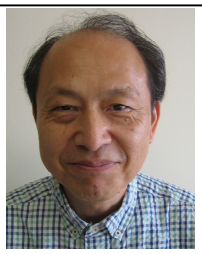
Send Orders for Reprints to reprints@benthamscience.ae

830 *Current Neuropharmacology,* **2015**, *13*, **830-835**

An Endogenous Tachykinergic NK_2/NK_3 Receptor Cascade System Controlling the Release of Serotonin from Colonic Mucosa

Shu-ichi Kojima[1,*], Atsushi Tohei[2], Masashi Ikeda[3] and Naohiko Anzai[1]

[1]*Department of Pharmacology and Toxicology, Dokkyo Medical University School of Medicine, Mibu, Tochigi 321-0293, Japan;* [2]*Laboratory of Experimental Animal Science Nippon Veterinary and Life Science University, Musashino, Tokyo 180-8602, Japan;* [3]*Institute of International Education and Research, Dokkyo Medical University School of Medicine, Mibu, Tochigi 321-0293, Japan*



S.I. Kojima

Abstract: 5-Hydroxytryptamine (5-HT) released from colonic mucosal enterochromaffin (EC) cells is a major signaling molecule, which participates in the pathophysiological regulation of colonic functions in gut disorder including irritable bowel syndrome (IBS), but the endogenous modulator system for the 5-HT release is not yet well elucidated. Our *in vitro* studies in guinea-pig colon have indicated that the cascade pathway of neuronal tachykinergic NK_3 receptors and NK_2 receptors on peptide YY (PYY)-containing endocrine L cells represents an endogenous modulator system for 5-HT release from EC cells and that melatonin, endogenous tachykinins and PYY play important roles in modulation of the release of 5-HT from EC cells *via* the endogenous NK_2/NK_3 receptor cascade system. This review aims at examining the potential role of the endogenous tachykinergic NK_2/NK_3 receptor cascade system controlling the release of 5-HT from EC cells, with special attention being paid to the pathophysiology of gut disorders including IBS.

Keywords: Colon, enterochromaffin cells, 5-HT (5-hydroxytryptamine, serotonin) release, melatonin, peptide YY, tachykinins, NK_2 and NK_3 receptors, Y_1 receptors.

1. INTRODUCTION

In the gastrointestinal (GI) tract, 5-hydroxytryptamine (serotonin, 5-HT) is a major signaling molecule, which mediates GI functions (motility and secretion), emesis, nausea and pain [1, 2]. Most of colonic 5-HT is synthesized and released from mucosal enterochromaffin (EC) cells; therefore, alterations in the release of 5-HT from EC cells affect both physiological and pathophysiological colonic functions [2].

Irritable bowel syndrome (IBS) is a GI chronic disorder characterized by abdominal discomfort or pain associated with altered bowel habits. Abnormal levels of chromogranin A (a marker for all endocrine cells) in the colon of IBS patients have been demonstrated [3], thus indicating a role of the colonic endocrine cells (namely, 5-HT-containg EC cells or peptide YY (PYY)-containing L cells), in the regulation of colonic function in IBS patients [4-6]. Indeed, a recent human study [7] suggests that the increased 5-HT release from colonic EC cells contributes to the development of abdominal pain in IBS patients. Therefore, an improved understanding of endogenous modulator system for 5-HT release from EC cells may provide novel insights into the etiology and pharmacotherapy of functional bowel disorders such as IBS.

Our *in vitro* studies have demonstrated that isolated guinea-pig colon is a helpful *in vitro*-preparation for studying the mechanism modulating the release of 5-HT from EC cells [8-10]. Experimental data from the *in vitro*-preparations indicate that melatonin, endogenous tachykinins (TKs; neuropeptides such as substance P and neurokinin A) and PYY play important roles in the modulation of the release of 5-HT from EC cells *via* a unique endogenous tachykinergic NK_2/NK_3 receptor cascade system [8-11]. This review focuses on the evidence establishing a potential role of the endogenous NK_2/NK_3 receptors cascade system controlling the release of 5-HT from EC cells, with special attention being paid to the pathophysiology of gut disorders including IBS.

