Pharmacology, Biochemistry and Behavior 99 (2011) 573–579

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

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

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ARTICLE INFO

Article history:
Received 29 March 2011
Received in revised form 16 May 2011
Accepted 22 May 2011
Available online 13 June 2011

Keywords:
5-HT_3 receptor
NK_1 receptor
2-methyl 5-HT
GR73632
Synergistic
Emesis
Least shrew
Cross-talk
Interaction

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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0091-3057/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2011.05.025

(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.

3. Results

3.1. Anti-emetic effects of tropisetron against 2-methyl 5-HT- and GR73632-induced emesis

Intraperitoneal administration of tropisetron (0, 0.5, 1, 2.5, 5, and 10 mg/kg, s.c.) attenuated the frequency of emesis induced by 2-methyl 5-HT (5 mg/kg, i.p.) [(KW (5, 47) = 18.88, $P<0.005$)] (Fig. 1A). Dunn's multiple comparisons post hoc test showed that significant reductions occurred at its 2.5 (79%, $P<0.005$), 5 (67%, $P<0.05$), and 10 mg/kg (70%, $P<0.05$) doses. The Fisher's exact test indicates that the percentage of shrews vomiting in response to 2-methyl 5-HT was reduced by tropisetron (0, 0.5, 1, 2.5, 5, and 10 mg/kg) in a U-shaped manner [(χ^2 (5, 47) = 17.64, $P<0.005$)] (Fig. 1B). Indeed, a significant reduction was seen only at its 2.5 mg/kg dose (62%, $P<0.005$). Tropisetron (0, 0.5, 1, 2.5, 5, and 10 mg/kg, i.p.) administration also attenuated the frequency of vomiting caused by GR73632 in a U-shaped dose–response manner [(KW (5, 41) = 18.96, $P<0.005$)] with a significant reduction occurring at its 2.5 mg/kg dose (76%, $P<0.005$) (Fig. 1C). However, the Fisher's exact test showed that the percentage of shrews vomiting in response to GR73632 was not affected by tropisetron (0, 0.5, 1, 2.5, 5, and 10 mg/kg) [(χ^2 (5, 41) = 8.92, $P.>0.5$)] (Fig. 1D). Since tropisetron produced its anti-emetic effects in a U-shaped manner, we examined whether higher doses of tropisetron would induce emesis by itself. As expected, larger doses of tropisetron (5, 10, and 20 mg/kg) induced emesis [(KW (2,15) = 8.755, $P<0.05$)] with a significant frequency of vomiting occurring at its 20 mg/kg dose (Fig. 1E). Likewise, the percentage of shrews vomiting in response to tropisetron was increased in a dose-dependent manner [(χ^2 (2, 13) = 8.88, $P<0.05$)] with a significant increase at its 20 mg/kg dose ($P<0.05$)(Fig. 1F).

3.2. Anti-emetic effects of CP99,994 against GR73632- and 2-methyl 5-HT-induced emesis

CP99,994 (0, 0.5, 1, 2.5, 5, and 10 mg/kg, i.p.) attenuated the frequency of vomiting caused by GR73632 in a dose dependent manner [(KW (5, 47) = 19.42, $P<0.005$)] (Fig. 2A). Dunn's multiple comparisons post hoc test showed that CP99,994 attenuated the frequency of vomits at its 10 mg/kg dose (89%, $P<0.001$). The Fisher's exact test showed that the percentage of shrews vomiting in response to GR73632 was also reduced by CP99,994 (0.5, 1, 2.5, 5, and 10 mg/kg) [(χ^2 (5, 47) = 13.90, $P<0.005$)], and a significant reduction was seen at its 10 mg/kg dose (62%, $P<0.001$) (Fig. 2B). CP99,994 (0, 0.5, 1, 2.5, 5, 10, and 20 mg/kg) also attenuated the frequency of 2-methyl 5-HT-induced vomiting [(KW (6, 41) = 15.28, $P<0.05$)] with a significant effect occurring at its 20 mg/kg dose (80% reduction, $P<0.005$) (Fig. 2C). However, CP99,994 administration failed to significantly reduce the number of shrews vomiting [(χ^2 (6, 41) = 11.36, $P>0.05$)] (Fig. 2D).

