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Short communication

Noradrenaline stimulates 5-hydroxytryptamine release from mouse ileal tissues via α_2-adrenoceptors

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Abstract

The effect of noradrenaline on 5-hydroxytryptamine (5-HT) release from isolated mouse ileal tissues was investigated. Noradrenaline, but not isoprenaline, at 1 μM stimulated 5-HT release, an effect which was inhibited by yohimbine, an α_2-adrenoceptor antagonist, but not by bunazosin, an α_1-adrenoceptor antagonist. α_2-Adrenoceptor agonists, UK 14,304 (5-bromo-6-(2-imidazolin-2-yl-amino)-quinoxaline) and clonidine at a higher concentration (10 μM) also stimulated 5-HT release, while α_1-adrenoceptor agonists, methoxamine and phenylephrine, had no effect. The effect of noradrenaline was completely abolished in ileal tissues isolated from mouse treated with pertussis toxin (100 μg/kg, i.v.) for 2 days. These results suggest that noradrenaline causes 5-HT release from enterochromaffin cells in mouse ileal tissues via α_2-adrenoceptor subtypes coupled to a pertussis toxin-sensitive G protein.

Keywords: Noradrenaline; 5-HT (5-hydroxytryptamine, serotonin); Enterochromaffin cell; Ileal tissue; α_2-Adrenoceptor; (Mouse)

1. Introduction

The gastrointestinal tract contains the largest amount of 5-hydroxytryptamine (5-HT) in the body. Approximately 95% of the intestinal 5-HT content is present in enterochromaffin cells in the intestinal mucosa (Erspamer and Asero, 1952). 5-HT released from enterochromaffin cells is important in diverse mechanisms regulating the physiological functions of the digestive tract, particularly the peristaltic motor activity of the gut (Foxx-Orenstein et al., 1996). Numerous pharmacological approaches using receptor agonists or antagonists have revealed that 5-HT release is triggered or modulated via multiple autoreceptors or heteroreceptors present on enterochromaffin cells (Racké et al., 1996; Hirafuji et al., 2000). Previous studies have suggested that adrenergic mechanisms also participate in the regulation of 5-HT release from the intestinal mucosa of the dog (Burks and Long, 1966), cat (Ahlman et al., 1976), rat (Pettersson et al., 1978) and rabbit (Kuemmerle et al., 1988). In the vascularly perfused ileum of the guinea-pig, the release of 5-HT is facilitated by activation of β-adrenoceptors and is inhibited via α_2-adrenoceptors present on enterochromaffin cells (Racké et al., 1988). In contrast, noradrenaline and isoprenaline have no effect on endogenous 5-HT release from isolated strips of rat caecum mucosa (Simon and Ternaux, 1990), while noradrenaline increases 5-HT release from suspensions of guinea-pig duodenal crypts (Lomax et al., 1999). Thus, it is still uncertain which receptor subtypes and what mechanisms are involved in the adrenergic regulation of 5-HT release from the intestinal mucosa. Therefore, in the present study, we investigated the effects of noradrenaline stimulation on 5-HT release from isolated mouse ileal tissue in vitro.

2. Materials and methods

2.1. Measurement of 5-HT release

5-HT release from isolated ileal tissues was evaluated as described previously (Minami et al., 1998). Mouse ileal tissues were isolated from 4- to 5-week-old male mice (ICR, Japan SLC, Hamamatsu, Japan) under ether anesthesia, and

