RESEARCH PAPER

A role for endogenous peptide YY in tachykinin NK₂ receptor-triggered 5-HT release from guinea pig isolated colonic mucosa

Shu-ichi Kojima[1]*, Atsushi Tohei[2]* and Naohiko Anzai[1]

[1]*Department of Pharmacology and Toxicology, Dokkyo Medical University School of Medicine, Mibu, Tochigi, Japan and [2]Laboratory Animal Research Center, Dokkyo Medical University School of Medicine, Mibu, Tochigi, Japan*

BACKGROUND AND PURPOSE

The colon-derived peptide hormone, peptide YY (PYY), regulates colonic motility, secretion and postprandial satiety; but little is known about the influence of endogenous PYY on 5-HT release from colonic mucosa. Tachykinin NK₂ receptor-selective agonist, βAla-NKA-(4-10) induces 5-HT release from guinea pig colonic mucosa via NK₂ receptors on the mucosal layer. The present study was designed to determine the influence of endogenous PYY on 5-HT release from guinea pig colonic mucosa, evoked by the NK₂ receptor agonist, βAla-NKA-(4-10).

EXPERIMENTAL APPROACH

Muscle layer-free mucosal preparations of guinea pig colon were incubated *in vitro* and the outflow of PYY or 5-HT and its metabolite, 5-HIAA, from these preparations were determined by enzyme immunoassays or HPLC with electrochemical detection respectively.

KEY RESULTS

βAla-NKA-(4-10) produced a tetrodotoxin-resistant sustained increase in the outflow of PYY and 5-HT from the mucosal preparations. The βAla-NKA-(4-10)-evoked 5-HT outflow was partially inhibited by Y_1 receptor antagonist, BIBO3304, and Y_2 receptor antagonist, BIIE0246, but with less potency. Exogenously-applied PYY also produced a sustained increase in the outflow of 5-HT that was inhibited by Y_1 blockade but not Y_2 blockade.

CONCLUSION AND IMPLICATIONS

Our findings support the view that the NK₂ receptor-selective agonist, βAla-NKA-(4-10) produces a long-lasting PYY release from guinea pig colonic mucosa via NK₂ receptors on L cells and βAla-NKA-(4-10)-evoked 5-HT release is in part mediated by endogenously released PYY, acting mainly on Y_1 receptors on EC cells. The PYY-containing L cells appear to play a role in controlling the release of 5-HT from colonic EC cells.

Abbreviations

5-HIAA, 5-hydroxyindoleacetic acid; βAla-NKA-(4-10), [β-Ala⁸]-neurokinin A4-10; EC, enterochromaffin; IBS, irritable bowel syndrome; PYY, peptide YY; TTX, tetrodotoxin

Introduction

5-Hydroxytryptamine (serotonin, 5-HT) has long been recognized as a crucial messenger substance, which regulates colonic motility, secretion or sensation by acting via multiple 5-HT receptor subtypes (Camilleri, 2002; Gershon and Tack, 2007). Most of the intestinal 5-HT is produced and stored in the mucosal enterochromaffin (EC) cells from which this

amine is released into the intestinal lumen and portal circulation (Racke and Schworer, 1991). The 5-HT-containing EC cells have been shown to play a key role in stimulating intrinsic primary afferent neurons; therefore, the 5-HT release from EC cells is regarded as an interesting target for the treatment of functional bowel disorders such as irritable bowel syndrome (IBS) (Costedio et al., 2007). However, the mechanism controlling the release of 5-HT from colonic mucosal EC cells is still not completely understood.

The colon-derived 36-amino-acid peptide, peptide YY (PYY), has long been recognized as an important peptide hormone, which regulates colonic motility, secretion or postprandial satiety by acting on different Y receptors (Sawa et al., 1995; Ferrier et al., 2000; Cox and Tough, 2001; Karra and Batterham, 2010). The colonic PYY is produced and stored in the mucosal L cells from which this peptide is released into the circulation in response to ingestion of food, and postprandial plasma PYY concentrations reach a peak level at 1–2 h and then remain elevated for several hours (Adrian et al., 1985; Batterham et al., 2003). Abnormal levels of PYY in the colonic mucosa have also been demonstrated in patients with IBS (Spiller et al., 2000; Simren et al., 2003), indicating a role of colonic PYY in the regulation of pathophysiological colonic functions. However, little is known about the influence of endogenous PYY on 5-HT release from colonic mucosa.

Our previous in vitro studies in guinea pig colon have demonstrated that the activation of tachykinin NK_2 receptors on the mucosal layer produces a sustained 5-HT release from the colonic mucosal EC cells, indicating a possible role of mucosal NK_2 receptor in the regulation of colonic endocrine cell functions (Kojima et al., 2004; 2005; 2009; 2011). The possibility therefore exists that the activation of mucosal NK_2 receptors also facilitates the release of PYY from the mucosal L cells, thereby affecting 5-HT release from colonic EC cells. Given that colonic mucosal 5-HT may play a crucial role in the regulation of gut function in IBS patients (Miwa et al., 2001; Coates et al., 2004; Cremon et al., 2011), it is important to elucidate the role of endogenous PYY in the regulatory mechanism of 5-HT release from the colonic mucosa in order to understand the pathophysiology of functional bowel disorders such as IBS.

In this study, we tested whether activation of mucosal NK_2 receptors facilitates the release of PYY from colonic mucosa and if so, whether the endogenously released PYY affects the enhancement of 5-HT release caused by NK_2 receptor stimulation, using isolated muscle layer-free mucosal preparations from the guinea pig colon.

