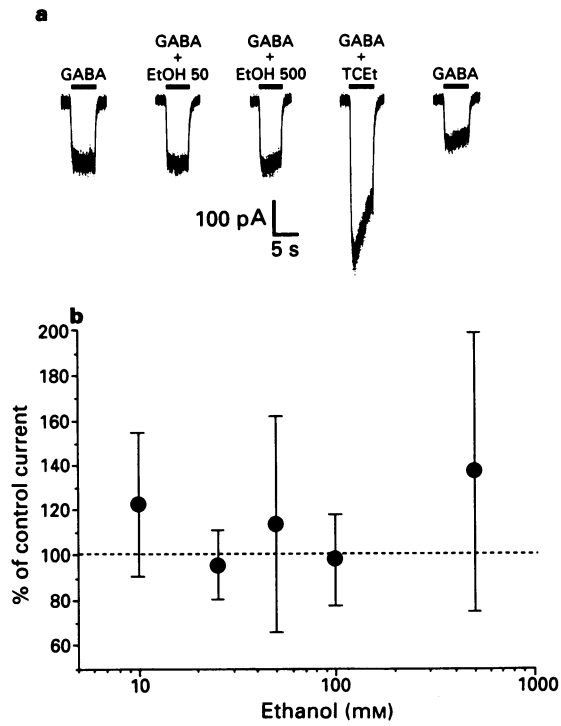

Figure 3 Comparison of the effects of ethanol and trichloroethanol on GABA-activated current in the same neurones. (a) Records of ion current activated by 1 μM GABA in the absence and presence of 50 mM ethanol (EtOH 50), 500 mM ethanol (EtOH 500), or 5 mM trichloroethanol (TCEt) in a single hippocampal neurone voltage-clamped at − 50 mV. Similar results were obtained in 5 other neurones tested. (b) Concentration-response for ethanol effect on current activated by 1 μM GABA. Data are means ± s.e. of 6 neurones voltage-clamped at − 50 mV. Ethanol at concentrations from 10 to 500 mM did not significantly affect GABA-activated current (analysis of variance and Student's Newman-Keuls ranges test, $P>0.05$). In the same neurones, in the presence of 5 mM trichloroethanol, current activated by 1 μM GABA was 310 ± 76% of control (analysis of variance and Student's Newman-Keuls ranges test, $P<0.01$).

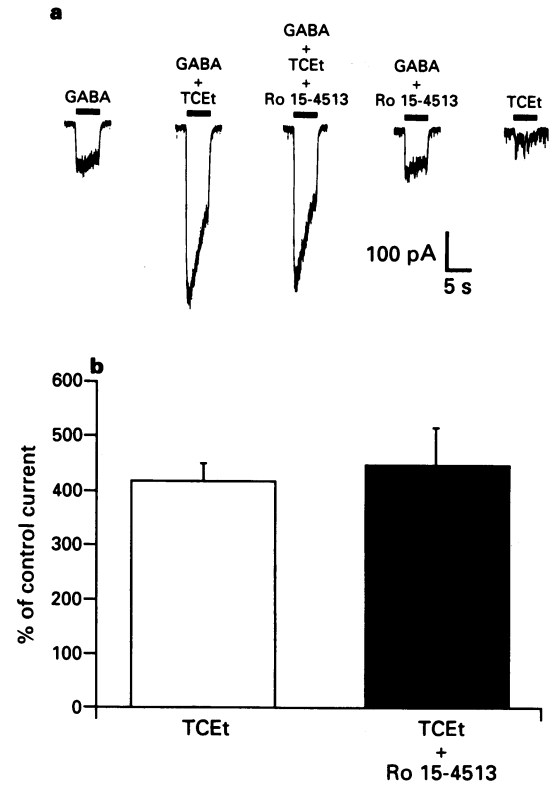

Figure 4 Effect of putative ethanol antagonist, Ro 15-4513, on trichloroethanol potentiation of GABA-activated current. (a) Records of current activated by 1 μM GABA in the absence and presence of 2.5 mM trichloroethanol (TCEt) and 1 μM Ro 15-4513, as labelled. Membrane holding potential was − 50 mV. (b) Comparison of average effect of 2.5 mM trichloroethanol (TCEt) on current activated by 1 μM GABA, plotted as percentage of control GABA-activated current, in the absence and presence of 1 μM Ro 15-4513, as labelled. Results are means ± s.e. of 5 neurones. Membrane holding potential was − 50 mV. There was no significant difference between the effect of trichloroethanol in the absence or presence of Ro 15-4513 (paired t test, $P>0.5$).

of GABA-activated current by trichloroethanol was similar in the presence of 1 μM Ro 15-4513. Ro 15-4513 at this concentration did not alter the amplitude of the GABA-activated current. On average, the enhancement of GABA-activated current by trichloroethanol did not differ significantly in the absence and presence of 1 μM Ro 15-4513 (414 ± 32 vs 444 ± 71% of control, respectively; paired t test, $P > 0.5$; $n = 5$).