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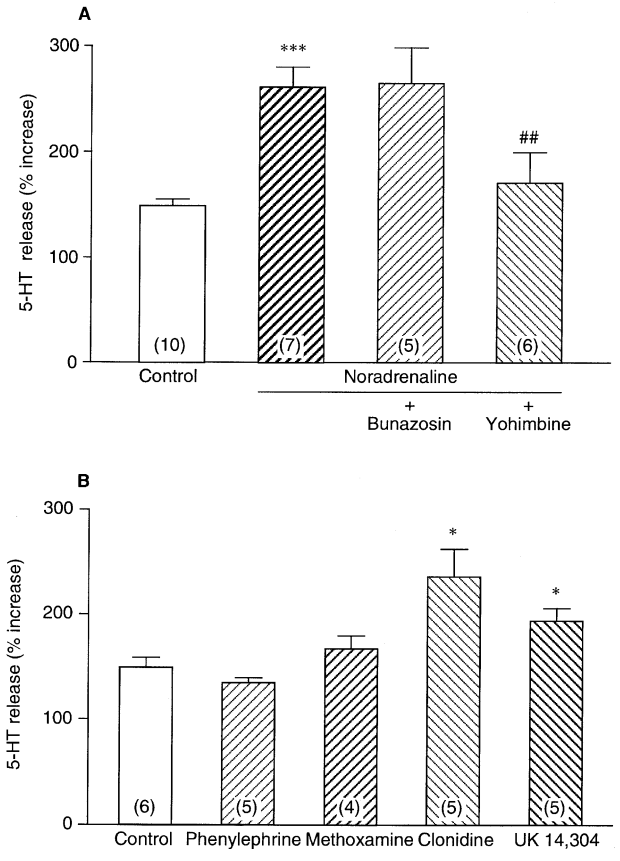

Fig. 1. Effects of α-adrenoceptor agonists and antagonists on 5-HT release from isolated ileal tissues of the mouse. A: Effects of α-adrenoceptor antagonists (1 μM) on noradrenaline (1 μM)-induced 5-HT release; B: effects of α-adrenoceptor agonists (10 μM) on 5-HT release. Results are expressed as percentage increase in 5-HT release for 60 min, taking 5-HT release during the 60-min equilibration period as 100% (15.17 ± 5.15 and 19.24 ± 8.03 ng/g wet tissue weight in each control of A and B, respectively). Each column represents mean ± S.E. of (*n*) experiments indicated in parentheses. $*P<0.05$, $***P<0.001$ versus control; $\#\#P<0.01$ versus noradrenaline alone.

dissected into approximately 3-cm long sections (0.2–0.3 g wet tissue). These ileal segments were placed in organ baths containing modified Krebs solution containing NaCl 120, KCl 5.0, $CaCl_2$ 2.5, $MgSO_4$ 1.0, $NaHPO_4$ 1.0, and glucose 11.0 mM (pH 7.4), which was aerated with 95% O_2 and 5% CO_2. After a 60-min equilibration period, the buffer solutions were collected in 20-min fractions. The amount of 5-HT released from the ileal segments was measured by using high-performance liquid chromatography (Eicom, EP-10, Japan) with an electrochemical detector (Eicom, ECD-100, Japan). 5-HT release during a 60-min period after the equilibration period is expressed as percentage (%) increase, taking the 5-HT amount released during the equilibration period as 100%.

When pertussis toxin was used, mice were treated with pertussis toxin (100 μg/kg, i.v.) dissolved in physiological saline for 2 days before experiments.

2.2. Materials

Noradrenaline, isoprenaline, yohimbine, phenylephrine, methoxamine, clonidine, 5-HT creatinine sulphate and pertussis toxin were purchased from Sigma, and UK 14,304 (5-bromo-6-(2-imidazolin-2-yl-amino)-quinoxaline) from Research Biochemicals International. Bunazosin was a kind gift from Eisai, Japan.

2.3. Statistical analysis

All values are given as means ± S.E. The significance of differences between two groups was assessed using Student's *t*-test. Analysis of variance was used for comparisons of more than two groups. A $P<0.05$ was considered as statistically significant.

3. Results

3.1. Effects of α-adrenoceptor agonists on 5-HT release

Fig. 1 shows the effect of some adrenoceptor agonists and antagonists on 5-HT release from isolated ileal tissues of the mouse. As shown in Fig. 1A, noradrenaline (1 μM) significantly stimulated the 5-HT release, an effect which was significantly inhibited by yohimbine (1 μM), an α_2-adrenoceptor antagonist, to the control level, but not by bunazosin (1 μM), an α_1-adrenoceptor antagonist. These antagonists alone had no significant effect (data not shown).