2. TACHYKININ RECEPTORS AND 5-HT RELEASE FROM COLONIC MUCOSA

A selective tachykinin NK_2 receptor agonist βAla-NKA-(4-10) (0.01-1 μM) evoked a long-lasting 5-HT release from isolated guinea-pig colon in a concentration-dependent manner [8-11]. The 5-HT-releasing action of βAla-NKA-(4-10) was unaffected by tetrodotoxin (TTX), and was also seen in myenteric plexus-free mucosal preparations, thus suggesting that the NK_2 agonist-evoked 5-HT release is mediated by activation of NK_2 receptors located on non-neuronal cells in the mucosal layer [8]. This agrees with a previous report which showed tachykinin NK_2 receptor expression on the surfaces of enterocytes of guinea-pig colon [12]. The L-type calcium channel blocker nicardipine or the syntaxin inhibitor botulinum toxin type C also reduced the long-lasting 5-HT release evoked by βAla-NKA-(4-10) [9], thus suggesting that the NK_2 receptor-triggered 5-HT release is mediated *via* syntaxin-related exocytosis mechanisms. However, an

*Address correspondence to this author at the Depatrment of Pharmacology and Toxicology, Dokkyo Medical University School of Medicine, Mibu, Tochigi 321-0293, Japan; Fax: + 81-282-86-2915;
E-mail: s-kojima@dokkyomed.ac.jp

1570-159X/15 $58.00+.00

involvement of tachykinin NK_1 receptors in the evoked 5-HT release is unlikely because a selective tachykinin NK_1 receptor agonist, [Sar^9, met $(O_2)^{11}$]-substance P (100 nM) failed to affect basal 5-HT release [9].

A selective tachykinin NK_3 receptor agonist senktide also evoked a transient 5-HT release from the isolated guinea-pig colon implicating tachykinin NK_3 receptors, in addition to NK_2 receptors in modulating 5-HT release from colonic mucosa [8]. As summarized in Fig. **1**, the enhancing effect of senktide on 5-HT release was prevented by both TTX and the NK_2 receptor-selective antagonist SR48968, thus suggesting that senktide stimulates the release of neuronal TKs, which in turn enhances 5-HT release *via* mucosal NK_2 receptors. This agrees with previous findings in guinea-pig colon where NK_3 receptor activation releases TKs and 5-HT [13, 14]. The 5-HT-releasing action of senktide was also inhibited by hexamethonium, and was not seen in myenteric plexus-free mucosal preparations, thus suggesting that myenteric cholinergic interneurons also participate in the mechanism for NK_3 receptor-triggered 5-HT release [8]. Thus, these findings support the view that the cascade of neuronal NK_3 receptors and mucosal NK_2 receptors represents an endogenous modulator system for 5-HT release from EC cells.

These results are also in line with previous findings (1) in human colon where NK_3 receptors present predominantly on myenteric and submucosal plexus neurons, and NK_3 receptors come into a play only in pathological conditions such as rescue responses or inflammation [15], and (2) in healthy volunteers where the stimulation of NK_2 receptors appears to induce IBS-like symptoms [16]. Moreover, it has been shown that the 5-HT release from mucosal biopsy specimens of IBS patients is increased by 10-fold over controls, and the increased 5-HT release contributes to development of abdominal pain in IBS patients [7]. Therefore, the view that the endogenous tachykininergic NK_2/NK_3 receptor cascade system can regulate the release of 5-HT from colonic EC cells, has an important implication for the potential role of endogenous TKs in the generation of the symptoms associated with IBS; it can be speculated that endogenous TKs acting through the NK_2/NK_3 receptor cascade system facilitate the release of 5-HT which in turn affects the colonic function of IBS patients through the activation of 5-HT receptor subtypes.

A sensory nerve-derived neuropeptide, calcitonin gene-related peptide (CGRP, 1-100 nM) has been also shown to produce a TTX-sensitive but atropine-resistant 5-HT release *via* stimulation of myenteric plexus neurons in the isolated segments of guinea-pig colon [8]. The enhancing effect of CGRP on 5-HT release was also prevented by both the NK_2 receptor-selective antagonist SR48968 and the NK_3 receptor-selective antagonist SR142801, but not by the NK_1 receptor-selective antagonist L703606 [8]. Thus, these findings support the view that CGRP stimulates the release of neuronal TKs, which in turn enhances 5-HT release *via* the endogenous NK_2/NK_3 receptor cascade system. Thus, it appears that CGRP-sensitive TKs-releasing neurons play a role in activating the endogenous NK_2/NK_3 receptor cascade system. This conjecture is also supported by a previous report which showed that CGRP can stimulate intramural tachykinergic neurons in the guinea-pig colon [17].