Methods

Tissue preparation

All studies involving animals are reported in accordance with the ARRIVE guidelines for reporting experiments involving animals (Kilkenny et al., 2010; McGrath et al., 2010). All procedures were performed in accordance with the Dokkyo University School of Medicine animal care guidelines, which confirm to the Guide for the Care and Use of Laboratory animal (NIH publication no. 85-23, revised 1985). Male

Dunkin–Hartley guinea pigs (250–500 g body weight) were purchased from Shizuoka Laboratory Animal Center, Inc. (Shizuoka, Japan) and Saitama Experimental Animals Supply Co., Ltd. (Saitama, Japan). Guinea pigs were anaesthetized with enflurane and bled via the femoral artery. A segment of the proximal colon, 3–8 cm distal from the caecum, was removed; and the luminal contents were washed out with a modified Tyrode's solution (136.8 mM NaCl, 2.7 mM KCl, 1.8 mM $CaCl_2$, 1.05 mM $MgCl_2$, 0.42 mM NaH_2PO_4, 11.9 mM $NaHCO_3$, 5.56 mM glucose and 0.06 mM $EDTANa_2$). The colon was divided into three segments of circa 1.5 cm and opened longitudinally. Muscle layer (longitudinal/circular muscle layer)-free colonic mucosal preparation consisted of a sheet of submucosa/mucosa, which was obtained by removal of the muscle layer by careful dissection; this preparation is a convenient model as a bioassay for epithelial NK_2 receptor (Kojima et al., 2011). The tissue preparations were suspended in a longitudinal direction under a 4.9 mN load in 2 mL tissue baths filled with modified Tyrode's solution at 37°C and were aerated with 95% O_2/5% CO_2. The tissue preparations were allowed to equilibrate for 80 min with fresh replacement of the bathing medium every 10 min. Following the equilibration period, the experiments were conducted by collecting the bathing medium every 10 min (measurement of 5-HT) or 20 min (measurement of PYY). The medium obtained during the first 80–100 min was discarded. At the end of the collection period, the tissue preparations were blotted and weighed.

Measurement of PYY

The collected medium was lyophilized, dissolved in phosphate buffer (200 μL) containing 25 mM $EDTANa_2$/1% BSA and was centrifuged at 4°C, 10 000× g for 15 min. After centrifugation, the levels of PYY in the supernatants (25–75 μL) were measured in duplicate by a commercially available PYY-EIA kit, according to the manufacturer's instructions (Peptide institute, Inc., Osaka, Japan). The lower limit of detection of the assay is 0.14 ng·mL^{-1}. The intra- and inter-assay coefficients of variation were 4.5% and 7.8% for PYY respectively. The levels of PYY in the incubation medium are expressed as pmol·g^{-1}·20 min^{-1}. The results are expressed as a percentage of the mean outflow observed during the first two collection samples (100–140 min of incubation) of the individual experiments.

Measurement of 5-HT, 5-hydroxyindoleacetic acid (5-HIAA)

The collected medium was lyophilized, dissolved in 0.4 M perchloric acid (200 μL) and passed through a 0.45 μm filter (Dismic-13CP; Advantec, Tokyo, Japan). 5-HT and 5-HIAA in the filtrate were measured by an HPLC with electrochemical detection (ECD-300; Eicom, Tokyo, Japan) and a pen recticoder (SS250F; Sekonic, Tokyo, Japan), as described previously (Kojima et al., 2004). Known concentrations of 5-HT and 5-HIAA (Sigma, St Louis, MO) were used as standards. The separation of 5-HT and 5-HIAA was achieved by a reverse-phase column [length of 100 mm, inner diameter of 4.6 mm, C-18 (3 μm); Shiseido, Tokyo, Japan], using a mobile phase consisting of 0.1 M monochloroacetic acid, 1 mM EDTA, 55 mg·L^{-1} sodium octylsulphate and 5–10% acetonitrile (pH 3.2) at a flow rate of 0.5 mL·min^{-1}. Aliquots (20 μL) of the

filtrate were injected directly into the HPLC column. The limit of detection was between 142 and 283 fmol for 5-HT, between 131 and 261 fmol for 5-HIAA per injection.

Drugs and solutions

The following drugs were used: βAla-NKA-(4-10) (Sigma); GR 159897, BIBO 3304 trifluoroacetate, BIIE 0246 formate (Tocris, Bristol, UK); tetrodotoxin, peptide YY (rat, mouse, porcine, canine) (Phoenix Pharmaceuticals, Inc., Burlingame, CA, USA). All drugs were dissolved in distilled water with the following exceptions: GR 159897 (100 μM) was dissolved in 70% DMSO. All subsequent dilutions of the drugs were made with distilled water. The vehicles had no effects on βAla-NKA-(4-10)-evoked 5-HT or PYY outflow or basal 5-HT or PYY outflow.

Presentation of results and statistical analysis

Data are expressed as the means ± SEM from n experiments. In many cases, n = the number of colonic preparations from different animals. The significance of differences was evaluated by one-way ANOVA followed by Student's unpaired t-test, by the computer programme Prism (GraphPad Software Inc., San Diego, CA, USA), where necessary multiple comparisons were conducted using one-way ANOVA with Dunnett's testing. A value of $P < 0.05$ was considered statistically significant.