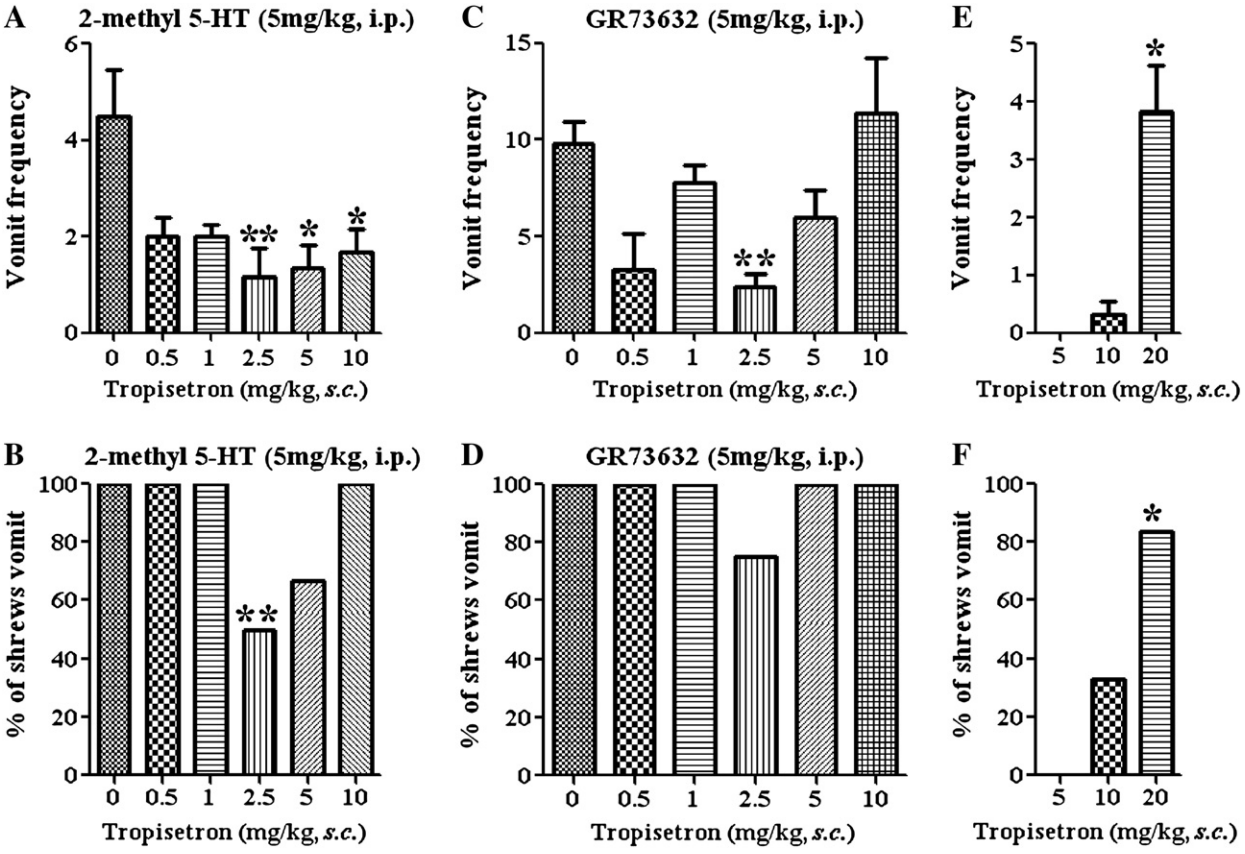

Fig. 1. Antiemetic effects of the 5-HT_3 receptor antagonist tropisetron against vomiting caused by either the selective 5-HT_3 (2-methyl 5-HT)- or NK_1 (GR73632)-receptor agonist in the least shrew. Varying doses of tropisetron was administered (s.c.) to different groups of shrews 30 min prior to an i.p.-injection of a 5 mg/kg maximal emetic dose of either 2-methyl 5-HT (graphs A and B) or GR73632 (graphs C and D). The frequency of vomits induced by 2-methyl 5-HT was significantly reduced by tropisetron at its 2.5, 5, and 10 mg/kg doses (graph A), while the percentage of shrews vomiting was reduced in a U-shaped dose–response manner and a significant reduction was observed at its 2.5 mg/kg dose (graph B). The frequency of vomits induced by GR73632 was also reduced by tropisetron in a U-shaped fashion and a significant reduction occurred at its 2.5 mg/kg dose (graph C), while the percentage of shrews vomiting was not significantly affected by any dose. At larger doses tropisetron by itself induced emesis in a dose-dependent manner and a significant increase in both vomit frequency (graph E) and percentage of shrews vomiting occurred at its 20 mg/kg dose. Frequency data are presented as mean (±SEM).