Effect of trichloroethanol on pentobarbitone-activated current

The $GABA_A$ receptor-ionophore complex can be activated in the absence of exogenous GABA by barbiturate anaesthetics at high concentrations (Mathers & Barker, 1980; Schulz & MacDonald, 1981). To assess whether trichloroethanol enhances pentobarbitone-activated current in a manner similar to GABA-activated current, current was activated by pentobarbitone in the absence and presence of trichloroethanol. Unlike the GABA-activated current, the current activated by low concentrations of pentobarbitone (10 and 100 μM) was not potentiated by trichloroethanol (repeated measures analysis of variance, $P > 0.05$, $n = 4$). In addition, the concentration-response curve for pentobarbitone-activated current was not significantly altered by 5 mM trichloroethanol (Figure 5). In the absence of trichloroethanol, pentobarbitone had an EC_{50} of 428 ± 38 μM, an E_{max} of 1305 ± 59 pA, and a slope factor of 1.5 ± 0.2, whereas in the presence of trichloroethanol, pentobarbitone had an EC_{50} of 441 ± 45 μM, an E_{max} of 1073 ± 53 pA, and a slope factor of 1.7 ± 0.3; these values did not differ significantly (analysis of variance; $P > 0.05$).

Ionic basis and pharmacology of current activated by trichloroethanol

As noted above, at concentrations of 2.5 mM or greater, trichloroethanol activated an inward current in the absence of exogenous GABA. We studied the ionic basis of the current activated by trichloroethanol by altering the ionic content of the patch-pipette solution, which dialyzes the interior of the neurones under study. The top set of records in Figure 6a illustrates that when CsCl was in the patch-pipette solution, the trichloroethanol-activated current was inward at a holding potential of − 20 mV and outward at + 20 mV. In contrast, when the patch-pipette solution contained Cs methanesulphonate in place of CsCl, the trichloroethanol-activated current was outward at both − 20 and + 20 mV (Figure 6a, bottom traces).

The graph in Figure 6b plots the average amplitude of currents activated by 25 mM trichloroethanol as a function of membrane holding potential in neurones internally dialyzed with a solution containing CsCl (open circles) or Cs methanesulphonate (closed circles). The reversal potential of trichloroethanol-activated current was − 0.8 mV when CsCl was in the patch-pipette solution, but shifted to − 50.6 mV when CsCl was replaced with Cs methanesulphonate in the intracellular solution. The reversal potential of GABA-activated current was − 46.0 mV when the recording pipette contained Cs methanesulphonate (not shown).

Figure 7 shows that current activated by trichloroethanol was reversibly inhibited by the $GABA_A$ antagonists, bicuculline methiodide (Figure 7a) or picrotoxin (Figure 7b). The average amplitude of the current activated by 10 mM trichloroethanol was 73 ± 18 pA under control conditions, and − 8 ± 5 pA in the presence of 50 μM bicuculline methiodide (paired t test, $P < 0.005$; $n = 6$ cells). The inhibition of trichloroethanol-activated current by picrotoxin was more gradual in onset but produced a similar degree of inhibition: the steady-state amplitude of the current activated by 25 mM trichloroethanol was 38 ± 16 pA under control conditions, and − 3 ± 5 pA in the presence of 100 μM picrotoxin (paired t test, $P < 0.05$; $n = 6$ cells). In 5 of 12 cells tested, 10 or 25 mM trichloroethanol in the presence of $GABA_A$ receptor antagonists induced a small-amplitude (5–30 pA) outward current (as indicated by the negative values above).

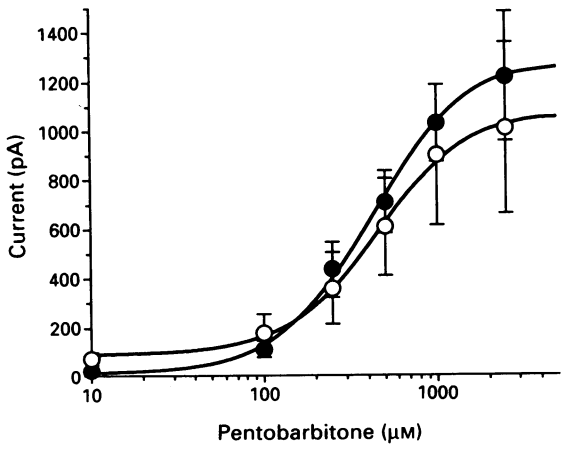

Figure 5 Effect of trichloroethanol on pentobarbitone-activated current. Concentration-response curves for pentobarbitone-activated current, without added GABA, in the absence (●) and presence (○) of 5 mM trichloroethanol. In the absence of trichloroethanol, pentobarbitone had an EC_{50} of 428 ± 38 μM, an E_{max} of 1305 ± 59 pA, and a slope factor of 1.5 ± 0.2, and in the presence of trichloroethanol, pentobarbitone had an EC_{50} of 441 ± 45 μM, an E_{max} of 1073 ± 53 pA, and a slope factor of 1.7 ± 0.3; these values were not significantly different (analysis of variance; $P > 0.05$). The curves shown are the best fits of the data to the logistic equation given in the methods. Data points are means ± s.e. of 4 neurones. Membrane holding potential was − 10 mV.

