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Inhibitory effect of ethanol on the 5-HT_3 receptor-mediated tachycardia in isolated guinea-pig atrium

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Abstract

The influence of ethanol on 5-hydroxytryptamine (5-HT)-induced tachycardia mediated by 5-HT_3 receptor activation in the isolated guinea-pig atrium was studied. Ethanol at 200 mM significantly inhibited 5-HT- but not isoproterenol- or histamine-induced tachycardia in the isolated guinea-pig atrium. The same inhibitory effect was observed in response to 2-propanol and chloral hydrate application. Both 2-propanol at 100 mM and chloral hydrate at 1 mM exhibited inhibitory effects on 5-HT-induced tachycardia as potent as those of ethanol at 200 mM. These alcohols did not inhibit the tachycardia induced by isoproterenol or histamine. The inhibitory effects of the alcohols seemed to be specific for 5-HT and to increase according to their lipophilicity. Our results suggest that the inhibitory effects of ethanol on 5-HT_3 receptor-mediated tachycardia are related to the direct effect of ethanol on 5-HT_3 receptors in the atrium.

Keywords: 5-HT_3 receptor; Atrium; Tachycardia; Ethanol; Alcohol

1. Introduction

Many studies have indicated that the well-known syndrome of acute intoxication following ethanol consumption is due to changes in certain neurotransmitter systems in the central nervous system (Samson and Harris, 1992). It has been shown that intoxicating concentrations of ethanol can potentiate ion currents mediated by $GABA_A$ receptors in some neuronal cells (Aguayo, 1990; Reynolds et al., 1992) and inhibit ion currents mediated by NMDA receptors (Lovinger et al., 1989). Ethanol has been shown to enhance the action of cholinergic agonists at nicotinic acetylcholine receptors in both electrophysiological and biochemical studies (Forman et al., 1989; Gage et al., 1975). Ethanol slows the decay rate of miniature endplate currents (Gage et al., 1975), and decreases the frequency of endplate power spectra, indicating that the rate of channel closing is slowed (Bradley et al., 1984). Thus, recent research has concentrated on ion channels as a site of action of ethanol (Gonzales and Hoffman, 1991; Starke, 1991). Furthermore, alterations in serotonergic function have been implicated in alcoholism (Samson and Harris, 1992; Sellers et al., 1992).

5-Hydroxytryptamine (5-HT) exerts its action via a number of 5-HT receptor subclasses, among which the 5-HT_1, 5-HT_2, 5-HT_3 and 5-HT_4 receptors have been best characterized (for review, see Saxena, 1995). In contrast to 5-HT_1, 5-HT_2 and 5-HT_4 receptors, which are linked to G proteins and second messenger signaling pathways, the 5-HT_3 receptor is a ligand-gated cation channel (Maricq et al., 1991). Several lines of evidence have suggested that 5-HT_3 receptors as well as $GABA_A$ and NMDA receptors are involved in the effects of ethanol. For example, it has been shown that 5-HT_3 receptor antagonists can block ethanol-induced dopamine release in the nucleus accumbens and striatum (Carboni et al., 1989; Wozniak et al., 1990), as well as ethanol intake and the discriminative stimulus properties of ethanol (Grant and Barett, 1991). In electrophysiological and neurochemical studies, however, contradictory results were also reported regarding the effects of ethanol on 5-HT_3 receptors (Barann et al., 1995).

It is generally accepted that 5-HT-induced positive chronotropic effects in guinea-pig atrium are mediated by mechanisms different from those active in other species and employ different receptor subtypes (Saxena and Villalon, 1991; Kaumann, 1991). It was initially reported that

