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Short communication

Patterns of serotonin- and 2-methylserotonin-induced pain may reflect 5-HT_3 receptor sensitization

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Abstract

Intraperitoneal injection of serotonin (5-HT; 0.5–4.0 mg/kg) produced dose-dependent nociceptive writhing responses, attenuated at all doses by pre-administration of tropisetron (1.0 mg/kg, i.p.). Administration of 2-methylserotonin (2-methyl-5-HT) alone was ineffective in inducing writhing. The effects of 5-HT (0.5 mg/kg, i.p.) were increased by subsequent injection of 2-methyl-5-HT (0.5–4.0 mg/kg, i.p.). The enhanced nociceptive responses produced by low dose of 5-HT and subsequently administered 2-methyl-5-HT were attenuated by pretreatment with tropisetron. These results suggest that the inflammatory cascade produced by peripheral administration of 5-HT evokes nociception by stimulating visceral 5-HT_3 receptors and that 5-HT-induced mechanisms appear to sensitize the 5-HT_3 receptor to subsequent pharmacologic activation.

Keywords: 5-HT_3 receptor; 5-HT (5-hydroxytryptamine, serotonin); 2-Methylserotonin; Writhing; Pain; Sensitization

1. Introduction

Serotonin 5-HT_3 receptors have been localized to primary C-fiber and non-C fiber nociceptive afferents in several peripheral loci, including the viscera (Fozard, 1984; Zeitz et al., 2002). The 5-HT_3 receptor has been shown to mediate a component of the nociceptive response to subacute and chronic inflammation produced by serotonin (5-HT) and other noxious agents (Giordano and Rogers, 1989), whereas the initial phase of inflammation and acute pain does not appear to be reliant upon 5-HT_3-mediated substrates (Sufka et al., 1992). However, administration of the 5-HT_3 receptor agonist 2-methylserotonin (2-methyl-5-HT) has been shown to be ineffective in eliciting a nociceptive response in tests of peripheral (Giordano and Rogers, 1989) and visceral pain (Moser, 1995). It may be that a rise in the local concentration of 5-HT (either exogenously administered or as a component of the inflammatory cascade) is important in activating and/or stimulating populations of 5-HT_3 receptors that mediate inflammatory nociception, and that this activation affects the pharmacologic sensitivity of the 5-HT_3 receptor.

This might explain why administration of 2-methyl-5-HT alone was ineffectual in evoking painful responses in such peripheral nociceptive assays. To address this possibility, the present study examined patterns of abdominal writhing produced by 5-HT and 2-methyl-5-HT, alone and in sequential combination.

2. Materials and methods

2.1. Subjects

All protocols were reviewed and approved by the Institutional Review Board for ethical treatment of experimental animal subjects in accordance with National Institutes of Health and European Community guidelines. Male CD mice (Charles River Farms, MA) (30–40 g) were used in all experiments and were housed in standard plastic cages, six per cage on a 12-h light/dark cycle. Food, water and conspecific contact were available ad libitum prior to experimentation. Separate animals

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were used at each dose, and each animal was used only once.

2.2. Drugs

All compounds were obtained from Sigma/RBI (St. Louis, MO). All drugs were dissolved in sterile saline solution and pH balanced prior to administration. Compounds were prepared freshly on the day of use and were administered in a total volume of 10 ml/kg. Doses were determined in preliminary experiments, based upon our previous work and review of the literature.

2.3. Nociceptive testing

Serotonin (0.5, 1.0, 2.0 and 4.0 mg/kg) or 2 methyl-5-HT (0.5, 1.0, 2.0 and 4.0 mg/kg) was intraperitoneally (i.p.) injected and abdominal writhes were quantified for a period from 5 to 25 min post-drug administration according to methods previously described (Moser, 1995). Time to peak pharmacologic effects were ascertained in pilot studies; mean peak effects occurred at 18 min post-injection. In the first set of experiments, 5-HT or 2-methyl-5-HT was administered alone. In a subsequent set of subjects, tropisetron (1.0 mg/kg; i.p.) was administered 2 min prior to injection of the agonist(s) in order to assess the effects of 5-HT_3 receptor antagonism on any observed nociceptive responses. In a second set of experiments, 5-HT (0.5 mg/kg, i.p.) was administered 1 min prior to administration of 2-methyl-5-HT (0.5-4.0 mg/kg, i.p.), or 2-methyl-5-HT (0.5–4.0 mg/kg, i.p.) was injected 1 min before 5-HT (0.5 mg/kg, i.p.) and writhes observed. In another group of animals, tropisetron (1.0 mg/kg, i.p.) was injected 2 min prior to agonist administration to assess 5-HT_3 receptor mediation of observed effects.