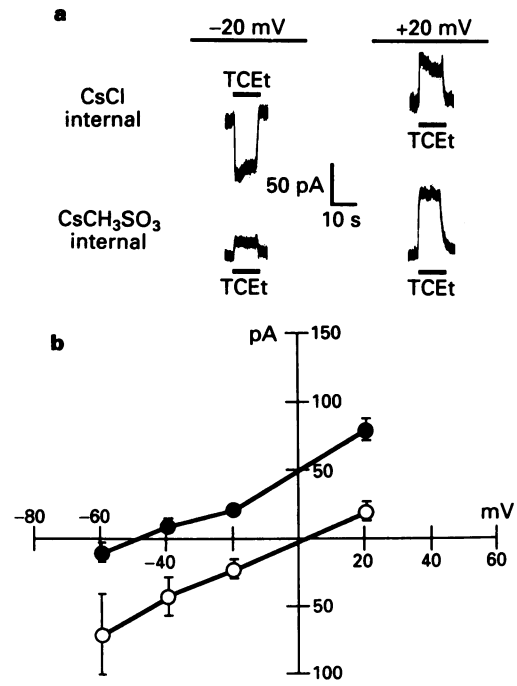

Figure 6 Effect of intracellular Cl^- concentration on trichloroethanol-activated current. (a) Records of current activated by 25 mM trichloroethanol (TCEt) in neurones internally perfused with solutions containing CsCl (upper traces) or Cs methanesulphonate ($CsCH_3SO_3$) (lower traces) at holding potentials of − 20 mV and + 20 mV, as indicated. The Cl^- concentration in the $CsCH_3SO_3$ recording pipette solution was 4 mM. (b) Current-voltage plot for trichloroethanol-activated current in neurones internally perfused with a solution containing CsCl (○) or $CsCH_3SO_3$ (●). Data points are the means ± s.e. obtained from 4 neurones. Note that the reversal potential of the trichloroethanol-activated current with $CsCH_3SO_3$ in the patch-pipette was approximately 50 mV negative to the reversal potential with CsCl in the patch-pipette.

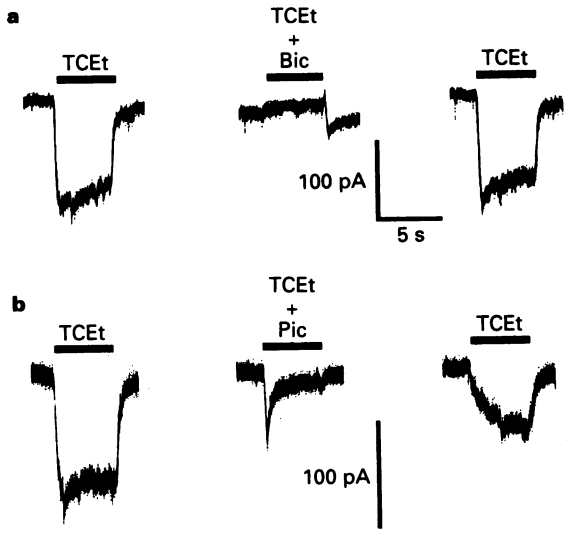

Figure 7 Effect of $GABA_A$ antagonists on trichloroethanol-activated current. (a) Records of current activated by 10 mM trichloroethanol (TCEt) in the absence and presence of 50 μM bicuculline methiodide (Bic). (b) Records of current activated by 25 mM trichloroethanol (TCEt) in the absence and presence of 100 μM picrotoxin (Pic). Records in (a) and (b) are from different neurones; holding potential of neurones was − 50 mV.