* $P<0.05$ vs. 0 mg/kg. ** $P<0.005$ vs. 0 mg/kg.

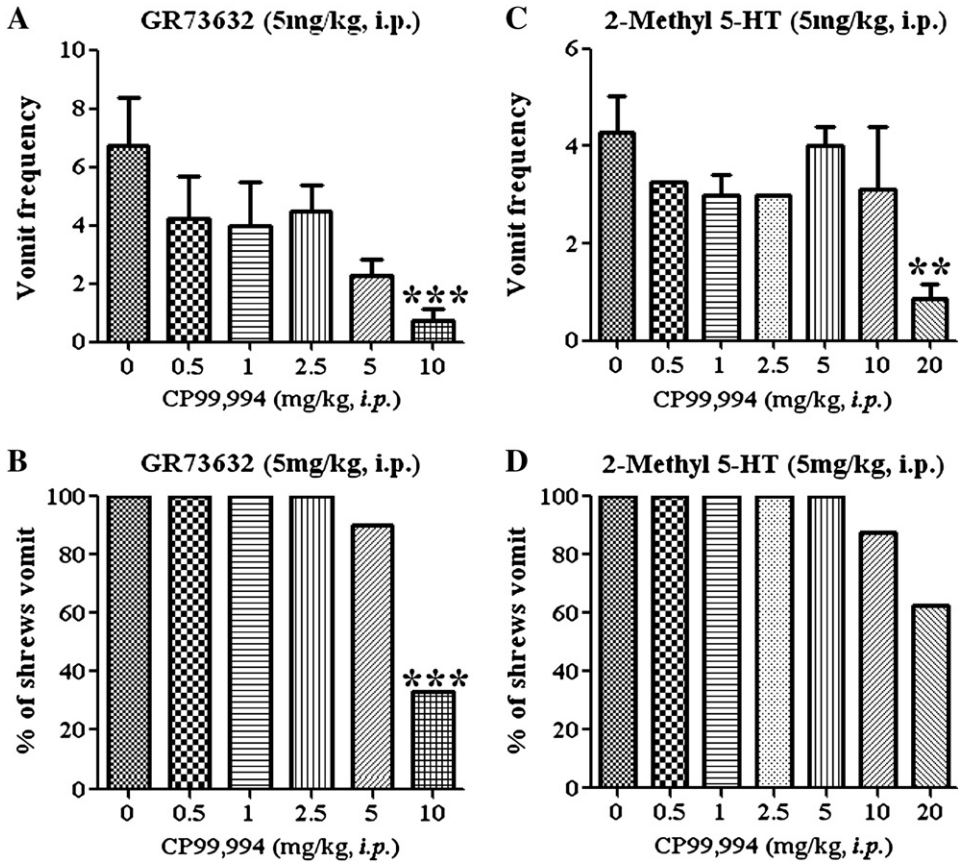

Fig. 2. Antiemetic effects of the NK_1 receptor antagonist CP99,994 against vomiting caused by either the selective NK_1 (GR73632)- or $5\text{-}HT_3$ (2-methyl 5-HT)- receptor agonist in the least shrew. Varying doses of CP99,994 were administered (i.p.) to different groups of shrews 30 min prior to an i.p.-injection of a 5 mg/kg maximal emetic dose of either GR73632 (graphs A and B) or 2-methyl 5-HT (graphs C and D). Both the frequency of vomits (graph A) induced by GR73632 and percentage of shrews vomiting (graph B) were significantly reduced by CP99,994 at its 10 mg/kg dose. The frequency of vomits induced by 2-methyl 5-HT was also reduced by CP99,994 at its 10 mg/kg dose (graph C), however the percentage of shrews vomiting was not significantly affected by any tested dose of CP99,994. Frequency data are presented as mean (±SEM).** $P<0.005$ vs. 0 mg/kg, *** $P<0.001$ vs. 0 mg/kg.

