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Original

5-HT_{2B} Receptors-triggered Serotonin Release from Guinea-pig Isolated Colonic Mucosa : a Role of Endogenous Peptide YY

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SUMMARY

Purpose : The effect of 5-HT_{2B} receptor-selective agonist BW723C86 was investigated on the outflow of 5-hydroxytryptamine (5-HT) from isolated muscle layer-free mucosal preparations of guinea-pig colon.

Methods : The mucosal preparations were incubated in vitro and the outflow of 5-HT from these preparations was determined by high-performance liquid chromatography with electrochemical detection.

Results : BW723C86 (1-10 μM) produced a sustained increase in the outflow of 5-HT from the mucosal preparations. The BW723C86-evoked 5-HT outflow was inhibited by the two different 5-HT_{2B} receptor antagonists, olanzapine (100 nM) and RS127445 (100 nM). The neuropeptide Y_1 receptor antagonist BIBO3304 (300 nM) markedly inhibited the BW723C86-evoked 5-HT outflow.

Conclusion : We found that 5-HT_{2B} receptor-triggered 5-HT release from guinea-pig colonic mucosa is mediated by the activation of 5-HT_{2B} receptors located at endocrine cells and that the 5-HT_{2B} receptor-triggered 5-HT release is mediated by endogenously released peptide YY, acting via Y_1 receptors. Given that both 5-HT and peptide YY are well-known mediators of nausea and vomiting, the 5-HT_{2B} receptors located at endocrine cells may play a role in the generation of nausea and vomiting.

Keywords : BW723C86, guinea-pig colon, 5-HT_{2B} receptor, 5-HT release, neuropeptide Y_1 receptor, peptide YY

INTRODUCTION

5-hydroxytryptamine (serotonin, 5-HT) released from enterochromaffin (EC) cells of colonic mucosa, plays important roles in motility, nausea, vomiting and pain sensation[1~3]. The 5-HT release from EC cells has been also reported to be increased in patient with irritable bowel syndrome[4]. Therefore, alterations in the release of 5-HT from EC cells might affect pathophysiological colonic functions. However, the endogenous modulator system controlling the release of 5-HT from the EC cells is not yet well elucidated.

Our previous *in vitro* studies have demonstrated that isolated muscle layer-free mucosal preparation of guinea-pig colon is a helpful *in vitro*-preparation for studying the mechanism modulating the release of 5-HT from EC cells[5~8]. Using the *in vitro*-preparation, we have indicated that an incretin hormone peptide YY (PYY) plays a role in controlling the release of 5-HT from EC cells *via* neuropeptide Y_1 receptors located on the colonic mucosa[9]. We have further demonstrated the role of Y_1 receptors in serotonin

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5-HT_3 receptors-triggered 5-HT release from guinea-pig colonic mucosa[10].

Several lines of experimental evidence suggest that serotonin 5-HT_{2B} receptors play a key role in regulation of colonic motility[11~12]. As it has been also shown that 5-HT_{2B} receptors contribute to the induction of visceral hypersensitivity[13], 5-HT_{2B} receptors may be regarded as an important target for the treatment of abdominal pain. However, there is still little information about the functional role of 5-HT_{2B} receptors in modulating the release of 5-HT from EC cells. We therefore designed the present study to determine the influence of 5-HT_{2B} receptor selective agonist, BW723C86[14] and two different 5-HT_{2B} receptor antagonists (olanzapine and RS127445) on spontaneous 5-HT release from EC cells, using the isolated muscle layer-free mucosal preparations of guinea-pig colon.

METHODS

Animals and tissue preparation

Male Hartley guinea-pigs (Shizuoka Laboratory Animal Center, Inc, Shizuoka, Japan) aged 4-9 weeks were used in this study. They were housed under controlled temperature and lighting (light on from 7 : 00 to 19 : 00 h) conditions and were provided with food and tap water *ad libitum*. Four to seven guinea-pigs were used in each experiment. All procedures were performed in accordance with the Dokkyo Medical 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). On the day of the experiment, guinea-pigs were anesthetized by inhalation of enflurane (1 ml/1 ml, 20 min) 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 two segments (2.2 cm in length) 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 the release of 5-HT from mucosal EC cells[5,6]. 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℃ and were aerated with 95% O_2/5% CO_2. The muscle layer-free mucosal preparations were allowed to equilibrate for 80 min with fresh replacement of the bathing medium (1 ml) every 10 min. Four to seven mucosal preparations were used in each experiment. Following the equilibration period, the experiments were conducted by collecting the bathing medium (1 ml) every 10 min. 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 5-HT, 5-hydroxyindoleacetic acid (5-HIAA)

