ELSEVIER

European Journal of Pharmacology 308 (1996) 173–180

ejp

5-HT_3 and 5-HT_4 receptors and cholinergic and tachykininergic neurotransmission in the guinea-pig proximal colon

Michel R. Briejer *, Jan A.J. Schuurkes

Department of Gastrointestinal Pharmacology, Janssen Research Foundation, B-2340 Beerse, Belgium

Received 12 October 1995; revised 2 April 1996; accepted 5 April 1996

Abstract

The pathways and possible transmitters involved in the contractile response to selective 5-HT_3 and 5-HT_4 receptor stimulation in the guinea-pig proximal colon were studied. In the presence of methysergide, 5-HT induced contractions, yielding a biphasic concentration-response curve that was changed into a monophasic curve in the presence of the 5-HT_3 receptor antagonist, granisetron (1 μM) (low-affinity phase blocked), or the 5-HT_4 receptor antagonist, SB 204070 ((1-butyl-4-piperidinyl methyl)-8-amino-7-chloro-1,4-benzodioxan-5-carboxylate) (10 nM) (high-affinity phase blocked) combination of the two antagonists abolished the contraction to 5-HT. The effectiveness and selectivity of both antagonists was confirmed by testing them against contractions in response to the 5-HT_3 receptor-selective agonist, 2-methyl-5-HT, and the 5-HT_4 receptor-selective agonist, 5-methoxytryptamine. Hexamethonium (100 μM) did not affect the 5-HT_3 receptor-mediated contractions, whereas tetrodotoxin (0.3 μM) caused only slight inhibition. Both in the absence and presence of tetrodotoxin, atropine (0.3 μM) inhibited the 5-HT_3 receptor-mediated contractions. Hence, the contractions to 5-HT are partly mediated by 5-HT_3 receptors that are localized on the nerve endings of the motor neurons. Hexamethonium halved the 5-HT_4 receptor-mediated contractions, whereas tetrodotoxin abolished them. The 5-HT_4 receptor-mediated contractions were inhibited by atropine (0.3 μM). Thus, the 5-HT_4 receptors seem to be localized in the soma of the motor neurons; they also occur on interneurons. The remaining contractions induced by 5-HT_3 and 5-HT_4 receptor stimulation in the presence of atropine were almost completely inhibited by the tachykinin NK_1 receptor antagonist, CP 96345 ((2*S*,3*S*)-*cis*-2-(diphenyl methyl)-*N*-[(2-methoxy phenyl)-methyl]-1-azabicyclo-[2.2.2]-octan-3-amine) (0.1 μM). CP 96345 also abolished or strongly inhibited contractions in response to substance P (10 nM) and to neurokinin A (30 nM), but neither granisetron nor SB 204070 affected them. Hence, stimulation of either 5-HT_3 or 5-HT_4 receptors induced contractions that are partially mediated by acetylcholine, and partially by a tachykinin NK_1 receptor-stimulating neurotransmitter, probably substance P and/or neurokinin A.

Keywords: 5-HT_3 receptor; 5-HT_4 receptor; Tachykinin; Colon, guinea-pig; Tetrodotoxin; Enteric nerve

1. Introduction

5-Hydroxytryptamine (5-HT) is an established neurotransmitter of the enteric nervous system, and is involved in the regulation of motility (Gershon and Erde, 1981; Furness and Costa, 1987; Gershon et al., 1989). It is generally assumed that, in the guinea-pig ileum and colon, the enteric nerves are endowed with excitatory 5-HT_3 and 5-HT_4 receptors. Stimulation of either receptor type causes a contraction or an amplification of field stimulation-induced cholinergic twitch contractions due to an increase in the release of acetylcholine (Craig and Clarke, 1990; Eglen et al., 1990; Elswood et al., 1991; Kilbinger and Wolf, 1992).

In the ileum, the contraction in response to 5-HT due to stimulation of 5-HT_4 receptors was fully suppressed by atropine and the neurotoxin, tetrodotoxin (Buchheit et al., 1985; Eglen et al., 1990; Fozard, 1990). In the proximal colon, 5-HT_4 receptor-induced contractions were abolished by tetrodotoxin, and inhibited only about 75% by atropine (Elswood et al., 1991; Briejer et al., 1993a). In longitudinal muscle-myenteric plexus preparations of the distal colon, tetrodotoxin and atropine completely prevented a 5-HT_4 receptor-mediated contraction (Wardle and Sanger, 1993). Hence, stimulation of 5-HT_4 receptors generally yields tetrodotoxin-sensitive cholinergic contractions which also seem to involve non-cholinergic transmitters.

5-HT_3 receptor-mediated responses in the ileum, proximal and distal colon were only partially sensitive to either tetrodotoxin or atropine (Buchheit et al., 1985; Butler et

* Corresponding author. Tel.: +32-14-605 104; fax: +32-14-605 737.

PII S0014-2999(96)00297-X

al., 1990; Eglen et al., 1990; Fozard, 1990; Briejer et al., 1993a). These findings suggest that 5-HT_3 receptors might be in part located on the nerve endings, where tetrodotoxin-insensitive release may occur. Further, they imply that non-cholinergic transmitters could be involved. Different conditions were used in each of the above described studies, and most of the findings mentioned were circumstantial data or deduced from curves where 5-HT_3 or 5-HT_4 receptor-mediated responses were not studied separately.

