British Journal of Pharmacology (1995) 114, 1641–1651 © 1995 Stockton Press All rights reserved 0007–1188/95 $12.00

The interaction of trichloroethanol with murine recombinant 5-HT$_3$ receptors

[2]David L. Downie, Anthony G. Hope, Delia Belelli, Jeremy J. Lambert, [1]John A. Peters, *Kim R. Bentley, *Lucinda J. Steward, *[3]Chong-Yang Chen & *Nicholas M. Barnes

Neuroscience Research Group, Department of Pharmacology and Clinical Pharmacology, Ninewells Hospital and Medical School, The University of Dundee, Dundee, DD1 9SY and *Department of Pharmacology, The Medical School, University of Birmingham, Edgbaston, Birmingham B15 2TT

1 The effects of ethanol, chloral hydrate and trichloroethanol upon the 5-HT$_3$ receptor have been investigated by use of electrophysiological techniques applied to recombinant 5-HT$_3$ receptor subunits (5-HT$_3$R-A or 5-HT$_3$R-A$_S$) expressed in *Xenopus laevis* oocytes. Additionally, the influence of trichloroethanol upon the specific binding of [^{3}H]-granisetron to membrane preparations of HEK 293 cells stably transfected with the murine 5-HT$_3$R-A$_S$ subunit and 5-HT$_3$ receptors endogenous to NG 108-15 cell membranes was assessed.

2 Ethanol (30–300 mM), chloral hydrate (1–30 mM) and trichloroethanol (0.3–10 mM), produced a reversible, concentration-dependent, enhancement of 5-HT-mediated currents recorded from oocytes expressing either the 5-HT$_3$R-A, or the 5-HT$_3$R-A$_S$ subunit.

3 Trichloroethanol (5 mM) produced a parallel leftward shift of the 5-HT concentration-response curve, reducing the EC$_{50}$ for 5-HT from 1 ± 0.04 μM ($n = 4$) to 0.5 ± 0.01 μM ($n = 4$) for oocytes expressing the 5-HT$_3$R-A. A similar shift, from 2.1 ± 0.05 μM ($n = 11$) to 1.3 ± 0.1 μM ($n = 4$), was observed in oocytes expressing the 5-HT$_3$R-A$_S$ subunit. Trichloroethanol (5 mM) had little or no effect upon the maximum current produced by 5-HT for either recombinant receptor.

4 Trichloroethanol (5 mM) similarly reduced the EC$_{50}$ for 2-methyl-5-HT from 13 ± 0.4 μM ($n = 4$) to 4.6 ± 0.2 μM ($n = 4$) and from 15 ± 2 μM ($n = 4$) to 5 ± 0.4 μM ($n = 4$) for oocytes expressing the 5-HT$_3$R-A and 5-HT$_3$R-A$_s$ subunit respectively. Additionally, trichloroethanol (5 mM) produced a clear enhancement of the maximal current to 2-methyl-5-HT (expressed as a percentage of the maximal current to 5-HT) from 63 ± 0.7% ($n = 4$) to 101 ± 1.6% ($n = 4$) and from 9 ± 0.2% ($n = 4$) to 74 ± 2% ($n = 4$) for oocytes expressing the 5-HT$_3$R-A and 5-HT$_3$R-A$_S$ subunit respectively.

5 Trichloroethanol (2.5 mM) had no effect upon the K_d, or B_{max}, of specific [^{3}H]-granisetron binding to membrane homogenates of NG 108-15 cells or HEK 293 cells. Similarly, competition for [^{3}H]-granisetron binding by the 5-HT$_3$ receptor antagonists ondansetron and tropisetron was unaffected. However, competition for [^{3}H]-granisetron binding by the 5-HT$_3$ receptor agonists, 5-HT, 2-methyl-5-HT and phenylbiguanide was enhanced by trichloroethanol (2.5 mM).

6 Unexpectedly, the competition for [^{3}H]-granisetron binding by the 5-HT$_3$ receptor antagonist, quipazine, was enhanced by 2.5 mM trichloroethanol. Quipazine (1 nM–0.3 μM) antagonized 5-HT-evoked currents recorded from oocytes expressing the 5-HT$_3$R-A subunit with an IC$_{50}$ of 18 ± 3 nM ($n = 4$). Additionally, quipazine (30 nM–0.3 μM) produced a small inward current which was greatly enhanced by 5 mM trichloroethanol and antagonized by 100 nM ondansetron. Collectively, these observations suggest that quipazine may act as a partial agonist.

7 The demonstration that a recombinant homo-oligomeric receptor, expressed in a foreign membrane, retains a sensitivity to alcohols, together with the sequencing of alcohol-insensitive 5-HT$_3$ receptor subunits, may lead to a better definition of the alcohol binding site(s).

Keywords: 5-HT$_3$ receptor; recombinant 5-HT$_3$ receptor; splice variants; *Xenopus laevis* oocytes; trichloroethanol; ethanol; chloral hydrate; ligand-gated ion channel

Introduction

The 5-HT$_3$ receptor is a ligand-gated ion channel that demonstrates functional properties similar to those of nicotinic acetylcholine receptors, including cation selectivity, cooperativity in agonist binding and rapid activation and desensitization kinetics (Lambert *et al.*, 1994; Peters *et al.*, 1994; Boess & Martin, 1994). The nucleotide sequence of a cDNA encoding a 5-HT$_3$ receptor subunit (5-HT$_3$R-A), cloned from the NCB-20 hybridoma cell line, predicts a protein which exhibits considerable structural commonality with nicotinic receptor subunits (Maricq *et al.*, 1991). Each subunit is thought to consist of a large extracellular amino terminal domain which contains the agonist binding site, four putative transmembrane regions (M$_1$–M$_4$), with M$_2$ probably forming the lining of the ion channel, and a large intracellular loop between M$_3$ and M$_4$. This model is supported by recent experiments investigating the properties of a chimaeric receptor. The chimaera which consisted of the amino terminal extracellular domain of the chick α_7 nicotinic receptor and the M$_1$ to carboxyl terminus of the 5-HT$_3$R-A subunit formed functional receptors gated by acetylcholine, but with the ion channel properties characteristic of the recombinant 5-HT$_3$R-A (Eiselé *et al.*, 1993).

Recently, a splice variant of the 5-HT$_3$R-A subunit, which lacks 6 consecutive amino acid residues within the large

[1] Author for correspondence.
[2] Present address: The Biophysics Section, The Blackett Laboratory, Imperial College, Prince Consort Road, London, SW7 2BZ.
[3] Present address: Department of Pharmacology, Guilin Medical College, Guilin, Guangxi 541001, China.

intracellular loop between M_3 and M_4 (the 5-HT_3R-A_s, s = short form) has been obtained from N1E-115 cells (Hope *et al.*, 1993). Both forms have been detected in N1E-115, NCB-20 and NG 108-15 cells, and the cerebral cortex, hippocampus and superior cervical ganglion of the mouse (Hope *et al.*, 1993; Werner *et al.*, 1993). A species homologue of the short form has additionally been found in rat superior cervical ganglion (Isenberg *et al.*, 1993). Either subunit forms functional, presumably homo-oligomeric, receptors when expressed in *Xenopus laevis* oocytes, or mammalian cell lines which reproduce many of the pharmacological and biophysical properties of the receptor native to the murine neuroblastoma cell lines (Maricq *et al.*, 1991; Gill *et al.*, 1993; Hope *et al.*, 1993; Yakel *et al.*, 1993; Downie *et al.*, 1994; Sepúlveda & Lummis, 1994; Uetz *et al.*, 1994). A detailed comparison of the agonist and antagonist pharmacology of the two subunits expressed in *Xenopus* oocytes revealed little difference between them, with the exception that the maximal effect of the agonist, 2-methyl-5-HT, was reduced for the 5-HT_3R-A_S subunit (Downie *et al.*, 1994; see also Sepúlveda & Lummis, 1994).

Little information exists concerning the influence of potential allosteric regulators upon recombinant 5-HT_3 receptors. For receptors endogenous to peripheral neurones and neuronal cell lines, such agents may include the dissociative anaesthetic ketamine (Peters *et al.*, 1991), indole derivatives (Kooyman *et al.*, 1994) and certain alcohols (e.g. Lovinger, 1991). Reports describing the potentiation of 5-HT_3 receptor-mediated currents recorded from NCB-20 cells and rat nodose ganglion neurones by ethanol (Lovinger, 1991; Lovinger & White, 1991) and trichloroethanol (Lovinger & Zhou, 1993) prompted the present investigation of the effects of these agents upon recombinant 5-HT_3 receptors. Primarily, it was of interest to determine whether the heterologously expressed subunits were susceptible to modulation by ethanol and trichloroethanol and if so, whether the splice variants of the 5-HT_3R-A would respond differentially to these agents. The latter consideration arose from work indicating the alternatively spliced forms of the $GABA_A$ receptor γ_2 subunit (γ_{2S} and γ_{2L}) to be an important determinant of the ethanol sensitivity of hetero-oligomeric $GABA_A$ receptor complexes expressed in *Xenopus* oocytes (Wafford *et al.*, 1991). Notably, the γ_{2S} subunit, which lacks a consensus sequence for phosphorylation by protein kinase C due to the deletion of 8 consecutive amino acids located within the large intracellular loop between M_3 and M_4, does not support the enhancement by ethanol of GABA-evoked currents observed with receptors containing the γ_{2L} subunit. In this regard, it is interesting that the 6 amino acids absent from the intracellular loop of the 5-HT_3R-A_S have been suggested to form a site for phosphorylation by casein kinase II (Werner *et al.*, 1993).

Species differences in the agonist and antagonist pharmacology of the 5-HT_3 receptor are well known (e.g. Butler *et al.*, 1990; Newberry *et al.*, 1991; Peters *et al.*, 1992) and it would appear that such differences extend to allosteric modulators. For example, trichloroethanol blocks, rather than potentiates, 5-HT_3 receptor-mediated currents recorded from nodose ganglion neurones of the guinea-pig (Gill *et al.*, 1994). Comparisons of the primary amino acid sequence of 5-HT_3 receptor subunits displaying differential responses to alcohol may lead to the definition of sites involved in allosteric regulation. In the present study, electrophysiological techniques were employed to investigate the interaction of ethanol, trichloroethanol and chloral hydrate (which is reduced to trichloroethanol *in vivo*; Garrett & Lambert, 1973) with the alternatively spliced variants of the 5-HT_3R-A expressed in *Xenopus* oocytes. In addition, the interaction of trichloroethanol with recombinant 5-HT_3 receptors in stably transfected HEK 293 cells (Hope, 1993) and 5-HT_3 receptors native to NG 108-15 cells (Neijt *et al.*, 1988) was examined in ligand binding assays employing the antagonist [^{3}H]-granisetron. A preliminary account of a part of this work has appeared in abstract form (Downie *et al.*, 1993).