Statistical analyses were conducted using analysis of variance (ANOVA) with post hoc, pairwise comparisons made with the Mann–Whitney *U*-test. Significance was considered at a level of $P<0.05$.

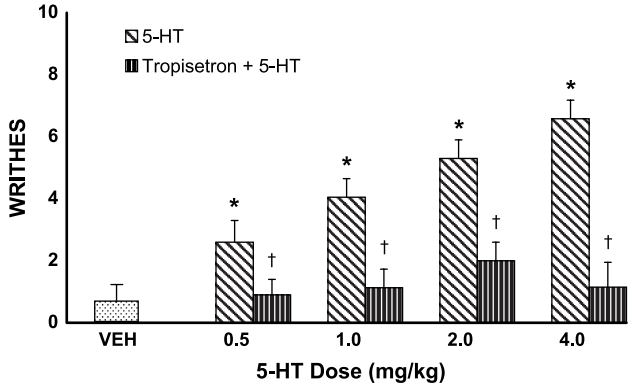

Fig. 1. Effects of 5-HT (0.5–4.0 mg/kg, i.p.) on eliciting the abdominal writhing response. Pretreatment with tropisetron (1.0 mg/kg) 2 min prior to administration of 5 HT doses significantly attenuated 5 HT induced effects. Columns represent mean scores from five determinations (± S.D.). *Significant difference from vehicle, $P<0.05$; $^{+}$significant effect of antagonist upon 5-HT-induced effect(s), $P<0.05$.

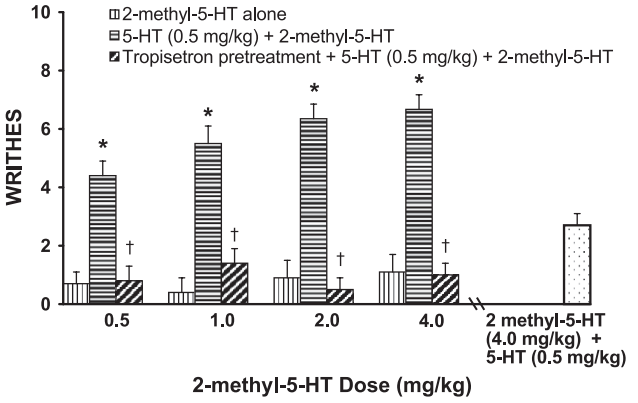

Fig. 2. Effects of 2-methyl-5-HT (0.5–4.0 mg/kg, i.p.) on abdominal writhing produced either alone or following pre-administration of a low dose of 5-HT (0.5 mg/kg/i.p.). 2-Methyl-5-HT was administered 1 min following injection of 5-HT. Pretreatment with tropisetron (1.0 mg/kg, i.p.) 2 min preceding administration of 5 HT attenuated any nociceptive effects produced. The far right column depicts the lack of effects of 2-methyl 5-HT (4.0 mg/kg, i.p.) administered 1 min prior to 5 HT (0.5 mg/kg, i.p.). Columns represent means of four to five determinations (± S.D.). *Significant difference from 5-HT alone, $P<0.05$; $^{+}$significant effect of antagonist vs. 5-HT and respective dose of 2-methyl-5-HT, $P<0.05$.

3. Results

Fig. 1 depicts that when administered alone, 5-HT produced significant dose-dependent visceral nociception as indicated by the increase in abdominal writhing ($P<0.05$). At all doses tested, 5-HT-induced nociception was attenuated by pretreatment with tropisetron ($P<0.05$). As shown in Fig. 2, when administered alone, 2-methyl-5-HT failed to produce any significant writhing responses at all doses tested. In contrast, 2-methyl-5-HT produced significant enhancement of the nociceptive response elicited by a low dose of 5-HT (0.5 mg/kg, i.p.; $P<0.05$ in all cases; see Fig. 2). All nociceptive responses were significantly reduced by tropisetron pretreatment ($P<0.05$). When administered prior to 5-HT (0.5 mg/kg, i.p.), 2 methyl-5-HT (0.5–4.0 mg/kg, i.p) did not produce any significant increase in the number of writhes produced by 5-HT alone (only the highest dose of 2-methyl-5-HT is illustrated on the far right panel of Fig. 2 for comparison).

