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5-Hydroxytryptamine$_3$ (5-HT$_3$) receptor-mediated depolarisation of the rat isolated vagus nerve: modulation by trichloroethanol and related alcohols

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Abstract

The ability of 2,2,2-trichloroethanol (TCE) and related alcohols to modify the 5-hydroxytryptamine$_3$ (5-HT$_3$) receptor-mediated depolarisation of the rat isolated cervical vagus nerve were investigated by extracellular electrophysiological recording using the 'grease gap' technique. TCE at millimolar concentrations increased the magnitude of the 5-HT$_3$ receptor-mediated depolarisations of the rat vagus nerve by a number of agonists (5-HT, phenylbiguanide (PBG), quipazine). Concentration response curves generated for the 5-HT$_3$ receptor agonists, 5-HT and PBG, in the absence and presence of TCE (5 mM) indicated that the potentiation in agonist-induced depolarisation was due to an increase in both agonist potency and apparent efficacy. Following apparent complete 5-HT$_3$ receptor desensitisation (induced by either 5-HT or PBG; 100 μM for 90 min), application of TCE (5 mM) in the continued presence of either agonist induced a depolarisation of the vagus nerve. In addition to TCE, a number of related alcohols (tribromoethanol, isopentanol and 5-chloropentanol but not ethanol) at millimolar concentrations also potentiated depolarisation of the vagus nerve induced by 5-HT. Combined application of both TCE (0.1–20 mM) and isopentanol (20 mM) indicated that the potentiation of the 5-HT$_3$ receptor-mediated depolarisation by these alcohols was not additive. The present studies indicate that the 5-HT$_3$ receptor expressed on the cervical vagus nerve is susceptible to allosteric modulation by a number of alcohols including the anaesthetic agent TCE. Such an interaction may have relevance to the nausea and vomiting experienced by some patients following recovery from general anaesthesia.

Keywords: 5-HT$_3$ receptor; Allosteric modulation; Trichloroethanol; Alcohol; Nausea; Vomiting

1. Introduction

The 5-hydroxytryptamine$_3$ (5-HT$_3$) receptor is a member of the fast-acting ligand-gated ion channel family and primarily conducts monovalent cations (for review see Peters et al., 1992). Functional 5-HT$_3$ receptors appear to be exclusively expressed by elements of the nervous system, mediating excitation in both peripheral nerves and central nervous system neurones (for review see Jackson and Yakel, 1995).

In addition to the recognition site for 5-HT, it is becoming increasingly clear that the 5-HT$_3$ receptor complex possesses additional, pharmacologically distinct sites through which alcohols and certain anaesthetic agents can allosterically modulate the function of this receptor (for review see Parker et al., 1996). Indeed, potentiation of 5-HT$_3$ receptor-mediated responses by some of these agents may exacerbate the nausea and vomiting that is evident during recovery from general anaesthesia which is alleviated by 5-HT$_3$ receptor antagonists (e.g., Russell and Kenny, 1992). Given the relatively high level of 5-HT$_3$ receptor expression on the vagus nerve (both in the periphery and on vagal terminals within the chemoreceptor trigger zone; e.g., Hoyer et al., 1989; Kilpatrick et al., 1989; Pratt et al., 1990), and the generally accepted importance of these vagal 5-HT$_3$ receptors in the mediation of the emesis following cancer chemo- and radiation therapy (e.g., Andrews et al., 1988), allosterically mediated potentiation of vagal 5-HT$_3$ receptors may exacerbate postoperative nausea and vomiting (e.g., Parker et al., 1996). While previous studies have demonstrated that the 5-HT$_3$ recep-

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tor expressed by nodose ganglion cells can be modulated allosterically by certain alcohols and anaesthetic agents (Lovinger and White, 1991; Peters et al., 1991; Gill et al., 1994), the present study investigated whether the 5-HT_3 receptor located on peripheral portions of the vagus nerve was susceptible to allosteric modulation assessed by extracellular recording of rat isolated vagus nerve using the grease-gap technique (e.g., Ireland and Tyers, 1987). The active metabolite of the general anaesthetic agent chloral hydrate, trichloroethanol (e.g., Garrett and Lambert, 1973) was the principle agent investigated since this compound has received more detailed attention with respect to its ability to allosterically modulate the 5-HT_3 receptor (for review see Parker et al., 1996).

2. Methods

2.1. Tissue preparation

Female Wistar rats (150–250 g) were killed by cervical dislocation and segments of cervical vagus nerve (15–20 mm), without the associated nodose ganglion, were gently dissected away from the attached carotid artery and placed in gassed (95% O_2/5% CO_2) chilled Krebs buffer (mM: NaCl 120, KCl 4.75, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25, glucose 11). The connective tissue sheath was removed from the nerve and the desheathed nerve was transferred to an inclined lightly greased (Vaseline) heated Perspex block (27°C). The wick of a silver–silver chloride electrode, mounted in a Pasteur pipette containing Krebs buffer, was placed on one end of the isolated nerve. The grease barrier was formed by applying grease over the wick of the electrode thereby electrically isolating one end of the nerve. The remainder of the nerve was covered with a strip of filter paper soaked in Krebs buffer before the wick of a second silver–silver chloride electrode was placed on the filter paper to record the potential difference between the two portions of the vagus nerve either side of the grease barrier which was monitored using a Universal Oscillograph (Harvard).