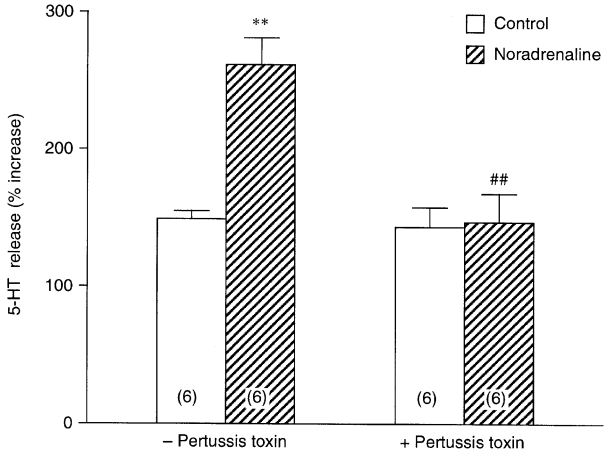

Fig. 2. Effect of pertussis toxin treatment on 5-HT release from isolated ileal tissues of the mouse. The ileal tissues were isolated from mice treated with 100 μg/kg, i.v. for 2 days, and the effect of noradrenaline (1 μM) on 5-HT release was determined. Results are expressed as percentage increase in 5-HT release for 60 min, taking 5-HT release during the equilibration period as 100% (19.77 ± 8.22 and 19.41 ± 7.54 ng/g wet tissue weight in each control of − and + pertussis toxin, respectively). Each column represents mean ± S.E. of (*n*) experiments indicated in parentheses. $**P<0.01$ versus control; $\#\#P<0.01$ versus − pertussis toxin.

Isoprenaline (1 μM) had no stimulatory effect (151.2 ± 17.5%, $n = 6$ versus 155.3 ± 8.2%, $n = 10$ for control). Subsequently, the effects of α_1-adrenoceptor agonists, methoxamine and phenylephrine, and α_2-adrenoceptor agonists, UK 14,304 and clonidine, were investigated. These four drugs at a concentration of 1 μM had no significant effects on the 5-HT release (data not shown). Fig. 1B shows the effects of these drugs at a higher concentration of 10 μM. UK 14,304 and clonidine at this concentration significantly stimulated 5-HT release, while methoxamine and phenylephrine again had no significant effect.

3.2. Effect of pertussis toxin treatment on 5-HT release

Ileal tissues were isolated from mice treated with pertussis toxin (100 μg/kg, i.v.) for 2 days, and the effect of noradrenaline on 5-HT release was investigated. As demonstrated in Fig. 2, pertussis toxin pretreatment completely abolished the stimulatory effect of noradrenaline (1 μM) on 5-HT release from mouse ileal tissues, whereas it had no effect on basal 5-HT release.

4. Discussion

The present study demonstrated that noradrenaline stimulated 5-HT release from mouse ileal tissue in vitro, an effect which was almost completely antagonized by yohimbine, a selective α_2-adrenoceptor antagonist, but not by bunazosin, a selective α_1-adrenoceptor antagonist. Phenylephrine and methoxamine, selective α_1-adrenoceptor agonists, even at a higher concentration had no stimulatory effect on 5-HT release, while clonidine and UK 14, 304, selective α_2-adrenoceptor agonists, showed a stimulatory effect on 5-HT release. Furthermore, the stimulatory effect of noradrenaline was completely inhibited in ileal tissues from pertussis toxin-pretreated mice. α_2-Adrenoceptors are receptors coupled to pertussis toxin-sensitive heterotrimeric $G_{i/o}$ protein (Docherty, 1998). Therefore, our results suggest that noradrenaline causes 5-HT release from mouse ileal tissues via α_2-adrenoceptors coupled to a pertussis toxin-sensitive G protein. This is the first report showing the presence of stimulatory α_2-adrenoceptors, most possibly on enterochromaffin cells, causing 5-HT release from the ileal mucosa. Stimulation of β-adrenoceptors by isoprenaline had no effect on 5-HT release from mouse ileal tissue. These results are in contrast to a previous report demonstrating that α_2-adrenoceptors on enterochromaffin cells inhibit 5-HT release from guinea-pig small intestine (Racké et al., 1988). Furthermore, catecholamines seem to stimulate 5-HT secretion from the intestines of cats, guinea pigs and rats, through the cyclic AMP-mediated mechanisms via β-adrenoceptors (Ahlman et al., 1976; Pettersson et al., 1978; Racké et al., 1988). These differences in the role of α_2- and β-adrenoceptors in 5-HT release from enterochromaffin cells could be due to species differences or receptor subtype heterogeneity.