In the gastrointestinal tract of guinea-pigs, substance P and neurokinin A, but not neurokinin B, immunoreactivity has been found (Theodorsson-Norheim et al., 1985; Deacon et al., 1987; Shuttleworth et al., 1991; Too et al., 1989). As substance P and neurokinin A share two of three precursor proteins (the third seems to play a minor role in the guinea-pig gastrointestinal tract), they are likely to be present in the same neurons (see Otsuka and Yoshioka, 1993; Holzer-Petsche, 1995). Many cholinergic neurons in the myenteric plexus also show substance P immunoreactivity (Brookes et al., 1992; see Otsuka and Yoshioka, 1993; Holzer-Petsche, 1995), and it is thus likely that substance P is co-released upon stimulation of cholinergic neurons. Therefore substance P and neurokinin A, but not neurokinin B, might be involved in 5-HT_3 and 5-HT_4 receptor-mediated responses of guinea-pig colon.

As highly selective and potent 5-HT_3 (e.g. granisetron, Butler et al., 1990) and 5-HT_4 (e.g. SB 204070, Wardle and Sanger, 1994) receptor antagonists have become available only in the last few years, a direct comparative study of 5-HT_3 and 5-HT_4 receptors and their respective pathway to contraction has not been done. The aims of the current functional study with the isolated guinea-pig proximal colon were therefore twofold. First, we wanted to obtain data regarding both the possible pathways involved after selective stimulation of either 5-HT_3 and 5-HT_4 receptors and the possible localization of these receptors. Second, we studied whether substance P and neurokinin A could be involved in the contraction due to selective 5-HT_3 or 5-HT_4 receptor stimulation. For the latter purpose we employed the selective and potent tachykinin NK_1 receptor antagonist, CP 96345 (Snider et al., 1991), which we have previously shown to selectively block contractions due to exogenous substance P in this preparation (Briejer et al., 1993b).

2. Materials and methods

2.1. Tissue preparation

Dunkin-Hartley guinea-pigs of either sex, weighing 400–600 g, were stunned by a blow on the head and then killed by decapitation. The proximal colon was removed, and the luminal contents were washed out with De Jalon's solution (composition in mM: KCl 5.6, $CaCl_2$ 0.5, $NaHCO_3$ 6.0, NaCl 155, glucose 2.78; in all but the tachykinin experiments methysergide 1 μM was included in the De Jalon solution in order to block responses mediated by 5-HT_1-like and 5-HT_2-like receptors). Starting at the proximal end, about 1 cm distal from the caecum, the colon was divided into 4 segments of 3 cm after removal of the mesentery. These intact segments were individually mounted vertically in an organ bath containing 20 ml De Jalon's solution for isotonic measurement of longitudinal muscle responses. This solution was kept at 37°C and gassed with carbogen (95% O_2-5% CO_2). The strips were subjected to a preload of 2 g and allowed to stabilize for half an hour. After stabilization, the contraction in response to methacholine (3 μM) was measured. The measurement was repeated after washing (i.e. replacing the bathing fluid twice) and 15-min stabilization. This last response was taken as 100% contraction.

2.2. Concentration-response curves

Agonists and antagonists were applied directly to the organ baths, except for methysergide (1 μM) which was permanently included in the organ bath solution to block neurogenic relaxation in response to the tryptamines (Briejer et al., 1995) and contractions mediated by 5-HT_{2A} receptors (Briejer et al., 1993a). Non-cumulative concentration-response curves were made with a 20-min dosing cycle, with refreshing of the buffer after 10 min. The indole agonist was washed out as soon as the peak contraction was reached (ca. 30 s). In preliminary experiments it was found that this wash-dosing cycle prevented desensitization. Agonist concentrations were applied in ascending order with 0.5-log concentration spacing. Antagonists were applied 20 min before the addition of the first concentration of indole agonist, and were re-added directly after each washout. Each preparation served for only one concentration-response curve. As four colon strips per guinea-pig were used, one was chosen randomly to serve as a control whereas the remaining three strips received antagonist treatment. Only one curve with a 5-HT receptor agonist was made per strip.

2.3. Substance P and neurokinin A contractions

For the evaluation of the effect of antagonists against contractions due to exogenous substance P (10 nM) and neurokinin A (30 nM), atropine (0.3 μM) was added after washout following the second contraction to methacholine (and was continuously re-added after every washout that was to follow). After 15 min, either substance P or neurokinin A was added to the bath, and the tissue was washed as soon as the maximum response was obtained. Then either antagonists or its solvent were added and 15 min later the contraction to the respective neurokinin was repeated. Only one antagonist was tested per strip.

2.4. Data analysis

For graphical representation means ± standard error of the means were calculated. Mean values were compared using an analysis of variance (ANOVA) followed by the Bonferroni/Dunn test for multiple comparisons. For the tachykinin experiments, a two-way ANOVA for repeated measures was used to test the significance of the effects due to the treatment. A level of $P < 0.05$ was considered to indicate a significant difference. The number of animals used for an experiment is denoted by n.