After placing of the recording electrodes, strips of filter paper were positioned to direct a flow of Krebs buffer (4–5 ml/min; 27°C, constantly gassed with 95% O_2/5% CO_2) over the portion of vagus nerve covered by the filter paper. The whole procedure was usually completed within 15 min of dissection.

2.2. Experimental protocol

All experiments were performed in the presence of the selective 5-HT_4 receptor antagonist GR113808A (100 nM; Gale et al., 1994) to eliminate the known 5-HT_4 receptor-mediated depolarisation of the rat vagus nerve (Rhodes et al., 1992), which was also evident in our preparation in the absence of GR113808A (data not shown).

2.2.1. Agonists

Noncumulative concentration response curves to agonists were obtained by including the agonist in the perfusing Krebs buffer for sufficient time to reach a maximum response (routinely about 90 s) with a 10–15 min 'washout' period between exposure to each agonist concentration.

2.2.2. Antagonists

An agonist concentration–response curve was constructed in the absence of a 5-HT_3 receptor antagonist before application of an antagonist via the perfusing Krebs buffer for at least 30–60 min (equilibration assumed to have been reached when two successive applications of a submaximal agonist concentration resulted in equal depolarisations to within 10%) before a second agonist concentration–response curve was constructed in the continued presence of the perfusing antagonist.

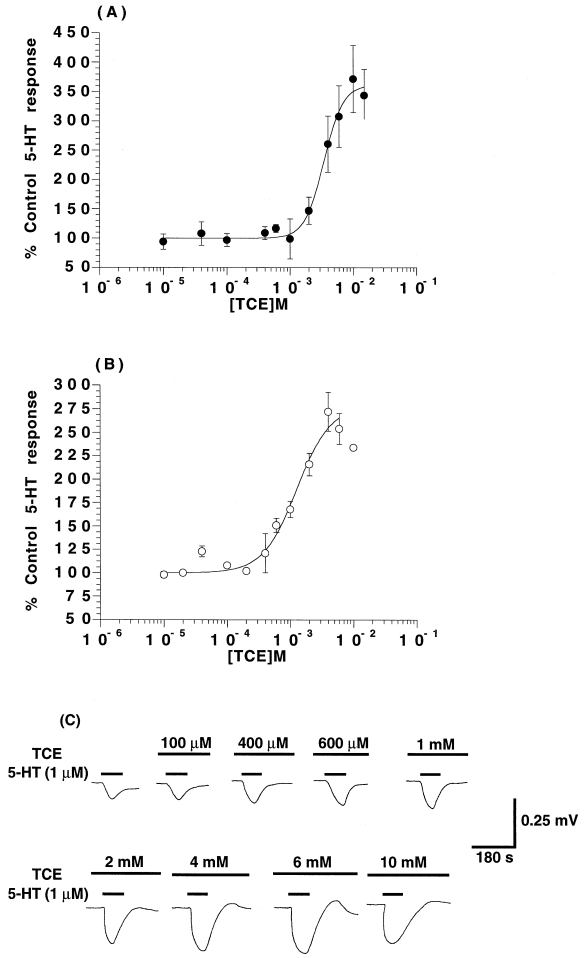

Fig. 1. Concentration–response relationship of TCE to enhance the depolarisations induced by 5-HT (●, A; 0.3 μM and ○, B; 1.0 μM) recorded from the rat isolated vagus nerve. (C) Example depolarisations evoked by 5-HT (1 μM) in the absence and presence of increasing concentrations of TCE (bars represent application of the indicated drug, not corrected for void volume. NB, a downward deflection represents depolarisation). Data (A and B) represents normalised values compared to the maximal response to 5-HT in the absence of TCE (mean ± SEM, $n = 4–5$). Experiments performed in the presence of the 5-HT_4 receptor antagonist GR113808A (100 nM).

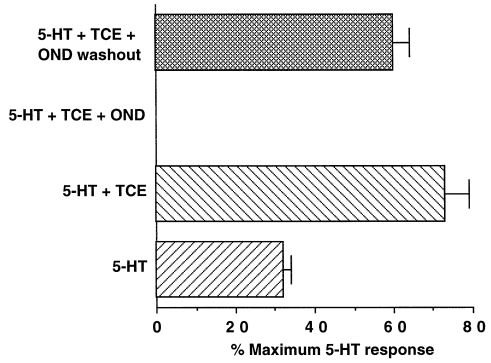

Fig. 2. Ability of ondansetron (OND; 50 nM) to antagonise the TCE (10 mM)-induced potentiation of the 5-HT (1 μM)-induced depolarisation of the rat isolated vagus nerve and the reversibility of the ondansetron antagonism on washout. Data are normalised to maximal response obtained from a 5-HT concentration response curve performed on the same tissue (mean ± SEM, $n = 3$). Experiments performed in the presence of the 5-HT_4 receptor antagonist GR113808A (100 nM).