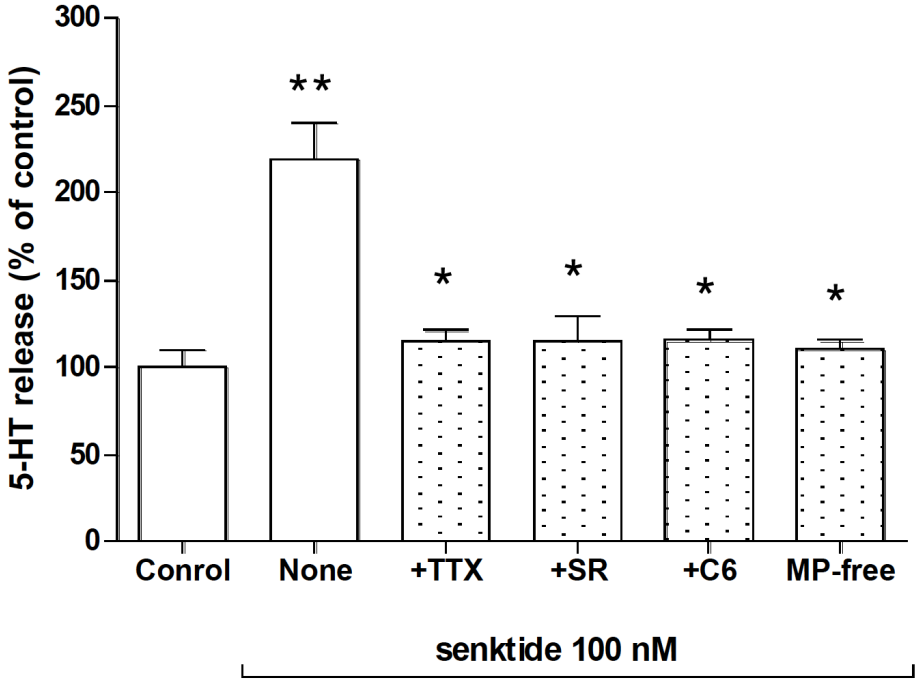


Fig. (1). Effects of tetrodotoxin (+TTX, 1 μM), SR48968 (+SR, 1 μM) and hexamethonium (+C6, 100 μM) on the 5-HT release from isolated guinea-pig colon by senktide (100 nM). Senktide had no effect on the 5-HT release from myenteric plexus-free mucosal preparations (MP-free). Height of columns: 5-HT release expressed as % of the control. * $P < 0.01$, significantly different from the None (no antagonists); ** $P < 0.01$, significantly different from the control.