2.5. Compounds

The following compounds were used: tetrodotoxin, 5-hydroxytryptamine creatinine sulphate (5-HT), neurokinin A, substance P (Serva, Germany), atropine sulphate, 5-methoxytryptamine HCl (Janssen Chimica, Belgium), hexamethonium bromide, SB 204070 (1-butyl-4-piperidinyl methyl)-8-amino-7-chloro-1,4-benzodioxan-5-carboxylate, granisetron, CP 96,345 ((2*S*,3*S*)-*cis*-2-(diphenyl methyl)-*N*-[(2-methoxy phenyl)-methyl]-1-azabicyclo-[2.2.2]-octan-3-amine), 2-methyl-5-hydroxytryptamine (2-methyl-5-HT) (Janssen Research Foundation, Belgium), methacholine HCl (Merck, Germany), methysergide maleate (Sandoz, Switzerland). All compounds were dissolved in distilled water, except for CP 96,345 (dissolved in distilled water acidified with tartaric acid pH < 3 in the stock solution) and the tryptamines (distilled water with ascorbic acid 0.25 μM in the stock solution); these solvents had no effects per se. All compounds were dissolved freshly, except for tetrodotoxin, neurokinin A and substance P, which were kept frozen (−30°C) as small aliquots.

3. Results

5-HT induced contractions from 10 nM onwards, yielding a biphasic concentration-response curve, with a maximum response (78.7 ± 6.9%) at 30 μM 5-HT (Fig. 1). In the presence of the 5-HT_4 receptor antagonist, SB 204070 (10 nM), the first phase of the concentration-response curve to 5-HT was suppressed, yielding a steep curve (Fig. 1). The maximum effect (at 10 and 30 μM) was not significantly altered as compared to the control (Fig. 1). The 5-HT_3 receptor antagonist, granisetron (1 μM), did not significantly affect the first, high-affinity, phase of the curve for 5-HT, but suppressed the second phase (significant from 10 μM onwards) (Fig. 1). The combination of the two antagonists abolished all the contractions to 5-HT up to 30 μM 5-HT (higher concentrations were not tested) (Fig. 1).

2-Methyl-5-HT, an agonist at 5-HT_3 but not 5-HT_4 receptors, induced contractions from 3 μM onwards, yielding a steep concentration-response curve (maximum effect of 66.8 ± 4.2% at 100 μM). The 2-methyl-5-HT-induced

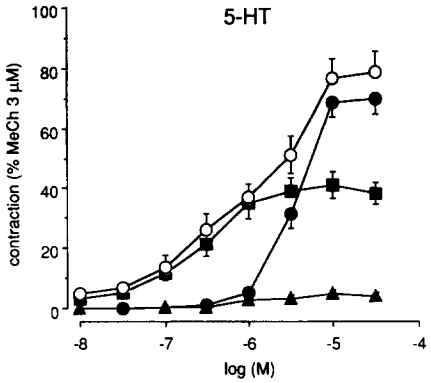

Fig. 1. Concentration-response curves of contractions in response to 5-hydroxytryptamine (5-HT): in the absence of antagonists (○), and in the presence of 1 μM granisetron (■), 10 nM SB 204070 (●) or both SB 204070 and granisetron (▲). Means and the standard errors are depicted ($n = 6–14$), and the results are expressed as percentages of contractions to 3 μM methacholine (MeCh). Methysergide (1 μM) was present in the bath solution during all experiments.

contractions were abolished by granisetron (1 μM) (Fig. 2), but were not affected by SB 204070 (10 nM) ($n = 4$; results not shown). 5-Methoxytryptamine, an agonist at 5-HT_4 but not 5-HT_3 receptors, induced contractions from 30 nM onwards, yielding a monophasic curve with a maximum response (42.1 ± 3.0%) at 30 μM 5-methoxytryptamine. The 5-methoxytryptamine-induced contractions were abolished by SB 204070 (10 nM) (Fig. 2), but were not affected by granisetron (1 μM) ($n = 4$; results not shown). Hence, under these circumstances, granisetron (1 μM) and SB 204070 (10 nM) can serve as tools for selective pharmacological isolation of either 5-HT_3 or 5-HT_4 receptors.

3.1. Block of ganglionic cholinergic transmission

The ganglionic nicotinic cholinoceptor blocker, hexamethonium (100 μM), did not significantly affect the 5-HT-induced contractions in the presence of SB 204070 (10 nM) or the 2-methyl-5-HT-induced contractions (Fig. 2). On the other hand, the 5-HT-induced contractions in the presence of granisetron (1 μM), and the 5-MeOT-induced contractions were approximately halved in the presence of hexamethonium (100 μM), causing a depression of their respective concentration-response curves (Fig. 2).

3.2. Block of nervous conductance and muscarinic cholinergic transmission

Tetrodotoxin (0.3 μM) did not significantly affect the contractions in response to 5-HT (Fig. 3) in the presence of SB 204070 (10 nM) or those in response to 2-methyl-5-HT (except at 10 μM 2-methyl-5-HT) (Fig. 2). In contrast, tetrodotoxin abolished the 5-HT-induced contractions in the presence of granisetron (1 μM) (Fig. 3) as well as those to 5-MeOT (Fig. 2). Atropine (0.3 μM) inhibited the