Much pharmacological evidence indicates that 5-HT release is regulated by multiple receptor-mediated mechanisms (Racké et al., 1996). However, the intracellular regulatory mechanisms of 5-HT release from enterochromaffin cells in response to receptor stimulation are still largely unknown and complicated (Hirafuji et al., 2000). Usually, 5-HT release seems to occur via exocytosis and depends on intracellular Ca^{2+} (Racké et al., 1996). Recently, a digital imaging analysis of intracellular Ca^{2+} dynamics in ileal epithelial cells including enterochromaffin cells present in isolated mouse ileal crypts revealed that noradrenaline, but not isoprenaline, induced a transient elevation of intracellular Ca^{2+} concentration (Satoh et al., 1995). Our preliminary study demonstrated that the noradrenaline-induced Ca^{2+} mobilization in mouse enterochromaffin cells is antagonized by yohimbine, suggesting that the effect of noradrenaline is mediated via the α_2-adrenoceptor subtype (Hirafuji et al., 2000). The functional response (5-HT release) observed in the present study is completely consistent with these results. Therefore, it appears that the α_2-adrenoceptors causing 5-HT release are coupled to a pertussis toxin-sensitive G protein, which possibly links to the increase in intracellular Ca^{2+} concentration of enterochromaffin cells in mouse ileal tissue. α_2-Adrenoceptors are coupled to a pertussin toxin-sensitive $G_{i/o}$ protein primarily linked to inhibition of adenylate cyclase (Docherty, 1998), but it is unlikely that this mechanism accounts for the stimulatory effect of noradrenaline on 5-HT release. Interestingly, it has been reported that stimulation of α_2-adrenoceptors, most likely of the α_{2B}-subtype, causes 5-HT release directly from neuroendocrine epithelial cells of the rabbit tracheal mucosa (Freitag et al., 1996).

In some species, such as rats and mice, 5-HT may be released from intestinal mast cells (Erspamer, 1966), but mast cells are nearly absent in the intestinal tract of normal mice (Guy-Grand et al., 1984). Noradrenaline has been shown not to cause, but rather to suppress, degranulation in rat peritoneal mast cells (Guirgis and Townley, 1976; Alm and Bloom, 1979). 5-HT is also present in and is released from serotonergic neurons of the enteric nervous system such as the myenteric plexus (Gershon, 1999). Therefore, there is a possibility that adrenoceptor stimulation by noradrenaline could release various neurotransmitters including 5-HT from enteric neurons, which may indirectly cause 5-HT release from enterochromaffin cells (Gershon, 1999). However, it seems unlikely that neuronal α_2-adrenoceptors stimulate the release of neurotransmitters (Docherty, 1998), although it cannot be ruled out that α_2-adrenoceptor stimulation causes the release from other enteric endocrine cells of certain mediators that directly stimulate enterochromaffin cells. Direct evidence for the presence of α_2-adrenoceptors stimulating 5-HT release from enterochromaffin cells can be obtained only by using isolated single enterochromaffin cells that have retained their functional responses. The stimulus-secretion coupling and

intracellular signalling pathways leading to 5-HT release in enterochromaffin cells still remain to be clarified.

In conclusion, the present study suggests that noradrenaline causes 5-HT release from enterochromaffin cells in mouse ileal tissues via α_2-adrenoceptor subtypes coupled to a pertussis toxin-sensitive G protein.

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