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Modulation by 5-HT_3 and 5-HT_4 receptors of the release of 5-hydroxytryptamine from the guinea-pig small intestine

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Summary. The effects of agonists and antagonists of 5-hydroxytryptamine (5-HT) receptors on the release of endogenous 5-HT from enterochromaffin cells were studied in the vascularly perfused isolated guinea-pig small intestine. The experiments were done in the presence of tetrodotoxin in order to exclude a neuronally mediated influence on 5-HT release.

The 5-HT_3 receptor agonist 2-methyl-5-HT increased 5-HT release, and this effect was antagonized by 1 nmol/l tropisetron. Nanomolar concentrations of tropisetron, MDL 72 222 and granisetron decreased 5-HT release. Ondansetron (0.1 and 1 μmol/l) did not modify 5-HT release.

5-Methoxytryptamine, BIMU8 and cisapride concentration-dependently inhibited 5-HT release. BIMU8 was more potent than 5-methoxytryptamine. Micromolar concentrations of tropisetron (1 and 10 μmol/l) enhanced the release, whilst methiothepine (0.1 μmol/l) did not affect the release of 5-HT.

The results suggest that enterochromaffin cells of the guinea-pig ileum do not contain 5-HT_1 and 5-HT_2 receptors, but are endowed with 5-HT_3 and 5-HT_4 autoreceptors. Activation of the 5-HT_3 receptors triggers a positive feedback mechanism leading to an increase of 5-HT release. The 5-HT_3 receptors on the enterochromaffin cell differ from neuronal 5-HT_3 receptors on guinea-pig myenteric plexus by their high affinity for tropisetron and MDL 72 222, and their very low affinity for ondansetron. Stimulation of 5-HT_4 receptors causes inhibition of release; the inhibitory 5-HT_4 receptor mechanism appears to predominate.

Key words: Enterochromaffin cells – 5-Hydroxytryptamine release – 5-HT_3 receptors – 5-HT_4 receptors

Introduction

Most of the intestinal 5-hydroxytryptamine (5-HT) is stored in the enterochromaffin cells of the mucosa (Erspamer 1966) from which 5-HT is released into the gut lumen and into the portal circulation. The mechanisms of 5-HT release from enterochromaffin cells have been thoroughly studied using the isolated vascularly perfused guinea-pig intestine as a model system, and it has been found that the spontaneous outflow of 5-HT into the portal circulation reflects an exocytotic release from enterochromaffin cells (see review by Racké and Schwörer 1991). This release can be modulated by a variety of heteroreceptors located on the enterochromaffin cell. Thus, release is increased by stimulation of nicotinic and muscarinic receptors (Schwörer et al. 1987a) and β-adrenoceptors (Racké et al. 1988), and it is inhibited via α_2-adrenoceptors (Racké et al. 1988) and $GABA_A$ and $GABA_B$ receptors (Schwörer et al. 1989).

The aim of the present study was to find out whether 5-HT autoreceptors are also involved in the regulation of 5-HT release from the vascularly perfused small intestine. For this purpose the effects of 5-HT agonists and antagonists on spontaneous release of endogenous 5-HT were studied. The compounds used are partially selective for 5-HT_3 receptors (*agonists*: 2-methyl-5-HT, phenylbiguanide; *antagonists*: MDL 72 222, nanomolar concentrations of tropisetron, ondansetron, granisetron) and 5-HT_4 receptors (*agonists*: 5-methoxytryptamine, BIMU8, cisapride; *antagonists*: micromolar concentrations of tropisetron) (for literature see Fozard 1990, and Bockaert et al. 1992). In addition, the 5-HT_1/5-HT_2 antagonist methiothepine (Hoyer and Schoeffter 1991) was studied. The experiments were performed in the presence of tetrodotoxin in order to exclude a neuronally mediated influence on 5-HT release. Tetrodotoxin blocks the neuronal input to the enterochromaffin cell but does not prevent the receptor mediated modulation of 5-HT release from enterochromaffin cells (Racké and Schwörer 1991). Parts of this work have been given to the German Pharmacological Society (Gebauer 1991; Kilbinger et al. 1992).