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the action of 5-HT is tyramine-like (Trendelenburg, 1960). Recently, however, a secondary mechanism, such as liberation of calcitonin gene-related peptide (Saito et al., 1986), was suggested to explain the effect of 5-HT (Dhasmana et al., 1988). Possible involvement of 5-HT_4-like (or 5-HT_3?) receptors in the mediation of the positive chronotropic effects of 5-HT was also suggested (Kaumann, 1991). Thus, we re-examined the pharmacological properties of 5-HT-induced tachycardia in isolated guinea-pig atrium (Nishio et al., 1996). We found that the effect of 5-HT was mimicked by 5-HT_3 receptor agonists, such as 2-methyl-5-HT, quipazine and phenylbiguanide. The effect of 5-HT was selectively inhibited by 5-HT_3 receptor antagonists, such as tropisetron, granisetron, ondansetron, cisapride and zacopride. We concluded that 5-HT can produce direct positive chronotropic effects which are not due to β-adrenergic stimulation, but which are mediated by activation of 5-HT_3 receptors (Nishio et al., 1996). We proposed the use of 5-HT-induced tachycardia in the isolated guinea-pig atrium as a simple and potentially useful experimental system for investigating 5-HT_3 receptor-mediated cellular responses as well as its functions.

The aim of the present study was to examine the influence of ethanol and other alcohols on 5-HT-induced tachycardia mediated by 5-HT_3 receptors located on the guinea-pig atrium. To determine the specificity of ethanol-induced antagonism, additional experiments were performed in which the influence of ethanol on isoproterenol- and histamine-induced positive chronotropic effects was also studied.

2. Materials and methods

2.1. Experiments on spontaneously beating atria

The paired atria were dissected out from the hearts of freshly killed guinea-pigs and suspended in Ringer-Locke solution (composition in g/l: NaCl, 9.0; KCl, 0.42; $CaCl_2$, 0.24; glucose 1.0; $NaHCO_3$, 0.2) gassed with 95% O_2 and 5% CO_2 at 30°C. The atrium was connected under a resting tension of 0.5 g to an isometric force transducer connected to an amplifier (EF-601G, Nihon Kohden, Japan) and a heart rate counter (AT-601G, Nihon Kohden). Contractile strength and heart rate were recorded continuously on a microcomputer system equipped with an A/D converter. Each atrium was treated with agonist (5-HT in the presence of 10 μM atropine, isoproterenol of histamine) in a cumulative manner (control experiment), then the effects of alcohols were estimated by addition 5 min before the second agonist stimulation. The agonists were applied at 1 h intervals. Positive chronotropic effects (tachycardia) of the agonists are expressed as percentages of the maximal increase in heart rate caused by the agonists in each control experiment. The maximum response obtained with 5-HT was approximately 45% and 40% of that to isoproterenol and histamine, respectively.

2.2. Statistical analyses

Results are expressed as means ± S.E.M., and the statistical significance of differences was determined by one-way analysis of variance followed by Dunnett's test.