3.3. Synergistic anti-emetic effects of sub-effective combination doses of tropisetron and CP99,994 against 2-methyl 5-HT and GR73632-induced emesis

Combination doses of tropisetron/CP99,994 (0.5/0.5, 1/1, 2.5/2.5, and 5/5 mg/kg) attenuated the frequency of 2-methyl 5-HT-induced emesis [(KW (4, 33) = 12.81, $P<0.05$)] (Fig. 3A). Dunn's multiple comparisons post hoc test showed that the combination doses attenuated the frequency of vomits at 2.5/2.5 mg/kg (82%, $P<0.05$) and 5/5 mg/kg (82%, $P<0.05$). Fisher's exact test showed that the percentage of shrews vomiting in response to 2-methyl 5-HT was reduced by the combination doses of tropisetron/CP99,994 [(χ^2 (4, 43) = 16.02, $P<0.05$)] (Fig. 3B). Indeed, significant reductions were observed at their 2.5/2.5 mg/kg (67%, $P<0.05$) and 5/5 mg/kg (67%, $P<0.05$) doses. Tropisetron/CP99,994 combination also attenuated the frequency of GR73632-induced emesis, but in a U-shaped manner [(KW (4, 39) = 22.27, $P<0.0005$)] (Fig. 3C). In fact, a significant reduction (94%, $P<0.0005$) in the frequency of vomits only occurred at their 1/1 mg/kg dose (94%, $P<0.0005$). Fisher's exact test showed the percentage of shrews vomiting in response to 2-methyl 5-HT was also reduced by the combination doses of tropisetron/CP99,994 [(χ^2 (4, 39) = 13.44, $P<0.05$)] (Fig. 3D). Moreover, a significant reduction was only observed at their 1/1 mg/kg dose (63%, $P<0.05$).

3.4. Synergistic effects of sub-maximal emetic doses of 2-methyl 5-HT and GR73632

Varying sub-maximal emetic doses of both 2-methyl 5-HT and GR73632 were tested in combination. The greatest effects obtained were at the 0.5 mg/kg dose of 2-methl5-HT and 1 mg/kg dose of GR73632. These doses of emetogens alone respectively caused emesis in 17% (emesis frequency = 0.5 ± 0.5) and 17% (emesis frequency = 0.33 ± 0.33) of shrews, whereas their combination resulted in 63% of shrews vomiting with a mean frequency of 4.12 ± 1.6. However, due to large vomit variability in the combination dose, the observed effects failed to attain significance.

4. Discussion

Accumulating evidence suggest that chemotherapeutic agents such as cisplatin initiate CINV in the periphery by stimulating release of several emetic neurotransmitters including 5-HT and SP from the enterochromaffin cells in the GIT which subsequently increase vagal afferent neuronal activity via stimulation of corresponding $5\text{-}HT_3$ and NK_1 receptors (Darmani and Ray, 2009). Support for this notion comes from the findings that vagotomy attenuates CINV in ferrets (Hawthorn et al., 1988) and peripheral administration of either 5-HT or SP, increase ferret vagal afferent activity (Minami et al., 1998; 2001). The latter authors have further demonstrated that complex interactions occur in ferrets between the two emetic neurotransmitter systems in that: i) pretreatment with a selective $5\text{-}HT_3$ receptor antagonist reduces the efficacy of SP to increase abdominal vagal activity; and ii) administration of selective peripherally- or centrally-acting NK_1 receptor antagonists (e.g. sendide and CP99,994, respectively) attenuates the increase in vagal activity produced by both selective and non-selective $5\text{-}HT_3$ receptor agonists such as 2-methyl 5-HT or 5-HT. Since the ferret does not vomit in response to peripheral administration of either 5-HT or SP (Knox et al., 1993), lack of an