The collected medium was passed through a 0.45-μm filter (Dismic-13CP ; Advantec, Tokyo, Japan). 5-HT and 5-HIAA in the filtrate were measured by a high-performance liquid chromatography (HPLC) with electrochemical detection (ECD-300 ; Eicom, Tokyo, Japan), as described previously[5,6]. Known concentrations of 5-HT and 5-HIAA (Sigma, St Louis, MO, USA) were used as standards. The separation of 5-HT and 5-HIAA was achieved by a reverse-phase column [length of 150 mm, inner diameter of 4.6 mm, C-18 (3 μm) ; Gl Sciences Inc., Tokyo, Japan], using a mobile phase consisting of 0.1 M monochloroacetic acid, 1 mM EDTA, 55 mg/l sodium octylsulphate and 12 % acetonitrile (pH 3.2) at a flow rate of 0.5 ml/min. Aliquots (30 μl) of the filtrate were injected directly into the HPLC column. 5-HT outflow is expressed as a percentage of the mean outflow observed during the first two collections (110-120 min of incubation).

Drugs and solutions

The following drugs were used : BIBO 3304 trifluoroacetate, BW723C86 hydrochloride, RS127445 hydrochloride (Tocris, Bristol, UK) ; olanzapine hydrochloride (Eli Lilly, Japan). All drugs were dissolved in distilled water and all subsequent dilutions of the drugs were made with distilled water. The vehicles had no effects on BW723C86-evoked 5-HT outflow or

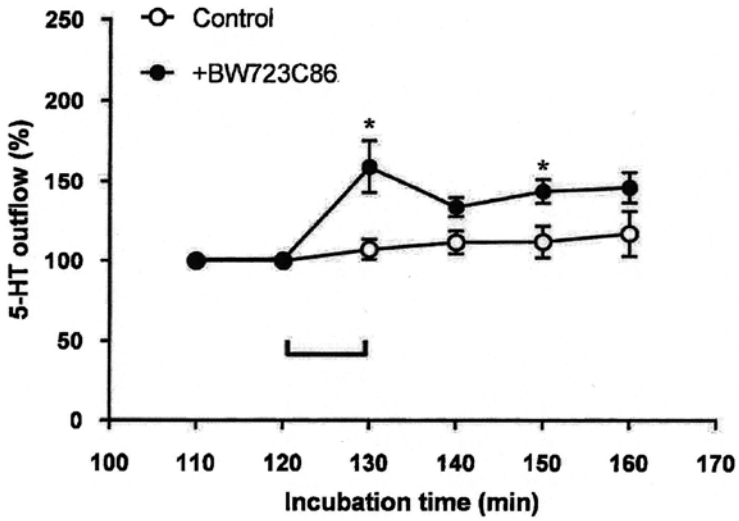

Fig. 1

Effect of 1 μM BW723C86 on the outflow of 5-HT from muscle layer-free mucosal preparations of guinea-pig colon. BW723C86 was present from 120 to 130 min of incubation, as indicated by the horizontal bar. The open circles (Control, ○) show 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 (at 110-120 min of incubation). Each point represents the means ± S.E.M. (vertical bars) from seven experiments. Abscissa : time after onset of collection of the incubation medium. $^{*}P<0.05$, significantly different from the paired BW723C86 alone.

basal 5-HT outflow.

Presentation of results and statistical analysis

Data are expressed as the means ± S.E.M. from n experiments. In many cases, n = the number of colonic preparations from different animals. The significance of differences was evaluated by one-way analysis of variance (ANOVA) followed by Dunnett's multi-comparison test. A value of $P<0.05$ was considered statistically significant.