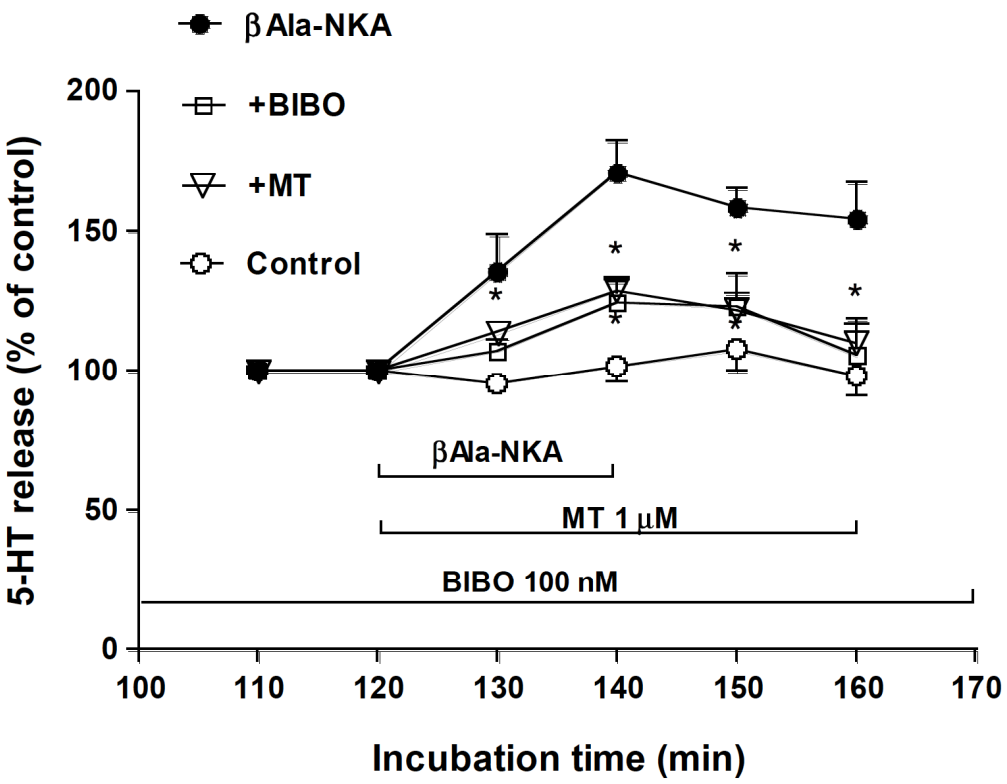


Fig. (2). Effect of βAla-NKA-(4-10) (100 nM, from 120 to 140 min of incubation), in the absence and presence of BIBO3304 (+BIBO, 100 nM, from the onset of incubation) or melatonin (+MT, 1μM, from 120 to 160 min of incubation) on the 5-HT release from isolated guinea-pig colonic mucosa. Ordinates: 5-HT release expressed as % of the control. Abscissae: time after onset of collection of the incubation medium. * $P < 0.05$, significantly different from the βAla-NKA.

Furthermore, the evidence for the elevation of the levels of endogenous CGRP and TKs in human diseased colonic mucosa has also been demonstrated [18, 19]; irritants, immunological and inflammatory mediators can release TKs and CGRP within the intestinal wall where these peptides may facilitate the release of 5-HT *via* the endogenous NK_2/NK_3 receptor cascade system. Thus, these findings suggest a possible role of the endogenous NK_2/NK_3 receptor cascade system controlling the release of 5-HT from EC cells in pathophysiological conditions that are associated with an upregulation of CGRP or TKs release.

3. NK_2 RECEPTOR-TRIGGERED 5-HT RELEASE IS MEDIATED BY ENDOGENOUS PYY

The 36 amino-acid long peptide, peptide YY (PYY) is known as a major peptide hormone, which regulates colonic functions (motility and secretion) and appetite by acting *via* different neuropeptide Y receptor subtypes [20, 21]. In the colon, PYY is mainly synthesized and released into the blood from mucosal L cells. The blood PYY levels have been shown to increase rapidly in response to food ingestion, and the postprandial blood PYY levels remain elevated for several hours [21]. Abnormal levels of PYY in large intestinal endocrine cells have also been reported in patients with IBS [6, 22], indicating a possible role of PYY in the generation of the symptoms associated with IBS.

The NK_2 receptor agonist, βAla-NKA-(4-10) (10-100 nM) also evoked a long-lasting and TTX-resistant PYY release from isolated guinea-pig colonic mucosa [11], thus suggesting that activation of NK_2 receptors on the mucosal layer induces a long-lasting PYY release from L cells. As shown in Fig. **2**, pretreatment with the neuropeptide Y_1 receptor-selective antagonist BIBO3304 attenuated the βAla-NKA-(4-10)-evoked 5-HT release [11]. These findings support the hypothesis that activation of NK_2 receptors on PYY-containing L cells provokes the release of PYY, which in turn activates mainly Y_1 receptors located on EC cells to induce 5-HT release (Fig. **3**). This hypothesis is further corroborated by findings that exogenously applied PYY (10 nM) evoked a long-lasting and BIBO3304-sensitive 5-HT release with a time course similar to that found for the NK_2 agonist-evoked 5-HT release [11]. Overall, the above observations indicate that endogenously released PYY play a fundamental role in the NK_2 receptor-triggered 5-HT release from EC cells; PYY-containing L cells appears to control 5-HT release from EC cells (Fig. **3**). Given that food intake is a major and physiologically significant factor stimulating the release of PYY from mucosal L cells [21] and both PYY and 5-HT are well-known mediators of nausea and vomiting [23], it is hypothesized that the cascade of NK_2 receptors on PYY-containing L cells and Y_1 receptors on EC cells (Fig. **3**) might play a role in the generation of postprandial symptoms (nausea and vomiting) in patients with IBS; in fact, a postprandial increase in plasma 5-HT levels have been shown to be related to the generation of postprandial symptoms in diarrhea-predominant IBS [24, 25]. Therefore, the NK_2 receptor-triggered PYY release from colonic mucosa may be