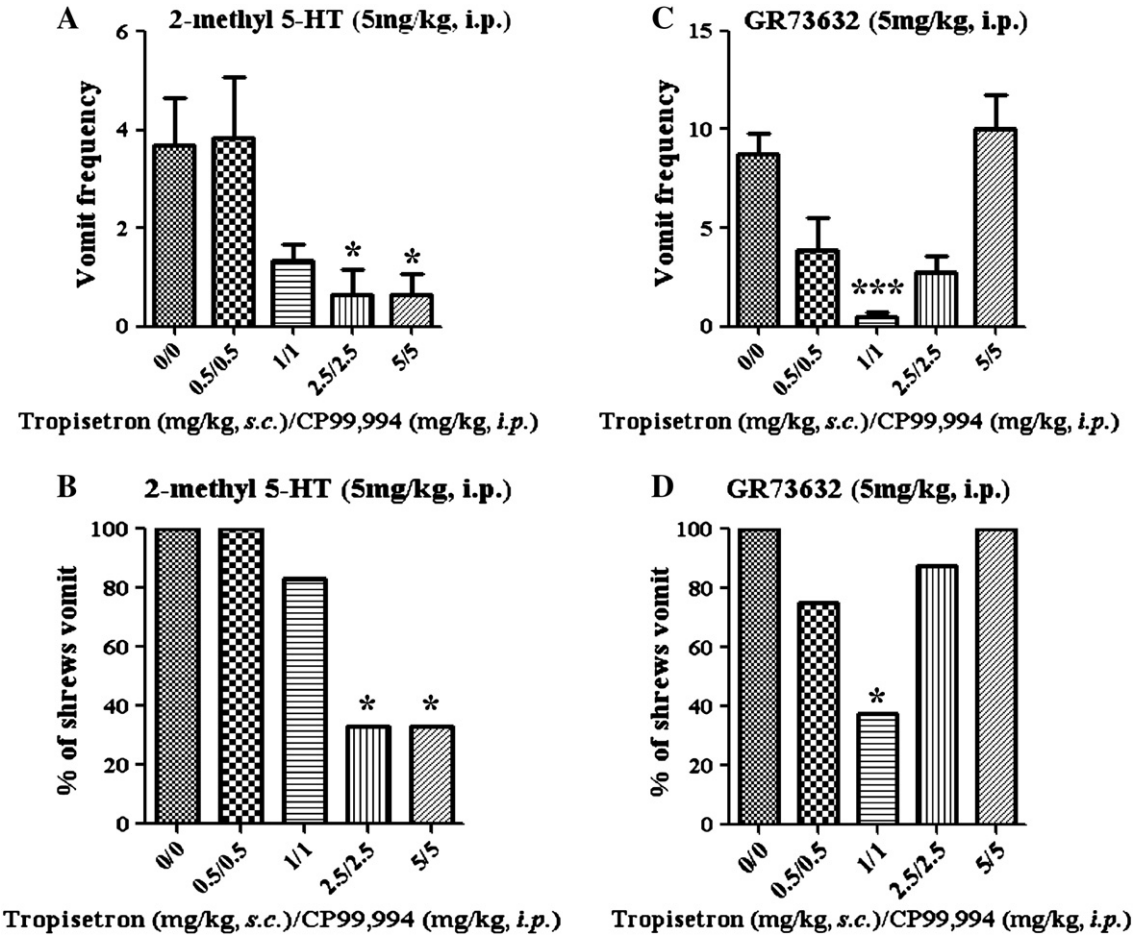

Fig. 3. Synergistic anti-emetic effects of combination doses of a selective 5-HT_3 (tropisetron)- and an NK_1(CP99,994)-receptor antagonist on vomiting caused by a 5 mg/kg dose of either a selective 5-HT_3 (2-methyl 5-HT)- or an NK_1 (GR73632)-receptor agonist. Varying combination doses of tropisetron (s.c.) and CP99,994 (i.p.), were administered concomitantly 30 min prior to an injection of a 5 mg/kg (i.p.) maximally effective emetic dose of either 2-methyl 5-HT (graphs A and B) or GR73632 (graphs C and D). Both the frequency of vomits and the percentage of shrews vomiting induced by 2-methyl 5-HT were significantly reduced by the 2.5/2.5 and 5/5 mg/kg combination doses of tropisetron/CP99,994. Both the frequency of vomits and the percentage of shrews vomiting induced by GR73632 were reduced by the combination doses of tropisetron/CP99994 in a U-shaped dose–response fashion and significant reductions in both emetic parameters occurred at their 1/1 mg/kg dose. Frequency data are presented as mean (±SEM).*** $P<0.001$ vs. 0/0 mg/kg. * $P<0.05$ vs. 0/0 mg/kg.

emetic model to demonstrate such an interaction on a functional behavioral level eluded us until the validation of the least shrew emesis model, which exhibits profuse vomiting in response to intraperitoneal injection of both 5-HT and SP (Darmani, 1998; Darmani et al., 2008).

Although ferrets do not vomit in response to peripheral injection of serotonin, its 5-HT_3-receptor selective analog 2-methyl 5-HT, can induce emesis in several species including ferrets (Sancillio et al., 1991), house musk shrews (Torii et al., 1991a,b,c), and least shrews (Darmani, 1998). Further, the 2-methyl 5-HT (5 mg/kg, i.p.)