Results

Effect of βAla-NKA-(4-10) on PYY outflow

The mean spontaneous outflow of PYY from the muscle layer-free mucosal preparations incubated in modified Tyrode's solution in the absence of test compounds (determined between 100 and 140 min of incubation) amounted to 1.21 ± 0.17 pmol·g⁻¹·20 min⁻¹ (n = 28). In control experiments, the spontaneous outflow of PYY from the muscle layer-free mucosal preparations did not change significantly during the period of observation up to 200 min (Figure 1). Addition of the tachykinin NK$_2$ receptor-selective agonist, βAla-NKA-(4-10) to the incubation medium (100 nM, the maximally effective concentration, from 140 to 160 min of incubation) produced a gradual increase in the outflow of PYY (n = 7, PYY outflow was enhanced to 184.1 ± 18.3% at 160 min, compared with the initial outflow, $P < 0.01$). Thereafter, PYY outflow remain elevated until the end of incubation period up to 200 min despite the absence of βAla-NKA-(4-10) (Figure 1). The βAla-NKA-(4-10)-evoked sustained PYY outflow was completely inhibited by the presence of a tachykinin NK$_2$ receptor-selective antagonist, GR159897 (100 nM, from the onset of incubation, n = 6) (Figure 1), but GR159897 alone (100 nM, from 140 to 200 min of incubation, n = 5) did not significantly affect the basal outflow of PYY (data not shown). The enhancing effect of βAla-NKA-(4-10) (10–1000 nM) on the PYY outflow showed a bell-shaped concentration–response relationship with the maximum effect at 100 nM (Figure 2). Tetrodotoxin (TTX, 1 μM, from the onset of incubation, n = 6) did not modify significantly the βAla-NKA-(4-10)-evoked PYY outflow (between 140 and 180 min of incubation), but the following late phase (between 180 and 200 min of incubation) of PYY outflow was markedly attenuated by the presence of TTX (Figure 1).
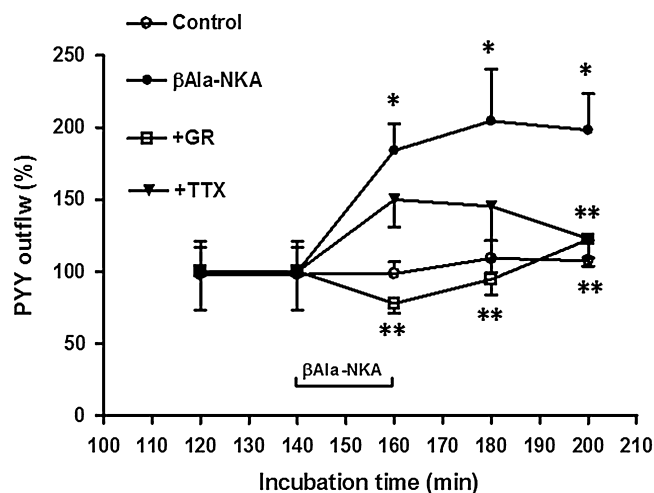

Figure 1

Effect of 100 nM βAla-NKA-(4-10), in the absence and presence of the NK$_2$ receptor antagonist, GR 159897 (+GR, 100 nM) or tetrodotoxin (+TTX, 1 μM) on the outflow of PYY from muscle layer-free mucosal preparations of guinea pig colon. βAla-NKA-(4-10) was present from 140 to 160 min of incubation, as indicated by the horizontal bar. Control results represent the spontaneous PYY outflow in the absence of any test compounds. Ordinates: outflow of PYY, expressed as % of the mean outflow of first two collections (100–140 min of incubation). Each point represents the means ± SEM from five to seven experiments. Abscissa: time after onset of collection of the incubation medium. *$P < 0.01$, significantly different from the paired control; **$P < 0.01$, significantly different from the βAla-NKA alone.
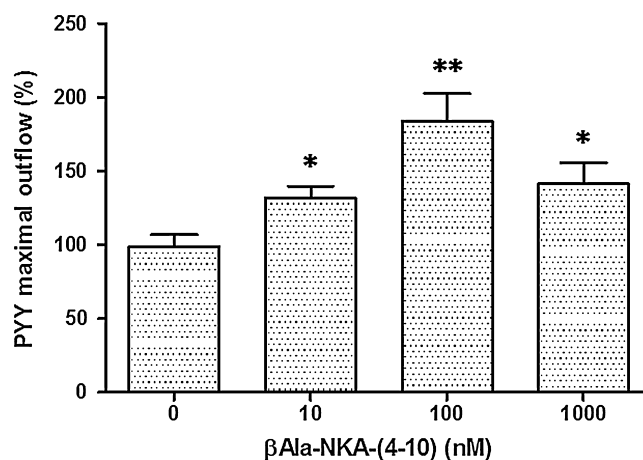