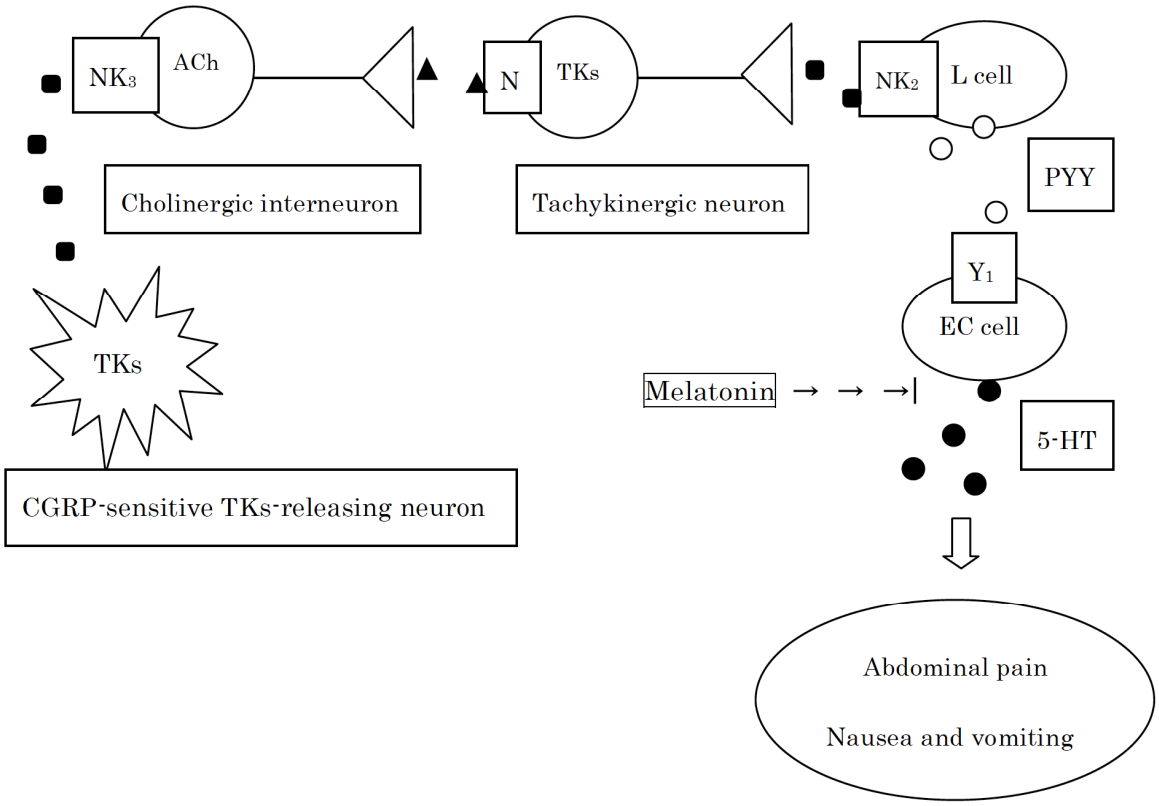


Fig. (3). An illustration of the working model of the endogenous tachykinergic NK_2/NK_3 receptor cascade system controlling the release of 5-HT/PYY from colonic mucosa: Interactions between enteric neurons (cholinergic interneuron and postganglionic tachykinergic neuron) and endocrine cells (PYY-containing L cell and 5-HT-containning EC cell) are present, where their activation potentially induces a long-lasting 5-HT/PYY release from colonic mucosa. We propose that NK_3 receptor on cholinergic interneuron is located upstream of nicotinic receptor (N) on postganglionic tachykinergic neuron, and activation of NK_2 receptor on L cell can provoke a long-lasting release of PYY, which in turn activate Y_1 receptor on EC cell to induce a long-lasting 5-HT release (Melatonin inhibits the 5-HT release). The evoked long-lasting 5-HT release could participate in the generation of abdominal pain or nausea and vomiting in functional bowel disorders including IBS. CGRP-sensitive TKs-releasing neurons appear to play a role in activating the endogenous tachykinergic NK_2/NK_3 receptor cascade system.

expected as a pharmacological target for postprandial symptoms in functional bowel disorders including IBS. Activation of tachykinin NK_3 receptors on enteric cholinergic neurons has also been shown to provoke the release of PYY from colonic mucosa [26]. Thus, to elucidate whether the endogenous tachykinergic NK_2/NK_3 receptor cascade system participates in the mechanism for food intake-induced PYY release, is an interesting topic for future study.