2.2.3. Allosteric modulators

Cumulative concentration–response curves for potential allosteric modulators were constructed by including increasing concentrations of the potential allosteric modulator in the perfusing Krebs buffer for 15 min (sufficient for maximal effect) prior to the application of agonist.

Where relevant, variations in these protocols are outlined in Section 3.

2.3. Data analysis

Amplitudes of the agonist-evoked depolarisations were measured manually from chart recorder traces. The EC_{50} values and Hill coefficients were derived from full log concentration–response curves plotted by computer assisted iterative curve fitting according to the logistic equation: $r = (E_{max}[L]^n)/([L]^n + (EC_{50})^n)$, where r = response to a given concentration of agonist, E_{max} = maximal response to the agonist, EC_{50} = the concentration of the agonist required to evoke a 50% maximal response, $[L]$ = the concentration of the agonist required to evoke the response r, and n = Hill coefficient. The apparent dissociation constant for each antagonist (apparent pA_2) was estimated by the equation: apparent pA_2 = log (CR-1)-log [antagonist], where; CR (concentration ratio) = EC_{50} of agonist in the presence of antagonist/EC_{50} of agonist in the absence of antagonist. All data represent mean ± SEM.

2.4. Drugs

Granisetron (HCl, SmithKline Beecham), 5-HT (HCl, Sigma), ondansetron (HCl, Glaxo), phenylbiguanide (PBG; Aldrich), quipazine (dimaleate, RBI) and (*S*)-zacopride (HCl; Delalande) were dissolved in distilled water and were diluted in Krebs buffer. 5-Chloropentanol (Lancaster), ethanol (BDH), isopentanol (Sigma), 2,2,2-tribromoethanol (Aldrich) and 2,2,2-trichloroethanol (TCE; Sigma) were dissolved directly into Krebs buffer. All drugs were prepared daily and stored on ice (protected from light).

3. Results

3.1. Pharmacology of 5-HT-induced depolarisations of the rat isolated vagus nerve

In the presence of the 5-HT_4 receptor antagonist GR113808 (100 nM), 5-HT (1 nM–30 μM) evoked depolarisations of the rat isolated vagus nerve in a concentration dependent manner ($pEC_{50} = 5.76 \pm 0.07$; $n = 3$) that were antagonised by the specific 5-HT_3 receptor antagonists ondansetron (10 nM; apparent $pA_2 = 8.7 \pm 0.3$, $n = 3$) and granisetron (5 nM; apparent $pA_2 = 9.3 \pm 0.4$, $n = 3$) in an apparently competitive manner (i.e., parallel rightward shift of the 5-HT concentration response curve with no suppression of the maximal response achieved in the presence of the antagonist). Maximal 5-HT-induced depolarisations ranged in magnitude between 0.6 and 1.1 mV.

3.2. Potency of TCE to enhance 5-HT_3 receptor-mediated depolarisation of the rat isolated vagus nerve

TCE (10 μM–15 mM) administered alone failed to evoke a response in the vagus nerve preparation but increased the magnitude of the 5-HT (0.3 μM and 1 μM)-in-

Table 1
Modulation of 5-HT- and PBG-induced depolarisations of the rat isolated vagus nerve by TCE (5 mM)

	5-HT		PBG	
	− TCE	+ TCE	− TCE	+ TCE
pEC_{50}	5.87 ± 0.03	6.21 ± 0.05 *	5.93 ± 0.06	6.51 ± 0.06 * *
Hill coefficient	1.58 ± 0.1	1.54 ± 0.4	1.79 ± 0.6	1.42 ± 0.1
E_{max} (mV)	0.69 ± 0.03	0.89 ± 0.03 *	0.60 ± 0.17	0.83 ± 0.20 *
% E_{max} (5-HT)[a]	100	129 ± 4	84 ± 7	114 ± 10

Experiments performed in the presence of the 5-HT_4 receptor antagonist GR113808A (100 nM). Data represents the mean ± SEM, $n = 3$.
* $P < 0.05$, * * $P < 0.01$, significantly different to the corresponding value in the absence of TCE (Student's paired *t*-test).
[a] Percent E_{max} relative to the E_{max} for 5-HT in the absence of TCE.

duced depolarisations in a concentration-dependent manner (pEC_{50} 2.44 ± 0.06 and 2.89 ± 0.04, (5-HT) 0.3 and 1.0 μM, respectively, $n = 3$, $P > 0.05$ comparison between the EC_{50}'s Student's *t*-test; Fig. 1). At the highest concentrations of TCE investigated, the potentiation of the 5-HT-induced response was submaximal (i.e., TCE generated a bell-shaped concentration–response curve; Fig. 1). Ondansetron (50 nM) completely antagonised the 5-HT (1 μM)-mediated depolarisations in both the absence and presence of TCE (10 mM); an effect which was reversed on washout of the antagonist (Fig. 2).