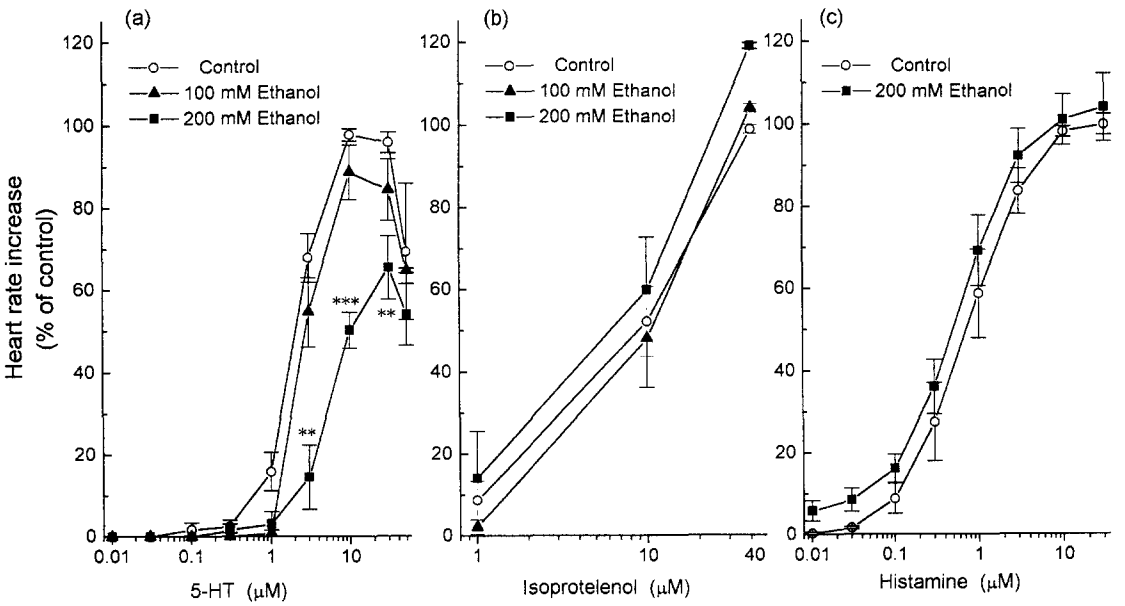

Fig. 1. Concentration–effect curves for (a) 5-HT, (b) isoproterenol and (c) histamine in the absence (Control) and presence of ethanol (100 mM, 200 mM) in isolated guinea-pig atrium. Chronotropic effects of 5-HT, isoproterenol or histamine are expressed as a percentage of the maximal increase in heart rate caused by the agonists in each control experiment. Each point represents the mean ± S.E.M. of four independent experiments. Significance: ** $P < 0.01$, *** $P < 0.001$ (vs. control).

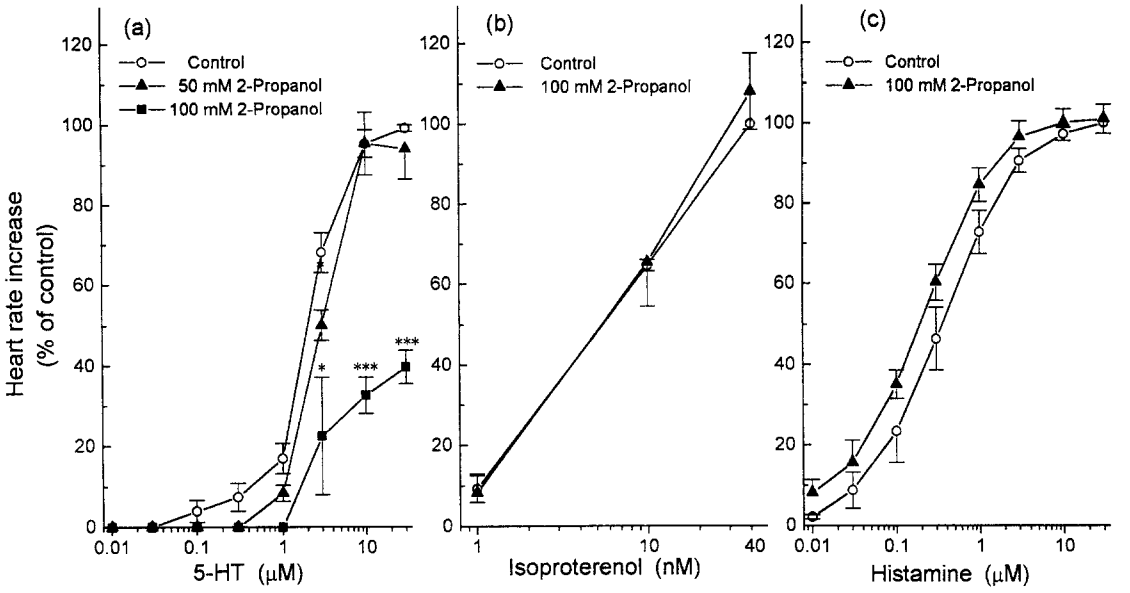

Fig. 2. Concentration–effect curves for (a) 5-HT, (b) isoproterenol and (c) histamine in the absence (Control) and presence of 2-propanol (50 mM, 100 mM) in isolated guinea-pig atrium. Chronotropic effects of 5-HT, isoproterenol or histamine are expressed as a percentage of the maximal increase in heart rate caused by the agonists in each control experiment. Each point represents the mean ± S.E.M. of four independent experiments. Significance: * $P < 0.05$, *** $P < 0.001$ (vs. control).

2.3. Chemicals

The following reagents were obtained from the sources indicated: 5-HT (5-hydroxytryptamine creatinine sulfate) was from E. Merck (Darmstadt, Germany); histamine 2HCl and (−)-isoproterenol HCl were from Sigma (St. Louis, MO, USA); chloral hydrate was from Tokyo Chemical Industry (Tokyo, Japan). All other chemicals used in the study were of analytical grade and were obtained from commercial sources.