4. INHIBITION OF NK_2 RECEPTOR-TRIGGERED 5-HT RELEASE BY MELATONIN

The 5-HT-derived neurohormone, melatonin (MT) is mainly synthesized in the pineal gland as well as in 5-HT-rich EC cells of the intestinal mucosa [27]. It has been shown that exogenous MT modulates colonic motility and pain in IBS patients [28, 29]; therefore, MT is considered as a possible therapeutic agent for IBS [29, 30]. In the isolated guinea-pig colonic mucosa, MT has been shown to inhibit the NK_2 receptor-triggered 5-HT release by acting through MT_3 melatonin receptors located on the mucosal layer, thus suggesting a possible role of MT as a negative-regulator for the NK_2 receptor-triggered 5-HT release from EC cells [10] (Fig. **2**). It is not yet known whether or not endogenous MT participates in the pathogenesis of IBS, but oral MT has been shown to reduce abdominal pain and the sensation of bowel urgency in IBS patients [29]. It is therefore to be expected that if low MT concentrations in the colonic mucosa facilitate 5-HT release from EC cells, the enhanced 5-HT release leads to abdominal pain in IBS patients. In fact, it has been shown that IBS patients have lower MT levels compared with healthy controls [31]. Thus, to elucidate whether the endogenous NK_2/NK_3 receptor cascade system participates in the modulatory effect of MT on colonic motility or pain, is an interesting topic for future study.

5. CONCLUDING REMARKS

The present review shows that the cascade pathway of NK_3 receptors on cholinergic interneurons and NK_2 receptors on PYY-containing endocrine L cells represents an endogenous modulator system for 5-HT release from colonic EC cells, and that melatonin, endogenous TKs and PYY play important roles in the modulation of the release of 5-HT from EC cells *via* the endogenous NK_2/NK_3 receptor cascade system (Fig. **3**). Given that TKs, 5-HT-containing EC cells,

PYY-containing L cells and melatonin play important roles in the pathophysiological regulation of colonic functions in the diseased gut including IBS, the potential role of the endogenous NK_2/NK_3 receptor cascade system controlling the release of 5-HT from EC cells, will undoubtedly be a focus of future investigations.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

Declared none.

REFERENCES

[1] Camilleri, M. Serotonergic modulation of visceral sensation: lower gut. *Gut*, **2002**, *51*(Suppl 1), i81. http://dx.doi.org/10.1136/gut.51.suppl_1.i81

[2] Costedio, M.M.; Hyman, N.; Mawe, G.M. Serotonin and its role in colonic function and in gastrointestinal disorders. *Dis. Colon. Rectum*, **2007**, 50, 376-388. http://dx.doi.org/10.1007/s10350-006-0763-3

[3] El-Salhy, M.; Lomholt-Beck, B.; Hausken, T. Chromogranin A as a possible tool in the diagnosis of irritable bowel syndrome. *Scand. J. Gastroenterol*., **2010**, *45*, 1435-1439. http://dx.doi.org/10.3109/00365521.2010.503965

[4] Miwa, J.; Echizen, H.; Matsueda, K.; Umeda, N. Patients with constipation-predominant irritable bowel syndrome (IBS) may have elevated serotonin concentrations in colonic mucosa as compared with diarrhea-predominant patients and subjects with normal bowel habits. *Digestion*, **2001**, *63*, 188-194. http://dx.doi.org/10.1159/000051888

[5] EI-Salhy, M.; Gundersen, D.; Ostgaard, H.; Lomholt-Beck, B.; Hatlebakk, J.G.; Hausken, T. Low densities of serotonin and peptide YY cells in the colon of patients with irritable bowel syndrome. *Dig. Dis. Sci*., **2012**, *57*, 873-878. http://dx.doi.org/10.1007/s10620-011-1948-8

[6] EI-Salhy, M.; Gundersen, D.; Hatlebakk, J.G.; Gilja, O.H.; Hausken, T. Abnormal rectal endocrine cells in patient with irritable bowel syndrome. *Regul. Pept*., **2014**, *88*, 60-65.