Discussion

In this investigation, we found that trichloroethanol could potentiate ion current activated by GABA or the $GABA_A$ agonist muscimol in cultured hippocampal neurones. Trichloroethanol increased the amplitude of the current activated by 1 μM GABA in a concentration-dependent manner, with an EC_{50} of 3 mM and an E_{max} of 576% of control. These results are in accord with the observations of Lovinger *et al.* (1993), who reported that 0.2–10 mM trichloroethanol increased the amplitude and duration of current activated by pressure application of GABA in cultured hippocampal neurones and prolonged inhibitory postsynaptic currents in hippocampal neurones in culture or in brain slices. However, this action of trichloroethanol appears to differ from the actions of other sedative/hypnotic agents on the $GABA_A$ receptor. Other sedative/hypnotic or anaesthetic agents that modulate $GABA_A$ responses, including benzodiazepines (Choi *et al.*, 1981; Little, 1984, Yu *et al.*, 1988), barbiturates (Barker & Ransom, 1978; Akaike *et al.*, 1985), ethanol (Mehta & Ticku, 1988), and hypnotic steroids (Harrison & Simmonds, 1984; Morrow *et al.*, 1988; 1990) in most cases shift the concentration-response curves for $GABA_A$ agonists to the left without altering the maximal response, suggesting an increase in the affinity of the receptor for agonist. Studies on $GABA_A$ receptors expressed in *Xenopus* oocytes suggest that volatile anaesthetics may also act by shifting the concentration-response curve for the $GABA_A$ receptor to the left (Lin *et al.*, 1993). In some cases, barbiturates (Higashi & Nishi, 1982; Yu *et al.*, 1988; Horne *et al.*, 1993), benzodiazepines (Biscoe & Duchen, 1985), and anaesthetic steroids (Horne *et al.*, 1993) have also been reported to increase the maximal response to $GABA_A$ agonists. In the present study, however, enhancement of GABA-evoked ion current by trichloroethanol was observed only at concentrations of GABA < 10 μM, with the result that trichloroethanol did not significantly alter either the EC_{50} or the E_{max} of the GABA concentration-response curve. There are several possible reasons for the preferential action of trichloroethanol at low agonist concentrations. First, increasing agonist concentrations in the presence of trichloroethanol may result in a very rapid onset of receptor desensitization that attenuates the amplitude of GABA-activated current. Second, trichloroethanol may enhance the rate of agonist-activated channel opening from the closed state, but not from the desensitized state. Thus, trichloroethanol might augment currents activated by low GABA concentrations, at which many of the ion channels are in the closed, non-desensitized state, but would not augment currents activated by higher GABA concentrations, when many of the channels are in the closed, desensitized state. This mechanism has been proposed to explain the potentiation of $5\text{-}HT_3$ receptor-mediated current by trichloroethanol in nodose ganglion neurones (Lovinger & Zhou, 1993). Third, the $GABA_A$ receptor-ion channel complex can enter into multiple open states, depending upon the concentration of GABA (MacDonald *et al.*, 1989). The selectivity of trichloroethanol for currents activated by low GABA concentrations may be due to a preferential action of trichloroethanol upon a kinetic substate of the receptor favoured at low GABA concentrations.

The observation in the present study that trichloroethanol did not potentiate currents activated by concentrations of GABA at or above 10 μM might seem to render implausible an effect of trichloroethanol on GABAergic synaptic currents, since GABA concentrations in the synapse appear to approach the millimolar range (Maconochie *et al.*, 1994). Enhancement of inhibitory postsynaptic currents by trichloroethanol has been demonstrated, however, in hippocampal neurones both in culture and in brain slices (Lovinger *et al.*, 1993). At least two explanations for this apparent discrepancy are possible. As mentioned previously, an effect of trichloroethanol to enhance currents activated by high concentrations of GABA may have been obscured in the present study by attenuation of peak current due to rapid desensitization. Alternatively, trichloroethanol could act primarily during the falling phase of the postsynaptic current, following the decline in the GABA concentration below the threshold for potentiation. Thus, trichloroethanol would have little or no effect on the peak amplitude of the postsynaptic current, when the synaptic concentration of GABA is high, but would prolong the falling phase of the current, when the synaptic concentration of GABA is low. These predicted results agree well with the observation that trichloroethanol increases the duration, but not the peak amplitude, of GABA-mediated postsynaptic currents (Lovinger *et al.*, 1993).

If the potentiation of GABA-activated current by trichloroethanol results from a general conformational alteration of the $GABA_A$ receptor-ionophore complex, one would expect that current would also be augmented if the channel were activated by an agent acting at another site. Our observation that trichloroethanol did not enhance pentobarbitone-activated current in the absence of exogenous GABA suggests that the action of trichloroethanol on the channel protein does not involve such a conformational change. The observation that trichloroethanol did not enhance pentobarbitone-activated current at a concentration that markedly enhanced GABA-activated current also implies that trichloroethanol does not modulate the function of the pentobarbitone site in a manner similar to its action at the GABA site.

The enhancement of GABA-mediated responses by volatile anaesthetic agents has been attributed to elevation of intracellular Ca^{2+}, as the effect was not observed when the neurones were dialyzed internally with a solution containing 11 mM BAPTA and 1 mM Ca^{2+}, which was calculated to buffer intracellular free Ca^{2+} to 10–100 nM (Mody *et al.*, 1991). In the present study, despite using an intracellular solution containing 10 mM BAPTA and no added Ca^{2+} (calculated intracellular free Ca^{2+} concentration < 1 nM), we observed a potentiation of GABA-activated current of over five fold by trichloroethanol. Thus, trichloroethanol does not appear to enhance GABA-activated current by elevating intracellular Ca^{2+}.

Benzodiazepines, barbiturates, and ethanol do not augment GABA responses in all central neurones (Study &

Barker, 1981; Biscoe & Duchen, 1985; Aguayo, 1990). Variation among neurones in sensitivity to modulation of $GABA_A$ receptor function by these sedative/hypnotic agents has been attributed to variation in the subunit composition of the receptor and/or in the biochemical state of the receptor by processes such as phosphorylation. Different combinations of $GABA_A$ receptor subunits yield receptors that differ in their functional properties, including sensitivity to modulation by barbiturates, benzodiazepines, and anaesthetic steroids (Levitan *et al.*, 1988; Pritchett *et al.*, 1989; Sigel *et al.*, 1990; Verdoorn *et al.*, 1990; Horne *et al.*, 1993), and perhaps also to ethanol (Lüddens *et al.*, 1990; Wafford *et al.*, 1991; Korpi *et al.*, 1993). In addition, Leidenheimer *et al.* (1993) recently reported that the phosphorylation state of the $GABA_A$ receptor-ion channel complex influences its sensitivity to modulation by benzodiazepines and barbiturates. Our observation that trichloroethanol potentiated GABA-activated current in all neurones tested may indicate that the determinants of the $GABA_A$ receptor responsible for sensitivity to trichloroethanol are less stringent than those responsible for its sensitivity to other modulators.