3.3. Effect of TCE on the potency with which 5-HT$_3$ receptor agonists depolarise the rat isolated vagus nerve

In a subsequent set of experiments, 5-HT and PBG evoked depolarisations of the vagus nerve with micromolar potency (Table 1; Fig. 3). Maximal depolarisations induced by PBG were smaller than those induced by 5-HT (Table 1; Fig. 3). In the presence of TCE (5 mM), the potencies of 5-HT and PBG were increased (two- to four-fold; Table 1; Fig. 3) as were the maximum responses induced by either agonist (Table 1; Fig. 3).

TCE (5 mM) also enhanced the depolarisation induced by the low intrinsic activity 5-HT$_3$ receptor agonist quipazine (300 nM; Fig. 4). The 5-HT$_3$ receptor antagonist (*S*)-zacopride (300 nM) failed to evoke a response either in the absence or presence of TCE (5 mM; Fig. 4).

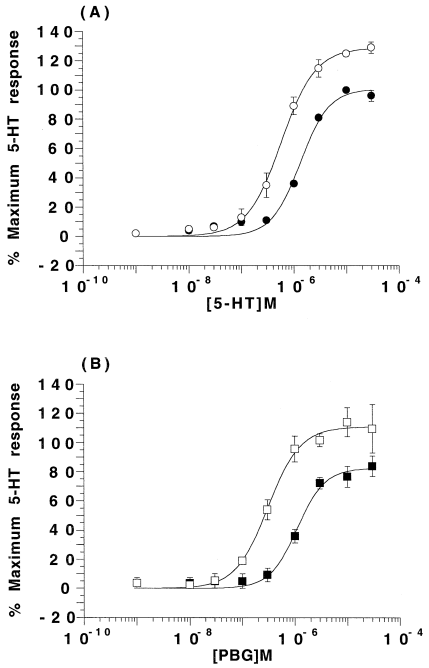

Fig. 3. Ability of the 5-HT$_3$ receptor agonists 5-HT and PBG to depolarise the rat isolated vagus nerve in the absence and presence of TCE (5 mM). (A) 5-HT in the absence (●) and presence (○) of TCE and (B) PBG in the absence (■) and presence (□) of TCE. Data represents normalised values compared to the maximal response to 5-HT in the absence of TCE (mean ± SEM, $n = 3$). Experiments performed in the presence of the 5-HT$_4$ receptor antagonist GR113808A (100 nM).

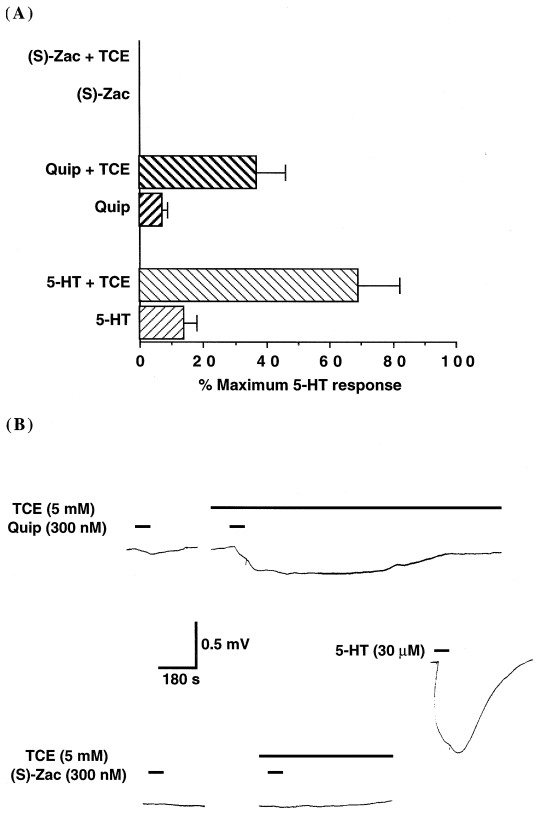

Fig. 4. Ability of TCE (5 mM) to potentiate the depolarisation of the rat isolated vagus nerve by either 5-HT (1 μM) or the 5-HT$_3$ receptor partial agonist quipazine (Quip; 300 nM) but failure of the 5-HT$_3$ receptor antagonist (*S*)-zacopride ((*S*)-Zac; 300 nM) to induce a response either in the absence or presence of TCE (5 mM). Data (A) represents normalised values compared to the maximal response to 5-HT in the absence of TCE (mean ± SEM, $n = 3$). (B) Examples of responses (bars represent application of the indicated drug, not corrected for void volume. NB, a downward deflection represents depolarisation). Experiments performed in the presence of the 5-HT$_4$ receptor antagonist GR113808A (100 nM).