Methods

Guinea pigs of either sex, weighing 200–500 g, were anaesthesized with pentobarbital (40 mg/kg, i.p.), the trachea cannulated and the animal

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artificially respirated. The preparation of the vascularly perfused small intestine was done as described previously (Holzer and Lembeck 1979; Schwörer et al. 1987b). The superior mesenteric artery and the portal vein were cannulated and the intestine was arterially perfused with a physiological salt solution at a rate of 1 ml per min using a peristaltic pump. The physiological salt solution contained (mmol/l): NaCl 137, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.0, $NaHCO_3$ 11.9, NaH_2PO_4 0.4, D-glucose 5.6, (+)-ascorbic acid 0.057, EDTA 0.03 and was gassed with 95% O_2 +5% CO_2. The preparation, consisting of two or three loops of the small intestine, was placed in an organ bath (200 ml capacity) which contained warmed (37 °C) physiological salt solution gassed with 95% O_2+5% CO_2. The oral end of the intestinal segment was connected to a Mariotte bottle with two inlet tubes containing physiological salt solution (37 °C). The aboral end was connected to an outflow cannula. During the first 45 min of a 75 min equilibration period peristalsis was induced every 15 min by raising the intraluminal pressure by 500 Pa for 1 min. The solution in the organ bath was renewed 45 min before the collection of the venous perfusate. Tetrodotoxin (1 µmol/l) was added to the perfusion medium 30 min before the collection period started.

After the 75 min equilibration period, the effluent of the portal vein was collected in 5 min fractions. Each glass tube used for collection contained 50 µl (+)-ascorbic acid (57 mmol/l), 50 µl EDTA (10 mmol/l) and 65 µl perchloric acid (1 mol/l). 5-HT receptor agonists and antagonists were present in the perfusion medium from 85 min onwards. At the end of the collection period, the preparation was freed of its mesentery. The tissues were blotted between filter paper and weighed. Their weight was between 0.8 and 2.5 g.

5-HT was measured by high pressure liquid chromatography (HPLC) with electrochemical detection (Schwörer et al. 1987b). 5-HT was separated on a reversed phase column (length 125 mm, inner diameter 4.6 mm, prepacked with ODS-Hypersil 5 µm) using a mobile phase of 0.09 mol/l NaH_2PO_4, sodium EDTA (0.3 mmol/l) and methanol (10%, v/v), adjusted to pH 3. Quantitation was achieved with a Waters 460 electrochemical detector equipped with a glass carbon working electrode and an Ag/AgCl reference electrode. The potential was set at +0.7 V. Portions of 50 µl were injected onto the HPLC-column. The amount of 5-HT in the portal venous outflow is given in pmol/g·min. The results are expressed as percentage of the mean outflow observed during the first two collection samples (75–85 min of perfusion) of the individual experiments.

Statistics. Mean values of n experiments are given ±SEM. Significance of differences between control and drug groups was evaluated by the Newman-Keuls-test with help from the computer program of Tallarida and Murray (1987).

Drugs. 2-Methyl-5-hydroxytryptamine, 5-methoxytryptamine (both RBI, Natick, Mass., USA); BIMU8 (endo-N-(8-methyl-8-azabicyclo[3.2.1]-oct-3-yl)-2,3-dihydro-(1-methyl)ethyl-2-oxo-1H-benzimidazole-1-carboxamide HCl; Boehringer Ingelheim Italia, Milano, Italy); cisapride (Janssen, Beerse, Belgium); granisetron (SmithKline Beecham, Betchworth, UK); MDL 72222 (1αH,3α, 5αH-tropan-3-yl-3,5-dichlorobenzoate, Merell Dow Research Institute, Strasbourg, France); methiothepine (Hoffmann-La Roche, Basel, Switzerland); ondansetron (Glaxo, Ware, UK); phenylbiguanide (Aldrich, Steinheim, FRG); tetrodotoxin (Sigma, München, FRG); tropisetron (Sandoz, Basel, Switzerland).

Results

Effects of antagonists

The spontaneous outflow of 5-HT determined between 75 and 85 min of perfusion was 27±1 pmol/g·min (n = 40). In control experiments the outflow did not change significantly during the following 50 min (Fig. 1).

Methiothepine (0.1 µmol/l; n = 3) perfused from 85 min of perfusion onwards had no effect on basal outflow of 5-HT. MDL 72 222 and nanomolar concentrations of tropisetron decreased basal outflow of 5-HT (Fig. 1). Concentration-response curves for both drugs are shown in Fig. 2. The $-\log EC_{50}$ value for the inhibitory effect of tropisetron was 9.6 and that for MDL 72 222 was 9.3. Granisetron, (0.1 µmol/l) also inhibited significantly the 5-HT outflow (Fig. 2). Micromolar concentrations of tropisetron significantly increased the outflow (Figs. 1 and 2), and the $-\log EC_{50}$ for this effect was 6.1. Ondansetron (0.1 and 1 µmol/l; n = 2 and 6) did not modify 5-HT outflow. Only a concentration of 10 µmol/l ondansetron reduced the outflow by 50±9% (n = 6; peak inhibition after 10 min of perfusion).