Methods

Preparation of in vitro *transcripts*

The 5-HT_3R-A cDNA (Maricq *et al.*, 1991) and the 5-HT_3R-A_S cDNA (Hope *et al.*, 1993) were linearised by use of the restriction enzymes *Not I* and *Bam HI* respectively. A Riboprobe System II transcription kit (Promega Limited, U.K.) was employed to synthesize capped *in vitro* transcripts from individual template cDNA using either an SP6 (5-HT_3R-A) or T_3 (5-HT_3R-A_S) RNA polymerase. The integrity of the RNA transcripts was determined by electrophoresis through a denaturing 1% agarose formaldehyde gel (Sambrook *et al.*, 1989), alongside standard RNA size markers (Gibco BRL, U.K.).

Expression and electrical recordings

Xenopus laevis oocytes were isolated as described by Hope *et al.* (1993) and incubated in Barth's saline comprising in (mM): NaCl 88, KCl 1, $NaHCO_3$ 2.4, $MgSO_4$ 1, $CaCl_2$ 0.5, $Ca(NO_3)_2$ 0.5; HEPES 15 (pH 7.5). Stage VI oocytes were identified and injected with 20–100 nl of nuclease free water containing 10–40 ng of the appropriate cRNA, using a Drummond Digital Microdispenser 510 (Drummond Scientific Co., Broomhall, PA, U.S.A.). Injected oocytes were individually stored in wells of 96 well microtitre plates containing 200 µl of Barth's saline supplemented with penicillin (50 iu ml^{-1}), streptomycin (50 µg ml^{-1}) and gentamycin (100 µg ml^{-1}). Oocytes were maintained at 19–20°C for 2 to 10 days prior to use.

Electrical recordings were made in modified Barth's solution containing (in mM): NaCl 88, KCl 1, $NaHCO_3$ 2.4, $MgCl_2$ 1, $CaCl_2$ 1 and HEPES 15 (pH 7.5; adjusted with NaOH). Agonist-induced currents were recorded at a holding potential of −30 mV with an Axoclamp 2A (Axon Instruments, Foster City, CA, U.S.A.) voltage-clamp amplifier in the twin electrode voltage-clamp mode. The voltage-sensing and current-passing microelectrodes were filled with 3 M KCl and 3 M CsCl respectively and had resistances of 0.4–1.5 MΩ when determined in the standard extracellular saline. Oocytes were continuously superfused at a rate of 5–6 ml min^{-1}. Antagonist, agonist and modulatory compounds were applied via the superfusion system. In the case of modulatory and antagonist compounds, the substances were preapplied for 1 min prior to their simultaneous application with the appropriate agonist for a further 20–60 s. Membrane currents were low-pass filtered at 100 Hz (8-pole Bessel filter; Fylde Instruments U.K.), recorded onto video tape via a PCM 2 VCR adapter (Medical Systems Corporation, Greenvale, N.Y., U.S.A.) or onto magnetic tape using a Racal Store 4 DS F.M. tape recorder and simultaneously displayed on a chart recorder (Multitrace 2, Lectromed, Jersey, C.I.). All recordings were performed at ambient temperature (18–23°C).

Cell culture

The neuroblastoma-glioma hybrid cell line NG 108-15 (N18TG2 mouse neuroblastoma × rat glioma C6BY-1; Klee & Nirenberg, 1974) was grown in Dulbecco's modified Eagle's medium containing 10% (v/v) foetal calf serum supplemented with hypoxanthine (100 µM), aminopterin (1.0 µM) and thymidine (16 µM) (i.e. HAT supplement). HEK 293 cells stably transfected with the 5-HT_3R-A_S subunit (Hope, 1993) were grown under similar conditions although the growth medium additionally contained geneticin (200 µg ml^{-1}) and lacked the HAT supplement. NG 108-15 and HEK 293 cells were harvested by agitation in Ca^{2+}- and Mg^{2+}-free Dulbecco's phosphate buffered saline (between passage 30 and 40 for NG 108-15 and 4 and 45 for HEK 293 cells) and pelleted by centrifugation (900 g, 10 min, 4°C). Pelleted cells were

immediately frozen at −80°C and stored for periods of up to 2 months with no loss of binding activity.

Preparation of cell homogenate for radioligand binding

To prepare membranes for radioligand binding assays, cells were thawed and then homogenized in 20 ml of ice-cold Tris/PMSF/EGTA buffer (in mM: Tris 50, phenylmethyl sulphonyl fluoride (PMSF) 0.1, ethylene glycol-*bis*(β-aminoethyl ether) N,N,N',N'-tetraacetic acid (EGTA) 5.0, final pH 7.4) using a Polytron blender (full power, 10 s) and centrifuged (48000 *g*, 10 min, 4°C). The resultant pellet was gently resuspended in 20 ml of Tris-Krebs buffer (in mM: Tris 50, NaCl 118, KCl 4.75, KH_2PO_4 1.2, $MgSO_4$ 1.2; $CaCl_2$ 2.5; $NaHCO_3$ 25 and glucose 11; final pH 7.4) and recentrifuged. The final pellet was gently resuspended in Tris-Krebs buffer to form the radioligand binding homogenate at a concentration of approximately 0.2 mg protein ml^{-1}. Protein content was assayed by the Bio-Rad Coomassie Brilliant Blue method (Bradford, 1976) with bovine serum albumin used as standard.

[^{3}H]-granisetron binding assay

For [^{3}H]-granisetron binding, assay tubes (in triplicate) contained 500 μl of competing drug, or vehicle (Tris-Krebs buffer), 150 μl trichloroethanol, or vehicle (Tris-Krebs buffer) and 100 μl [^{3}H]-granisetron (final concentration 0.9–1.1 nM for competition studies, or at a range of concentrations between 0.1–12.6 nM for saturation studies; radioligand concentrations calculated by assay of the incubation solutions). The assay tubes were preincubated for 2 min at 37°C before the addition of 250 μl cell homogenate to initiate binding, which was allowed to proceed at 37°C for 60 min before termination by rapid filtration under vacuum through prewet (0.1% v/v polyethyleneimine in Tris-Krebs buffer) Whatman GF/B filters followed by washing with ice-cold Tris-Krebs buffer (wash time 8 s). Bound radioactivity remaining on the filters was assayed in 10 ml of Ecoscint A (National Diagnostics) by liquid scintillation spectroscopy at an efficiency of approximately 47%.

Data analysis

For radioligand binding experiments, saturation and competition data were analysed by computer assisted iterative curve fitting according to the equation:

$$\frac{B}{B_{max}} = \frac{[L]^n}{[L]^n + [K]^n}$$

where B = bound radioligand; B_{max} = maximum binding at equilibrium; for saturation studies K = molar equilibrium dissociation constant, or for competition studies K = molar concentration of competing compound to reduce the specific binding by 50%; for saturation studies L = free molar concentration of radioligand, or for competition studies L = molar concentration of competing compound, n = Hill coefficient.

For electrophysiological studies, the peak amplitudes of agonist-evoked currents were measured manually from chart recorder traces. EC_{50} values of agonists, together with the Hill coefficient or slope factor, were derived from full log concentration-effect relationships. Each of these was fitted iteratively with a sigmoidal function equivalent to that given above by use of Fig P version 6.0c (Biosoft, U.K.) to estimate the appropriate parameters. Data are reported as the arithmetic mean ± the standard error of the mean (s.e.mean).

Drugs

Reagents were obtained from the following sources: 5-HT maleate, 5-HT creatinine sulphate, metoclopramide hydrochloride, cocaine hydrochloride, (+)-tubocurarine chloride, trichloroethanol, chloral hydrate (Sigma Chemical Co.), ethanol (BDH-Analar grade), 2-methyl-5-HT maleate, quipazine maleate (Research Biochemicals Inc.), phenylbiguanide (Aldrich Chemical Co.), ondansetron hydrochloride (Glaxo), granisetron hydrochloride and BRL 46470 (endo-*N*-(8-methyl-8-azabicyclo-[3,2,1] oct-3-yl)-2,3-dihydro-3,3-dimethyl-indol-1-carboxamide hydrochloride; SmithKline Beecham); tropisetron hydrochloride (Sandoz). [^{3}H]-granisetron (84.5 Ci mmol^{-1} New England Nuclear) was supplied in ethanol and diluted into Tris-Krebs buffer solution. With the exception of the latter, all drugs were freshly prepared and dissolved as concentrated stock solutions in either twice distilled water or the appropriate buffer solution.

Results

Previous studies have demonstrated that the potentiation of 5-HT$_3$ receptor-mediated currents by ethanol and trichloroethanol is most pronounced at low agonist concentrations (Lovinger & White, 1991; Lovinger & Zhou, 1993). In the present study, concentration effect relationships for ethanol, chloral hydrate and trichloroethanol were constructed using 5-HT concentrations of 0.5 μM and 1.0 μM in recordings performed on oocytes expressing the 5-HT$_3$R-A and 5-HT$_3$R-A$_S$ subunits respectively. On average, these concentrations of agonist correspond to the EC_{20} of the relevant 5-HT concentration response curve (Downie *et al.*, 1994).

Ethanol

Relatively high concentrations of ethanol enhance the 5-HT$_3$ receptor-mediated current recorded from murine NCB-20 hybridoma cells (Lovinger, 1991) and rat nodose ganglion neurones (Lovinger & White, 1991). In the present study, very high concentrations of ethanol (100 mM–300 mM) clearly potentiated inward current responses to 5-HT recorded from all oocytes expressing either the 5-HT$_3$R-A, or the 5-HT$_3$R-A$_S$ subunit (Figure 1). For example, at the highest concentration of ethanol tested (300 mM), responses elicited by 5-HT (1 μM) acting at the 5-HT$_3$R-A$_S$ were increased to 186 ± 6% (n = 4) of control. Similarly, currents evoked by 0.5 μM 5-HT at the 5-HT$_3$R-A were increased to

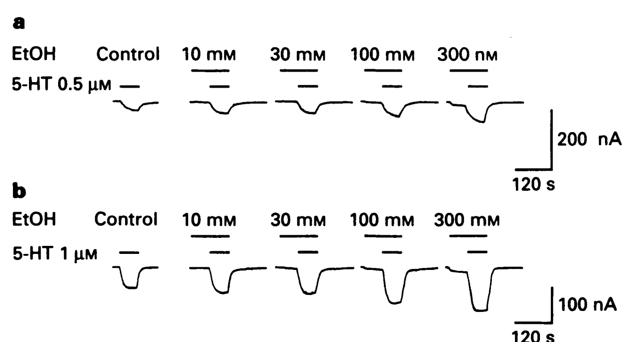

Figure 1 Ethanol (EtOH) potentiates 5-HT$_3$ receptor-mediated currents. All records illustrated are from *Xenopus laevis* oocytes voltage-clamped at −30 mV and expressing the 5-HT$_3$R-A (a) or the 5-HT$_3$ R-A$_S$ (b) subunit. Inward currents elicited by bath-applied 5-HT (0.5 μM for 5-HT$_3$R-A; 1 μM for 5-HT$_3$R-A$_S$) are enhanced in a concentration-dependent manner by ethanol (30 mM–300 nM). The concentration of 5-HT used approximates to the EC_{20} of the relevant concentration-effect relationship for 5-HT. In this and all subsequent figures, the periods of drug application are indicated by the horizontal bars above each current record. In all illustrations currents were low pass filtered at 100 Hz.