-induced vomiting in house musk shrews was shown to be completely blocked by small doses (<1 mg/kg, s.c.) of the selective 5-HT_3 receptor antagonist, tropisetron. Likewise, a 1 mg/kg dose (s.c.) of tropisetron was effective in preventing vomiting caused by a 10 mg/kg oral dose of 2-methyl 5-HT in ferrets (Sancillio et al., 1991). However, in the least shrew tropisetron, up to 10 mg/kg (s.c.) doses, attenuated the vomit frequency only by 67–70%, while completely protecting shrews from vomiting in a U-shaped dose–response manner with maximal blockade (62%, $P<0.05$) occurring at its 2.5 mg/kg dose. These data suggest that either tropisetron does not effectively block 5-HT_3 receptors in the least shrew, or tropisetron is a 5-HT_3 receptor partial agonist and least shrews are sensitive to its agonist emetic action at higher doses. We believe the latter two notions are correct since in the present study larger doses of tropisetron by itself caused dose-dependent vomiting in least shrews. In fact, at high doses structurally diverse 5-HT_3 receptor antagonists (e.g. tropisetron, zacopride, BRL 43694), act as partial agonists and cause vomiting or other behaviors in various species including ferrets, house musk shrews, humans and rodents (Dukat et al., 2000; King, 1990; Minami, 2003; Sancillio et al., 1991; Torii et al., 1991a). Moreover, the least shrew is more sensitive than rodents to 5-HT_{2A} receptor serotonergic agonists (Darmani et al., 1994). Our behavioral studies further demonstrate that tropisetron's blockade of 5-HT_3 receptors also significantly attenuates the frequency of vomiting induced by an intraperitoneal injection of the NK_1 receptor selective agonist GR73632. However, the observed reduction in the vomit dose–response frequency was U-shaped, and the tested doses of tropisetron failed to completely protect shrews from vomiting. The observed reduction in GR73632-induced vomit frequency is supported by electrophysiological findings since another 5-HT_3 receptor antagonist (palonosetron) can inhibit cisplatin-induced enhancement of nodose ganglion responses to SP (Rojas et al., 2010).