Figure 2

Effect of increasing concentrations of βAla-NKA-(4-10) on the outflow of PYY from the muscle layer-free mucosal preparations. Height of columns: βAla-NKA-(4-10)-evoked PYY maximal outflow (at 160 min of incubation), expressed as % of the mean outflow of first two collections (100–140 min). Results are the means ± SEM from seven experiments. *$P < 0.05$; **$P < 0.01$, significantly different from the control [the absence of βAla-NKA-(4-10)].
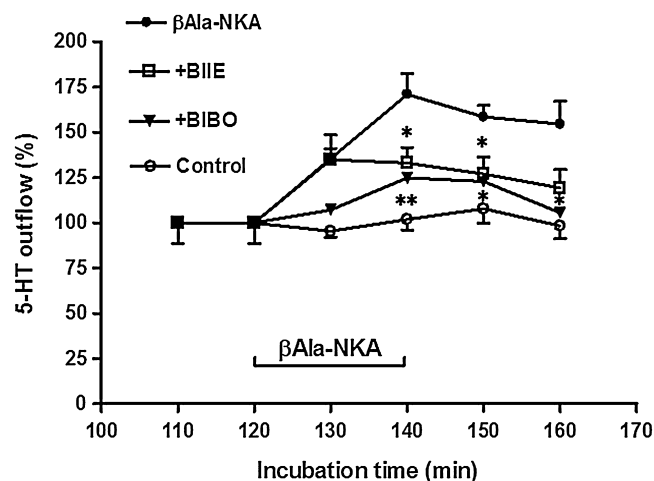
Figure 3

Effect of 100 nM βAla-NKA-(4-10), in the absence and presence of BIBO3304 (+BIBO, 100 nM) or BIIE0246 (+BIIE, 1 μM) on the outflow of 5-HT from the muscle layer-free mucosal preparations. βAla-NKA-(4-10) was present from 120 to 140 min of incubation, as indicated by horizontal bar. Control results represent the spontaneous 5-HT outflow in the absence of any test compounds. Ordinates: outflow of 5-HT, expressed as % of the mean outflow of first two collections (100–120 min). Each point represents the means $\pm$ SEM from seven to eight experiments. $*P < 0.05$; $**P < 0.01$, significantly different from the paired control.

Effect of βAla-NKA-(4-10) on 5-HT outflow

The mean spontaneous outflow of 5-HT and 5-HIAA from the muscle layer-free mucosal preparations incubated in modified Tyrode's solution in the absence of test compounds (determined between 100 and 120 min of incubation) amounted to 98.6 ± 14.1 and 262.9 ± 20.7 pmol·g^{-1}·10 min^{-1} respectively ($n = 16$). In control experiments, the spontaneous outflow of 5-HT from the muscle layer-free mucosal preparations did not change significantly during the period of observation up to 160 min (Figure 3). Likewise, the spontaneous outflow of 5-HIAA from the muscle layer-free preparations also did not change significantly during the period of observation up to 160 min (data not shown). As in previous studies (Kojima *et al.*, 2004; 2005; 2009; 2011), addition of the NK$_2$-agonist, βAla-NKA-(4-10) to the incubation medium (100 nM, from 120 to 140 min of incubation) caused a sustained increased in the outflow of 5-HT ($n = 8$, 5-HT outflow was enhanced to $171.0 \pm 11.3\%$ at 140 min, compared with the initial outflow, $P < 0.01$) and then remained significantly elevated compared with the initial outflow after washout of the NK$_2$ agonist ($P < 0.05$; Figure 3), but did not affect the outflow of 5-HIAA ($106.1 \pm 7.4\%$ at 140 min, $n = 8$, compared with the initial outflow). Similar to previous observations (Kojima *et al.*, 2004), when TTX (1 μM, from the onset of incubation) was present, the enhancing effect of 100 nM βAla-NKA-(4-10) was not significantly affected ($186.4 \pm 23.6\%$ at 140 min, $n = 6$).

Effect of Y$_1$/Y$_2$-atagonists on βAla-NKA-(4-10)-evoked 5-HT outflow

Y$_1$ receptor antagonist, BIBO3304, and Y$_2$ receptor antagonist, BIIE0246, were tested against the enhancing effect of βAla-
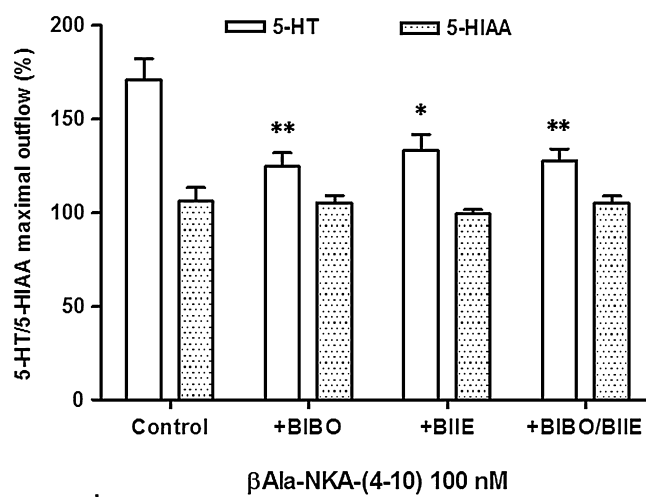

Figure 4

Effects of BIBO3304 (100 nM), BIIE0246 (1 μM), the combined application of BIBO3304 and BIIE0246 on the maximal outflow of 5-HT and 5-HIAA from the muscle layer-free mucosal preparations, evoked by 100 nM βAla-NKA-(4-10). Height of columns: βAla-NKA-(4-10)-evoked 5-HT/5-HIAA maximal outflow (at 140 min of incubation), expressed as % of the mean outflow of first two collections (100–120 min). Results are the means $\pm$ SEM from seven to eight experiments. $*P < 0.05$; $**P < 0.01$, significantly different from the control.