[7] Cremon, C.; Carini, G.; Wang, B.; Vasina, V.; Cogliandro, R.F.; De Giorgio, R.; Stanghellini, V.; Grundy, D.; Tonini, M.; De Ponti, F.; Corinaldesi, R.; Barbara, G. Intestinal serotonin release, sensory neuron activation, and abdominal pain in irritable bowel syndrome. *Am. J. Gastroenterol*., **2011**, *106*, 1290-1298. http://dx.doi.org/10.1038/ajg.2011.86

[8] Kojima, S.; Ueda, S.; Ikeda, M.; Kamikawa, Y. 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*., **2004**, *141*, 385-390. http://dx.doi.org/10.1038/sj.bjp.0705624

[9] Kojima, S.; Ikeda, M.; Kamikawa, Y. Further investigation into the mechanism of tachykinin NK_2 receptor-triggered serotonin release from guinea-pig proximal colon. *J. Pharmacol. Sci*., **2009**, *110*, 122-126. http://dx.doi.org/10.1254/jphs.09032SC

[10] Kojima, S.; Tohei, A.; Ikeda, M. Melatonin inhibits tachykinin NK_2 receptor-triggered 5-HT release from guinea-pig isolated colonic mucosa. *Br. J. Pharmacol*., **2011**, *162*, 1179-1185. http://dx.doi.org/10.1111/j.1476-5381.2010.01122.x

[11] Kojima, S.; Tohei, A.; Anzai, N. A role for endogenous peptide YY in tachykinin NK_2 receptor-triggered 5-HT release from guinea-pig isolated colonic mucosa. *Br. J. Pharmacol*., **2012**, *167*, 1362-1368. http://dx.doi.org/10.1111/j.1476-5381.2012.02094.x

[12] Portbury, A.L.; Furness, J.B.; Southwell, B.R.; Wong, H.; Walsh, J.H.; Bunnett, N.W. Distribution of neurokinin-2 receptors in the guinea-pig gastrointestinal tract. *Cell Tissue Res.*, **1996**, *286*, 281-292. http://dx.doi.org/10.1007/s004410050698

[13] Goldhill, J.; Angel, I. Mechanism of tachykinin NK3 receptor-mediated colonic ion transport in the guinea-pig. *Eur. J. Pharmacol*., **1998**, *363*, 161-168. http://dx.doi.org/10.1016/S0014-2999(98)00797-3

[14] Goldhill, J.; Porquet, M.F.; Selve, N. Antisecretory and relaxatory effects of tachykinin antagonists in the guinea-pig intestinal tract. *J. Pharm. Pharmacol*., **1999**, *51*, 1041-1048. http://dx.doi.org/10.1211/0022357991773375

[15] Sanger, G.J. Neurokinin NK1 and NK3 receptors as targets for drugs to treat gastrointestinal motility disorders and pain. *Br. J. Pharmacol.*, **2004**, *141*, 1303-1312. http://dx.doi.org/10.1038/sj.bjp.0705742

[16] Lordal, M.; Navalesi, G.; Theodorsson, E.; Maggi, C.A.; Hellstrom, P.M. A novel tachykinin NK2 receptor antagonist prevents motility-stimulating effects of neurokinin A in small intestine. *Br. J. Pharmacol.*, **2001**, *134*, 215-223. http://dx.doi.org/10.1038/sj.bjp.0704217

[17] Kojima, S.; Shimo, Y. Calcitonin gene-related peptide (CGRP)-enhanced non-adrenergic non-cholinergic contraction of guinea-pig proximal colon. *Br. J. Pharmacol*., **1995**, *115*, 1290-1294. http://dx.doi.org/10.1111/j.1476-5381.1995.tb15038.x

[18] Johansson, M.; Jonsson, M.; Norrgard, O.; Forsgren, S. New aspects concerning ulcerative colitis and colonic carcinoma: analysis of levels of neuropeptides, neurotrophins, and TNFalpha/TNF receptor in plasma and mucosa in parallel with histological evaluation of the intestine. *Inflamm. Bowel Dis*., **2008**, *14*, 1331-1340. http://dx.doi.org/10.1002/ibd.20487

