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Synergistic antiemetic interactions between serotonergic 5-HT_3 and tachykininergic NK_1-receptor antagonists in the least shrew (*Cryptotis parva*)

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ABSTRACT

Significant electrophysiological and biochemical findings suggest that receptor cross-talk occurs between serotonergic 5-HT_3- and tachykininergic NK_1-receptors in which co-activation of either receptor by ineffective doses of their corresponding agonists (serotonin (5-HT) or substance P (SP), respectively) potentiates the activity of the other receptor to produce a response. In contrast, selective blockade of any one of these receptors attenuates the increase in abdominal vagal afferent activity caused by either 5-HT or SP. This interaction has important implications in chemotherapy-induced nausea and vomiting (CINV) since 5-HT_3- and NK_1-receptor antagonists are the major classes of antiemetics used in cancer patients receiving chemotherapy. The purpose of this study was to demonstrate whether the discussed interaction produces effects at the behavioral level in a vomit-competent species, the least shrew. Our results demonstrate that pretreatment with either a 5-HT_3 (tropisetron)- or an NK_1 (CP99,994)-receptor specific antagonist, attenuates vomiting caused by a selective agonist (2-methyl 5-HT or GR73632, respectively) of both emetic receptors. In addition, relative to each antagonist alone, their combined doses were 4–20 times more potent against vomiting caused by each emetogen. Moreover, combined sub-maximal doses of the agonists 2-methyl 5-HT and GR73632, produced 8–12 times greater number of vomits relative to each emetogen tested alone. However, due to large variability in vomiting caused by the combination doses, the differences failed to attain significance. The antiemetic dose–response curves of tropisetron against both emetogens were U-shaped probably because larger doses of this antagonist behave as a partial agonist. The data demonstrate that 5-HT_3- and NK_1-receptors cross-talk to produce vomiting, and that synergistic antiemetic effects occur when both corresponding antagonists are concurrently used against emesis caused by each specific emetogen.

1. Introduction

Serotonin (5-hydroxytryptamine = 5-HT) is a monoamine neurotransmitter present in both the central and peripheral nervous systems (Darmani and Ray, 2009). 5-HT produces its diverse effects via stimulation of seven different classes of serotonergic receptors (5-HT_1–5-HT_7) many of which possess multiple subtypes. In regard to vomiting, both serotonin 5-HT_3 (an ion-gated channel) and 5-HT_4 (a G protein-coupled receptor) receptor agonists have emetic efficacy, while 5-HT_3 receptor antagonists are the main defense against the acute phase of chemotherapy-induced nausea and vomiting (CINV) in cancer patients receiving chemotherapy (Andrews and Rudd, 2004; Darmani and Ray, 2009; Feyer and Jordan, 2011). The established dogma regarding emetic neurotransmitters involved in CINV suggests that chemotherapeutics agents such as cisplatin induce their acute vomiting phase by releasing 5-HT from enterochromaffin cells in the gastro-intestinal tract (GIT) to stimulate local 5-HT_3 receptors found on the GIT vagal afferents, which subsequently activate the brainstem dorsal vagal complex (DVC) emetic nuclei [area postrema (AP), nucleus of the solitary tract (NTS) and the dorsal motor nucleus of the vagus (DMNX)] to complete the vomiting reflex (Rudd and Andrews, 2005).

The delayed CINV phase has been assumed to be due to activation of brainstem tachykininergic NK_1 receptors subsequent to the release of SP in the DVC (Andrews and Rudd, 2004). The mammalian tachykinins include the peptides substance P (SP), neurokinin A (NKA) and neurokinin B (NKB) (Darmani and Ray, 2009). These peptides activate three tachykininergic receptors (NK_1, NK_2 and NK_3) in both the CNS and periphery. The latter receptors belong to the family of G protein-coupled receptors that are respectively recognized with moderate selectivity by endogenous SP, NKA and NKB. While NK_1 receptor-selective agonists induce vomiting (Darmani et al., 2008), selective NK_1 antagonists not only prevent vomiting caused by NK_1 receptor agonists (Darmani et al., 2008), but also act as broad-spectrum antiemetics against a diverse array of centrally- and peripherally-acting emetogens in several animal models of emesis