3.4. Effect of TCE on the apparent 5-HT$_3$ receptor agonist-induced desensitisation

TCE (5 mM) was able to reverse the apparent desensitisation of the 5-HT$_3$ receptor induced by either 5-HT or PBG. Thus, continuous application of 5-HT (100 μM; 90

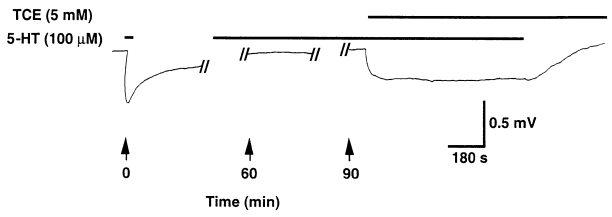

Fig. 5. Example recording demonstrating the apparent ability of TCE (5 mM) to reverse the 5-HT (100 μM)-induced 5-HT$_3$ receptor desensitisation of the rat isolated vagus nerve. Bars represent application of the indicated drug (not corrected for void volume. NB, a downward deflection represents depolarisation). Experiments performed in the presence of the 5-HT$_4$ receptor antagonist GR113808A (100 nM).

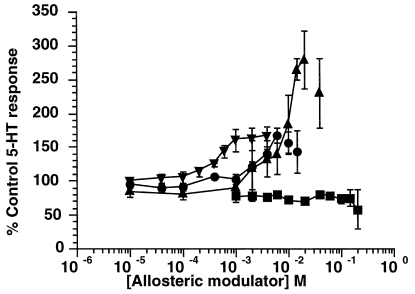

Fig. 6. Ability of a range of alcohols to modify the 5-HT (1 μM)-induced depolarisation of the rat isolated vagus nerve. 5-Cloropentanol (●), ethanol (■), isopentanol (▲) and tribromoethanol (▼). Data represents normalised values compared to the response to 5-HT (1 μM) in the absence of the relevant alcohol (mean ± SEM, $n = 3$). Experiments performed in the presence of the 5-HT_4 receptor antagonist GR113808A (100 nM).

min) initially induced a maximal 5-HT-induced depolarisation of the vagus nerve (0.67 ± 0.03 mV, $n = 3$) which subsequently diminished back to baseline. In the continued presence of 5-HT (100 μM), subsequent application of TCE (5 mM) produced a depolarisation (0.48 ± 0.10 mV, $n = 3$) of the 'desensitised' vagus nerve (Fig. 5). In a similar experiment, TCE (5 mM) also induced depolarisation (0.59 ± 0.15 mV, $n = 3$) when applied with PBG (100 μM; continuously perfused for 90 min prior to application of TCE, maximal depolarisation in the absence of TCE 0.67 ± 0.09 mV, $n = 3$) to the vagus nerve.

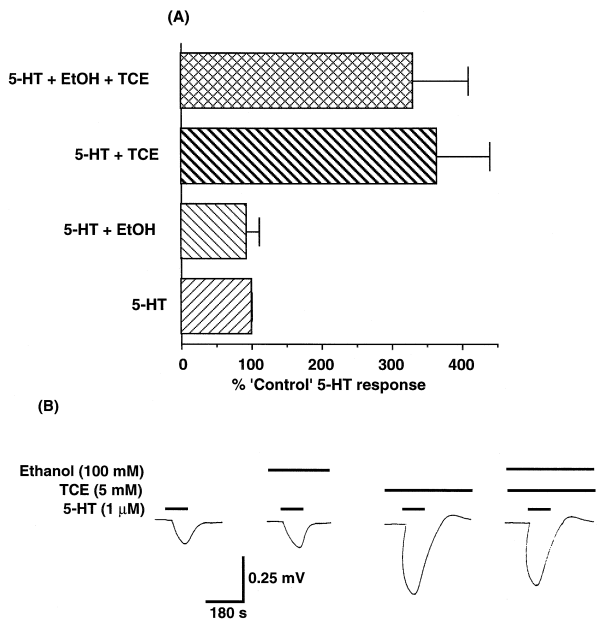

Fig. 7. Failure of ethanol (100 mM; EtOH) to modify the 5-HT (1 μM)-induced depolarisation of the rat isolated vagus nerve in the absence or presence of TCE (5 mM). Data (A) represents normalised values compared to the response to 5-HT (1 μM) in the absence of any alcohol (mean ± SEM, $n = 3$). (B) examples of responses (bars represent application of the indicated drug, not corrected for void volume. NB, a downward deflection represents depolarisation). Experiments performed in the presence of the 5-HT_4 receptor antagonist GR113808A (100 nM).

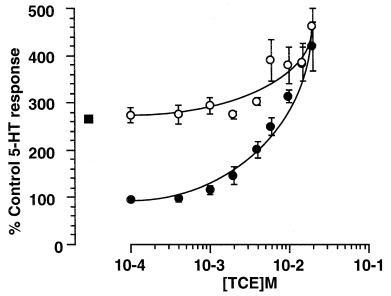

Fig. 8. Failure of isopentanol (20 mM) to prevent TCE from potentiating 5-HT (1 μM)-induced depolarisation of the rat isolated vagus nerve. 5-HT (1 μM) in the presence of isopentanol (20 mM; ■) and effect of increasing concentrations of TCE on the 5-HT (1 μM)-induced response in the absence (●) and presence (○) of isopentanol (20 mM). Data represents normalised values compared to the response to 5-HT (1 μM) in the absence of any alcohol (mean ± SEM, $n = 3$). Experiments performed in the presence of the 5-HT_4 receptor antagonist GR113808A (100 nM).