NKA-(4-10). Neither BIBO3304 (100 nM) nor BIIE0246 (1 μM) affected the basal outflow of 5-HT or 5-HIAA (data not shown). As shown in Figure 3, in the presence of BIBO3304 (100 nM, from the onset of incubation) or BIIE0246 (1 μM, from the onset of incubation), the βAla-NKA-(4-10)-evoked sustained 5-HT outflow was partially reduced by 65% (at 140 min, $P < 0.01$) and 53% (at 140 min, $P < 0.05$) respectively. The combined application of BIBO3304 (100 nM) and BIIE0246 (1 μM) showed no additive inhibitory effect (Figure 4). Neither BIBO3304 (100 nM) nor BIIE0246 (1 μM) affected the outflow of 5-HIAA in the presence of βAla-NKA-(4-10) (Figure 4).

Effect of PYY on 5-HT outflow

Next, we examined whether exogenously applied PYY mimics the enhancing effect of βAla-NKA-(4-10). As shown in Figure 5, exogenous PYY (10 nM, from 120 to 140 min of incubation) caused a sustained increase in the outflow of 5-HT ($n = 8$, 5-HT outflow was enhanced to $146.1 \pm 9.9\%$ at 140 min, compared with the initial outflow, $P < 0.01$). Similar to that of βAla-NKA-(4-10), PYY-evoked 5-HT outflow remain elevated until the end of incubation period up to 160 min despite the absence of PYY (Figure 5). The enhancing effect of PYY (1–100 nM) was concentration-dependent with the maximum effect at 10 nM, but PYY did not affect the outflow of 5-HIAA (Figure 6). In the presence of BIBO3304 (100 nM, from the onset of incubation), the PYY-evoked maximal 5-HT outflow was reduced by 81% ($P < 0.01$) (Figure 7). BIIE0246 (1 μM, from the onset of incubation) showed a tendency to reduce the PYY-evoked maximal 5-HT outflow, but these changes did not reach statistical significance (Figure 7). The
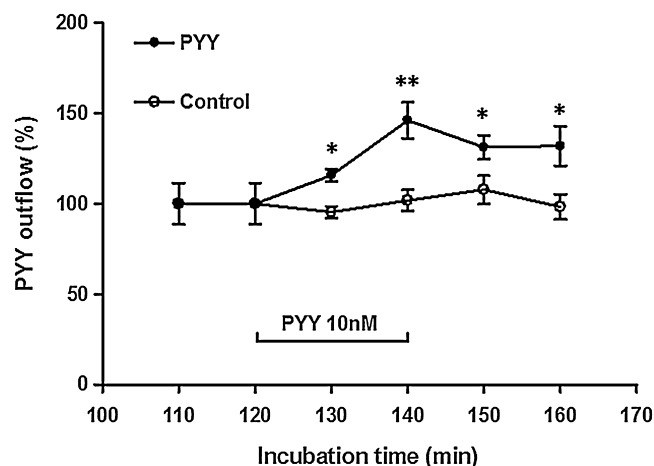
Figure 5

Effect of 10 nM PYY on the outflow of 5-HT from the muscle layer-free mucosal preparations. PYY was present from 120 to 140 min of incubation, as indicated by horizontal bar. Ordinates: outflow of 5-HT, expressed as % of mean outflow of first two collections (100–120 min of incubation). Each point represents the means ± SEM of eight experiments. *$P < 0.05$; **$P < 0.01$, significantly different from the paired control.
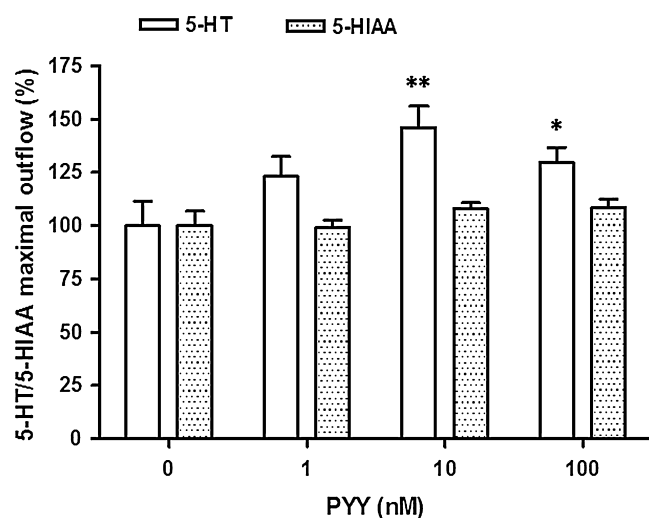

Figure 6

Effect of PYY (1–100 nM) on the outflow of 5-HT/5-HIAA from the muscle layer-free mucosal preparations. Height of columns: maximal outflow of 5-HT/5-HIAA (at 140 min of incubation), expressed as % of the mean outflow of first two collections (100–120 min). Results are the means ± SEM of six to eight experiments. *$P < 0.05$; **$P < 0.01$, significantly different from the control (the absence of PYY).

combined application of BIBO3304 (100 nM) and BIIE0246 (1 μM) showed no additive inhibitory effect (Figure 7).