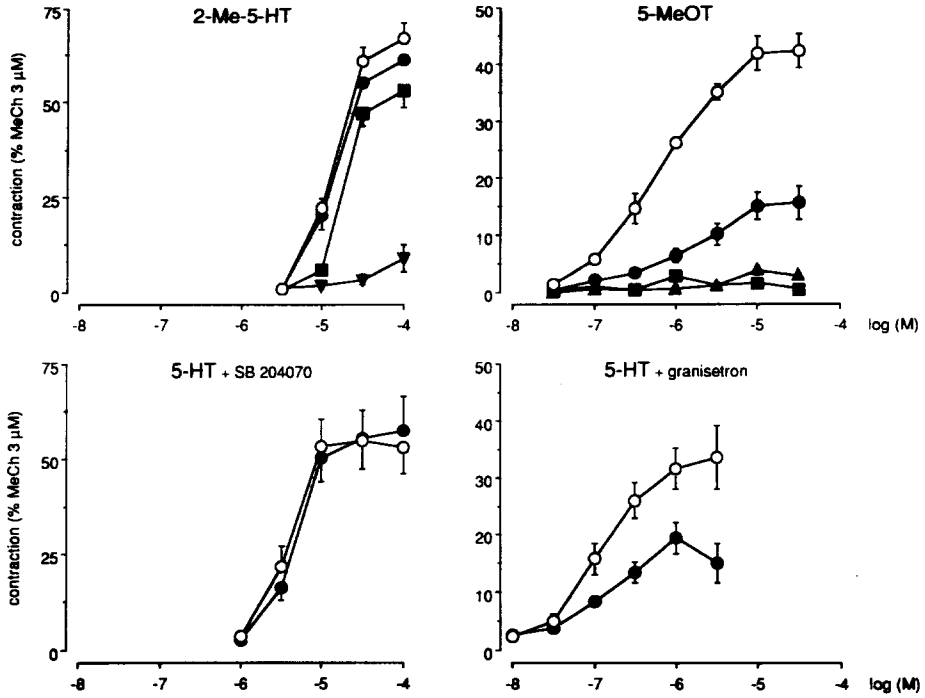

Fig. 2. Concentration-response curves of contractions in response to 2-methyl-5-hydroxytryptamine (2-methyl-5-HT; *upper left panel*), 5-methoxytryptamine (*upper right panel*) and 5-hydroxytryptamine (5-HT; + 10 nM SB 204070, *lower left panel*; + 1 μM granisetron, *lower right panel*), in the absence of antagonists (○), and in the presence of 0.3 μM tetrodotoxin (■), 100 μM hexamethonium (●), 10 nM SB 204070 (▲) or 1 μM granisetron (▼). Means and the standard errors are depicted ($n = 6$), and the results are expressed as percentages of contractions to 3 μM methacholine (MeCh). Methysergide (1 μM) was present in the bath solution during all experiments.

5-HT-induced contractions in the presence of either SB 204070 (10 nM) or granisetron (1 μM) by about 50% (Fig. 3). In the presence of SB 204070 (10 nM) and tetrodotoxin (0.3 μM), atropine (0.3 μM) still significantly inhibited the contractions in response to 5-HT by about 75% (Fig. 3). Conversely, in the presence of SB 204070 (10 nM) and atropine (0.3 μM), tetrodotoxin (0.3 μM) tended to depress the contractions to 5-HT further, but this was never statistically significant (Fig. 3).

3.3. Putative involvement of substance P or neurokinin A

The remaining contractions to 5-HT in the presence of atropine (0.3 μM) and SB 204070 (10 nM) together were

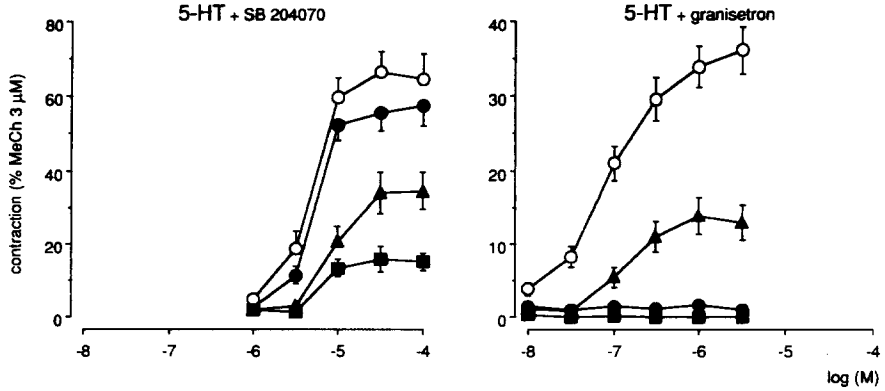

Fig. 3. Concentration-response curves of contractions in response to 5-hydroxytryptamine (5-HT; + 10 nM SB 204070, *left panel*; + 1 μM granisetron, *right panel*): in the absence of antagonists (○), and in the presence of 0.3 μM atropine (▲), 0.3 μM tetrodotoxin (●), or tetrodotoxin and atropine (■). Means and the standard errors are depicted ($n = 6$–8), and the results are expressed as percentages of contractions to 3 μM methacholine (MeCh). Methysergide (1 μM) was present in the bath solution during all experiments.