[19] Liu, L.; Markus, I.; Saghire, H.E.; Perera, D.S.; King, D.W.; Burcher, E. Distinct differences in tachykinin gene expression in ulcerative colitis, Crohn's disease and diverticular disease: a role for hemokinin-1? *Neurogastroenterol. Motil*., **2011**, *23*, 475-e180. http://dx.doi.org/10.1111/j.1365-2982.2011.01685.x

[20] Tough, I.R.; Forbes, S.; Tolhurst, R.; Ellis, M.; Herzog, H.; Bornstein, J.C.; Cox, H.M. Endogenous peptide YY and neuropeptide Y inhibit colonic ion transport, contractility and transit differentially *via* Y_1 and Y_2 receptors. *Br .J. Pharmacol*., **2011**, *164*, 471-484. http://dx.doi.org/10.1111/j.1476-5381.2011.01401.x

[21] Karra, E.; Batterham, R.L. The role of gut hormones in the regulation of body weight and energy homeostasis. *Mol. Cell. Endocrinol.*, **2010**, *316*, 120-128. http://dx.doi.org/10.1016/j.mce.2009.06.010

[22] EI-Salhy, M.; Gundersen, D.; Ostgaard, H.; Lomholt-Beck, B.; Hatlebakk, J.G.; Hausken, T. Low densities of serotonin and peptide YY cells in the colon of patients with irritable bowel syndrome. *Dig. Dis. Sci*., **2012**, *57*, 873-878. http://dx.doi.org/10.1007/s10620-011-1948-8

[23] Wu, W.; Bates, M.A.; Bursian, S.J.; Flannery, B.; Zhou, H.R.; Link, J.E.; Zhang, H.; Pestka, J.J. Peptide YY3-36 and 5-hydroxytryptamine mediate emesis induction by trichothecene deoxynivalenol (Vomitoxin). *Toxicol. Sci*., **2013**, *133*, 186-195. http://dx.doi.org/10.1093/toxsci/kft033

[24] Bearcroft, C.P.; Perrett, D.; Farthing, M.J.G. Postprandial plasma 5-hydroxytryptamine in diarrhea predominant irritable bowel syndrome: a pilot study. *Gut*, **1998**, *42*, 42-46. http://dx.doi.org/10.1136/gut.42.1.42

[25] Houghton, L.A.; Atkinson, W.; Whitaker, R.P.; Whorwell, P.J.; Rimmer, M.J. Increased platelet depleted plasma 5-hydroxytryptamine concentration following meal ingestion in symptomatic female subjects with diarrhea predominant irritable bowel syndrome. *Gut*, **2003**, *52*, 663-670. http://dx.doi.org/10.1136/gut.52.5.663

[26] Kojima, S.; Tohei, A.; Kojima, K.; Anzai, N. Evidence for tachykinin NK_3 receptors-triggered peptide YY release from isolated guinea-pig distal colon. *Eur. J. Pharmacol*., **2014**, *740*, 121-126. http://dx.doi.org/10.1016/j.ejphar.2014.06.062

[27] Bubenik, G.A. Thirty four years since the discovery of gastrointestinal melatonin. *J. Physiol. Pharmacol*., **2008**, *59*, 33-51.

[28] Lu, W.Z.; Song, G.H.; Gwee, K.A.; Ho, K.Y. The effects of melatonin on colonic transit time in normal control and IBS patients. *Dig. Dis. Sci*., **2009**, *54*, 1087-1093. http://dx.doi.org/10.1007/s10620-008-0463-z

[29] Song, G.H.; Leng, P.H.; Gwee, K.A.; Moochhala, S.M.; Ho, K.Y. Melatonin improves abdominal pain in irritable bowel syndrome patients who have sleep disturbances – a randomized double blind placebo controlled study. *Gut*, **2005**, *54*, 1402-1407. http://dx.doi.org/10.1136/gut.2004.062034

[30] Andresen, V.; Camilleri, M. Irritable bowel Syndrome. *Drugs*, **2006**, *66*, 1073-1088. http://dx.doi.org/10.2165/00003495-200666080-00004

[31] Radwan, P.; Skrzydlo-radomanska, B.; Radwan-kwiatek, K.; Burak-czapiuk, B.; Strzemecka, J. Is melatonin involved in the irritable bowel syndorome? *J. Physiol. Pharmacol*., **2009**, *60*, Suppl 3, 67-70.

Received: December 08, 2014 Revised: March 13, 2015 Accepted: August 20, 2015