5-HT$_4$ receptors on cholinergic nerves involved in contractility of canine and human large intestine longitudinal muscle

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1 5-HT$_4$ receptors mediate circular muscle relaxation in both human and canine large intestine, but this phenomenon alone can not explain the improvement in colonic motility induced by selective 5-HT$_4$ receptor agonists *in vivo*. We set out to characterize 5-HT$_4$ receptor-mediated effects in longitudinal muscle strips of canine and human large intestine.

2 Electrical field stimulation (EFS) was applied providing submaximal isotonic contractions. L-NOARG (0.1 mM) was continuously present in the organ bath to preclude nitric oxide-induced relaxation to EFS.

3 The selective 5-HT$_4$ receptor agonist prucalopride (0.3 μM) enhanced EFS-evoked contractions, that were antagonized in both preparations by the selective 5-HT$_4$ receptor antagonist GR 113808 (0.1 μM). The prucalopride-induced increase was present in canine ascending and descending colon, but absent in rectum. Regional differences in response to prucalopride were not observed in human ascending and sigmoid colon and rectum. Incubation with atropine (1 μM) or tetrodotoxin (0.3 μM) inhibited EFS-induced contractions, which were then unaffected by prucalopride (0.3 μM) in both tissues.

4 In the presence of methysergide (3 μM; both tissues) and granisetron (0.3 μM; only human tissues), 5-HT (0.3 μM) enhanced EFS-induced contractions, an effect that was antagonized by GR 113808 (0.1 μM). In the presence of atropine or tetrodotoxin, EFS-induced contractions were inhibited, leaving 5-HT (0.3 μM) ineffective in both preparations.

5 This study demonstrates for the first time that in human and canine large intestine, 5-HT$_4$ receptors are located on cholinergic neurones, presumably mediating facilitating release of acetylcholine, resulting in enhanced longitudinal muscle contractility. This study and previous circular muscle strip studies suggest that 5-HT$_4$ receptor agonism facilitates colonic propulsion *via* a coordinated combination of inhibition of circumferential resistance and enhancement of longitudinal muscle contractility.

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Abbreviations: EFS, electrical field stimulation; TTX, tetrodotoxin

Introduction

Since their discovery in mouse colliculi neurones (Dumuis *et al.*, 1988), the study of 5-HT$_4$ receptors in various tissues, cell lines and cell structures has provided a bulk of locations and actions of, and hypotheses around, 5-HT$_4$ receptors. To start with, the 5-HT$_4$ receptor subtype may be a generic term for up to 14 splice variants, that in theory may have different localization, transductional mechanisms and even operational characteristics (Gerald *et al.*, 1995; Blondel *et al.*, 1998; Bender *et al.*, 2000). It remains to be determined, however, whether the observed differences at the protein level in cells and in distribution of splice variant mRNA in tissues correlate to functional responses across tissues or even across intact species. Nevertheless, if one considers 5-HT$_4$ receptors in bioassays, their distributions and actions do differ markedly across species and across tissues.

In bioassays, it was demonstrated that 5-HT$_4$ receptors are abundantly present in the gut (for review: Hegde & Eglen, 1996), but were also found in human brain (Reynolds *et al.*, 1995; by means of binding studies), heart (Kaumann, 1993), adrenal cortex (Lefebvre *et al.*, 1998) and bladder (Candura *et al.*, 1996). In the gut, rat oesophagus muscularis mucosae contains smooth muscle 5-HT$_4$ receptors mediating relaxation (Baxter *et al.*, 1991), an effect suggested to be absent in the guinea-pig and dog (Cohen *et al.*, 1994). In the human lower oesophageal sphincter, there is evidence for the presence of excitatory 5-HT$_4$ receptors on the cholinergic nerves (Tomita *et al.*, 1997). In the gastric area, contractile 5-HT$_4$ receptors have been found on the cholinergic nerves in the guinea-pig (Buchheit & Buhl, 1994), dog (Bingham *et al.*, 1995) and, based on preliminary data, man (Schuurkes *et al.*, 1991). In the small intestine, 5-HT$_4$ receptors mediate mucosal secretion (Budhoo *et al.*, 1996) and smooth muscle relaxation (Kuemmerle *et al.*, 1995). Coming down to the large intestine, 5-HT$_4$ receptors were found on circular smooth muscle, mediating relaxation (Meulemans *et al.*, 1995; McLean *et al.*, 1995; Tam *et al.*, 1994; Prins *et al.*, 2000), and on the mucosa mediating secretion (Borman & Burleigh, 1996).