3. Results

In the isolated guinea-pig atrium, 5-HT-induced tachycardia was partially inhibited by 100 mM ethanol, and significantly inhibited by 200 mM ethanol (Fig. 1a). The

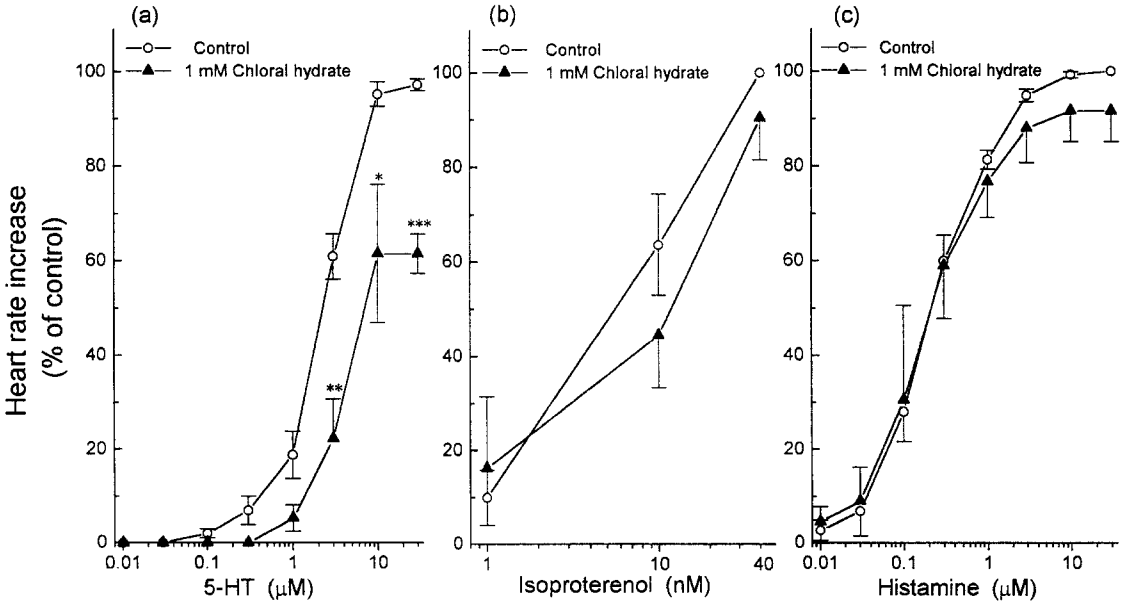

Fig. 3. Concentration–effect curves for (a) 5-HT, (b) isoproterenol and (c) histamine in the absence (Control) and presence of 1 mM chloral hydrate in isolated guinea-pig atrium. Chronotropic effects of 5-HT, isoproterenol or histamine are expressed as a percentage of the maximal increase in heart rate caused by the agonists in each control experiment. Each point represents the mean ± S.E.M. of three to four independent experiments. Significance: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (vs. control).

tachycardia induced by isoproterenol, 1–40 nM, was not inhibited by ethanol. Rather, 200 mM ethanol showed a tendency to stimulate the isoproterenol-induced tachycardia (Fig. 1b). Futhermore, histamine-induced tachycardia was not affected by 200 mM ethanol (Fig. 1c).

The same inhibitory effects were observed in response to 2-propanol and chloral hydrate. Thus, the inhibitory effect of 100 mM 2-propanol on 5-HT-induced tachycardia was stronger than that observed with 200 mM ethanol (Fig. 2a). Chloral hydrate (1 mM) potently inhibited 5-HT-induced tachycardia in isolated guinea-pig atria (Fig. 3a). The inhibition caused by chloral hydrate, as well as that caused by ethanol and 2-propanol, was not overcome by 5-HT at the highest doses tested. Non-competitive inhibition might be suggested in the effects of the alcohols. Furthermore, both 100 mM 2-propanol and 1 mM chloral hydrate did not cause any significant inhibition of both isoproterenol- and histamine-induced tachycardia (Fig. 2b,c; Fig. 3b,c).