176 ± 8% ($n = 4$) of control. A modest potentiation of 5-HT-induced responses, to 119 ± 3% ($n = 4$) and 115 ± 5% ($n = 5$) of control, was observed with 30 mM ethanol acting at the 5-HT$_3$R-A$_S$ and 5-HT$_3$-R-A respectively. At concentrations between 1 and 10 mM, ethanol had little or no effect upon currents elicited by 5-HT acting at either subunit ($n = 3-4$; Figure 1). At concentrations > 100 mM, ethanol induced a direct inward current. Such currents were not blocked by the 5-HT$_3$ receptor antagonist, ondansetron (10 nM), and were also observed on non-injected oocytes. Therefore, this direct current is unlikely to involve 5-HT$_3$ receptor activation.

Chloral hydrate

Trichloroethanol has been shown to enhance the 5-HT$_3$ receptor-mediated current of rat nodose ganglion neurones (Lovinger & Zhou, 1993). Trichloroethanol is an active metabolite of the sedative and anaesthetic chloral hydrate (Garrett & Lambert, 1973). The effect of chloral hydrate (0.1–30 mM) was studied using the experimental conditions employed for ethanol. For either subunit, clear concentration-dependent enhancement of 5-HT-induced currents was observed with concentrations of chloral hydrate ≥ 3 mM

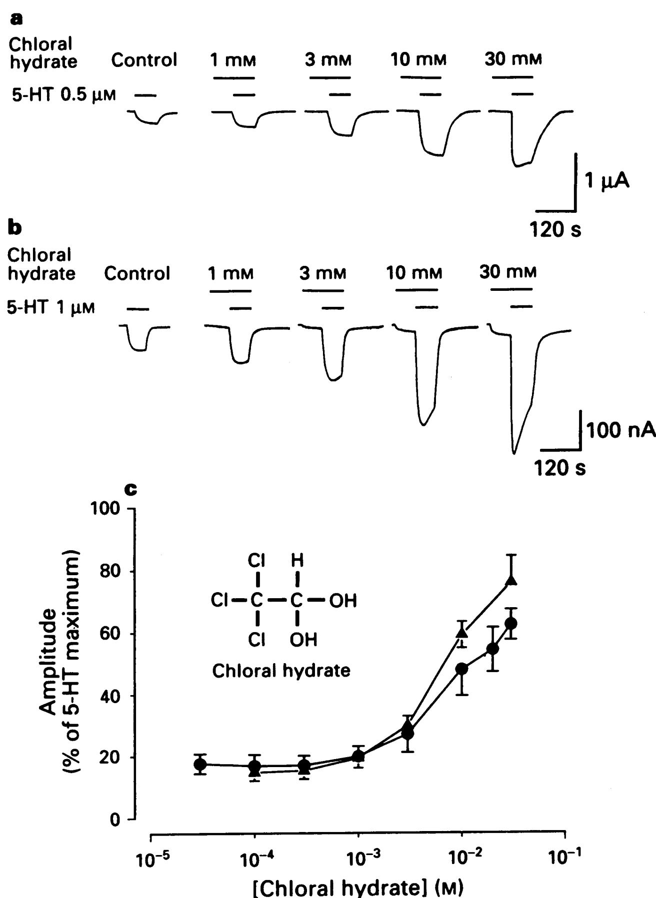

Figure 2 Chloral hydrate potentiates 5-HT$_3$ receptor-mediated currents. All records illustrated are from *Xenopus laevis* oocytes voltage-clamped at −30 mV and expressing the 5-HT$_3$R-A (a) or the 5-HT$_3$R-A$_S$ (b) subunit. The inward current produced by bath application of 5-HT (0.5 μM for 5-HT$_3$R-A; 1 μM for 5-HT$_3$R-A$_S$) is enhanced in a concentration-dependent manner by chloral hydrate (1 mM–30 mM). The concentrations of 5-HT used produce a response approximating to 20% of maximum attainable with a saturating concentration of 5-HT (i.e. EC$_{20}$ values). (c) The relationship between the concentration of bath-applied chloral hydrate (log scale; x-axis) and the magnitude of the potentiation of the 5-HT-induced current (expressed as a percentage of the maximum response to a saturating concentration of 5-HT, y-axis) for the 5-HT$_3$R-A (●) and 5-HT$_3$R-A$_S$ (▲) subunit. Each point represents the mean ± s.e.mean of data obtained from 4 oocytes.

(Figure 2). The maximal effect of chloral hydrate could not be determined because at concentrations > 30 mM the compound proved toxic to both injected and non-injected oocytes. Clearly, chloral hydrate is more potent than ethanol and is effective upon both splice variants of the 5-HT$_3$R-A. On some oocytes chloral hydrate (> 3 mM) alone induced an inward current. Such currents were not antagonized by ondansetron (10 nM) and were also observed on uninjected oocytes. Therefore, this direct effect of chloral hydrate is not mediated through 5-HT$_3$ receptor activation.

Trichloroethanol

Trichloroethanol (0.3–10 mM) produced a concentration-dependent enhancement of the 5-HT-induced current recorded from oocytes expressing either splice variant of the 5-HT$_3$R-A (Figures 3 and 4). In both cases, enhancement was evident at 0.3 mM, and the maximal effect of trichloroethanol occurred at 10 mM. At higher concentrations of trichloroethanol, the magnitude of the potentiation was reduced and currents evoked by low concentrations of 5-HT (i.e. 1 μM) demonstrated a rapid decay from their peak value in the continued presence of the agonist (Figure 3). This phenomenon, which was not apparent in control responses elicited by 1 μM 5-HT, or those recorded in the presence of lower concentrations of trichloroethanol, might reflect either enhancement of desensitization, or a channel blocking action, that becomes evident at high concentrations of the compound. Concentrations of trichloroethanol > 0.3 mM induced a direct current which was not blocked by ondansetron (10–100 nM; see Figure 7c for example) and was observed on uninjected oocytes. Thus this direct action is unrelated to 5-HT$_3$ receptor activation and was not studied further.

Previous work has established that the potentiation of the 5-HT$_3$ receptor-mediated current of rat nodose ganglion neurones by trichloroethanol is associated with an increase in the apparent affinity of the receptor for 5-HT (Lovinger & Zhou, 1993). Consistent with this observation, experiments performed on oocytes expressing the 5-HT$_3$R-A subunit demonstrated 5 mM trichloroethanol to produce a parallel leftward shift of the 5-HT concentration-response curve with no change of the maximal current produced (Figure 3). Analysis of these concentration-response curves revealed the 5-HT EC$_{50}$ to be reduced from 1 ± 0.04 μM ($n = 4$) to 0.5 ± 0.01 μM ($n = 4$) in the presence of 5 mM trichloroethanol (Figure 3). Similarly, for oocytes expressing the 5-HT$_3$R-A$_S$ subunit, trichloroethanol (5 mM) reduced the 5-HT EC$_{50}$ from 2.1 ± 0.05 μM ($n = 11$) to 1.3 ± 0.1 μM ($n = 4$) although additionally a small increase of the 5-HT maximum response was evident (Figure 4).

Radioligand binding studies

To investigate further the interaction of trichloroethanol with the 5-HT$_3$ receptor, its influence upon the specific binding of the radiolabelled antagonist [^{3}H]-granisetron to membrane preparations made from NG 108-15 cells and HEK 293 cells (stably transfected with the 5-HT$_3$R-A$_S$ subunit; Hope, 1993) was determined. [^{3}H]-granisetron (0.1–12.6 nM) bound to a saturable population of binding sites (non-specific binding being defined by the presence of 10 μM tropisetron) in HEK 293 and NG 108-15 cell homogenates (Table 1). Trichloroethanol, at a concentration (2.5 mM) producing a substantial enhancement of the 5-HT$_3$ receptor-mediated current (Figures 3 and 4), had little or no effect upon the K_d, B_{max}, or the Hill coefficient of [^{3}H]-granisetron binding to homogenates of either cell line (Table 1).

The 5-HT$_3$ receptor agonists, 5-HT, 2-methyl-5-HT and phenylbiguanide and the 5-HT$_3$ receptor antagonist tropisetron competed for a similar proportion of [^{3}H]-granisetron (0.9–1.1 nM) binding in both the HEK 293 and NG 108-15 cell preparations (approximately 90–95%). In both prepara-