3.5. Ability of a range of alcohols to modify 5-HT_3 receptor-mediated depolarisation of the rat isolated vagus nerve

Tribromoethanol (10 μM–3 mM), isopentanol (1 μM–30 mM) and 5-chloropentanol (1 μM–10 mM) increased the magnitude of depolarisation of the vagus nerve induced by 5-HT (0.3 or 1.0 μM) in a concentration-dependent manner ($pEC_{50} = 3.09 \pm 0.03$, 2.03 ± 0.07, and 2.70 ± 0.21, respectively, $n = 3$; Fig. 6). In contrast, ethanol (1–200 mM) failed to enhance 5-HT (1 μM)-induced depolarising responses (Figs. 6 and 7). Furthermore, ethanol (100 mM) failed to modify the 5-HT (1 μM)-induced depolarisation in the presence of TCE (5 mM; Fig. 7).

3.6. Ability of isopentanol to modify the TCE-induced allosteric modulation of the 5-HT_3 receptor-mediated depolarisation of the rat isolated vagus nerve

Isopentanol (20 mM) potentiated 5-HT (1 μM)-induced depolarisation of the vagus nerve (Fig. 8). In the presence of isopentanol (20 mM), TCE (0.1–30 mM) still potentiated 5-HT (1 μM)-induced depolarisation although the maximal potentiations induced by TCE in the absence and presence of isopentanol (20 mM) were similar (Fig. 8).

4. Discussion

The 5-HT-evoked depolarisation of the rat isolated vagus nerve is mainly mediated via 5-HT_3 receptors (e.g., Ireland and Tyers, 1987), although a small component of the response is mediated by the 5-HT_4 receptor (Rhodes et al., 1992; Bley et al., 1994). Consequently, in the present study, experiments were performed in the presence of the high-affinity and selective 5-HT_4 receptor antagonist, GR113808A (Gale et al., 1994) to pharmacologically isolate the 5-HT_3 receptor-mediated depolarisation.

At millimolar concentrations, TCE potentiated 5-HT_3 receptor agonist-induced depolarisation of the rat isolated vagus nerve. The magnitude of TCE-induced potentiation decreased with increasing 5-HT concentrations, suggesting that the mechanism of enhancement appears to involve an increase in agonist potency, consistent to that found in other preparations (for review see Parker et al., 1996). However, with the present preparation, an increase in efficacy of the endogenous agonist 5-HT, in addition to that of partial agonists (PBG, quipazine), was also apparent. While this phenomenon has been reported previously for partial agonists, it would appear less marked for the full endogenous agonist, 5-HT (for review see Parker et al., 1996). This difference with respect to the increase in efficacy of 5-HT induced by TCE may be a consequence of the different preparations rather than differences between the molecular actions of TCE at the 5-HT_3 receptors expressed in different preparations.

While the results of the present study largely correlate with results from electrophysiological studies performed on isolated rat and rabbit nodose ganglion neurones (Lovinger and Zhou, 1993; Gill et al., 1994) and heterologously expressed 5-HT_3 receptors (Lovinger and Zhou, 1994; Gill et al., 1994; Downie et al., 1995), there are some differences relating to desensitisation. For instance, Lovinger and Zhou (1993) proposed that the sensitivity to TCE was decreased for desensitised receptors since in their studies, agonist pretreatment reduced the ability of TCE to potentiate 5-HT_3 receptor-mediated current. In apparent contrast, in the present study TCE could still facilitate a response by 5-HT after the 5-HT_3 receptors on the isolated vagus nerve had been desensitised with the continuous perfusion (90 min) of a saturating concentration (eliminating any mechanism involving an increase in agonist potency) of 5-HT. Therefore, in the present study, TCE appeared to reverse agonist-induced desensitisation of the 5-HT_3 receptor. The collective results of the present study would suggest that TCE enhances 5-HT_3 receptor function by at least two mechanisms: (i) an increase in agonist potency and (ii) a reversal of agonist-induced desensitisation. This latter action may account for the apparent increase in agonist efficacy due to the likely presence of desensitised 5-HT_3 receptors, particularly at relatively high agonist concentrations. Consistent with these findings, it is of interest that a recent report suggested that alcohols potentiate 5-HT_3 receptor-mediated responses by stabilising the open state of the receptor complex by decreasing the rate of channel deactivation and desensitisation (and also by increasing the rate of channel activation; Zhou et al., 1998).