In addition to the potentiation of GABA-activated current, we found that trichloroethanol concentrations $\geqslant 2.5$ mM activated a current in the absence of exogenous GABA. The observations that the reversal potential for this current shifted when the intracellular Cl^- concentration was changed and that this shift was coincident with the shift in reversal potential of GABA-activated current, suggest that the trichloroethanol-activated current is carried predominantly by Cl^-. In addition, the antagonism of this current by either bicuculline or picrotoxin suggests that the trichloroethanol-activated Cl^- current involves GABA-gated ion channels. Whether the current activated by trichloroethanol in the absence of exogenous GABA results from a direct stimulation of the $GABA_A$ receptor-ionophore complex or from potentiation of low concentrations of endogenous GABA is not clear. The observation that the $GABA_A$ competitive antagonist, bicuculline, inhibits the trichloroethanol-activated current could be interpreted as support for the latter hypothesis. However, it remains possible that bicuculline inhibits the trichloroethanol-activated current not by displacing endogenous GABA bound to the receptor, but by stabilizing the receptor-ionophore complex so that it is less sensitive to activation by trichloroethanol. It is of interest to note that barbiturates and hypnotic steroids also activate a Cl^- current in the absence of exogenous GABA (Mathers & Barker, 1980; Schulz & MacDonald, 1981; Higashi & Nishi, 1982; Jackson *et al.*, 1982; Akaike *et al.*, 1985; Majewska *et al.*, 1986; Barker *et al.*, 1987), and that these currents are also antagonized by bicuculline and picrotoxin (Higashi & Nishi, 1982; Akaike *et al.*, 1985; Majewska *et al.*, 1986; Barker *et al.*, 1987).

GABA is now considered to be the major inhibitory neurotransmitter in the mammalian brain, and the major presynaptic inhibitory neurotransmitter in the spinal cord (Barker & Owen, 1986; Roberts, 1986; McCormick, 1989). Previous studies have shown that benzodiazepines, barbiturates, hypnotic steroids, and inhalational anaesthetics can potentiate inhibitory synaptic responses in brain and presynaptic inhibition in spinal cord, and it has been suggested that potentiation of GABA-mediated synaptic responses may contribute to the sedative/hypnotic actions of those agents (Nicoll, 1972; Nicoll *et al.*, 1975; Majewska *et al.*, 1986; Jones *et al.*, 1992; Tanelian *et al.*, 1993). In contrast, the cellular actions of chloral derivative anaesthetics have been less well characterized. Chloral anaesthetics are rapidly metabolized *in vivo* to trichloroethanol, which is believed to be responsible for the CNS effects of these agents (Rall, 1990). We estimate the anaesthetic concentration of trichloroethanol to be in the range of 2–5 mM, based on data from Breimer (1977), Owen & Taberner (1980), and Pringle *et al.* (1981). Our observations that trichloroethanol could potentiate GABA-activated current in all neurones studied, and that the EC_{50} for this effect was 3 mM, suggest that potentiation of GABA-mediated responses may contribute to the sedative/hypnotic actions of chloral derivative anaesthetics.

In addition to potentiating GABA-activated current, we have also found that trichloroethanol can inhibit ion currents activated by excitatory amino acids (Peoples *et al.*, 1990). Given the important role of excitatory amino acid neurotransmitters in regulating excitability in the CNS (Mayer & Westbrook, 1987; Collingridge & Lester, 1989), both inhibition of excitatory amino acid-mediated responses and potentiation of GABA-mediated responses in the CNS by chloral derivatives may contribute to their sedative/hypnotic actions.

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References

AGUAYO, L.G. (1990). Ethanol potentiates the $GABA_A$-activated Cl^- current in mouse hippocampal and cortical neurons. *Eur. J. Pharmacol.*, **187**, 127–130.

AKAIKE, N., HATTORI, K., INOMATA, N. & OOMURA, Y. (1985). γ-Aminobutyric-acid- and pentobarbitone-gated chloride currents in internally perfused frog sensory neurones. *J. Physiol.*, **360**, 367–386.

BARKER, J.L., HARRISON, N.L., LANGE, G.D. & OWEN, D.G. (1987). Potentiation of γ-aminobutyric-acid-activated chloride conductance by a steroid anaesthetic in cultured rat spinal neurones. *J. Physiol.*, **386**, 485–501.

BARKER, J.L. & OWEN, D.G. (1986). Electrophysiological pharmacology of GABA and diazepam in cultured CNS neurons. In *Benzodiazepine/GABA Receptors and Chloride Channels: Structural and Functional Properties*. ed. Olsen, R.W. & Venter, J.C. pp. 135–165. New York: Alan R. Liss.

BARKER, J.L. & RANSOM, B.R. (1978). Pentobarbitone pharmacology of mammalian central neurones grown in tissue culture. *J. Physiol.*, **280**, 355–372.