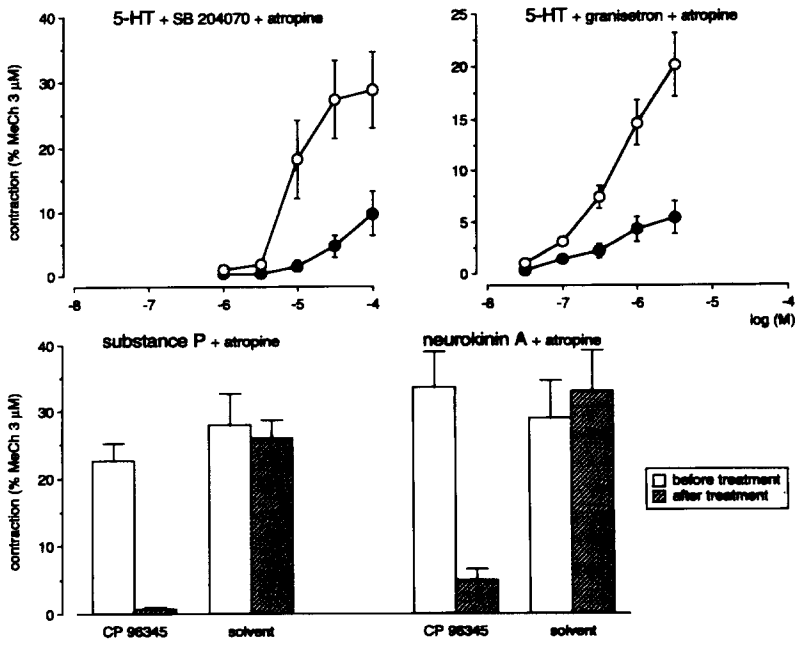

Fig. 4. Concentration-response curves of contractions in response to 5-hydroxytryptamine (5-HT; +10 nM SB 204070 + 0.3 μM atropine, *left upper panel*; +1 μM granisetron +0.3 μM atropine, *right upper panel*): in the absence of antagonists (○), and in the presence of 0.1 μM CP 96345 (●). In the *lower panel*, the bars represent the contraction, in the presence of atropine, in response to 10 nM substance P (*left*) or 30 nM neurokinin A (*right*) before (open bars) and after (hatched bars) treatment with 0.1 μM CP 96345 or solvent. Means and the standard errors are depicted ($n = 6–8$), and the results are expressed as percentages of contractions to 3 μM methacholine (MeCh). Methysergide (1 μM) was present in the bath solution during the experiments with 5-HT.

almost completely inhibited by CP 96345 (0.1 μM; optimum specific concentration for this tissue, see Briejer et al., 1993b) (Fig. 4). CP 96345 (0.1 μM) had similar effects against the remaining contractions to 5-HT in the presence of atropine (0.3 μM) and granisetron (1 μM) together. Substance P (10 nM) and neurokinin A (30 nM) both induced contractions that had a slightly higher amplitude than the remaining contractions due to 5-HT_3 or 5-HT_4 stimulation in the presence of atropine (0.3 μM) (Fig. 4). These contractions were reproducible (Fig. 4). In some cases, the contraction in response to neurokinin A was preceded by a small relaxation. CP 96345 (0.1 μM) abolished the contractions to substance P (10 nM) (Fig. 4). The contractions in response to neurokinin A (30 nM) were strongly inhibited by CP 96345, and the preceding relaxation was much more prominent. In the presence of atropine (0.3 μM), neither granisetron (1 μM) nor SB 204070 (10 nM) affected the contractions to substance P (10 nM) or neurokinin A (30 nM) ($n = 6$; results not shown), demonstrating the lack of interference of these 5-HT receptor antagonists with the tachykinin receptors involved.

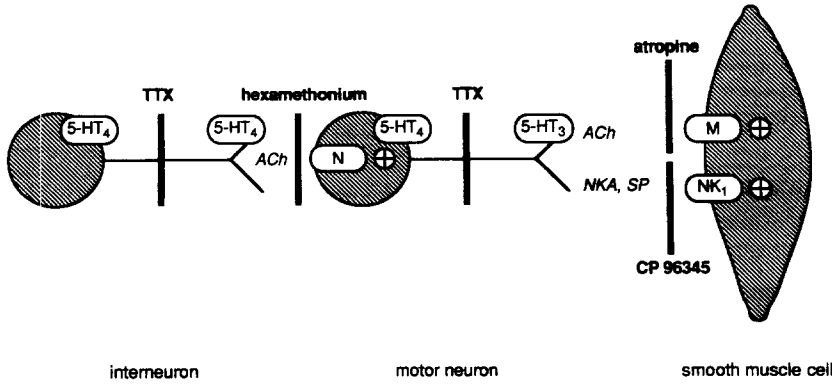

Fig. 5. Schematic representation of the localization of 5-HT_3 and 5-HT_4 receptors, and the pathways involved in the contraction following their stimulation, based upon an interpretation of the current findings. An interneuron and a motor neuron, presumably of the myenteric plexus, and a longitudinal smooth muscle cell are depicted. The bold bars represent a block due to pharmacological intervention in this study. See Discussion for an explanation of the depicted (deduced) findings. Abbreviations: TTX, tetrodotoxin; ACh, acetylcholine; N, nicotinic cholinoceptor; NKA, neurokinin A; SP, substance P.

4. Discussion

The current study revealed some interesting differences between the pathway to contraction upon either 5-HT_3 or 5-HT_4 receptor selective stimulation.

Given the lack of effect of hexamethonium, 5-HT_3 receptors seem to be located primarily on motor neurons as they do not seem to depend upon interneurons which synapse in the myenteric ganglia. Tetrodotoxin was generally ineffective to inhibit the 5-HT_3 receptor-mediated responses, although there was some inhibition at one concentration of 2-methyl-5-HT and a tendency to inhibit 5-HT-induced 5-HT_3 receptor-mediated contractions in the presence of atropine. The concentration of tetrodotoxin (0.3 μM) was sufficient however, as tetrodotoxin at the same concentration did abolish the 5-HT_4 receptor-mediated contractions. Action-potential propagation along axons is Na^+-mediated and can therefore be blocked by the concentration of tetrodotoxin used. However, tetrodotoxin-insensitive (Ca^{2+}-mediated) action potentials have been observed in a small subset of myenteric neurons (Hirst and Spence, 1973; North, 1973). It is also conceivable that tetrodotoxin-insensitive release occurs due to stimulation of receptors localized on the nerve endings (Al-Humayyd and White, 1985). Indeed, in the presence of tetrodotoxin, atropine was still able to strongly inhibit the remaining contraction upon 5-HT_3 receptor stimulation, which demonstrates that tetrodotoxin-insensitive acetylcholine release can be elicited (at the concentration that was used atropine does not bind to 5-HT_3 receptors: pK_i at 5-HT_3 = 6.2; Schiavi et al., 1994). It is thus proposed (analogous with a previous study: Briejer et al., 1993a) that the 5-HT_3 receptor-mediated contraction is largely due to stimulation of 5-HT_3 receptors that are localized on the nerve endings of the motor neurons (see Fig. 5), although also a small population of 5-HT_3 receptors may be localized proximal to the nerve endings.