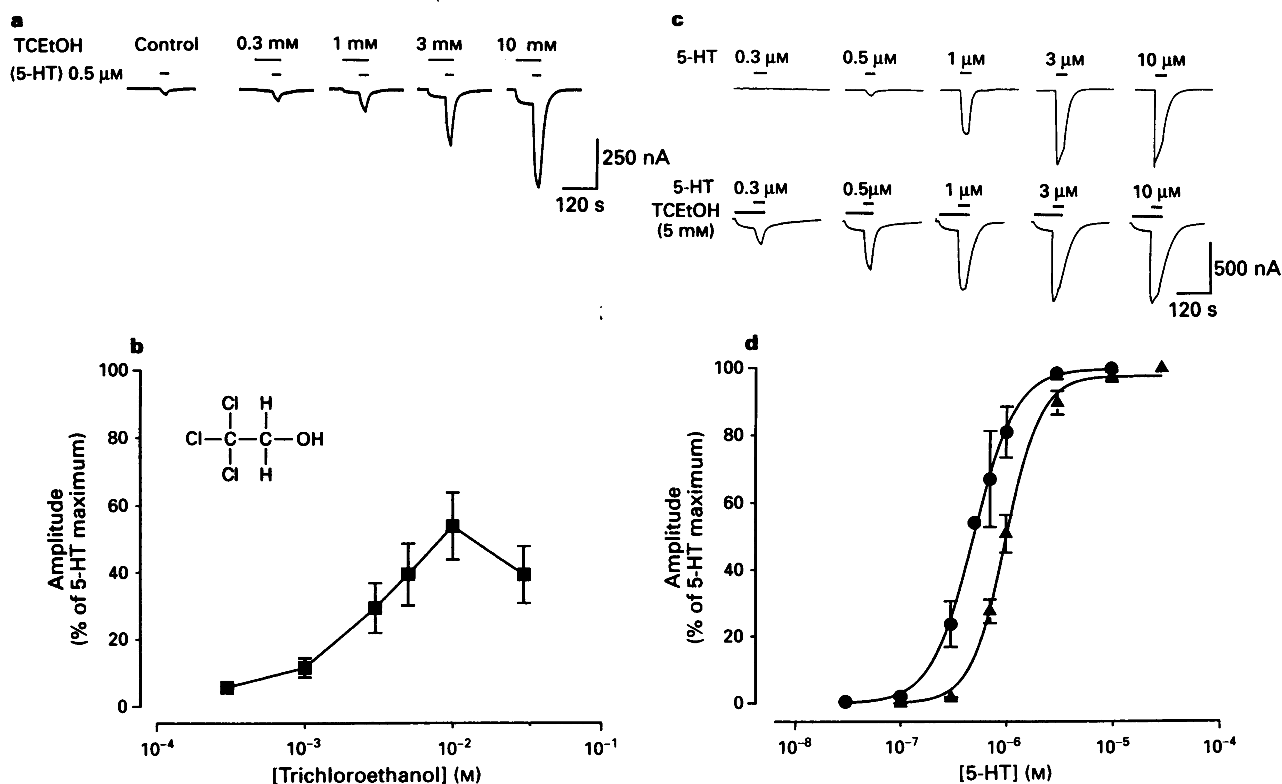

Figure 3 Trichloroethanol (TCEtOH) potentiates currents mediated by the 5-HT$_3$R-A subunit. All records illustrated are from *Xenopus laevis* oocytes voltage-clamped at a holding potential of −30 mV. (a) Inward currents evoked by a bath applied 5-HT (0.5 μM) are enhanced in a concentration-dependent manner by trichloroethanol. Note that the inward current elicited by trichloroethanol (⩾1.0 mM) alone was resistant to antagonism by 10 nM ondansetron and was evident on control (non-injected) oocytes (not illustrated). (b) The relationship between the concentration of bath-applied trichloroethanol (log scale, x-axis) and the potentiation of the 5-HT-induced current (expressed as a percentage of the maximum response to 5-HT, y-axis). (c) The traces illustrate the concentration-dependent inward current response to bath applied 5-HT (0.3–10 μM) in the presence and absence of 5 mM trichloroethanol. (d) The relationship between the concentration of bath applied 5-HT (log scale; x-axis) and peak current amplitude (expressed as a percentage of the current produced by a saturating concentration (30 μM) of 5-HT, y-axis) in the presence (●) and absence (▲) of 5 mM trichloroethanol. The curves were fitted to the data points as described in the Methods. In control, the EC_{50} and Hill slope were found to be 1.0 ± 0.04 μM and 2.6 ± 0.3 (n = 4) respectively. In the presence of 5 mM trichloroethanol, an EC_{50} of 0.5 ± 0.01 μM and a Hill slope of 2.2 ± 0.1 (n = 4) were estimated. In both (b) and (d), each point represents the mean ± s.e.mean of data obtained from 4 oocytes.

tions, the agonists were effective in the micromolar range, whereas the potent 5-HT$_3$ receptor antagonist, tropisetron, was active at nanomolar concentrations. Analysis of the competition curves indicated that generally the agonists competed for [^{3}H]-granisetron binding with Hill coefficients greater than unity, although the Hill coefficients were higher with the NG 108-15 than the HEK 293 cell preparation (Figure 5, Table 2). Competition with the antagonist tropisetron generated Hill coefficients of approximately unity in both preparations (Figure 5, Table 2).

The presence of trichloroethanol (2.5 mM) enhanced the affinity with which 5-HT, 2-methyl-5-HT and phenylbiguanide competed for the [^{3}H]-granisetron-labelled 5-HT$_3$ receptor in both the HEK 293 and NG 108-15 cell preparations (increases in affinity were between 2–4 fold, Figure 5, Table 2). However, the presence of trichloroethanol (2.5 mM) did not change the affinity with which tropisetron or ondansetron competed for the radiolabelled receptor in both preparations (Figure 5, Table 2). In addition, trichloroethanol (2.5 mM) modified the nature of the agonist competition curves for the [^{3}H]-granisetron-labelled receptor in the NG 108-15 cell preparation (Figure 5, Table 2). Thus, the presence of trichloroethanol (2.5 mM) reduced the Hill coefficients with which each of the agonists competed such that they approached unity (Figure 5, Table 2). This phenomenon was not apparent with the HEK 293 cell preparation (Figure 5, Table 2). In both the HEK 293 and NG 108-15 cell preparation, the presence of trichloroethanol (2.5 mM) did not change the nature in which tropisetron competed for the [^{3}H]-granisetron-labelled receptor (Figure 5, Table 2).

2-Methyl-5-HT and trichloroethanol

As discussed above, trichloroethanol enhanced the displacement of [^{3}H]-granisetron binding by 2-methyl-5-HT (Table 2). We have previously demonstrated that 2-methyl-5-HT acts as a partial agonist on the 5-HT$_3$R-A and 5-HT$_3$R-A$_S$ subunits (Hope *et al.*, 1993; Downie *et al.*, 1994). Hence, it was of interest to determine the influence of trichloroethanol on currents induced by 2-methyl-5-HT. For oocytes expressing the 5-HT$_3$R-A subunit, 2-methyl-5-HT (1 μM–0.3 mM) induced a concentration-dependent inward current with an estimated EC_{50} of 13 ± 0.4 μM (n = 4) and a maximal effect amounting to 63 ± 0.7% (n = 4) of the maximal current produced by a saturating concentration of 5-HT. Trichloroethanol (5 mM) reduced the 2-methyl-5-HT EC_{50} to 4.6 ± 0.2 μM (n = 4), but additionally increased the maximum to 101 ± 1.6% (n = 4) of that produced by 5-HT (Figure 6). Similarly, for oocytes expressing the 5-HT$_3$R-A$_S$ subunit, 2-methyl-5-HT (1 μM–0.3 mM) evoked a concentration-dependent inward current with an estimated EC_{50} of 15 ± 2 μM (n = 4), but with a maximal effect that was only 9 ± 0.2% (n = 4) of that elicited by a maximally effective concentration of 5-HT. Trichloroethanol (5 mM) reduced the EC_{50} for 2-methyl-5-HT to 5 ± 0.4 μM (n = 4) and greatly increased the

maximal response to 74 ± 2% ($n = 4$) of that produced by 5-HT (Figure 6).

Quipazine and trichloroethanol

Unusually, the competition for [^{3}H]-granisetron binding by the antagonist quipazine was enhanced by trichloroethanol, whereas the competition by the antagonists ondansetron and tropisetron was unaffected (Figure 5; Table 2). In this respect, the influence of trichloroethanol on quipazine is similar to its effect on the 5-HT_3 receptor agonists. Therefore, the interaction of quipazine and trichloroethanol was investigated further on *Xenopus* oocytes expressing the 5-HT_3R-A subunit. Quipazine (1–300 nM) produced a concentration-dependent inhibition of the current elicited by a submaximal concentration of 5-HT (1.5 μM) giving an IC_{50} value of 18 ± 3 nM ($n = 4$; Figure 7). In the absence of 5-HT, quipazine (10 nM–0.3 μM) induced a small inward current, amounting to 0.7–1.6% of the maximal current that could be elicited with a saturating concentration of 5-HT (see Figure 7). However, in the presence of 5 mM trichloroethanol, the quipazine-induced current response was increased substantially, such that 0.3 μM quipazine now induced a response approximately 40% of that produced by a maximal concentration of 5-HT (Figure 7). The current elicited by quipazine in the presence of trichloroethanol was