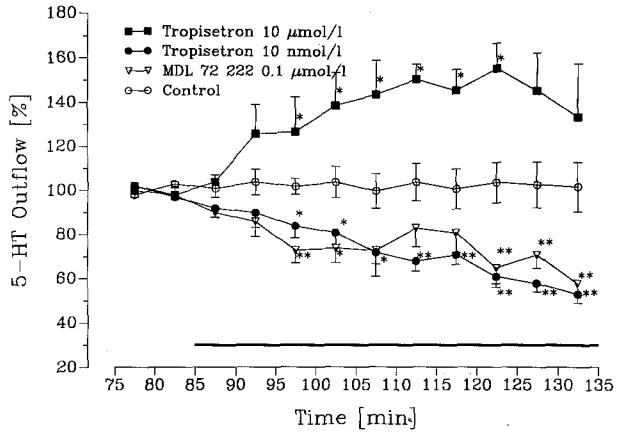

Fig. 1. Effects of tropisetron (10 nmol/l, n = 5; 10 µmol/l, n = 3) and MDL 72 222 (100 nmol/l, n = 4) on the outflow of 5-HT in the presence of tetrodotoxin (1 µmol/l). Tropisetron and MDL 72 222 were present in the arterial perfusate as indicated by the *horizontal bar.* Tetrodotoxin was added to the perfusate 40 min before these drugs. *Abscissa:* perfusion time. *Ordinate:* outflow of 5-HT into the portal venous effluent expressed as percent of the mean outflow from 75–85 min of perfusion. Mean values±SEM. Significance of difference from corresponding control value (n = 8): $*P<0.05$, $**P<0.01$

Effects of 5-HT$_3$ receptor agonists

2-Methyl-5-HT (30 and 60 µmol/l) significantly enhanced the outflow of 5-HT. This effect was maximal within

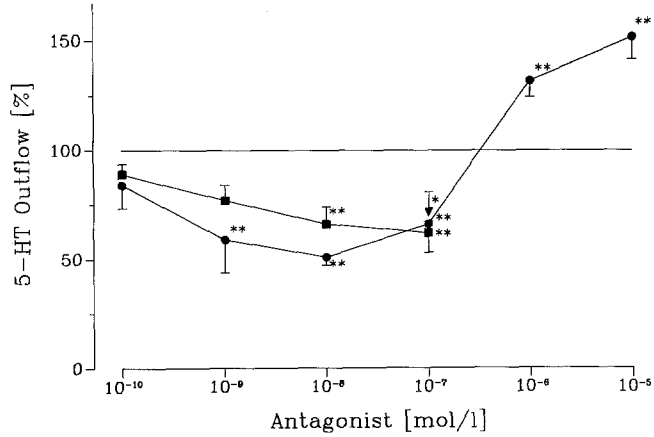

Fig. 2. Concentration-dependent effects of tropisetron, MDL 72 222 and granisetron on the outflow of 5-HT in the presence of tetrodotoxin (1 µmol/l). Given are the mean values of the maximal changes observed in the individual experiments±SEM; n = 3–5. Significance of difference from the controls: $*P<0.05$, $**P<0.01$. ● – ● Tropisetron, ■ – ■ MDL 72222, ▼ Granisetron

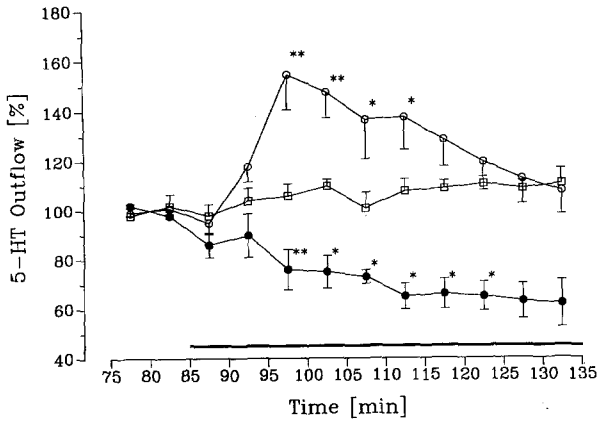

Fig. 3. Effects of 2-methyl-5-HT ($n = 5$), phenylbiguanide ($n = 4$) and 5-methoxytryptamine (5-MOT; $n = 3$) on the outflow of 5-HT in the presence of tetrodotoxin (1 μmol/l). The agonists were present in the perfusate as indicated by the *horizontal bar*. Tetrodotoxin was added 40 min before these drugs. *Abscissa:* perfusion time. *Ordinate:* outflow of 5-HT expressed as percent of the mean outflow from 75–85 min of perfusion. Mean values ± SEM. Significance of difference from corresponding control value: $*P < 0.05$, $**P < 0.01$. ○–○ 2-Methyl-5-HT 30 μmol/l, □–□ Phenylbiguanide 30 μmol/l, ■–■ 5-MOT 1 μmol/l