BISCOE, T.J. & DUCHEN, M.R. (1985). Actions and interactions of GABA and benzodiazepines in the mouse hippocampal slice. *Q. J. Exp. Physiol.*, **70**, 313–328.

BREIMER, D.D. (1977). Clinical pharmacokinetics of hypnotics. *Clin. Pharmacokinet.*, **2**, 93–109.

CHOI, D.W., FARB, D.H. & FISCHBACH, G.D. (1977). Chlordiazepoxide selectively augments GABA action in spinal cord cell cultures. *Nature*, **269**, 342–344.

CHOI, D.W., FARB, D.H. & FISCHBACH, G.D. (1981). Chlordiazepoxide selectively potentiates GABA conductance of spinal cord and sensory neurons in cell culture. *J. Neurophysiol.*, **45**, 621–631.

COLLINGRIDGE, G.L. & LESTER, R.A.J. (1989). Excitatory amino acid receptors in the vertebrate central nervous system. *Pharmacol. Rev.*, **40**, 143–210.

DELEAN, A., MUNSON, P.J. & RODBARD, D. (1978). Simultaneous analysis of families of sigmoidal curves: application to bioassay, radioligand assay, and physiological dose-response curves. *Am. J. Physiol.*, **235**, E97–E102.

FORSYTHE, I.D. & WESTBROOK, G.L. (1988). Slow excitatory postsynaptic currents mediated by *N*-methyl-D-aspartate receptors on cultured mouse central neurones. *J. Physiol.*, **396**, 515–533.

GAGE, P.W. & ROBERTSON, B. (1985). Prolongation of inhibitory postsynaptic currents by pentobarbitone, halothane and ketamine in CA1 pyramidal cells in rat hippocampus. *Br. J. Pharmacol.*, **85**, 675–681.

HAEFELY, W., KULCSAR, A., MÖHLER, H., PIERI, L., POLC, P. & SCHAFFNER, R. (1975). Possible involvement of GABA in the central actions of benzodiazepines. *Adv. Biochem. Psychopharmacol.*, **14**, 131–151.

HARRIS, R.A., ALLAN, A.M., DANIELL, L.C. & NIXON, C. (1988). Antagonism of ethanol and pentobarbital actions by benzodiazepine inverse agonists: neurochemical studies. *J. Pharmacol. Exp. Ther.*, **247**, 1012–1017.

HARRISON, N.L., MAJEWSKA, M.D., HARRINGTON, J.W. & BARKER, J.L. (1987). Structure-activity relationships for steroid interaction with the γ-aminobutyric acid$_A$ receptor complex. *J. Pharmacol. Exp. Ther.*, **241**, 346–353.

HARRISON, N.L. & SIMMONDS, M.A. (1984). Modulation of the GABA receptor complex by a steroid anaesthetic. *Brain Res.*, **323**, 287–292.

HIGASHI, H. & NISHI, S. (1982). Effect of barbiturates on the GABA receptor of cat primary afferent neurones. *J. Physiol.*, **332**, 299–314.

HORNE, A.L., HARKNESS, P.C., HADINGHAM, K.L., WHITING, P. & KEMP, J.A. (1993). The influence of the γ_{2L} subunit on the modulation of responses to GABA$_A$ receptor activation. *Br. J. Pharmacol.*, **108**, 711–716.

ISHIZUKA, S., SIKDAR, S.K., YASUI, S., OYAMA, Y. & AKAIKE, N. (1989). α-Chloralose opens the chloride channel of frog isolated sensory neurons. *Brain Res.*, **498**, 181–184.

JACKSON, M.B., LECAR, H., MATHERS, D.A. & BARKER, J.L. (1982). Single channel currents activated by γ-aminobutyric acid, muscimol and (−)-pentobarbital in cultured mouse spinal neurons. *J. Neurosci.*, **2**, 889–894.

JONES, M.V., BROOKS, P.A. & HARRISON, N.L. (1992). Enhancement of γ-aminobutyric acid-activated Cl^- currents in cultured rat hippocampal neurones by three volatile anaesthetics. *J. Physiol.*, **449**, 279–293.

KORPI, E.R., KLEINGOOR, C., KETTENMANN, H. & SEEBURG, P.H. (1993). Benzodiazepine-induced motor impairment linked to point mutation in cerebellar GABA$_A$ receptor. *Nature*, **361**, 356–359.

LEIDENHEIMER, N.J., WHITING, P.J & HARRIS, R.A. (1993). Activation of calcium-phospholipid-dependent protein kinase enhances benzodiazepine and barbiturate potentiation of the GABA$_A$ receptor. *J. Neurochem.*, **60**, 1972–1975.

LEVITAN, E.S., SCHOFIELD, P.R., BURT, D.R., RHEE, L.M., WISDEN, W., KÖHLER, M., FUJITA, N., RODRIGUEZ, H.F., STEPHENSON, A. & DARLISON, M.G. (1988). Structural and functional basis for GABA$_A$ receptor heterogeneity. *Nature*, **335**, 76–79.