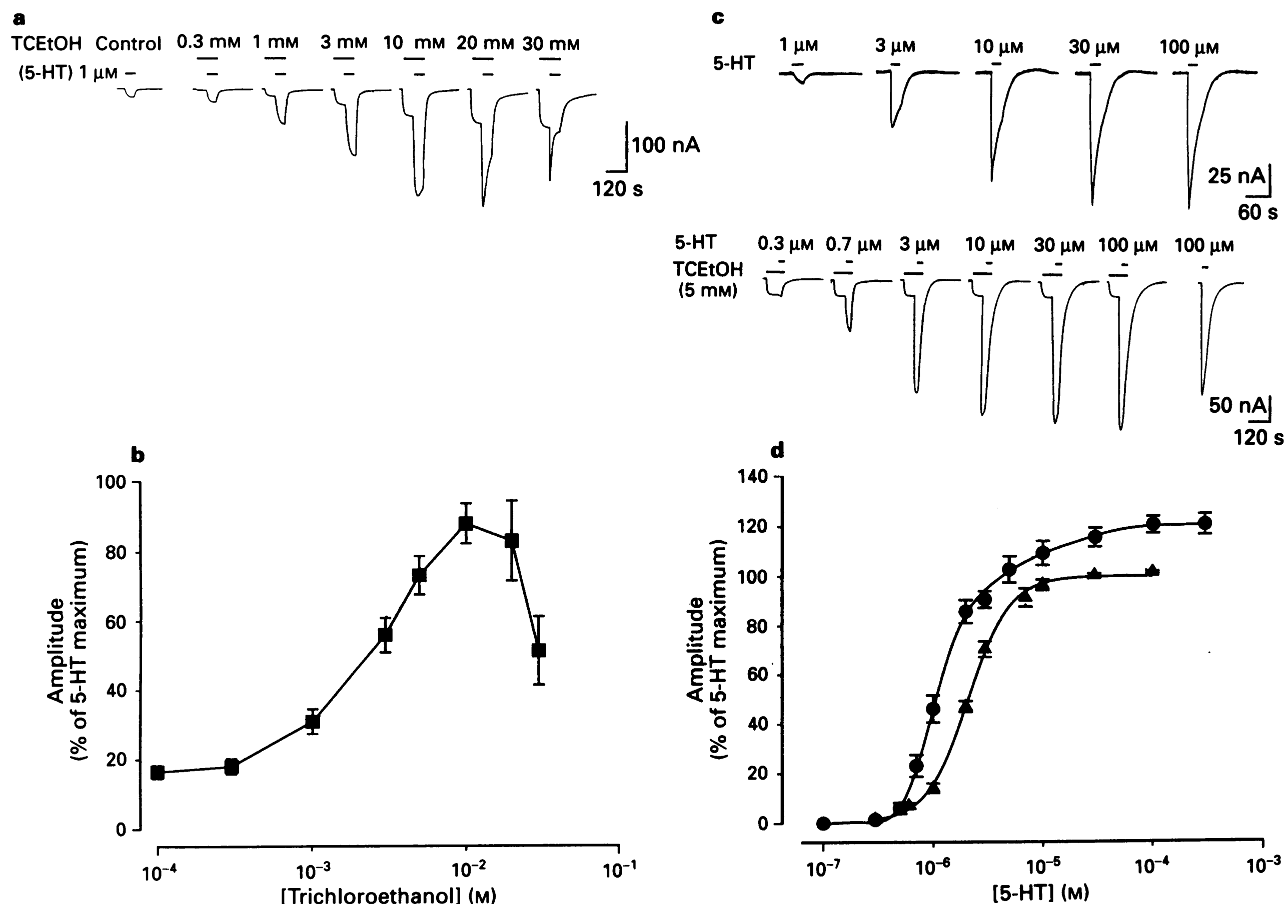

Figure 4 Trichloroethanol (TCEtOH) potentiates currents mediated by the 5-HT_3R-A_S subunit. All records illustrated are from *Xenopus laevis* oocytes voltage-clamped at −30 mV. (a) The inward current produced by bath applied 5-HT (1 μM) is enhanced in a concentration-dependent manner by trichloroethanol (0.3–30.0 mM). The inward current produced by trichloroethanol alone was not blocked by 10 nM ondansetron and was evident on control (non-injected) oocytes. (b) The relationship between the concentration of bath-applied trichloroethanol (log scale, x-axis) and the potentiation of the 5-HT-induced current (expressed as a percentage of the maximum response to 5-HT, y-axis). (c) Records illustrating the concentration-dependent inward current response to bath applied 5-HT (0.3–100 μM) in the presence and absence of 5 mM trichloroethanol. (d) The relationship between the concentration of bath-applied 5-HT (log scale; x-axis) is plotted against the current amplitude (expressed as a percentage of the current produced by a saturating concentration (100 μM) of 5-HT) in the presence (●) and absence (▲) of 5 mM trichloroethanol. The curves were fitted to the data points as described in the Methods and gave a control EC_{50} of 2.1 ± 0.0.5 μM and a Hill slope of 2.2 ± 0.1 ($n = 4$). In the presence of 5 mM trichloroethanol an EC_{50} of 1.3 ± 0.1 μM and a Hill slope of 1.9 ± 0.2 ($n = 4$) were estimated. For both (b) and (d), each point represents the mean ± s.e.mean of data obtained from 4 oocytes.

Table 1 Trichloroethanol (2.5 mM) has no effect upon the K_d, B_{max} or Hill coefficient (n_H) of [^{3}H]-granisetron binding to membrane homogenates prepared from either NG 108-15 cells or HEK 293 cells expressing the 5-HT_3R-A_S

	Without trichloroethanol			*With 2.5 mM trichloroethanol*		
Tissue	K_d (nM)	B_{max} (fmol mg^{-1})	n_H	K_d (nM)	B_{max} (fmol mg^{-1})	n_H
HEK 293 cells	2.01 ± 0.39	913 ± 163	1.06 ± 0.11	1.86 ± 0.46	912 ± 99	1.13 ± 0.05
NG 108-15 cells	2.43 ± 0.68	786 ± 133	1.11 ± 0.06	1.67 ± 0.27	728 ± 80	1.18 ± 0.04

Binding data were obtained with concentrations of [^{3}H]-granisetron in the range 0.1–12.6 nM. Non-specific binding was defined by the inclusion of tropisetron (10 μM). Data represent the mean ± s.e.mean from three independent experiments.

Table 2 The influence of trichloroethanol (2.5 mM) upon the affinity of agonist and antagonist ligands for the [^{3}H]-granisetron-labelled 5-HT$_3$ receptor endogenous to NG 108-15 cells and the 5-HT$_3$R-A$_S$ expressed in HEK 293 cells

NG 108-15 cells ([^{3}H]-granisetron concentration = 0.98–1.51 nM)	*Without trichloroethanol*		*With trichloroethanol* (2.5 mM)	
Drug	IC$_{50}$ (nM)	n_H	IC$_{50}$ (nM)	n_H
5-HT	752 ± 140	1.67 ± 0.08	170 ± 24	0.99 ± 0.03
2-Me-5-HT	2113 ± 195	2.10 ± 0.16	907 ± 122	1.29 ± 0.13
PBG	1790 ± 283	2.47 ± 0.90	620 ± 89	1.32 ± 0.13
Quipazine	4.88 ± 0.94	1.62 ± 0.13	2.32 ± 0.49	1.25 ± 0.22
Tropisetron	3.85 ± 0.83	0.98 ± 0.05	5.23 ± 1.04	0.99 ± 0.07
Ondansetron	34.3 ± 6.8	1.10 ± 0.17	35.3 ± 4.2	0.90 ± 0.06
HEK 293 cells ([^{3}H]-granisetron concentration = 0.90–1.10 nM)				
Drug	IC$_{50}$ (nM)	n_H	IC$_{50}$ (nM)	n_H
5-HT	548 ± 21	1.35 ± 0.11	229 ± 35	1.17 ± 0.05
2-Me-5-HT	2847 ± 590	1.30 ± 0.03	1097 ± 114	1.36 ± 0.12
PBG	2907 ± 753	1.10 ± 0.08	837 ± 73	1.34 ± 0.13
Tropisetron	3.80 ± 1.13	0.88 ± 0.03	4.03 ± 1.25	0.97 ± 0.05

Data are the mean ± s.e.mean of 3–6 independent experiments.
Abbreviations: 2-Me-5-HT, 2-methyl-5-HT; PBG, 1-phenylbiguanide.

abolished by ondansetron (100 nM) indicating that it was due to 5-HT$_3$ receptor activation (Figure 7). Under identical recording conditions, no such current was observed with the 5-HT$_3$ receptor antagonists, granisetron (1 nM), BRL 46470 (1 nM), ondansetron (10 nM), (+)-tubocurarine (30 nM), metoclopramide (1 μM) or cocaine (1 μM) applied alone, or in the presence of 5 mM trichloroethanol (results not shown).

Discussion

Many of the properties of 5-HT$_3$ receptors endogenous to neurones and neuronal cell lines are faithfully represented by the recombinant 5-HT$_3$R-A subunit, or its splice variant, 5-HT$_3$R-A$_S$ (Maricq *et al.*, 1991; Hope *et al.*, 1993; Yakel *et al.*, 1993; Downie *et al.*, 1994). However, the actions of putative positive allosteric modulators of the 5-HT$_3$ receptor (Peters *et al.*, 1992) on these recombinant subunits have not been determined. Previous electrophysiological studies have demonstrated that ethanol and trichloroethanol enhance 5-HT$_3$ receptor-mediated currents recorded from NCB-20 cells and rat nodose ganglion neurones (Lovinger, 1991; Lovinger & White, 1991; Lovinger & Zhou, 1993). By contrast, the 5-HT$_3$ receptor-mediated uptake of [^{14}C]-guanidinium into NG 108-15 cells is markedly suppressed by 100 mM ethanol (Emerit *et al.*, 1993). The present study demonstrates that ethanol, trichloroethanol and chloral hydrate potentiate 5-HT-induced currents recorded from *Xenopus laevis* oocytes expressing either the 5-HT$_3$R-A, or the 5-HT$_3$R-A$_S$ splice variants. Splice variants of the γ_2 GABA$_A$ receptor subunit (γ_{2S} and γ_{2L}) have been identified, which in some studies appear to impart a differential sensitivity to ethanol upon the receptor complex (Wafford *et al.*, 1991 cf. Sigel *et al.*, 1993). Here, homo-oligomeric receptors formed from either splice variant of the 5-HT$_3$R-A subunit were enhanced by ethanol and more potently by chloral hydrate and trichloroethanol.

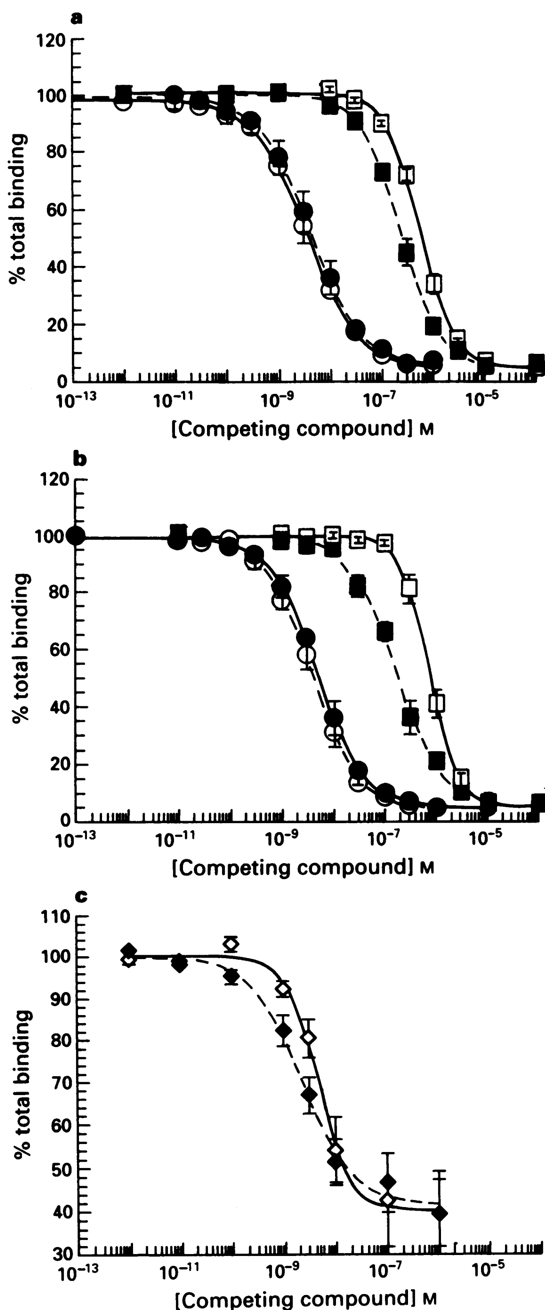

Figure 5 Competition for [^{3}H]-granisetron binding by 5-HT (□, ■), tropisetron (○, ●) and quipazine (◇, ◆) in the absence (open symbols) or presence (closed symbols) of trichloroethanol (2.5 mM). Experiments were performed with homogenates prepared from NG 108-15 cells (a,c) or HEK 293 cells (b) expressing the 5-HT$_3$R-A$_S$ receptor subunit. Data represent the mean ± s.e.mean (in some cases, s.e.mean smaller than the size of the symbol) from at least three independent experiments.