Discussion and conclusion

The present study first deals with the question of whether activation of tachykinin NK_2 receptors on the mucosal layer
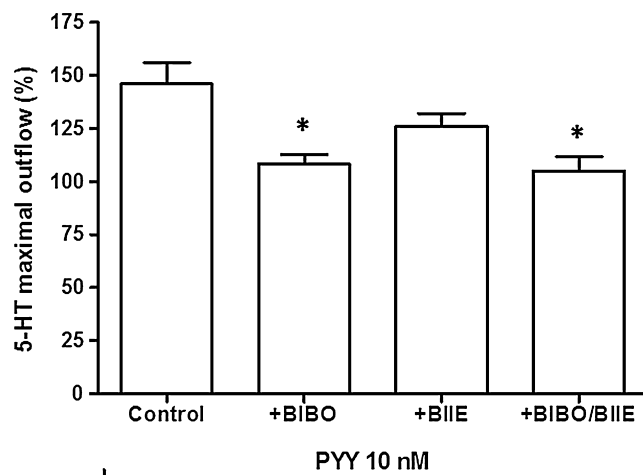
Figure 7

Effects of BIBO3304 (100 nM), BIIE0246 (1 μM), and the combined application of BIBO3304 and BIIE0246 on the maximal outflow of 5-HT from the muscle layer-free mucosal preparations, evoked by 10 nM PYY. Height of columns: PYY-evoked 5-HT maximal outflow (at 140 min of incubation), expressed as % of the mean outflow of first two collections (100–120 min). Results are the means ± SEM from six to eight experiments. *$P < 0.01$, significantly different from the control.

affects the outflow of PYY from guinea pig colonic mucosa. The muscle layer-free mucosal preparation of the guinea pig proximal colon is a useful *in vitro* preparation as a bioassay for mucosal NK_2 receptors (Kojima *et al*., 2011). In our initial experiments, we showed for the first time that the outflow of PYY from the muscle layer-free mucosal preparations was markedly increased in a sustained manner, in the presence of the NK_2 receptor-selective agonist, βAla-NKA-(4-10), suggesting that βAla-NKA-(4-10) produces a long-lasting PYY release from mucosal store sites. The L cells have commonly been addressed as a candidate for the PYY storage sites in the colonic mucosa; PYY-immunoreactive endocrine cells are demonstrated in the colonic mucosa of rat, guinea pig and human (Onaga *et al*., 2002). Thus, the mucosal PYY storage sites are most likely to be within the L cells. Since the enhancing effect of βAla-NKA-(4-10) was completely inhibited by the NK_2 receptor-selective antagonist, GR159897, we suggest that the βAla-NKA-(4-10)-evoked long-lasting PYY release is mediated by activation of NK_2 receptors. Moreover, the βAla-NKA-(4-10)-evoked long-lasting PYY outflow remained unaffected by the presence of TTX, supporting the hypothesis that the βAla-NKA-(4-10)-evoked PYY release is mediated via NK_2 receptors located on L cells. This concept is further corroborated by the observation that in the guinea pig proximal, tachykinin NK_2 receptor immunoreactivity is predominantly found on the surfaces of enterocytes at the bases of crypts (Portbury *et al*., 1996). Consistent with this observation, a similar pattern of PYY release has been observed previously in isolated, vascularly perfused rat colon; a neuropeptide bombesin produced a TTX-resistant sustained increase in the portal PYY level from this tissue (Plaisancie *et al*., 1995). Unexpectedly, the following late phase of PYY outflow (between 180 and 200 min of incubation) was sensitive to

TTX, suggesting that the presence of neuronal input to the L cells is required to maintain the long-lasting PYY release. Indeed, it has been shown that in healthy humans, postprandial PYY release from gut is controlled by the cholinergic system (Maier *et al.*, 2008). However, since the late phase of PYY outflow persisted following pretreatment with βAla-NKA-(4-10), the involvement of neuronal NK_2 receptors is doubtful. In our previous *in vitro* study in the guinea pig colon, we demonstrated that endogenous tachykinins play a messenger role at the interface between the enteric nervous system and the mucosal endocrine cells (Kojima *et al.*, 2004). Thus, our findings are congruent with the idea that neuronal tachykinins acting through the mucosal NK_2 receptors play a role in controlling the long-lasting release of PYY from colonic mucosal L cells. PYY is an anorectic gut hormone; circulating levels of PYY are low in the fasted state but are high in the fed state, and postprandial plasma PYY concentrations remain elevated for several hours (Adrian *et al.*, 1985; Batterham *et al.*, 2003). Since tachykinin-containing neurons are found in the submucosal layer of guinea pig intestine (Holzer, 1998), whether the tachykinin-containing neurons participate in the mechanisms triggering the food-induced long-lasting PYY release, remain an open question that requires further detailed work.

Our previous *in vitro* studies have repeatedly indicated that the NK_2 receptor agonist, βAla-NKA-(4-10) is capable of inducing a sustained 5-HT release from EC cells of the guinea pig colonic mucosa via NK_2 receptors on the mucosal layer (Kojima *et al.*, 2004; 2005; 2009; 2011). However, the underlying mechanism of the NK_2 receptor-triggered sustained 5-HT release is still not completely understood. On the other hand, it has been shown that in human isolated colonic mucosal preparations, PYY and related peptides (neuropeptide Y and pancreatic polypeptide) exert their effects through three different Y receptor subtypes (Y_1, Y_2 and Y_4 receptors, nomenclature follows Alexander *et al.*, 2011), and PYY can co-stimulate Y_1 and Y_2 receptors at nanomolar concentrations (Cox and Tough, 2001; Tough *et al.*, 2011). We, therefore, examined the effects of the Y_1 receptor antagonist, BIBO3304 (Wieland *et al.*, 1998), and the Y_2 receptor antagonist, BIIE0246 (Cox and Tough, 2001), to obtain a better understanding of the underlying mechanism of the NK_2 receptor-triggered sustained 5-HT release. As the second main result of the present study, we found that the Y_1 receptor antagonist, BIBO3304, and the Y_2 receptor antagonist, BIIE0246, with less potency, produced a sustained decline in the βAla-NKA-(4-10)-evoked sustained 5-HT outflow without affecting the outflow of 5-HT's metabolite 5-HIAA; however, the combined application of Y_1 and Y_2 antagonists did not completely abolish the βAla-NKA-(4-10)-evoked 5-HT outflow. Thus, these results support the hypothesis that the NK_2 receptor-triggered sustained 5-HT release is in part mediated by endogenously released PYY, acting via Y_1 and slightly by Y_2 receptors, without affecting the 5-HT degradation processes. This concept is further corroborated by the third present finding that exogenous applied PYY produces a sustained 5-HT release with a time course similar to that found for βAla-NKA-(4-10)-evoked 5-HT release, but failed to affect the outflow of 5-HIAA. Moreover, the enhancing effect of PYY was inhibited by Y_1 blockade, but not Y_2 blockade, suggesting that the PYY-evoked 5-HT outflow is mediated mainly via Y_1 receptors on EC cells. This may also support the idea that the