LIN, L.-H., WHITING, P. & HARRIS, R.A. (1993). Molecular determinants of general anesthetic action: role of GABA$_A$ receptor structure. *J. Neurochem.*, **60**, 1548–1553.

LITTLE, H.J. (1984). The effects of benzodiazepine agonists, inverse agonists and Ro 15-1788 on the responses of the superior cervical ganglion to GABA *in vitro*. *Br. J. Pharmacol.*, **83**, 57–68.

LITTLE, H.J. (1991). Mechanisms that may underlie the behavioural effects of ethanol. *Prog. Neurobiol.*, **36**, 171–194.

LOVINGER, D.M. & ZHOU, Q. (1993). Trichloroethanol potentiation of 5-hydroxytryptamine$_3$ receptor-mediated ion current in nodose ganglion neurons from the adult rat. *J. Pharmacol. Exp. Ther.*, **265**, 771–776.

LOVINGER, D.M., ZIMMERMAN, S.A., LEVITIN, M., JONES, M.V. & HARRISON, N.L. (1993). Trichloroethanol potentiates synaptic transmission mediated by γ-aminobutyric acid$_A$ receptors in hippocampal neurons. *J. Pharmacol. Exp. Ther.*, **264**, 1097–1103.

LÜDDENS, H., PRITCHETT, D.B., KÖHLER, M., KILLISCH, I., KEINÄNEN, K., MONYER, H., SPRENGEL, R. & SEEBURG, P.H. (1990). Cerebellar GABA$_A$ receptor selective for a behavioral alcohol antagonist. *Nature*, **346**, 648–651.

MACDONALD, R.L. & BARKER, J.L. (1978). Benzodiazepines specifically modulate GABA mediated postsynaptic inhibition in cultured mammalian neurones. *Nature*, **271**, 563–564.

MACDONALD, R.L., ROGERS, C.J. & TWYMAN, R.E. (1989). Kinetic properties of the GABA$_A$ receptor main conductance state of mouse spinal cord neurones in culture. *J. Physiol.*, **410**, 479–499.

MACONOCHIE, D.J., ZEMPEL, J.M. & STEINBACH, J.H. (1994). How quickly can GABA$_A$ receptors open? *Neuron*, **12**, 61–71.

MAJEWSKA, M.D., HARRISON, N.L., SCHWARTZ, R.D., BARKER, J.L. & PAUL, S.M. (1986). Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor. *Science*, **232**, 1004–1007.

MATHERS, D.A. & BARKER, J.L. (1980). (−)Pentobarbital opens ion channels of long duration in cultured mouse spinal neurons. *Science*, **209**, 507–509.

MAYER, M.L. & WESTBROOK, G.L. (1987). The physiology of excitatory amino acids in the vertebrate central nervous system. *Prog. Neurobiol.*, **28**, 197–276.

MCCORMICK, D.A. (1989). GABA as an inhibitory neurotransmitter in human cerebral cortex. *J. Neurophysiol.*, **62**, 1018–1027.

MEHTA, A.K. & TICKU, M.K. (1988). Ethanol potentiation of GABAergic transmission in cultured spinal cord neurons involves γ-aminobutyric acid$_A$-gated chloride channels. *J. Pharmacol. Exp. Ther.*, **246**, 558–564.

MODY, I., TANELIAN, D.L. & MACIVER, M.B. (1991). Halothane enhances tonic neuronal inhibition by elevating intracellular calcium. *Brain Res.*, **538**, 319–323.

MORROW, A.L., PACE, J.R., PURDY, R.H. & PAUL, S.M. (1990). Characterization of steroid interactions with γ-aminobutyric acid receptor-gated chloride ion channels: evidence for multiple steroid recognition sites. *Mol. Pharmacol.*, **37**, 263–270.

MORROW, A.L., SUZDAK, P.D. & PAUL, S.M. (1988). Benzodiazepine, barbiturate, ethanol and hypnotic steroid hormone modulation of GABA-mediated chloride ion transport in rat brain synaptoneurosomes. *Adv. Biochem. Psychopharmacol.*, **45**, 247–261.

NAKAHIRO, M., YEH, J.Z., BRUNNER, E. & NARAHASHI, T. (1989). General anesthetics modulate GABA receptor channel complex in rat dorsal root ganglion neurons. *FASEB J.*, **3**, 1850–1854.

NESTOROS, J.N. (1980). Ethanol specifically potentiates GABA-mediated neurotransmission in feline cerebral cortex. *Science*, **209**, 708–710.

NICOLL, R.A. (1972). The effects of anaesthetics on synaptic excitation and inhibition in the olfactory bulb. *J. Physiol.*, **223**, 803–814.

NICOLL, R.A., ECCLES, J.C., OSHIMA, T. & RUBIA, F. (1975). Prolongation of hippocampal inhibitory postsynaptic potentials by barbiturates. *Nature*, **258**, 625–627.

OWEN, B.E. & TABERNER, P.V. (1980). Studies on the hypnotic effects of chloral hydrate and ethanol and their metabolism *in vivo* and *in vitro*. *Biochem. Pharmacol.*, **29**, 3011–3016.