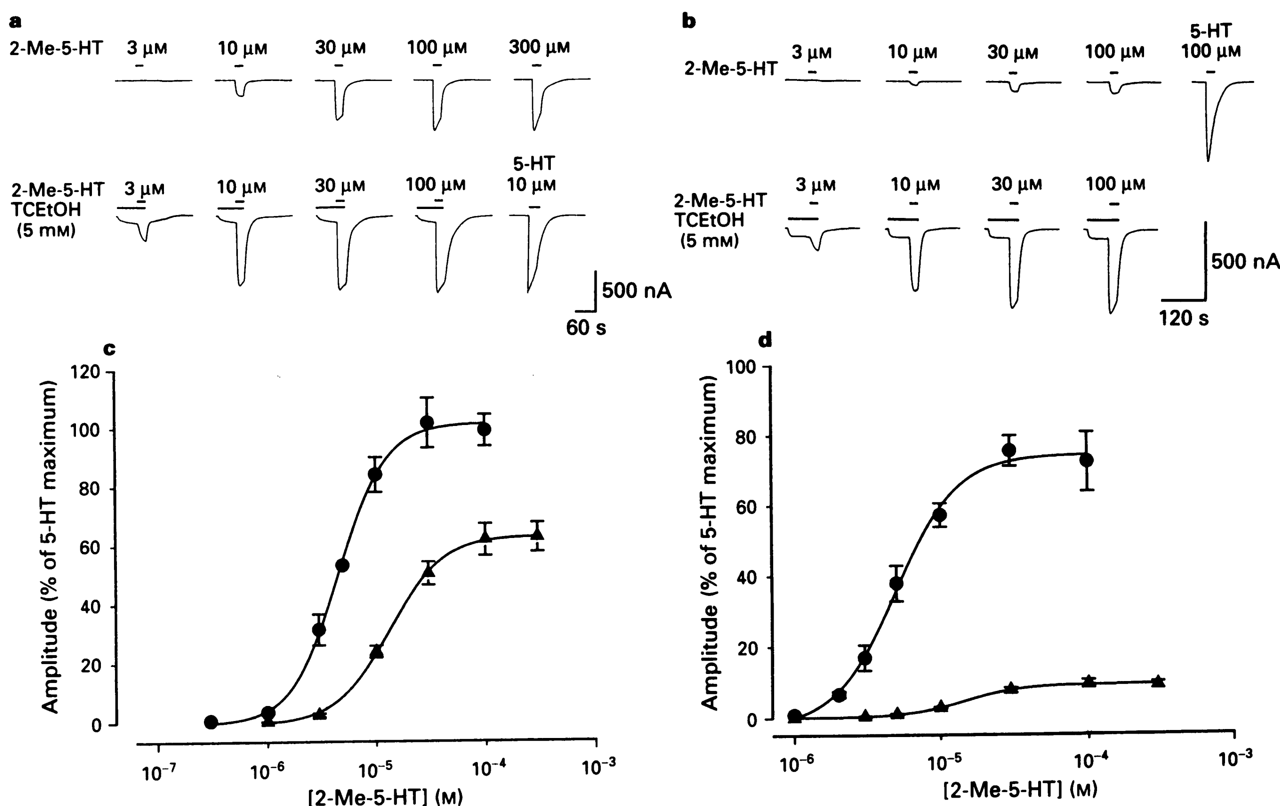

Figure 6 The interaction of trichloroethanol (TCEtOH) with 2-methyl-5-HT (2-Me-5-HT). All records illustrated are from *Xenopus laevis* oocytes voltage-clamped at −30 mV and expressing the 5-HT_3R-A (a) or the 5-HT_3R-A_S subunit (b). The traces illustrate the concentration-dependent inward current response to bath applied 2-methyl-5-HT (3–300 μM) in the presence and absence of 5 mM trichloroethanol. In each set of traces, the current response evoked by a maximal concentration of 5-HT is illustrated for comparison. Note that in both cases, the maximal current produced by 2-methyl-5-HT is less than that elicited by 5-HT and that the maximal effect for the 5-HT_3R-A_S is much less than for the 5-HT_3R-A subunit. For both subunits, trichloroethanol clearly increases the amplitude of the maximum response to 2-methyl-5-HT. (c and d) The concentration of bath-applied 2-methyl-5-HT (log scale, x-axis) is plotted against the current amplitude (expressed as a percentage of the current produced by a maximum concentration of 5-HT) in the presence (●) and absence (▲) of 5 mM trichloroethanol for the 5-HT_3R-A (c) and the 5-HT_3R-A_S (d) subunit. The curves were fitted to the data points as described in the Methods. For the 5-HT_3R-A (c), the control 2-methyl-5-HT EC_{50} is 13 ± 0.4 μM with a calculated maximum amounting to 63 ± 0.7% of that produced by 5-HT ($n = 4$). In the presence of 5 mM trichloroethanol, the 2-methyl-5-HT EC_{50} is reduced to 4.6 ± 0.2 μM and the calculated maximum is increased to 101 ± 1.6% of that produced by 5-HT ($n = 4$). For the 5-HT_3R-A_S (d), the control 2-methyl-5-HT EC_{50} is 15 ± 1 μM and the caclulated maximum is only 9 ± 0.2% of that produced by 5-HT ($n = 4$). In the presence of 5 mM trichloroethanol, the 2-methyl-5-HT EC_{50} is reduced to 5 ± 0.4 μM and the maximum is augmented to 74 ± 2% of that produced by 5-HT ($n = 4$). In both (c) and (d), each point represents the mean ± s.e.mean of data obtained from 4 oocytes.

A number of studies have reported 5-HT_3 receptor antagonists to reduce ethanol intake in animals and man (see Sellers *et al.*, 1992). The concentrations of ethanol necessary to produce clear potentiation of 5-HT-induced currents recorded from oocytes expressing either splice variant are broadly consistent with previous results obtained with NCB-20 cells (Lovinger, 1991) and rat nodose ganglion neurones (Lovinger & White, 1991). However, such concentrations are relatively high in relation to the behavioural effects of ethanol. Here, threshold effects were observed with 30 mM ethanol, which corresponds to a plasma concentration in man that is associated with gross intoxication. A robust potentiation of 5-HT-induced currents occurred only at concentrations (100–300 mM) of ethanol known to produce marked neurological impairment, coma and death due to respiratory depression. Furthermore, a variety of ligand-gated ion channels are influenced by similar concentrations of ethanol including the NMDA (Lovinger *et al.*, 1989), AMPA (Lovinger, 1993), $GABA_A$ (Aguayo, 1990; Nakahiro *et al.*, 1991; Wafford *et al.*, 1991; Kurata *et al.*, 1993 cf. Sigel *et al.*, 1993) and nicotinic (Dilger & Brett, 1991) receptor channel complexes. Hence, the behavioural actions of ethanol probably involve multiple molecular targets.

The actions of trichloroethanol were investigated in detail. In rat nodose ganglion neurones, 5 mM trichloroethanol has been shown to shift the 5-HT-concentration-response curve to the left, but to have little effect on the maximum current produced by 5-HT (Lovinger & Zhou, 1993). In the present study, 5 mM trichloroethanol, acting at either the 5-HT_3R-A or 5-HT_3R-A_S subunits, produced an approximately 2 fold sinistral shift in the concentration-response curve to 5-HT, indicating an increase in the apparent affinity of the receptor for the agonist. Consistent with this observation, the agonists 5-HT, phenylbiguanide and 2-methyl-5-HT displaced [^{3}H]-granisetron binding from NG 108-15 and HEK 293 cell homogenates with enhanced potency in the presence of trichloroethanol (2.5 mM). This effect is unlikely to involve an alteration in the affinity of the receptor for the radiolabelled antagonist, since trichloroethanol (2.5 mM) had no effect on the binding of [^{3}H]-granisetron *per se*, nor the displacement of this binding by the 5-HT_3 receptor antagonists, tropisetron and ondansetron. Collectively, these observations suggest a selective influence of trichloroethanol upon agonist binding and subsequent receptor activation.

The effect of trichloroethanol upon the agonist action of 2-methyl-5-HT was of particular interest. In voltage-clamp studies made on NG 108-15, N1E-115 and PC12 cell lines, frog dorsal root ganglion neurones and *Xenopus* oocytes