RESULTS

Effect of BW723C86

After an equilibration period, 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 110 and 120 min of incubation) amounted 106.6 ± 13.8 and 294.8 ± 27.6 pmol/g tissue/10 min, respectively (n = 28). Similar to previous reports[8,9,10], in control experiments, the outflow of 5-HT from the muscle layer-free mucosal preparations did not change significantly during the period of observation up to 160 min (Fig. 1). Application of a 5-HT$_{2B}$ receptor-selective agonist, BW723C86 to the incubation medium (1 μM, from 120 to 130 min of incubation) produced a sustained increase in the outflow of 5-HT (n = 7, 5-HT outflow was enhanced to 159.2 ± 16.1% at 130 min, compared with initial outflow, $P<0.05$) (Fig. 1). BW723C86 (1 μM) had no significant effect on 5-HIAA outflow from the mucosal preparations (n = 7, 110.6 ± 5.9% at 130 min, compared with initial outflow). The enhancing effect of BW723C86 (0.1, 1 and 10 μM) on the 5-HT outflow showed a bell-shaped concentration-response relationship with the maximum effect at 1 μM (Fig. 2).

Effect of antagonists

Next, we have examined the effect of two different 5-HT$_{2B}$ receptor antagonists (olanzapine and RS127445) on the BW723C86 (1 and 10 μM) -evoked 5-HT outflow. As shown in Fig. 3, olanzapine (+ORZ, 100 nM, from the start of incubation, n = 6, $P<0.05$) produced the significant depression of the maximum in the concentration-response curve to BW723C86. Likewise, RS127445 (+RS127, 100 nM, from the start

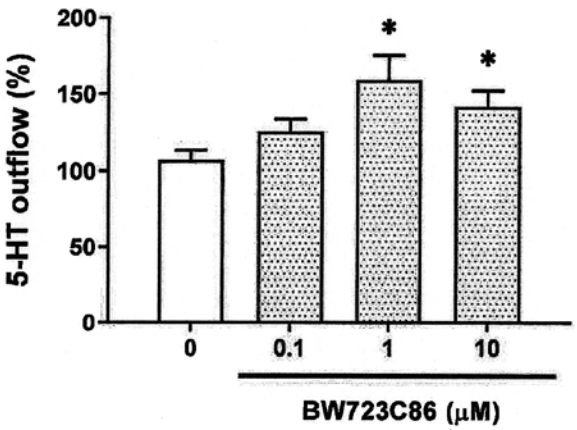

Fig. 2

Effects of increasing concentrations of BW723C86 on the outflow of 5-HT from the muscle layer-free mucosal preparations. Height of columns : BW723C86-evoked 5-HT maximal outflow (at 130 min of incubation), expressed as % of the mean outflow of first two collections (at 110-120 min). Results are the means ± S.E.M. (vertical bars) from seven experiments. $^{*}P<0.05$, significantly different from the control.

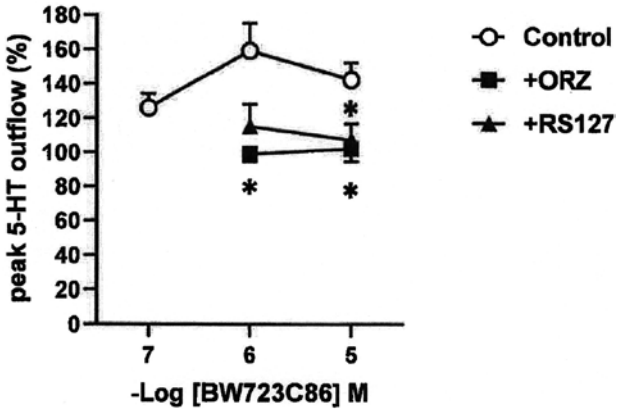

Fig. 3

Effect of increasing concentrations of BW723C86, in the absence (Control, ○) or presence of olanzapine (100 nM, ■, +ORZ) or RS127445 (100 nM, ▲, +RS127) on the outflow of 5-HT from the mucosal preparations. Ordinates : peak outflow of 5-HT (at 130 min of incubation), expressed as % of the mean outflow of first two collections (at 110-120 min). Each point represents the means ± S.E.M. (vertical bars) from four to seven experiments. $^{*}P<0.05$ significantly different from the control preparations given BW723C86 alone.

of incubation, n = 4, $P<0.05$) also produced the significant depression of maximum in the concentration-response curve to BW723C86 (Fig. 3).

To determine the role of Y_1 receptor in the BW723C86-evoked 5-HT outflow, we further tested the effect of Y_1 receptor antagonist, BIBO3304. The enhancing effect of BW723C86 (1 and 10 μM) was markedly depressed by BIBO3304 (+ BIBO, 300 nM, from the start of incubation, n = 6) (Fig. 4).