15 min and then declined slowly during the following 35 min (Fig. 3). Since only small amounts of 2-methyl-5-HT were available, higher concentrations than 60 μmol/l could not be tested. Under the assumption that 60 μmol/l caused the maximum increase, a $-\log EC_{50}$ of 4.7 was calculated from the concentration-response curve in Fig. 4. The increase by 30 μmol/l 2-methyl-5-HT was significantly ($p < 0.05$) reduced by tropisetron (1 nmol l/l) applied to the perfusion medium 40 min before 2-methyl-5-HT (Fig. 4). In control experiments in which tropisetron alone was present the outflow of 5-HT determined between 75 and 85 min of perfusion was 24 ± 2 pmol/g·min; $n = 6$), and remained constant during the observation time. Phenylbiguanide (1, 10 and

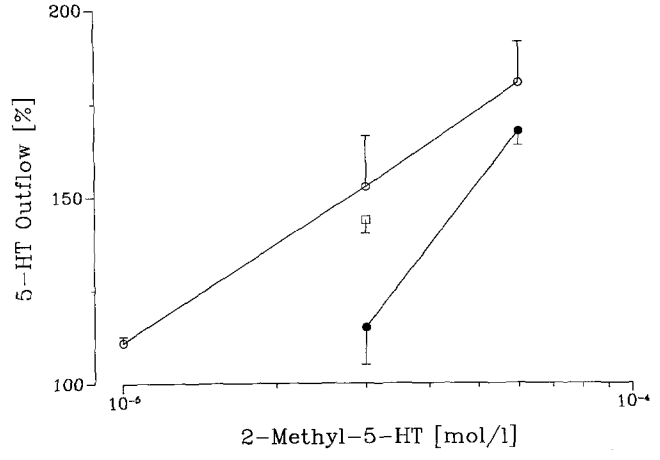

Fig. 4. Effects of tropisetron on the stimulation by 2-methyl-5-HT of the outflow of 5-HT in the presence of tetrodotoxin. 2-Methyl-5-HT was present in the perfusate from the 85th min onwards. Tropisetron was added together with tetrodotoxin 40 min before 2-methyl-5-HT. Given are the means ± SEM of the peak effects in individual experiments ($n = 3–5$). ○–○ 2-M-5-HT, ●–● 2-M-5-HT+Tropisetron 1 nmol/l, □ 2-M-5-HT+Tropisetron 0.1 nmol/l

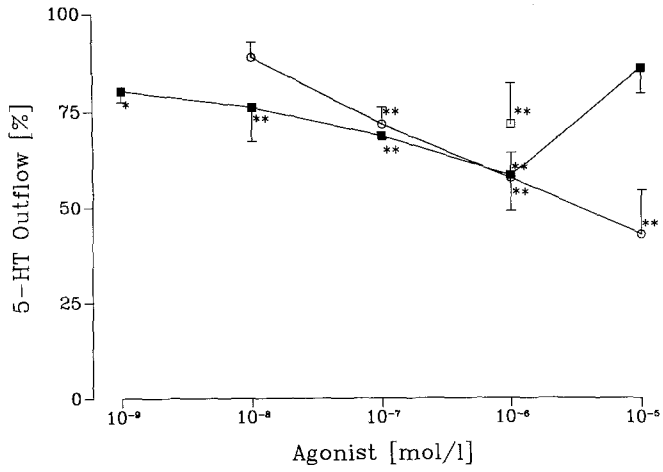

Fig. 5. Inhibition of 5-HT outflow by 5-methoxytryptamine (5-MOT), BIMU8 and cisapride in the presence of tetrodotoxin (1 μmol/l). Given are the means ± SEM of the peak effects ($n = 3–5$). Significance of inhibition of 5-HT outflow: $*P < 0.05$, $**P < 0.01$. ■–■ BIMU 8, □ Cisapride, ○–○ 5-MOT

30 μmol/l; $n = 4–6$) did not significantly change the outflow of 5-HT (Fig. 3).

Effects of 5-HT$_4$ receptor agonists

BIMU8, 5-methoxytryptamine and cisapride inhibited the outflow of 5-HT. The maximum inhibitory effect was reached within 30 min and was maintained for the following 20 min (see Fig. 3 for 5-methoxytryptamine). Concentration-response curves of the agonists are shown in Fig. 5. BIMU8 was more potent than 5-methoxytryptamine (Fig. 5).