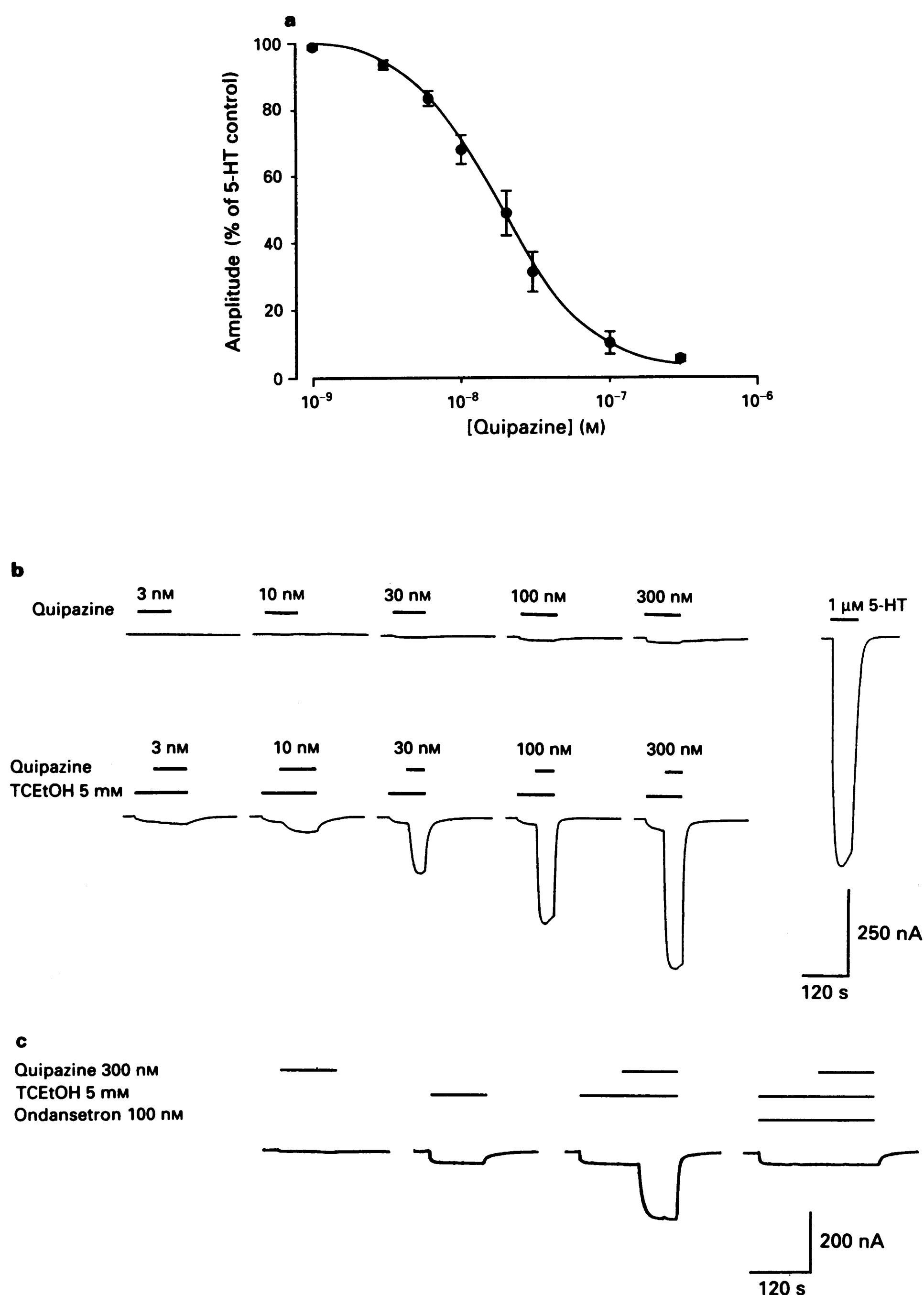

Figure 7 The interaction of trichloroethanol (TCEtOH) with quipazine. (a) Concentration-inhibition curve for bath applied quipazine (log scale, x-axis) as an antagonist of inward current responses evoked by bath applied 5-HT (1.5 μM, y-axis) on *Xenopus* oocytes expressing the 5-HT$_3$R-A subunit. The fitted curve (see Methods) yields an IC_{50} for quipazine of 18 ± 3 nM. Data points are the mean ± s.e.mean of 4 observations made on different oocytes. (b, upper row of traces) Bath applied quipazine (3 nM–300 nM) elicits concentration-dependent inward current responses in a *Xenopus* oocyte expressing the 5-HT$_3$R-A subunit. To emphasize the low amplitude of the quipazine-induced response, a current evoked by 5-HT (1 μM) is illustrated for comparison. In the same oocyte (lower row of traces), responses to quipazine are greatly potentiated in amplitude by trichloroethanol (5 mM). (c) Quipazine-induced currents recorded in the presence of trichloroethanol (5 mM) are abolished by ondansetron (100 nM). Note that the direct effect of trichloroethanol is resistant to antagonism by ondansetron. All recordings were performed at a holding potential of −30 mV.

expressing the 5-HT$_3$R-A or 5-HT$_3$R-A$_S$ subunit, the maximal current response to 2-methyl-5-HT is less than that produced by saturating concentrations of 5-HT (Sepúlveda *et al.*, 1991; Boess *et al.*, 1992; Yakushiji & Akaike, 1992; Furukawa *et al.*, 1992; Hope *et al.*, 1993; Downie *et al.*, 1994). This effect is subunit-dependent, with 2-methyl-5-HT being far less effective at the 5-HT$_3$R-A$_S$ than the 5-HT$_3$R-A subunit (Hope *et al.*, 1993; Downie *et al.*, 1994; Sepúlveda & Lummis, 1994). One interpretation of these data is that 2-methyl-5-HT acts as a partial agonist, but with greater efficacy at the 5-HT$_3$R-A splice variant, a notion supported by experiments investigating the interaction of 2-methyl-5-HT and 5-HT (Downie *et al.*, 1994). For both 5-HT$_3$R-A and 5-HT$_3$R-A$_S$ subunits, trichloroethanol (5 mM) produced an approximately 3 fold sinistral shift of the concentration-response curve to 2-methyl-5-HT. Moreover, in contrast to the results obtained with 5-HT, the maxima of the 2-methyl-5-HT concentration-response curves were greatly enhanced.

In preliminary studies (D.L.D., J.A.P. & J.J.L., unpublished observations), the 5-HT$_3$ receptor agonist, SR5722A (Bachy *et al.*, 1993) was found to act as a partial agonist at the 5-HT$_3$R-A expressed in oocytes. Interestingly, the maximal effect of this compound, like that of 2-methyl-5-HT, was greatly enhanced by trichloroethanol. Hence, trichloroethanol may selectively enhance the maximal effect of at least some partial agonists (see below also). Single channel studies are required to determine the molecular mechanism of this intriguing interaction. Unfortunately, this approach is hampered by the extremely low single channel conductance (< 1pS) of the murine recombinant 5-HT$_3$ subunits (Gill *et al.*, 1995) and the 5-HT$_3$ receptors of the cell lines from which these cDNAs were derived (see Peters *et al.*, 1992; 1994). Future experiments utilizing solutions designed to increase the single channel conductance (van Hooft *et al.*, 1994), or employing preparations with larger single channel conductances (Peters *et al.*, 1992; 1994) may be instructive.

In contrast to the other antagonists tested, the competition for [^{3}H]-granisetron by quipazine was enhanced by trichloroethanol. In this respect, the actions of quipazine resembled those of an agonist. The majority of functional studies indicate that quipazine behaves as a 5-HT$_3$ receptor antagonist (e.g Lansdown *et al.*, 1980; Ireland & Tyers, 1987; Round & Wallis, 1987). However, in ligand binding studies, quipazine competes for [^{3}H]-GR65630 (Kilpatrick *et al.*, 1987), [^{3}H]-granisetron, [^{3}H]-LY 274,584 and [^{3}H]-GR 67330 (Barnes *et al.*, 1992) binding in rat cortical membranes with Hill coefficient greater than unity, an effect that is shared with 5-HT$_3$ receptor agonists, but not commonly with other antagonists. More directly, it has recently been reported that quipazine causes the uptake of [^{14}C]-guanidinium into NG 108-15 cells via 5-HT$_3$ receptor activation (Emerit *et al.*, 1993).

In the present study, quipazine produced a concentration-dependent antagonism of the 5-HT-induced current recorded from *Xenopus* oocytes expressing the 5-HT$_3$R-A subunit. During these experiments, quipazine (10–300 nM) was noted to induce a small inward current, the magnitude of which was greatly enhanced by trichloroethanol (5 mM). Such potentiated currents were abolished by ondansetron (100 nM) indicating them to be 5-HT$_3$ receptor-mediated. The most parsimonious interpretation of these data is that quipazine is a partial agonist with very low efficacy and that the weak agonist effect, like that of 2-methyl-5-HT, is amplified in the presence of trichloroethanol. This interaction may be relevant to *in vivo* studies. For example, quipazine has been reported to evoke a 5-HT$_3$-receptor-mediated bradycardia and hypotension in ritanserin pretreated rats anaesthetized with chloral hydrate (which is reduced to trichloroethanol *in vivo*; Garrett & Lambert, 1973) (Vayssettes-Courchay *et al.*, 1990).

Whether the effects of alcohols on ligand gated ion channels represent a specific interaction with the receptor-channel protein, or are secondary to a perturbation of the surrounding membrane is not known. The present demonstration that a recombinant homo-oligomeric receptor expressed in a foreign membrane (HEK 293 cells or oocytes) retains the sensitivity to alcohols exhibited by native receptors is, therefore, of interest. The pharmacological properties of the 5-HT$_3$ receptor are highly species-dependent (Peters *et al.*, 1992). Although trichloroethanol enhances 5-HT$_3$ receptor-mediated currents recorded from murine neuroblastoma cells (Lovinger & White, 1991), HEK 293 cells stably transfected with the murine 5-HT$_3$R-A subunit (Gill *et al.*, 1994) and rat (Lovinger & Zhou, 1993) and rabbit nodose ganglion neurones (Gill *et al.*, 1994), we have recently demonstrated that it inhibits those of guinea-pig nodose ganglion neurones (Gill *et al.*, 1994). Hence, the sequencing of 5-HT$_3$ cDNAs encoding 5-HT$_3$ receptors exhibiting such differential effects (Lambert *et al.*, 1994) may lead to a better definition of the alcohol binding site(s).

We thank the Wellcome Trust, the M.R.C. and SmithKline Beecham for financial support. Dr David Julius for the generous gift of the 5-HT$_3$R-A cDNA, Drs Tom Blackburn and Brian Jones for helpful discussion and Gillian Thomson for typing the manuscript. We are grateful to Mrs Margaret West and Mr Phillip McWilliams for help with the cell culture.

References

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

BACHY, A., HÉAULME, M., GUIDICE, A., MICHAUD, J.-C., LEFEVRE, I.A., SOUILHAC, J., MANARA, L., EMERIT, M.B., GOZLAN, H., HAMON, M., KEANE, P.E., SOUBRIÉ, P. & LE FUR, G. (1993). SR 5722A: a potent and selective agonist at central and peripheral 5-HT$_3$ receptors *in vitro* and *in vivo*. *Eur. J. Pharmacol.*, **237**, 299–309.

BARNES, J.M., BARNES, N.M., COSTALL, B., JAGGER, S.M., NAYLOR, R.J., ROBERTSON, D.W. & ROE, S.Y. (1992). Agonist interactions with 5-HT$_3$ receptor recognition sites in the rat entorhinal cortex labelled by structurally diverse ligands. *Br. J. Pharmacol.*, **105**, 500–504.

BOESS, F.G. & MARTIN, I.L. (1994). Molecular biology of 5-HT receptors. *Neuropharmacology*, **33**, 275–317.

BOESS, F.G., SEPÚLVEDA, M.-I., LUMMIS, S.C.R. & MARTIN, I.L. (1992). 5-HT$_3$ receptors in NG 108-15 neuroblastoma × glioma cells: effect of the novel agonist 1-(m-chlorophenyl)-biguanide. *Neuropharmacology*, **31**, 561–564.

BRADFORD, M.M. (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, **72**, 248–254.

BUTLER, A., ELSWOOD, C.J., BURRIDGE, J., IRELAND, S.J., BUNCE, K.T., KILPATRICK, G.J. & TYERS, M.B. (1990). The pharmacological characterization of 5-HT$_3$ receptors in three isolated preparations derived from guinea-pig tissues. *Br. J. Pharmacol.*, **94**, 591–598.

DILGER, J.P. & BRETT, R.S. (1991). Actions of volatile anaesthetics and alcohols on cholinergic receptors channels. In *Molecular and Cellular Mechanisms of Alcohol and Anaesthetics*, ed. Rubin, E., Miller, K.W. & Roth, S.H. *Ann. N.Y. Acad. Sci.*, **625**, 616–627.

DOWNIE, D.L., HOPE, A.G., LAMBERT, J.J., PETERS, J.A., BLACKBURN, T.P. & JONES, B.J. (1994). Pharmacological characterization of the apparent splice variants of the murine 5-HT$_3$ R-A subunit expressed in *Xenopus laevis* oocytes. *Neuropharmacology*, **33**, 473–482.

DOWNIE, D.L., HOPE, A.G., LAMBERT, J.J., PETERS, J.A., BURCHELL, B. & JULIUS, D. (1993). Enhancement by trichloroethanol of agonist-induced currents mediated by a cloned murine 5-HT$_3$ receptor subunit (5-HT$_3$R-A) expressed in *Xenopus* oocytes. *Br. J. Pharmacol.*, **109**, 53P.

EISELÉ, J.-L., BERTRAND, S., GALZI, J.-L., DEVILLERS-THIÉRY, A., CHANGEUX, J.-P. & BERTRAND, D. (1993). Chimaeric nicotinic-serotonergic receptor combines distinct ligand binding and channel specificities. *Nature*, **366**, 479–483.