The 5-HT_4 receptor-mediated contraction seems to involve interneurons as well as motor neurons, as a partial block due to hexamethonium was seen. Tetrodotoxin completely prevented the 5-HT_4 receptor-mediated effects. This suggests that, on the motor neurons, 5-HT_4 receptors must be localized at the soma (more precisely: proximal to the nerve endings) (see Fig. 5). With respect to the 5-HT_4 receptors on the interneurons, it cannot be definitely concluded whether they are present on the nerve endings or on the soma.

Electrophysiological experiments with myenteric neurons of the guinea-pig ileum and stomach have revealed three types of response upon 5-HT application: fast depolarization and a decrease in membrane resistance due to 5-HT_3 receptor stimulation, slow depolarization and an increase in membrane resistance due to putative 5-HT_{1P} receptor stimulation and hyperpolarization with concomitant decrease in membrane resistance due to 5-HT_{1A} receptor activation (Nemeth et al., 1985; Mawe et al., 1986; Pan and Galligan, 1994, 1995). No effects on input resistance or membrane potential have been observed that appeared to be mediated by 5-HT_4 receptors. However, focal stimulation of interganglionic (nicotinergic) cholinergic fiber tracts induces fast excitatory post-synaptic potentials (EPSPs) that can be ascribed to 5-HT_4 receptor stimulation (Tonini et al., 1989; Pan and Galligan, 1994). From these results it has been concluded that the 5-HT_4 receptors are not present on the nerve cell bodies of either interneurons or motor neurons, but rather on the nerve endings. This view does not entirely overlap with our findings which suggest a somal rather than a nerve ending localization (at least with respect to the motor neurons). Furthermore, electrophysiology predicts that at least a large part of the 5-HT_3 receptors are on the nerve cell body of interneurons and motor neurons, but our experiments suggest a preferential nerve ending localization mainly on motor neurons. These differences between results of functional and electrophysiological experiments might indicate that, in functional experiments, effects on motor neurons are much more important and might mask effects on interneurons, whereas in electrophysiological experiments interneurons are probably much more in evidence than motor neurons. Furthermore, the electrophysiological data were obtained using myenteric neurons from the ileum and to a lesser extent the stomach. The colon might differ with respect to the distribution of 5-HT receptor subtypes.

Previously, we have shown that exogenous substance P causes a contraction of the colon longitudinal muscle by direct activation of tachykinin NK_1 receptors on the smooth muscle (the pA_2 of CP 96345 was found to be 8.9); tetrodotoxin and atropine had no effect (Briejer et al., 1993b). Although neurokinin A is tachykinin NK_2 receptor preferring, it also has affinity and activity at tachykinin NK_1 and NK_3 receptors. Indeed, it was found that, in the presence of atropine, the contraction in response to neurokinin A was strongly inhibited by CP 96345 at a concentration of 0.1 μM, a concentration previously found to be devoid of non-specific inhibitory effects in this tissue (Briejer et al., 1993b). However, not all of the contraction to neurokinin A was inhibited, which could be due either to an effect mediated by tachykinin NK_2 or NK_3 receptors, or an insufficient concentration of CP 96345. The latter possibility can be excluded, as contractions induced by substance P exceeding those seen to 5-HT in the presence of atropine, were blocked by this concentration of CP 96345. The relaxation response to neurokinin A was due to an effect on the nerves, as tetrodotoxin abolished these relaxations. The receptor that was involved was not further investigated (but was not a tachykinin NK_1 receptor as CP 96345 did not inhibit the remaining response), but there was a similar observation for the guinea-pig ileum (Burcher and Stamatakos, 1994). Nevertheless, the effectiveness of CP 96345 against substance P- and neurokinin A-induced contractions on one hand, and against the 5-HT-induced contractions (all in the presence of

atropine) on the other hand, does not allow us to conclude that only one of the tachykinins is involved. Conversely, it is likely that one or more tachykinins are involved in the contraction to 5-HT_3 and 5-HT_4 receptor stimulation in the colon, acting on smooth muscle tachykinin NK_1 receptors.