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(Andrews and Rudd, 2004; Darmani and Ray, 2009). Further, such antagonists are used in the clinic in cancer patients against the delayed phase of CINV (Andrews and Rudd, 2004; Darmani and Ray, 2009). More recently, the discussed simple dogma of one neurotransmitter in a given emetic locus per emetic phase, was revised by us (Darmani and Ray, 2009) to suggest that: i) not only is simultaneous release of 5-HT and SP involved in both emetic phases of CINV, but also other emetic transmitters (e.g. dopamine, prostaglandins) contribute to their manifestations, and ii) many of these emetogens act concomitantly via their corresponding emetic receptors present in both the GIT and the DVC emetic loci to induce CINV.

The proposed multi-transmitter/emetic loci notion of CINV is further complicated by findings that receptor cross-talk occurs among diverse receptor systems, particularly between 5-HT_3 and NK_1 receptors both in the CNS and periphery. For example, NK_1 receptors in the brainstem at the level of NTS, contribute downstream to the 5-HT_3 receptor-mediated inhibition of the aortic, but not carotid, baroreflex response during defense reaction in rats (Comet et al., 2005). Further, pharmacological blockade of the NK_1 receptor or its genetic deletion increases both the neuronal activity of dorsal raphe neurons and 5-HT release in some of its terminal fields which could subsequently activate different serotonergic receptors (Gobbi et al., 2007; Guiard et al., 2007). On the other hand, intra-raphe injection of SP reduces serotonergic terminal field 5-HT levels. At the GIT level, it has been demonstrated that NK_1 receptor desensitization (Ramirez et al., 1994) or antagonism of NK_1 receptors (Briejer and Schuurkes, 1996), attenuates the contractile effect of a "selective" 5-HT_3 receptor agonist (2-methyl 5-HT) in the presence of atropine in both the guinea pig longitudinal muscle-myenteric plexus preparation and in guinea pig proximal colon. At the level of vagal afferents, it has been demonstrated that prior treatment with a peripherally acting (Sendide) or a CNS-penetrating (CP99,994) NK_1 receptor antagonist, reduces the ability of 5-HT or its brain-penetrating analog 2-methyl 5-HT to increase abdominal vagal nerve activity in a vomit-competent species, the ferret (Minami et al., 1998; Minami et al., 2001). Furthermore, the latter authors have also shown that pretreatment with a 5-HT_3 receptor antagonist can attenuate the efficacy of SP to increase vagal afferent activity in ferrets. In line with these findings, SP has been shown to potentiate the 5-HT-induced inward currents through 5-HT_3 receptor ion-channels in the rat trigeminal ganglion neurons via the activation of NK_1 receptors (Hu et al., 2004).

The discussed receptor cross-talk has important implications in CINV since specific emetogens may affect each others' vomiting efficacy and use of a combination of their selective antagonists could lead to synergistic antiemetic potential. Thus, the purpose of the current study was to demonstrate in a emesis-competent species, the least shrew (*Cryptotis parva*) (Darmani, 1998; Darmani et al., 2008), whether: i) utilization of a combination of a 5-HT_3 (tropisetron) (Darmani, 1998)- and an NK_1 (CP99,994) (Darmani et al., 2008)-receptor antagonist would exhibit synergistic antiemetic efficacy against a maximally effective emetic dose of either a selective 5-HT_3 (2-methyl 5-HT)- or a selective NK_1 (GR73632)-receptor agonist; and ii) sub-maximal doses of 2-methyl 5-HT and GR73632 could potentiate each other's emetic potential.