4. Discussion

Intraperitoneal injection of 5-HT produced a dose-dependent nociceptive writhing response that was attenuated at all doses by pre-administration of tropisetron. However, administration of the 5-HT_3 receptor agonist, 2-methyl-5-HT alone, was ineffective in inducing writhing. Yet, when a low dose of 5-HT was intraperitoneally pre-injected, 2-

methyl-5-HT produced significant increases in writhing. The enhanced nociceptive responses produced by low dose of 5-HT and subsequently administered 2-methyl-5-HT were attenuated by tropisetron pretreatment.

Several possibilities might explain such findings. First, the possibility of simple volume distention produced by the addition of 2-methyl-5-HT following 5-HT administration causing increased writhing is improbable in that total administered volumes were constant, and adjusted for serial injections. Second, as 2-methyl-5HT has been shown to have approximately half the functional potency of 5-HT at the 5-HT_3 receptor (Richardson et al., 1985), the patterns of nociception may represent additive effects of these agonists. However, this is unlikely in that pretreatment with 2-methyl-5-HT prior to administration of low dose of 5-HT failed to produce the enhanced nociceptive responses as seen when 2-methyl-5-HT was preceded by 5-HT administration.

A third possibility is that 5-HT_3 receptor-mediated nociception is dependent, at least partly, upon other mechanisms of inflammation that stimulate the receptor. This is certainly feasible in that 5-HT (but not 2-methyl-5-HT) has been shown to engage heterogeneous pro-inflammatory and pronocisponsive substrates that act through both serotonergic (e.g. 5-HT_1, 5-HT_2, 5-HT_4 receptor-mediated; Sufka et al., 1992; Doak and Sawynok, 1997) and non-serotonergic (e.g. platelet and mast cell degranulation, retrograde release of substance P, etc.) processes. The relatively long-lasting effects produced by 5-HT in the writhing (Moser, 1995) and other inflammatory pain tests (Sufka et al., 1992) substantiate the involvement of such multiple, more durable mechanisms. These events might modulate domains of the 5-HT_3 receptor (and/or the plasma membrane) and thus affect subsequent sensitivity to pharmacologic agonists. Serotonin may invoke a cascade of pro-inflammatory chemical events that could result in differential 5-HT receptor (e.g. 5-HT_1, 5-HT_2, 5-HT_3, 5-HT_4) activation and communication, change the physiochemical stability of the membrane and/or induce reciprocal interactions between these substrates. Although this possibility is speculative, it is supported by studies that have shown that the 5-HT_3 receptor can be indirectly and allosteric modulated (Barann et al., 1993; Wu et al., 2000). Such effects might account for the increase in 2-methyl-5-HT-induced nociception following a low dose of 5-HT. The efficacy of tropisetron, a 5-HT_3 and 5-HT_4 receptor antagonist, in attenuating these responses strengthens the hypothesis that 5-HT_3-mediated nociception may be reliant, at least in part, upon substrates of the inflammatory response that are subserved by the 5-HT_4 receptor (Doak and Sawynok, 1997).

Finally, it may be that an increase in the local concentration of 5-HT directly sensitizes the 5-HT_3 receptor, through either "unmasking" of viable binding domains, initiating availability of sequestered non-membrane pools of receptor, or both. The physiologic quiesence of 5-HT_3 receptors under non-inflammatory conditions has been suggested (Green et al., 2000), and the present results appear to support this hypothesis. The question arises, however, whether 5-HT-induced activation of the 5-HT_3 site involves movement of receptor fractions from one membrane or submembrane region to another, de novo synthesis of receptors or an alteration in the molecular structure and hence pharmacologic sensitivity of existing 5-HT_3 sites. Further study is important to clarify these possibilities and fortify an understanding of the role of peripheral 5-HT_3 receptors in mediating inflammatory pain.

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