Colocalization of tachykinins and acetylcholine implies that it should be possible to enhance both non-cholinergic and cholinergic contractions by stimulation of 5-HT_4 receptors in the guinea-pig gut. However, some conflicting data exist with respect to this issue. Reports on functional experiments with the guinea-pig colon show that 5-HT_4 receptor stimulation can enhance electrically induced non-cholinergic neurogenic contractions (Kojima and Shimo, 1995). The current experiments with colon also showed that 5-HT_4 receptor stimulation evokes a tachykinin-mediated contraction. In contrast, experiments done with ileum preparations did not provide evidence that 5-HT_4 receptors influence non-cholinergic electrically induced twitch contractions. In the presence of a 5-HT_3 receptor antagonist, neither 5-HT nor 5-carboxamidotryptamine (which is a full 5-HT_4 receptor agonist, approximately 30–100 times less potent than 5-HT; see Bockaert et al., 1992) induced any enhancement of electrically induced non-cholinergic twitch contractions, even after block of release-inhibiting 5-HT_{1A} receptors (Galligan, 1992). In similar experiments done in our laboratory, 5-methoxytryptamine also did not affect non-cholinergic twitch responses in a similar experiment on the ileum longitudinal muscle-myenteric plexus preparation (unpublished observations). In a subset of myenteric neurons, one can measure after interganglionic fiber tract stimulation, slow EPSPs that are mediated by tachykinins or 5-HT. In contrast to cholinergic fast EPSPs which can be measured in a different subset of neurons, 5-HT_4 receptor stimulation could not enhance the non-cholinergic slow EPSPs (Pan and Galligan, 1995). However, Ramírez et al. (1994) have reported that, in a similar ileum preparation, both 2-methyl-5-HT and 5-methoxytryptamine induced contractions that were only partially blocked by atropine. It is not clear why stimulation of 5-HT_4 receptors in some but not in other cases, induces an enhancement of both cholinergic and non-cholinergic transmission.

A thorough understanding of the precise role of 5-HT_3 and 5-HT_4 receptors in gastrointestinal motility is essential. 5-HT_3 or 5-HT_4 agonists and antagonists have organ-specific and species-dependent effects on gastrointestinal motility. Investigations such as the current study might eventually contribute to the development of future prokinetic drugs, possibly with organ specificity.

It can be concluded that, in the guinea-pig colon, 5-HT_3 receptors are probably localized primarily on the nerve endings of the motor neurons. 5-HT_4 receptors are present on both the interneurons and motor neurons; they are localized on the soma of the motor neurons, but no such conclusion could be drawn for the interneurons on the basis of the current findings. Stimulation of either 5-HT receptor elicited a contraction that was mediated by acetylcholine and to a lesser extent by a tachykinin acting on smooth muscle tachykinin NK_1 receptors. This tachykinin-mediated response could be ascribed to substance P and/or neurokinin A.

References

Al-Humayyd, M. and T.D. White, 1985, 5-Hydroxytryptamine releases adenosine 5′-triphosphate from nerve varicosities isolated from the myenteric plexus of the guinea-pig ileum, Br. J. Pharmacol. 84, 27.

Bockaert, J., J.R. Fozard, A. Dumuis and D.E. Clarke, 1992, The 5-HT_4 receptor: a place in the sun, Trends Pharmacol. Sci. 13, 141.

Briejer, M.R., L.M.A. Akkermans, A.L. Meulemans, R.A. Lefebvre and J.A.J. Schuurkes, 1993a, Pharmacological characterization of 5-hydroxytryptamine receptor types in the guinea-pig proximal colon, J. Gastroint. Motil. 5, 239.

Briejer, M.R., L.M.A. Akkermans, A.L. Meulemans, R.A. Lefebvre and J.A.J. Schuurkes, 1993b, Substance P-induced contractions of the guinea-pig proximal colon through stimulation of post-junctional tachykinin NK_1 receptors, Eur. J. Pharmacol. 250, 181.

Briejer, M.R., L.M.A. Akkermans, R.A. Lefebvre and J.A.J. Schuurkes, 1995, Novel 5-HT_2-like receptor mediates neurogenic relaxation of the guinea-pig proximal colon, Eur. J. Pharmacol. 279, 123.

Brookes, S.J.H., Z.-M. Song, P.A. Steele and M. Costa, 1992, Identification of motor neurons to the longitudinal muscle of the guinea pig ileum, Gastroenterology 103, 961.

Buchheit, K.-H., G. Engel, E. Mutschler and B. Richardson, 1985, Study of the contractile effect of 5-hydroxytryptamine (5-HT) in the isolated longitudinal muscle strip from guinea-pig ileum. Evidence for two distinct release mechanisms, Naunyn-Schmied. Arch. Pharmacol. 329, 36.

Burcher, E. and C. Stamatakos, 1994, Septide but not substance P stimulates inhibitory neurons in guinea-pig ileum, Eur. J. Pharmacol. 258, R9.

Butler, A., C.J. Elswood, J. Burridge, S.J. Ireland, K.T. Bunce, G.J. Kilpatrick and M.B. Tyers, 1990, The pharmacological characterization of 5-HT_3 receptors in three isolated preparations derived from guinea-pig tissues, Br. J. Pharmacol. 101, 591

Craig, D.A. and D.E. Clarke, 1990, Pharmacological characterization of a neuronal receptor for 5-hydroxytryptamine in guinea pig ileum with properties similar to the 5-hydroxytryptamine$_4$ receptor, J. Pharmacol Exp. Ther. 252, 1378.

Deacon, C.F., D.V. Agoston, R. Nau and J.M. Conlon, 1987, Conversion of neuropeptide K to neurokinin A and vesicular colocalization of neurokinin A and substance P in neurons of the guinea pig small intestine, J. Neurochem. 48, 141.

Elswood, C.J., K.T. Bunce and P.P.A. Humphrey, 1991, Identification of putative 5-HT_4 receptors in guinea-pig ascending colon, Eur. J. Pharmacol. 196, 149.

Eglen, R.M., S.R. Swank, L.K.M. Walsh and R.L. Whiting, 1990, Characterization of 5-HT_3 and 'atypical' 5-HT receptors mediating guinea-pig ileal contractions in vitro, Br. J. Pharmacol. 101, 513.