2. Materials and methods

2.1. Animals and drugs

Adult male and female least shrews, 45–60 days old weighing 4–6 g were used throughout the experiment. The feeding and maintenance of shrews are fully described elsewhere (Darmani, 1998). All experiments were performed between 11:00 and 17:30 h in accordance with the NIH guidelines and Western University IACUC standards. All drugs were purchased from Sigma-Aldrich (St. Louis, MO, USA) except GR73632 (Tocris Bioscience, Ellisville, MO, USA), and dissolved in distilled water. All drugs were administered at a volume of 0.1 ml/10 g of body weight and the doses and routes of administration used were based on our published studies (Darmani et al., 2008; Ray et al., 2009a).

2.2. Experimental protocols

The present protocols were based upon our preliminary dose–response studies as well as published findings in the least shrew (Darmani, 1998; Darmani et al., 2008). On the day of experimentation shrews were transferred to the experimental room and were allowed to acclimate to the laboratory conditions for one hour. During this period food was restricted, but not water. To habituate the shrews to the test environment, each animal was randomly selected and transferred to a $20\times18\times21$ cm clean plastic holding cage 30 min prior to experimentation. To determine whether 5-HT_3 (tropisetron)- or NK_1 (CP99,994)-receptor blockade can abolish the ability of either 2-methyl 5-HT or GR73632 to induce emesis, different groups of shrews were injected with either tropisetron (0, 0.5, 1, 2.5, 5 and 10 mg/kg, n = 4–8 per group, s.c.) or CP99,994 (0, 0.5, 1, 2.5, 5, 10, and 20 mg/kg, n = 4–14 per group, i.p.) and then each shrew was offered 4 meal worms (*Tenebrio sp.*). Thirty minutes following antagonist administration, the treated shrews were injected with a maximal emetic dose of either 2-methyl 5-HT (5 mg/kg. i.p.) (Darmani, 1998) or GR73632 (5 mg/kg, i.p.) (Darmani et al., 2008). Immediately following agonist injection, each shrew was placed in the observation cage and the frequency of emesis (mean ± SEM; oral ejections of food or liquid plus emetic episodes without expulsion of food) was recorded for the next 30 min. Since the dose–response antiemetic effect of tropisetron in preventing shrews from vomiting followed a U-shaped curve, the emetic potential of larger doses of tropisetron (5, 10 and 20 mg/kg, n = 4–6 per group, s.c.) was investigated in accord with our agonist-induced vomiting studies as described later.

Since tropisetron and CP99,994 pretreatment each alone attenuated the emetic ability of both 2-methyl 5-HT and GR73632, in the final antagonist experiment we investigated the synergistic antiemetic potential of these antagonists against the emetic efficacy of each of the tested emetogens. Thus, different groups of shrews were injected with either corresponding vehicles (i.p. and s.c.) or combination doses (0.5/0.5, 1/1, 2.5/2.5 and 5/5 mg/kg, n = 6–10 per group) of tropisetron (s.c.) plus CP99,994 (i.p.) 30 min prior to administration of a maximal emetic dose of either 2-methyl 5-HT (5 mg/kg, i.p.) or GR73632 (5 mg/kg, i.p.). Immediately following agonist injection, the frequency of emesis was recorded for the next 30 min as described above for the antagonist studies.

To determine whether combination of a 5-HT_3 (2-methyl 5-HT)- and an NK_1 (GR73632)- receptor agonist can cause synergistic emetic effects, different groups of shrews were i.p.-injected with sub-maximal emetic doses of either 2-methyl 5-HT (0.5 mg/kg, n = 6 per group) or GR73632 (1 mg/kg, n = 6 per group) alone, or with a combination of the same doses of the discussed agonists (n = 8 per dose). Immediately following injection, each shrew was placed in the observation cage and the frequency of emesis was recorded for the next 30 min as described earlier.

2.3. Statistical analyses

The data on the frequency of emesis were analyzed by Kruskal–Wallis (KW) nonparametric one-way analysis of variance (ANOVA) and post hoc analysis by Dunn's multiple comparisons test. The incidence of emesis (number of shrews vomiting) was analyzed by Fisher's exact test to identify differences between groups. When appropriate, pairwise comparisons were also made by this method. A P value of <0.05 was considered to be statistically significant.