While TCE potentiated depolarisation of the isolated vagus nerve induced by 5-HT, PBG and the relatively low intrinsic activity partial agonist quipazine, (*S*)-zacopride failed to induce a response either in the absence or presence of TCE. The rationale for testing (*S*)-zacopride in this preparation follows the speculation that (*S*)-zacopride induces emesis due to activation of 5-HT_3 receptors (King, 1990; King and Landauer, 1990). However, an additional report indicates that this action of (*S*)-zacopride is due to agonistic action at the 5-HT_4 receptor (Bhandari and Andrews, 1991). Since we have found no evidence that (*S*)-zacopride behaves as a 5-HT_3 receptor agonist, even in the presence of an allosteric modulator, such as TCE, we concur with the belief that (*S*)-zacopride does not evoke the emetic response by activation of 5-HT_3 receptors.

In addition to TCE, a number of other alcohols were tested for their ability to potentiate 5-HT-induced depolarisation of the rat isolated vagus nerve. Tribromoethanol potentiated the 5-HT_3 receptor-mediated response with slightly higher potency than TCE, although tribromoethanol displayed an apparently lower level of efficacy. Isopentanol and 5-chloropentanol also potentiated the 5-HT_3 receptor-mediated response although more weakly than TCE. In addition, 5-chloropentanol displayed an apparently lower level of efficacy, when compared with TCE.

While ethanol has been reported to enhance 5-HT_3 receptor function in various preparations expressing both native and recombinant 5-HT_3 receptors (Lovinger, 1991; Lovinger and White, 1991; Lovinger and Peoples, 1993; Lovinger and Zhou, 1994; Machu and Harris, 1994; Downie et al., 1995), the effects of ethanol reported in many of these studies were inconsistent with potentiation being evident in only 60–80% of recordings. To add to the inconsistency of the findings, the present study failed to detect potentiation by ethanol of 5-HT-induced depolarisations of the rat isolated vagus nerve. The detected variability of the effects of ethanol on 5-HT_3 receptor function is difficult to explain and is unlikely to arise from different 5-HT_3 receptor subunit compositions expressed by the different preparations (see Parker et al., 1996) as has been proposed for the differential ability of ethanol to modulate $GABA_A$ receptor function (Wafford et al., 1990).

In addition to the failure of ethanol to modulate 5-HT-induced depolarisation, a relatively high concentration of ethanol (100 mM) also failed to modify the potentiation of the 5-HT-induced depolarisation induced by a just-maximal concentration of TCE. This suggests that ethanol does not 'antagonise' the TCE recognition site on the 5-HT_3 receptor complex.

In further alcohol combination studies, a maximally effective concentration of isopentanol did not prevent TCE from further potentiating the 5-HT-induced depolarisation although the combined effects of the two alcohols was not additive, suggesting that their actions were mediated through the same recognition site. The lower level of apparent efficacy displayed by isopentanol to potentiate the 5-HT_3 receptor may indicate that this alcohol is a 'partial agonist' for the allosteric site. However, since the effective concentrations of TCE to enhance the 5-HT-induced response either in the absence or presence of isopentanol were similar, this suggests that isopentanol does not

behave as a 'partial agonist' for the TCE/isopentanol recognition site. Thus, the isopentanol-induced potentiation may not have been as large as that induced by TCE because higher concentrations of isopentanol were required which may have caused a greater inhibitory action as is evident with high concentrations of all the alcohols, i.e., the potentiation induced by isopentanol never reached a maximal effect due to the secondary inhibitory action of the alcohol at higher concentrations. Indeed, this may be the case for all the alcohols investigated to date. In a comparable study, the same concentration of isopentanol as used in the present study (20 mM) appeared to 'antagonise' the action of TCE to potentiate the 5-HT_3 receptor-mediated response in NCB-20 cells (Zhou and Lovinger, 1996), although in this latter study, this was not apparent at lower concentrations of isopentanol (Zhou and Lovinger, 1996).

In conclusion, the present studies have demonstrated that the 5-HT_3 receptor expressed by rat vagus nerve is susceptible to positive allosteric modulation by a number of alcohols including the general anaesthetic agent trichloroethanol. Similar interactions may contribute to the nausea and vomiting experienced by some patients recovering from general anaesthesia.

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References

Andrews, P.L.R., Rapeport, W.G., Sanger, G.J., 1988. Neuropharmacology of emesis induced by anti-cancer therapy. Trends Pharmacol. Sci. 9, 334–341.

Bhandari, P., Andrews, P.L.R., 1991. Preliminary evidence for the involvement of the putative 5-HT_4 receptor in zacopride-induced and copper sulphate-induced vomiting in the ferret. Eur. J. Pharmacol. 204, 273–280.

Bley, K.R., Eglen, R.M., Wong, E.H.F., 1994. Characterisation of 5-hydroxytryptamine-induced depolarisations in rat isolated vagus nerve. Eur. J. Pharmacol. 260, 139–147.

Downie, D.L., Hope, A.G., Belelli, D., Lambert, J.J., Peters, J.A., Bentley, K.R., Steward, L.J., Chen, C.-Y., Barnes, N.M., 1995. The interaction of trichloroethanol with murine recombinant 5-HT_3 receptors. Br. J. Pharmacol. 114, 1641–1651.