4. Discussion

Previously, we reported that 5-HT caused positive chronotropic effects (tachycardia) when cumulatively administered to isolated guinea-pig atrium, and suggested that this effect of 5-HT was mediated by direct stimulation of 5-HT_3 receptors (Nishio et al., 1996). In the present study, we found that ethanol at 200 mM significantly inhibited 5-HT- but not isoproterenol- or histamine-induced tachycardia in the isolated guinea-pig atrium. Thus, ethanol did not show any inhibitory effect on the function of G protein-coupled receptors, namely β-adrenoceptors and histamine H_2 receptors, which stimulate adenylate cyclase. These results indicate that the ethanol-induced inhibition of tachycardia is specific for ligand-gated cation channel receptors, namely 5-HT_3 receptors.

The same inhibitory effects were observed in response to 2-propanol and chloral hydrate. The inhibitory effect of 100 mM 2-propanol on 5-HT-induced tachycardia was stronger than that observed with 200 mM ethanol. Chloral hydrate (1 mM) potently inhibited 5-HT-induced tachycardia in isolated guinea-pig atria, while 1 mM chloral hydrate as well as 100 mM 2-propanol did not inhibit isoproterenol- or histamine-induced tachycardia. These results are in agreement with the observation that the magnitude of alcohol-induced effects increases with the lipophilicity of the alcohol (Li et al., 1994; Lovinger and Zhou, 1994; Barann et al., 1995; Downie et al., 1995). Our present study indicated that the antagonistic effect of ethanol, as well as that of 2-propanol and chloral hydrate, was not overcome by increasing the concentration of 5-HT, indicating that the antagonism might be non-competitive. The observation that the inhibitory effects of alcohols on 5-HT-induced tachycardia were more pronounced for alcohols with higher lipophilicity suggests that alcohols may interact with a hydrophobic region of the 5-HT_3 receptor channel to induce such stabilization (Li et al., 1994). However, the present results do not allow a definite conclusion to be reached concerning the mechanism underlying the effects of ethanol on 5-HT_3 receptors. No interference with the 5-HT recognition site of the 5-HT_3 receptor was reported in the effect of ethanol (Barann et al., 1995). 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 also not known. We cannot exclude the possibility that ethanol might affect the cellular mechanism after 5-HT_3 receptor activation.

There have been conflicting reports regarding the electrophysiological and neurochemical effects of ethanol on 5-HT_3 receptor function. Lovinger and White (1991), Lovinger (1991), Barann et al. (1995), Machu and Harris (1994), Lovinger and Zhou (1994) and Downie et al. (1995) reported stimulatory effects of ethanol on responses mediated via 5-HT_3 receptors in isolated adult mammalian neurons, NCB-20 neuroblastoma cells, N1E-115 mouse neuroblastoma cells and cells expressing recombinant 5-HT_3 receptors, respectively. However, ethanol has been shown to inhibit the noradrenaline release induced by activation of 5-HT_3 receptors on the sympathetic nerve endings of the isolated rabbit heart (Göthert et al., 1979), cation influx through the 5-HT_3 receptor channel of neuroblastoma-glioma cells (Emerit et al., 1993), and the 5-HT_3 receptor-mediated Bezold-Jarisch reflex (Malinowska et al., 1995). Since these studies were carried out under different experimental conditions, it remains to be established whether the discrepancies are due to experimental conditions (Barann et al., 1995) or are related to species variations (Peters and Lambert, 1989) or subtypes (or variants) of 5-HT_3 receptors (Hope et al., 1993). The present observation that 5-HT_3 receptors in the isolated guinea-pig atrium are sensitive to alcohols that are active in the peripheral nervous system, such as on the Bezold-Jarich reflex, is of interest.

In conclusion, the present study revealed that ethanol inhibits 5-HT- but not isoproterenol- or histamine-induced tachycardia in the isolated guinea-pig atrium. The inhibitory effects of the alcohol were shown to be dependent on their lipophilicity. Accordingly, the effects of ethanol seem to be related to its direct influence on 5-HT_3 receptors in the atrium. Functional investigation of 5-HT_3 receptors in guinea-pig isolated atria, which we recently re-evaluated (Nishio et al., 1996), is one of the most promising systems for studying the molecular basis of the action of alcohol on the 5-HT_3 receptor, a ligand-gated cation channel receptor.

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