Discussion

The release of 5-HT evoked by 2-methyl-5-HT can be attributed to stimulation of 5-HT$_3$ receptors, since 2-methyl-5-HT is a selective 5-HT$_3$ receptor agonist and since 1 nmol/l tropisetron antagonized the increase in release. The $-\log EC_{50}$ found in the present study (4.7) is similar to values obtained for its effects on acetylcholine release (5.0) and smooth muscle contraction (4.8) in the guinea-pig ileum (Fox and Morton 1990).

The 5-HT release evoked by 2-methyl-5-HT declined only slowly during the first 15 min after the peak increase (Fig. 3). In general, 5-HT$_3$ receptor agonists induce a fast response which is due to the opening of a cation channel and which desensitizes rapidly; this has for example been shown for the neuronal 5-HT$_3$ receptor of guinea-pig intestine (Derkach et al. 1989). There are, however, some exceptions to this: 5-HT$_3$ receptor stimulation by 2-methyl-5-HT increased basal dopamine release from rat striatal slices (Blandina et al. 1989) and suppressed firing of rat cortex neurones (Wang et al. 1991). Both effects showed little or no desensitization. Similarly, the time course of the 2-methyl-5-HT-evoked increase in 5-HT release suggests that the 5-HT$_3$ receptor of the enterochromaffin cell desensitizes far more slowly than 5-HT$_3$ receptors in most other tissues.

Phenylbiguanide has been recognized as a potent 5-HT$_3$ receptor agonist in the rat (see Fozard 1990), but

it was inactive in different tissues of the guinea-pig (Butler et al. 1990). Thus the failure of phenylbiguanide to modify 5-HT release in the present study corroborates the proposal of 5-HT_3 receptor subtypes in different species (Butler et al. 1990).

Our findings with the antagonists tropisetron, MDL 72 222 and granisetron support the assumption of the presence of 5-HT_3 autoreceptors on enterochromaffin cells. These drugs decreased 5-HT release, the maximal inhibition was about 50%. This suggests that endogenous 5-HT slightly activates these receptors and thus stimulates its own release. Release-modulating 5-HT_1 and 5-HT_2 receptors probably play no role, since methiothepine did not affect 5-HT release. Surprisingly, ondansetron did not modify 5-HT release although this compound is a selective antagonist of neuronal 5-HT_3 receptors in the guinea-pig intestine (Butler et al. 1990; Fox and Morton 1990). The obvious point to be made from all these data is that 5-HT_3 receptors on the enterochromaffin cells differ from neuronal 5-HT_3 receptors in guinea-pig intestine 1. by their high affinity for tropisetron and MDL 72 222, 2. by the failure of ondansetron to block these receptors, and 3. by the slow desensitization after receptor activation.

The inhibition of basal 5-HT release by 5-methoxytryptamine is attributable to stimulation of 5-HT_4 receptors as 5-methoxytryptamine is devoid of any effect at 5-HT_3 receptors (Fozard 1990; Bockaert et al. 1992). BIMU8 and cisapride are potent agonists at 5-HT_4 receptors and are in addition 5-HT_3 receptor antagonists (Bockaert et al. 1992). It is therefore difficult to say which of both effects causes the decrease in release. The mechanisms that lead to inhibition are certainly not additive since the maximal inhibition by BIMU8 was somewhat less than that caused by 5-methoxytryptamine. We assume that the inhibition of release by BIMU8 is due to stimulation of 5-HT_4 receptors since the differences in potencies between 5-methoxytryptamine and BIMU8 were comparable to those found for the 5-HT_4 receptor mediated increase in acetylcholine release from myenteric neurones (Kilbinger and Wolf 1992; Kilbinger et al. 1992).

Taken together, our findings suggest that endogenous 5-HT can stimulate 5-HT_3 and 5-HT_4 autoreceptors on enterochromaffin cells and thus facilitate and inhibit, respectively, its own release. For the following reasons the inhibitory 5-HT_4 receptor mechanism seems to predominate: 1. Micromolar concentrations of tropisetron which block both 5-HT_3 and 5-HT_4 receptors enhanced the release of 5-HT. It follows then that the biophase concentration of endogenous 5-HT activates more markedly the inhibitory than the facilitatory mechanism. 2. 5-HT is about 100times more potent at 5-HT_4 than at 5-HT_3 receptors (Buchheit et al. 1985; Kilbinger and Wolf 1992). Therefore, the release-enhancing 5-HT_3 receptor mechanism would be less operative than the release-inhibiting 5-HT_4 receptor mechanism at low biophase concentrations of 5-HT.

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