Neither olanzapine (100 nM) nor RS127445 (100 nM) nor BIBO3304 (300 nM) significantly altered the basal 5-HT and 5-HIAA outflow (data not shown).

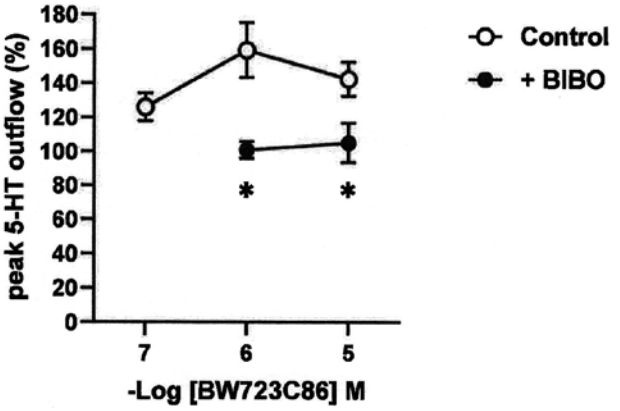

Fig. 4

Effects of increasing concentrations of BW723C86, in the absence (Control, ○) or presence of BIBO3304 (300 nM, ●, +BIBO) on the outflow of 5-HT from the mucosal preparations. Ordinates : peak outflow of 5-HT (at 130 min of incubation), expressed as % of the mean outflow of first two collections (at 110-120 min). Each point represents the means ± S.E.M. (vertical bars) from six to seven experiments. $^{*}P<0.05$ significantly different from the control preparations.

DISCUSSION

The isolated guinea-pig colonic mucosal preparation is a useful preparation to study the release of 5-HT from mucosal EC cells[5~8]. Using this preparation, we have first examined the effect of the 5-HT_{2B} receptor-selective agonist BW723C86[14] on the outflow of 5-HT from the mucosal preparations. In the present study, BW723C86 produced a sustained increase in the outflow of 5-HT without affecting the outflow of 5-HT's metabolite 5-HIAA. This suggests that BW723C86 produces a long-lasting 5-HT release from the mucosal EC cells, without affecting the 5-HT degradation.

Since the BW723C86-evoked sustained 5-HT release was inhibited by the 5-HT_{2B} receptor-selective antagonist (olanzapine and RS127445[13,15]), we suggest that the BW723C86-evoked sustained 5-HT release is mediated *via* the activation of 5-HT_{2B} receptors located on the colonic mucosa.

Our previous in vitro studies in the guinea-pig colon have demonstrated that endogenously formed, an incretin hormone peptide YY (PYY) facilitates the release of 5-HT from the muscle layer-free mucosal preparation, acting on neuropeptide Y_1 receptors on EC cells[9]. We have also demonstrated that 5-HT_3 receptor-triggered 5-HT release from the mucosal preparation is in part mediated by endogenously released PYY, acting *via* Y_1 receptor[10]. We have therefore examined the effect of Y_1 receptor antagonist in order to gain information on the role for endogenous PYY in the BW723C86-evoked 5-HT outflow. In the present experiments, the Y_1 receptor antagonist BIBO3304 markedly inhibited the BW723C86-evoked 5-HT outflow, suggesting that the 5-HT_{2B} receptor-triggered 5-HT release is mediated by endogenously released PYY, acting *via* Y_1 receptors. Accordingly, our data suggest that 5-HT_{2B} receptors located at the colonic mucosa play a role in paracrine signaling between EC cells and PYY-containing endocrine cells.

Both 5-HT and PYY are well-known mediators of nausea and vomiting[1,16], and further the efficacy of olanzapine for prevention of nausea and vomiting in patients receiving highly emetogenic chemotherapy has been demonstrated[17]. Therefore, as a possible therapeutic target in nausea and emesis, both 5-HT_{2B} receptors and Y_1 receptors may become a center of attention.

CONCLUSION

We have found in this study that the 5-HT_{2B} receptor agonist BW723C86 produces a long-lasting 5-HT release from guinea-pig colonic mucosa *via* 5-HT_{2B} receptors located at the mucosal endocrine cells and that the activation of Y_1 receptors is required to maintain the 5-HT_{2B} receptor-triggered 5-HT release. The 5-HT_{2B} receptors located at mucosal endocrine cells may play a role in the generation of nausea and vomiting.

Declaration of Interest

The authors report no conflicts of interest.

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