EMERIT, M.B., RIAD, M., FATTACINI, C.M. & HAMON, M. (1993). Characteristics of [^{14}C] guanidinium accumulation in NG 108-15 cells exposed to serotonin 5-HT$_3$ receptor ligands and substance P. *J. Neurochem.*, **60**, 2059–2067.

FURUKAWA, K., AKAIKE, N., ONODERA, H. & KOGURE, K. (1992). Expression of 5-HT$_3$ receptors in PC12 cells treated with NGF and 8-Br-cAMP. *J. Neurophysiol.*, **67**, 812–819.

GARRETT, E.R. & LAMBERT, H.J. (1973). Pharmacokinetics of trichloroethanol and metabolites and interconversions among variously referenced pharmacokinetic parameters. *J. Pharmaceut. Sci.*, **62**, 550–572.

GILL, C.H., LAMBERT, J.J. & PETERS, J.A. (1994). The actions of ketamine and trichloroethanol on 5-HT$_3$ receptors are species dependent. *Can. J. Physiol. Pharmacol.*, **72**, Suppl. 1, p 18.4.24.

GILL, C.H., PETERS, J.A. & LAMBERT, J.J. (1995). An electrophysiological investigation of the properties of a murine recombinant 5-HT$_3$ receptor stably expressed in HEK 293 cells. *Br. J. Pharmacol.* (in press).

GILL, C.H., PETERS, J.A., LAMBERT, J.J., HOPE, A.G. & JULIUS, D. (1993). Modulation by divalent cations of current responses mediated by a cloned murine 5-HT receptor (5-HT$_3$ R-A) expressed in HEK 293 cells. *Br. J. Pharmacol.*, **109**, 98P.

HOPE, A.G. (1993). The molecular cloning and functional expression of a murine 5-HT$_3$ receptor. *Ph.D. Thesis, The University of Dundee, Scotland.*

HOPE, A.G., DOWNIE, D.L., SUTHERLAND, L., LAMBERT, J.J., PETERS, J.A. & BURCHELL, B. (1993). Cloning and functional expression of an apparent splice variant of the murine 5-HT$_3$ receptor A subunit. *Eur. J. Pharmacol. (Mol. Pharmacol. Sect.)*, **245**, 187-192.

IRELAND, S.J. & TYERS, M.B. (1987). Pharmacological characterization of 5-hydroxytryptamine-induced depolarization of the rat isolated vagus nerve. *Br. J. Pharmacol.*, **90**, 229-238.

ISENBERG, K.E., UKHUN, I.A., HOLSTAD, S.G., JAFRI, S., UCHIDA, U., ZORUMSKI, C.F. & YANG, J. (1993). Partial cDNA cloning and NGF regulation of a rat 5-HT$_3$ receptor subunit. *Neuro-Report*, **5**, 121-124.

KILPATRICK, G.J., JONES, B.J. & TYERS, M.B. (1987). Identification and distribution of 5-HT$_3$ receptors in rat brain using radioligand binding. *Nature*, **330**, 746-748.

KLEE, W.B. & NIRENBERG, M. (1974). A neuroblasoma × glioma hybrid cell line with morphine receptors. *Proc. Natl. Acad. Sci. U.S.A.*, **71**, 3474-3477.

KOOYMAN, A.R., VAN HOOFT, J.A., VANDERHEIJDEN, P.M.L. & VIJVERBERG, H.P.M. (1994). Competitive and non-competitive effects of 5-hydroxyindole on 5-HT$_3$ receptors in N1E-115 neuroblastoma cells. *Br. J. Pharmacol.*, **112**, 541-546.

KURATA, K., MARSZALEC, W., HAMILTON, B.J., CARTER, D.B. & NARAHASHI, T. (1993). Alcohol modulation of cloned GABA$_A$ receptor-channel complex expressed in human kidney cell lines. *Brain Res.*, **631**, 143-146.

LAMBERT, J.J., PETERS, J.A. & HOPE, A.G. (1995). 5-HT$_3$ receptors. In *CRC Handbook of Ion Channels*, ed. North, R.A. pp. 177-211. Boca Raton: CRC Press.

LANSDOWN, M.J.R., NASH, H.L., PRESTON, P.R., WALLIS, D.I. & WILLIAMS, R.G. (1980). Antagonism of 5-hydroxytryptamine receptors by quipazine. *Br. J. Pharmacol.*, **68**, 525-532.

LOVINGER, D.M. (1991). Ethanol potentiation of 5-HT$_3$ receptor-mediated ion current in NCB-20 neuroblastoma cells. *Neurosci. Lett.*, **122**, 57-60.

LOVINGER, D.M. (1993). High ethanol sensitivity of recombinant AMPA-type glutamate receptors expressed in mammalian cells. *Neurosci. Lett.*, **159**, 83-87.

LOVINGER, D.M. & WHITE, G. (1991). Ethanol potentiation of 5-hydroxytryptamine$_3$ receptor-mediated ion current in neuroblastoma cells and isolated adult mammalian neurons. *Mol. Pharmacol.*, **40**, 263-270.

LOVINGER, D.M., WHITE, G. & WEIGHT, F.F. (1989). Ethanol inhibits NMDA-activated ion current in hippocampal neurons. *Science*, **243**, 1721-1724.

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.

MARICQ, A.V., PETERSON, A.S., BRAKE, A.J., MYERS, R.M. & JULIUS, D. (1991). Primary structure and functional expression of the 5-HT$_3$ receptor, a serotonin gated ion channel. *Science*, **254**, 432-437.

NAKAHIRO, M., ARAKAWA, O. & NARAHASHI, T. (1991). Modulation of γ-aminobutyric acid receptor-channel complex by alcohols. *J. Pharmacol. Exp. Ther.*, **259**, 235-240.

NEIJT, H.C,, KARPF, A., SCHOEFFTER, P., ENGEL, G. & HOYER, D. (1988). Characterisation of 5-HT$_3$ recognition sites in membranes of NG 108-15 neuroblastoma-glioma cells with [^{3}H]ICS 205-930. *Naunyn-Schmied. Arch. Pharmacol.*, **337**, 493-499.

NEWBERRY, N.R., CHESHIRE, S.H. & GILBERT, M.J. (1991). Evidence that the 5-HT$_3$ receptors of the rat, mouse and guinea-pig superior cervical ganglion may be different. *Br. J. Pharmacol.*, **102**, 615-620.

PETERS, J.A., LAMBERT, J.J. & MALONE, H.M. (1994). Electrophysiological studies of 5-HT$_3$ receptors. In *5-HT$_3$ Receptor Antagonists*, ed. Jones, B.J., King, F.D. & Sanger, G.J. pp. 115-153. Boca Raton: CRC Press.

PETERS, J.A., MALONE, H.M. & LAMBERT, J.J. (1991). Ketamine potentiates 5-HT$_3$ receptor mediated currents in rabbit nodose ganglion neurones. *Br. J. Pharmacol.*, **103**, 1623-1625.

PETERS, J.A., MALONE, H.M. & LAMBERT, J.J. (1992). Recent advances in the electrophysiological characterization of 5-HT$_3$ receptors. *Trends Pharmacol. Sci.*, **13**, 391-397.

ROUND, A. & WALLIS, D.I. (1987). Further studies on the blockade of 5-HT depolarizations of rabbit vagal afferent and sympathetic ganglion cells by MDL 72222 and other antagonists. *Neuropharmacology*, **26**, 39-48.

SAMBROOK, J., FRITSCH, E.F. & MANIATIS, T. (1989). *Molecular Cloning: A Laboratory Manual* (2nd Ed.). ed. Nolan, C., pp. 7.43-7.45. New York: Cold Spring Harbor Press.

SELLERS, E.M., HIGGINS, G.A. & SOBELL, M.B. (1992). 5-HT and alcohol abuse. *Trends Pharmacol. Sci.*, **13**, 69-75.

SEPÚLVEDA, M.-I. & LUMMIS, S.C.R. (1994). 5-HT$_3$ agonists can distinguish cloned 5-HT$_3$ receptors from NCB-20 and NG108-15 cell lines. *Br. J. Pharmacol.*, **112**, 316P.

SEPÚLVEDA, M.-I., LUMMIS, S.C.R. & MARTIN, I.L. (1991). The agonist properties of m-chlorophenylbiguanide and 2-methyl-5-hydroxytryptamine on 5-HT$_3$ receptors in N1E-115 neuroblastoma cells. *Br. J. Pharmacol.*, **104**, 536-540.

SIGEL, E., BAUR, R. & MALHERBE, P. (1993). Recombinant GABA$_A$ receptor function and ethanol. *FEBS Lett.*, **234**, 140-142.

UETZ, P., ABDELATTY, F., VILLAROEL, A., RAPPOLD, G., WEISS, B. & KOENEN, M. (1994). Organisation of the 5-HT$_3$ receptor gene and assignment to human chromosome 11. *FEBS Lett.*, **339**, 302-306.

VAN HOOFT, J.A., KOOYMAN, A.R., VERKERK, A., VAN KLEEF, R.G.D.M. & VIJVERBERG, H.P.M. (1994). Single 5-HT$_3$ receptor-gated ion channel events resolved in N1E-115 mouse neuroblastoma cells. *Biochem. Biophys. Res. Commun.*, **199**, 227-233.

VAYSSETTES-COURCHAY, C., BOUYSSET, F., VERBEUREN, T.J., LAUBIE, M. & SCHMITT, H. (1990). The cardiovascular effects of quipazine are mediated by peripheral 5-HT$_2$ and 5-HT$_3$ receptors in anaesthetised rats. *Eur. J. Pharmacol.*, **184**, 75-85.

WAFFORD, K.A., BURNETT, D.M., LEIDENHEIMER, N.J., BURT, D.R., WANG, J.B., KOJUJI, 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 gamma-2L subunit. *Neuron*, **7**, 27-33.

WERNER, P., HUMBERT, Y., BOESS, F.G., REID, J., JONES, K. & KAWASHIMA, E. (1993). Organisation of the murine 5-HT$_3$ receptor gene and investigation of its splice variants. *Soc. Neurosci. Abs.*, **19**, 1164.

YAKEL, J.L., LAGRUTTA, A., ADELMAN, J.P. & NORTH, R.A. (1993). Single amino acid substitution affects desensitization of the 5-hydroxytryptamine type 3 receptor expressed in *Xenopus* oocytes. *Proc. Natl. Acad. Sci. U.S.A.*, **90**, 5030-5033.

YAKUSHIJI, T. & AKAIKE, N. (1992). Blockade of 5-HT$_3$ receptor-mediated currents in dissociated frog sensory neurones by benzoxazine derivative, Y-25130. *Br. J. Pharmacol.*, **107**, 853-857.

(Received September 19, 1994
Revised November 21, 1994
Accepted December 21, 1994)