Fozard, J.R., 1990, Agonists and antagonists of 5-HT_3 receptors., in: Cardiovascular Pharmacology of 5-Hydroxytryptamine, eds. P.R. Saxena, D.I. Wallis, W. Wouters and P. Bevan (Kluwer Academic Publishers, Dordrecht) p. 101.

Furness, J.R. and M. Costa, 1987, The Enteric Nervous System (Churchill Livingstone, Edinborough).

Galligan, J.J., 1992, Differential inhibition of cholinergic and noncholinergic neurogenic contractions by 5-hydroxytryptamine$_{1A}$ receptor agonists in guinea pig ileum, J. Pharmacol. Exp. Ther. 260, 306.

Gershon, M.D. and S.M. Erde, 1981, The nervous system of the gut, Gastroenterology 80, 1571.

Gershon, M.D., G.M. Mawe and T.A. Branchek, 1989, 5-Hydroxy-

tryptamine and enteric neurones., in: The Peripheral Actions of 5-Hydroxytryptamine, ed. J.R. Fozard (Oxford University Press, Oxford, UK) p. 247.

Hirst, G.D.S. and I. Spence, 1973, Calcium action potentials in mammalian peripheral neurones, Nature New Biol. 243, 54.

Holzer-Petsche, U., 1995, Tachykinin receptors in gastrointestinal motility, Reg. Pept. 57, 19.

Kilbinger, H. and D. Wolf, 1992, Effects of 5-HT_4 receptor stimulation on basal and electrically evoked release of acetylcholine from guinea-pig myenteric plexus, Naunyn-Schmied. Arch. Pharmacol. 345, 270.

Kojima, S. and Y. Shimo, 1995, An enhancing effect of 5-hydroxytryptamine on electrically evoked atropine-resistant contraction of guinea-pig proximal colon, Br. J. Pharmacol. 114, 73.

Mawe, G.M., T.A. Branchek and M.D. Gershon, 1986, Peripheral neural serotonin receptors: identification and characterization with specific antagonists and agonists, Proc. Natl. Acad. Sci. USA 83, 9799.

Nemeth, P.R., C.A. Ort, D.H. Zafirov and J.D. Wood, 1985, Interactions between serotonin and cisapride on myenteric neurons, Eur. J. Pharmacol. 108, 77.

North, R.A., 1973, The calcium-dependent slow after-hyperpolarization in myenteric plexus neurones with tetrodotoxin-resistant action potentials, Br. J. Pharmacol. 49, 709.

Otsuka, M. and K. Yoshioka, 1993, Neurotransmitter functions of mammalian tachykinins, Physiol. Rev. 73, 229.

Pan, H. and J.J. Galligan, 1994, 5-HT_{1A} and 5-HT_4 receptors mediate inhibition and facilitation of fast synaptic transmission in enteric neurons, Am. J. Physiol. 266, G230.

Pan, H. and J.J. Galligan, 1995, Effects of 5-HT_{1A} and 5-HT_4 receptor agonists on slow synaptic potentials in enteric neurons, Eur. J. Pharmacol. 278, 67.

Ramírez, M.J., E. Cenarruzabeitia, J. Del Río and B. Lasheras, 1994, Involvement of neurokinins in the non-cholinergic response to activation of 5-HT_3 and 5-HT_4 receptors in guinea-pig ileum, Br. J. Pharmacol. 111, 419.

Schiavi, G.B., S. Brunet, C.A. Rizzi and H. Ladinsky, 1994, Identification of serotonin 5-HT_4 recognition sites in the porcine caudate nucleus by radioligand binding, Neuropharmacology 33, 543.

Shuttleworth, C.W.R., R. Murphy, J.B. Furness and S. Pompolo, 1991, Comparison of the presence and actions of substance P and neurokinin A in guinea-pig taenia coli, Neuropeptides 19, 23.

Snider, M.R., J.A. Constantine, J.A. Lowe III, K.P. Longo, W.S. Lebel, H.A. Woody, S.E. Drozda, M.C. Desai, F.J. Vinick, R.W. Spencer and Hess H.-J., 1991, A potent nonpeptide antagonist of the substance P (NK1) receptor, Science 251, 435.

Theodorsson-Norheim, E., X. Hua, E. Brodin and J.M. Lundberg, 1985, Capsaicin treatment decreases tissue levels of neurokinin A-like immunoreactivity in the guinea-pig, Acta Physiol. Scand. 124, 129.

Tonini, M., J.J. Galligan and R.A. North, 1989, Effects of cisapride on cholinergic neurotransmission and propulsive motility in the guinea-pig ileum, Gastroenterology 96, 1257.

Too, H.-P., J.L. Cordova and J.E. Maggio, 1989, A novel radioimmunoassay for neuromedin K. I. Absence of neuromedin K-like immunoreactivity in guinea pig ileum and urinary bladder. II. Heterogeneity of tachykinins in guinea pig tissues, Reg. Pept. 26,93.

Wardle, K.A. and G.J. Sanger, 1993, The guinea-pig distal colon - a sensitive preparation for the investigation of 5-HT_4 receptor-mediated contractions, Br. J. Pharmacol. 110, 1593.

Wardle, K.A. and G.J. Sanger, 1994, The effects of SB 204070, a highly potent and selective 5-HT_4 receptor antagonist, on guinea-pig distal colon, Br. J. Pharmacol. 112, 789.