Gale, J.D., Grossman, C.J., Whitehead, J.W.F., Oxford, A.W., Bunce, K.T., Humphrey, P.P.A., 1994. GR113808: a novel, selective antagonist with high affinity at the 5-HT_4 receptor. Br. J. Pharmacol. 111, 332–338.

Garrett, E.R., Lambert, H.J., 1973. Pharmacokinetics of trichloroethanol and metabolites and interconversions among variously referenced pharmacokinetic parameters. J. Pharm. Sci. 62, 550–572.

Gill, C., 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.), p. 18.4.24.

Hoyer, D., Waeber, C., Karpf, A., Neijt, H., Palacios, J.M., 1989. [^{3}H]ICS 205–930 labels 5-HT_3 recognition sites in membranes of cat and rabbit vagus nerve and superior cervical ganglion. Naunyn-Schmiedeberg's Arch. Pharmacol. 340, 396–402.

Ireland, S.J., Tyers, M.B., 1987. Pharmacological characterisation of 5-hydroxytryptamine-induced depolarization of the rat isolated vagus nerve. Br. J. Pharmacol. 90, 229–238.

Jackson, M.B., Yakel, J.L., 1995. The 5-HT_3 receptor channel. Annu. Rev. Physiol. 57, 447–468.

Kilpatrick, G.J., Jones, B.J., Tyers, M.B., 1989. Binding of the 5-HT_3 ligand, [^{3}H]GR65630, to rat area postrema, vagus nerve and the brains of several species. Eur. J. Pharmacol. 159, 157–164.

King, G.L., 1990. Emesis and defecations induced by the 5-hydroxytryptamine (5-HT_3) receptor antagonist zacopride in the ferret. J. Pharmacol. Exp. Ther. 253, 1034–1041.

King, G.L., Landauer, M.R., 1990. Effects of zacopride and BMY25801 (batanapride) on radiation-induced emesis and locomotor behaviour in the ferret. J. Pharmacol. Exp. Ther. 253, 1026–1033.

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., 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., Peoples, R.W., 1993. Actions of alcohols and other sedative/hypnotic compounds on cation channels associated with glutamate and 5-HT_3 receptors. In: Alling, C., Diamond, I., Leslie, S., Wood, W.G. (Eds.), Alcohol, Cell Membranes and Signal Transduction in Brain. Plenum, New York, pp. 157–168.

Lovinger, D.M., Zhou, Q., 1993. Trichloroethanol potentiation of 5-hydroxytryptamine$_3$ receptor-mediated ion current in nodose ganglion neurons from adult rat. J. Pharmacol. Exp. Ther. 265, 771–776.

Lovinger, D.M., Zhou, Q., 1994. Alcohols potentiate ion current mediated by recombinant 5-HT_{3A} receptors expressed in a mammalian cell line. Neuropharmacology 33, 1567–1572.

Machu, T.K., Harris, R.A., 1994. Alcohols and anaesthetics enhance the function of 5-hydroxytryptamine$_3$ receptors expressed in *Xenopus laevis* oocytes. J. Pharmacol. Exp. Ther. 271, 898–905.

Parker, R.M.C., Bentley, K.R., Barnes, N.M., 1996. Allosteric modulation of 5-HT_3 receptors: focus on alcohols and anaesthetic agents. Trends Pharmacol. Sci. 17, 95–99.

Peters, J.A., Malone, H.M., Lambert, J.J., 1991. Ketamine potentiates 5-HT_3 receptor-mediated currents in rabbit nodose ganglion neurons. 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.

Pratt, G.D., Bowery, N.G., Kilpatrick, G.J., Leslie, R.A., Barnes, N.M., Naylor, R.J., Jones, B.J., Nelson, D.R., Palacios, J.M., Slater, P., Reynolds, J.M., 1990. The distribution of 5-HT_3 receptors in mammalian hindbrain: a consensus. Trends Pharmacol. Sci. 11, 135–137.

Rhodes, K.F., Coleman, J., Lattimer, N., 1992. A component of 5-HT-evoked depolarization of the rat isolated vagus nerve in mediated by a putative 5-HT_4 receptor. Naunyn-Schmiedeberg's Arch. Pharmacol. 346, 496–503.

Russell, D., Kenny, G.N.C., 1992. 5-HT_3 antagonists in postoperative nausea and vomiting. Br. J. Anaesth. 69 (Suppl 1), S63–S68.

Wafford, K.A., Burnett, D.M., Dunuriddie, T.V., Harris, R.A., 1990. Genetic differences in the ethanol sensitivity of GABA-A receptors expressed in *Xenopus* oocytes. Science 249, 291–293.

Zhou, Q., Lovinger, D.M., 1996. Pharmacological characteristics of potentiation of 5-HT_3 receptors by alcohols and diethyl ether in NCB-20 neuroblastoma cells. J. Pharmacol. Exp. Ther. 278, 732–740.

Zhou, Q., Verdoorn, T.A., Lovinger, D.M., 1998. Alcohols potentiate the function of 5-HT_3 receptor-channels on NCB-20 neuroblastoma cells by favouring and stabilizing the open channel state. J. Physiol. 507, 335–352.