As expected, pretreatment with 0.5–10 mg/kg doses of the NK_1 receptor antagonist CP99,994, significantly and dose-dependently reduced the frequency of vomiting induced by the selective NK_1 receptor agonist GR73632 in least shrews. However, only 62% of

shrews were completely protected from vomiting at the highest tested doses of CP99,994. Greater reductions in emesis frequency and even complete protection of shrews from the induced emesis can occur at the 20 mg/kg dose of CP99,994 (Darmani et al., 2008). Antagonism of NK_1 receptors by up to 20 mg/kg doses of CP99,994 failed to completely protect all tested shrews from vomiting caused by 2-methyl 5-HT. However, the latter dose of CP99,994 did significantly attenuate the mean frequency of 2-methyl 5-HT-induced emesis by 80%. Thus, at the whole-animal level, our emesis frequency data appear to support the reported: i) receptor interactions occurring in the periphery where blockade of NK_1 receptors attenuates the ability of 2-methyl 5-HT to increase both abdominal vagal activity (Minami et al., 1998, 2001) and intestinal contractility (Briejer and Schuurkes, 1996); and ii) brainstem NK_1- and 5-HT_3-receptors' functional interactions in control of the baroreceptor reflex response (Comet et al., 2005). Such interactions at both locations can be important in the modulation of emesis since both serotonin and SP induce vomiting via brainstem and gastrointestinal loci (Andrews and Rudd, 2004; Darmani et al., 2008; Darmani and Ray, 2009; Ray et al., 2009b,c).

The published and current findings clearly demonstrate that NK_1- and 5-HT_3-receptors cross talk, in that blockade of a particular receptor not only prevents its corresponding function but also can attenuate the performance of the other receptor in response to its corresponding agonist. Thus, we investigated the potential synergistic antiemetic effects of combined blockade of both 5-HT_3- and NK_1-receptors against vomiting induced by their respective corresponding selective agonists such as 2-metyl 5-HT and GR73632. Indeed, relative to each antagonist alone, the combination doses of tropisetron/CP99,994 were at least 4 times more potent in reducing the vomit frequency and providing total vomit protection against 2-methyl 5-HT-induced vomiting. Further, the combined doses of the NK_1 and 5-HT_3 antagonists were even more protective (i.e. at 1/1 mg/kg up to 20 times) against GR73632-induced emesis. However, the protection was U-shaped at larger doses. Indeed, the partial agonist emetic nature of tropisetron seems to be further unmasked at its lower doses (i.e. 2.5 and 5 mg/kg) when it is combined with CP99,994 against GR73632-induced emesis. One possible explanation for the latter observation could be pharmacokinetic interaction at the level of metabolism or plasma protein binding between the two antagonists in least shrews. The latter notion may provide a partial explanation as to why clinically relevant but relatively larger doses of tropisetron can become ineffective as antiemetics in cancer patients receiving multiple therapeutic agents (Gamse, 1990).

Alhough in the present investigation the mechanism(s) underlying the synergistic antiemetic efficacy of combined low doses of the 5-HT_3 and NK_1 receptor antagonists was not investigated, published literature points at the level of signal transduction. Indeed, SP potentiates serotonin-induced 5-HT_3-receptor-mediated inward currents in rat trigeminal ganglion neurons through stimulation of NK_1 receptors and is thought to involve protein kinase C activation (Hu et al., 2004). This latter enzyme regulates the magnitude and duration of NK_1-induced Ca^{2+} mobilization (Dery et al., 2001). Likewise, subthreshold inactive concentrations of serotonin have also been shown to induce a 10-fold synergistic increase in the potency of SP to increase Ca^{2+}-ion mobilization in NG108-15 cells (Rojas et al., 2010). These results suggest that the two emetogens used in the current study should exhibit synergistic emetogenicity. Indeed, low doses of 2-methyl 5-HT and GR73632, each capable of producing emesis in 17% of animals when tested alone, caused vomiting in 63% of shrews when combined. Even more remarkable, the combined agonist doses respectively produced 8 and 12 times greater number of vomits relative to each drug tested alone. However, due to large variability in the response to the combined doses, the attained results did not achieve significance.

In conclusion our behavioral findings, combined with published electrophysiological and biochemical data, support the notion of receptor cross-talk occurring between 5-HT_3 and NK_1 receptors whose concomitant antagonism can lead to synergistic antiemetic activity.

Acknowledgments

We would like to acknowledge COMP funds for supporting this study and Dr. J. Felton for proof reading the manuscript.

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