PENNEFATHER, P. & QUASTEL, D.M.J. (1980). Actions of anesthetics on the function of nicotinic acetylcholine receptors. In *Molecular Mechanisms of Anesthesia* (*Progress in Anesthesiology*, Vol. 2). ed. Fink, B.R. pp. 45–58. New York: Raven Press.

PEOPLES, R.W., LOVINGER, D.M. & WEIGHT, F.F. (1990). Inhibition of excitatory amino acid currents by general anesthetic agents. *Soc. Neurosci. Abstr.*, **16**, 1017.

PEOPLES, R.W. & WEIGHT, F.F. (1991). Modulation of amino acid-activated ion currents by trichloroethanol. *Third IBRO World Congress of Neurosci. Abstr.*, **3**, 63.

PRINGLE, M.J., BROWN, K.B. & MILLER, K.W. (1981). Can the lipid theories of anesthesia account for the cutoff in anesthetic potency of homologous series of alcohols? *Mol. Pharmacol.*, **19**, 49–55.

PRITCHETT, D.B., SONTHEIMER, H., SHIVERS, B.D., YMER, S., KETTENMANN, H., SCHOFIELD, P.R. & SEEBURG, P.H. (1989). Importance of a novel GABA$_A$ receptor subunit for benzodiazepine pharmacology. *Nature*, **338**, 582–585.

RALL, T.W. (1990). Hypnotics and sedatives; ethanol. In *The Pharmacological Basis of Therapeutics*. ed. Gilman, A.G., Rall, T.W., Nies, A.S. & Taylor, P. pp. 345–382. Elmsford, New York: Pergamon Press.

RANSOM, B.R. & BARKER, J.L. (1975). Pentobarbital modulates transmitter effects on mouse spinal neurones grown in tissue culture. *Nature*, **254**, 703–705.

ROBERTS, E. (1986). GABA: the road to neurotransmitter status. In *Benzodiazepine/GABA Receptors and Chloride Channels: Structural and Functional Properties*. ed. Olsen, R.W. & Venter, J.C. pp. 1–39. New York: Alan R. Liss.

SCHULZ, D.W. & MACDONALD, R.L. (1981). Barbiturate enhancement of GABA-mediated inhibition and activation of chloride ion conductance: correlation with anticonvulsant and anesthetic actions. *Brain Res.*, **209**, 177–188.

SIGEL, E., BAUR, R., TRUBE, G., MÖHLER, H. & MALHERBE, P. (1990). The effect of subunit composition of rat brain GABA$_A$ receptors on channel function. *Neuron*, **5**, 703–711.

STERZ, R., HERMES, M, PEPER, K. & BRADLEY, R.J. (1981). Effects of halogenated derivatives of ethanol on the nicotine acetylcholine receptor. *Brain Res.*, **230**, 434–438.

STUDY, R.E. & BARKER, J.L. (1981). Diazepam and (−)-pentobarbital: fluctuation analysis reveals different mechanisms for potentiation of γ-aminobutyric acid responses in cultured central neurons. *Proc. Natl. Acad. Sci. U.S.A.*, **78**, 7180–7184.

SUZDAK, P.D., GLOWA, J.R., CRAWLEY, J.N,. SCHWARTZ, R.D., SKOLNICK, P. & PAUL, S.M. (1986a). A selective imidazobenzodiazepine antagonist of ethanol in the rat. *Science*, **234**, 1243–1247.

SUZDAK, P.D., SCHWARTZ, R.D., SKOLNICK, P. & PAUL, S.M. (1986b). Ethanol stimulates γ-aminobutyric acid receptor-mediated chloride transport in rat brain synaptoneurosomes. *Proc. Natl. Acad. Sci. U.S.A.*, **83**, 4071–4075.

TANELIAN, D.L., KOSEK, P., MODY, I. & MACIVER, M.B. (1993). The role of the $GABA_A$ receptor/chloride channel complex in anesthesia. *Anesthesiol.*, **78**, 757–776.

VERDOORN, T.A., DRAGUHN, A., YMER, S., SEEBURG, P.H. & SAKMANN, B. (1990). Functional properties of recombinant rat $GABA_A$ receptors depend upon subunit composition. *Neuron*, **4**, 919–928.

WAFFORD, K.A., BURNETT, D.M., LEIDENHEIMER, N.J., BURT, D.R., WANG, J.B., KORUJI, P., DUNWIDDIE, T.V., HARRIS, R.A. & SIKELA, J.M. (1991). Ethanol sensitivity of the $GABA_A$ receptor expressed in Xenopus oocytes requires 8 amino acids contained in the γ_{2L} subunit. *Neuron*, **7**, 27–33.

WHITE, G., LOVINGER, D.M. & WEIGHT, F.F. (1990). Ethanol inhibits NMDA-activated current but does not alter GABA-activated current in an isolated adult mammalian neuron. *Brain Res.*, **507**, 332–336.

YU, O., CHIU, T.H & ROSENBERG, H.C. (1988). A comparison of the effects of midazolam and pentobarbital on the dose-response of GABA-gated Cl^- influx in rat brain microsacs. *Brain Res.*, **451**, 376–380.

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