Y_2 receptor-mediated minor inhibition of βAla-NKA-(4-10)-evoked 5-HT release is due to the subsequent release of other Y_2 receptor agonists (e.g. PYY3-36). Also, the possibility that Y_1 and Y_2 receptors-mediated facilitation of basal 5-HT release is evoked via endogenously released PYY is unlikely, because neither the Y_1 receptor antagonist, BIBO3304 nor the Y_2 receptor antagonist, BIIE0246 affected the basal 5-HT release. Taken together, these results indicate that under the conditions used in the present study, the NK_2 receptor-triggered sustained 5-HT release is in part mediated by the activation in the cascade of NK_2 receptors located on L cells and mainly Y_1 receptors located on EC cells. These findings are also congruent with the idea that the PYY-containing L cells play a role in controlling the release of 5-HT from colonic EC cells; PYY appears to exert a significant paracrine effect on colonic EC cells.

It has previously been shown that diarrhoea-predominant IBS (d-IBS) subjects exhibit higher postprandial plasma 5-HT concentrations compared with healthy subjects under fed conditions (Bearcroft *et al.*, 1998; Houghton *et al.*, 2003), indicating an abnormality in EC cell release of 5-HT to food intake in d-IBS. Given that food intake is a good stimulus for increased PYY release from colonic L cells (Batterham *et al.*, 2003; Neary and Batterham, 2009) and that colonic mucosal 5-HT may play an important role in the regulation of gut function in IBS patients (Miwa *et al.*, 2001; Coates *et al.*, 2004; Cremon *et al.*, 2011), our observation that NK_2 receptor stimulation facilitates the release of endogenous PYY, which in turn enhances 5-HT release via mainly Y_1 receptors on EC cells, may be important from a clinical perspective, as an excessive increase in plasma 5-HT after a meal appears to be related to expression of postprandial symptoms in d-IBS (Bearcroft *et al.*, 1998; Houghton *et al.*, 2003).

In conclusion, our findings support the view that the tachykinin NK_2 receptor-selective agonist, βAla-NKA-(4-10) can produce a long-lasting PYY release from guinea pig colonic mucosa via NK_2 receptors located on L cells and that βAla-NKA-(4-10)-evoked 5-HT release is in part mediated by endogenously released PYY, acting via Y_1 receptors located on EC cells. Thus, PYY-containing L cells appear to play a role in controlling the release of 5-HT from colonic EC cells. There is an abundance of evidence, which suggests that the blockade of peripheral tachykinin NK_2 receptors contributes to the treatment of IBS (Lecci *et al.*, 2004). Therefore, the tachykinin NK_2 receptor-triggered PYY release from colonic L cells might be regarded as an interesting target for the treatment of postprandial symptoms in functional bowel disorders such as IBS.

Acknowledgements

Conflict of interest

None.

References

Adrian TE, Ferri GL, Bacarese- Hamilton AJ, Fuessl HS, Polak JM, Bloom SR (1985). Human distribution and release of a putative new gut hormone, peptide YY. Gastroenterology 89: 1070–1077.

Alexander SPH, Mathie A, Peters JA (2011). Guide to receptors and channels, 5th edn. Br J Pharmacol 164 (Suppl. 1): S85.

Batterham RL, Cohen MA, Ellis SM, Le Roux CW, Withers DJ, Frost GS et al. (2003). Inhibition of food intake in obese subjects by peptide YY3-36. N Engl J Med 349: 941–948.

Bearcroft CP, Perrett D, Farthing MJG (1998). Postprandial plasma 5-hydroxytryptamine in diarrhea predominant irritable bowel syndrome: a pilot study. Gut 42: 42–46.

Camilleri M (2002). Serotonergic modulation of visceral sensation: lower gut. Gut 51 (Suppl. 1): i81–i86.

Coates MD, Mahoney CR, Linden DR, Sampson JE, Chen J, Blaszyk H et al. (2004). Molecular defects in mucosal serotonin content and decreased serotonin reuptake transporter in ulcerative colitis and irritable bowel syndrome. Gastroenterology 126: 1657–1664.

Costedio MM, Hyman N, Mawe GM (2007). Serotonin and its role in colonic function and in gastrointestinal disorders. Dis Colon Rectum 50: 376–388.

Cox HM, Tough IR (2001). Neuropeptide Y, Y_1, Y_2 and Y_4 receptors mediate Y agonist responses in isolated human colon mucosa. Br J Pharmacol 135: 1505–1512.

Cremon C, Carini G, Wang B, Vasina V, Cogliandro RF, De Giorgio R et al. (2011). Intestinal serotonin release, sensory neuron activation, and abdominal pain in irritable bowel syndrome. Am J Gastroenterol 106: 1290–1298.

Ferrier L, Segain JP, Pacaud P, Cherbut C, Loirand G, Galmiche JP et al. (2000). Pathways and receptors involved in peptide YY induced contraction of rat proximal colonic muscle in vitro. Gut 46: 370–375.

Gershon MD, Tack J (2007). The serotonin signaling system: from basic understanding to drug development for functional GI disorders. Gastroenterology 132: 397–414.

Holzer P (1998). Implications of tachykinins and calcitonin gene-related peptide in inflammatory bowel disease. Digestion 59: 269–283.

Houghton LA, Atkinson W, Whitaker RP, Whorwell PJ, Rimmer MJ (2003). Increased platelet depleted plasma 5-hydroxytryptamine concentration following meal ingestion in symptomatic female subjects with diarrhea predominant irritable bowel syndrome. Gut 52: 663–670.

Karra E, Batterham RL (2010). The role of gut hormones in the regulation of body weight and energy homeostasis. Mol Cell Endocrinol 316: 120–128.

Kilkenny C, Browne W, Cuthill IC, Emerson M, Altman DG (2010). NC3Rs Reporting Guidelines Working Group. Br J Pharmacol 160: 1577–1579.

Kojima S, Ueda S, Ikeda M, Kamikawa Y (2004). Calcitonin gene-related peptide facilitates serotonin release from guinea-pig colonic mucosa via myenteric neurons and tachykinin NK_2/NK_3 receptors. Br J Pharmacol 141: 385–390.

Kojima S, Ikeda M, Kamikawa Y (2005). Loperamide inhibits tachykinin NK_3 receptor-triggered serotonin release without affecting NK_2-receptor-triggered serotonin release from guinea-pig colonic mucosa. J Pharmacol Sci 98: 175–180.

Kojima S, Ikeda M, Kamikawa Y (2009). Further investigation into the mechanism of tachykinin NK_2 receptor-triggered serotonin release from guinea-pig proximal colon. J Pharmacol Sci 110: 122–126.

Kojima S, Tohei A, Ikeda M (2011). Melatonin inhibits tachykinin NK_2 receptor- triggered 5-HT release from guinea-pig isolated colonic mucosa. Br J Pharmacol 162: 1179–1185.

Lecci A, Capriati A, Maggi CA (2004). Tachykinin NK2 receptor antagonists for the treatment of irritable bowel syndrome. Br J Pharmacol 141: 1249–1263.

Maier C, Riedl M, Vila G, Nowotny P, Wolzt M, Clodi M et al. (2008). Cholinergic regulation of ghrelin and peptide YY release may be impaired in obesity. Diabetes 57: 2332–2340.

McGrath J, Drummond G, Kilkenny C, Wainwright C (2010). Guidelines for reporting experiments involving animals: the ARRIVE guidelines. Br J Pharmacol 160: 1573–1576.

Miwa J, Echizen H, Matsueda K, Umeda N (2001). Patients with constipation-predominant irritable bowel syndrome. Digestion 63: 188–194.

Neary MT, Batterham RL (2009). Peptide YY: food for thought. Physiol Behav 97: 616–619.

Onaga T, Zabielski R, Kato S (2002). Multiple regulation of peptide YY secretion in the digestive tract. Peptides 23: 279–290.

Plaisancie P, Bernard C, Chayvialle JA, Cuber JC (1995). Release of peptide YY by neurotransmitters and gut hormones in the isolated, vascularly perfused rat colon. Scand J Gastroenterol 30: 568–574.

Portbury AL, Furness JB, Southwell BR, Wong H, Walsh JH, Bunnett NW (1996). Distribution of neurokinin-2 receptors in the guinea-pig gastrointestinal tract. Cell Tissue Res 286: 281–292.

Racke K, Schworer H (1991). Regulation of serotonin release from the intestinal mucosa. Pharmacol Res 23: 13–25.

Sawa T, Mameya S, Yoshimura M, Itsuno M, Makiyama K, Niwa M et al. (1995). Differential mechanism of peptide YY and neuropeptide Y in inhibiting motility of guinea-pig colon. Eur J Pharmacol 276: 223–230.

Simren M, Stotzer PO, Sjovall H, Abrahamsson H, Bjornsson ES (2003). Abnormal levels of neuropeptide Y and peptide YY in the colon in irritable bowel syndrome. Eur J Gastroenterol Hepatol 15: 55–62.

Spiller RC, Jenkins D, Thornley JP, Hebden JM, Wright T, Skinner M et al. (2000). Increased rectal mucosal enteroendocrine cells, T lymphocytes, and increased gut permeability following acte Campylobacter enteritis and in post-dysenteric irritable bowel sundorome. Gut 47: 804–811.

Tough IR, Forbes S, Tolhurst R, Ellis M, Herzog H, Bornstein JC et al. (2011). Endogenous peptide YY and neuropeptide Y inhibit colonic ion transport, contractility and transit differentially via Y_1 and Y_2 receptors. Br J Pharmacol 164: 471–484.

Wieland HA, Engel W, Eberlein W, Rudolf K, Doods HN (1998). Subtype selectivity of the novel nonpeptide neuropeptide Y Y_1 receptor antagonist BIBO3304 and its effect on feeding in rodents. Br J Pharmacol 125: 549–555.