However, circular muscle relaxation alone can not only explain the improvement of propulsive large intestinal motility observed after administration of selective 5-HT$_4$ receptor agonists *in vivo*. In the dog chronically equipped with force

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transducers on the serosal side of the large intestine, a model frequently used to study the influence of candidate drugs on gastrointestinal motility, 5-HT$_4$ receptor stimulation resulted in enhancement of contractility in the ascending portion of the large intestine and an inhibition in the rectum (Briejer *et al.*, 1998a,b). Recently, we reported this gradual increase in 5-HT$_4$ receptor-mediated inhibition towards the distal large intestine to align our results with canine large intestinal circular muscle strips *in vitro*, where the relaxation was almost absent in the ascending colon and most pronounced in the rectal portion (Prins *et al.*, 1999). However, the enhancement of proximal contractility observed *in vivo* was not explained. In view of the positive coupling of 5-HT$_4$ receptors to adenylate cyclase, we hypothesized that an increase in colon muscle contractility could be due to 5-HT$_4$ receptors facilitating release of a contractile neurotransmitter.

In order to test these hypotheses in dogs and humans, we set out to characterize the responses to the endogenous ligand 5-HT and the selective 5-HT$_4$ receptor agonist prucalopride in electrical field-stimulated longitudinal muscle strips of canine and human large intestine.

Methods

Preparation

Fifteen Beagle dogs (13 female, 2 male, weighing 7–14 kg) were used. Ten of these dogs, that had been previously used for gastrointestinal *in vivo* studies, were allowed a two-week recovery period, after which they were sacrificed on the day of the experiment by decerebration and immediate desanguination through the carotid artery. The other five dogs had been used in cardiovascular *in vivo* studies (solvent treatment) the day before the experiment and were anaesthetized with pentobarbital (30 mg kg^{-1} i.v.), and, subsequently, sacrificed with KCl (150 mg kg^{-1} i.v.). There were no differences in any responses observed between tissue from dogs sacrificed either the day before or on the day of the experiment. The canine entire large intestine was isolated and approximate 3-cm sections of ascending colon (1 cm distal to the ileocolonic junction), rectum (at the point where the urethra (male) or vagina (female) is adhered to the rectum) and descending colon (in the middle between the ascending colonic and rectal portion) were dissected and mucosa and mesentery was cut away. Tissue taken from dogs sacrificed the day before the experiment was stored overnight in Krebs-Henseleit solution (containing (in mM) glucose 11.1; CaCl$_2$ 2.51; NaHCO$_3$ 25; MgSO$_4$ 1.18; KH$_2$PO$_4$ 1.18; KCl 4.69 and NaCl 118) at 4°C. Human large intestinal specimens were obtained from patients undergoing surgery for colonic cancer, untreatable constipation or diverticular disease (ascending colon $n=4$; sigmoid colon $n=13$; rectum $n=6$) with the approval of the local ethics committee. The specimens were cut open longitudinally and the luminal contents, if present, were rinsed out with Krebs-Henseleit solution. Mucosa and adhering mesentery were cut away and the remaining muscle tissue was stored overnight in Krebs-Henseleit solution at 4°C.

Muscle strips (of the *taeniae coli* in case of human tissue) were cut in the longitudinal direction, each measuring approximately 2–3 mm width and 2–3 cm length. The strips were mounted onto organ bath hooks equipped for electrical field stimulation (EFS) with coaxial, platina-wire electrodes, and placed in an organ bath set-up for measurement of isotonic displacement under a load of 2 g.

Protocol

The strips were allowed a stabilization period of 30 min, after which carbachol (10 μM) was added twice to contract the tissue. Each addition of carbachol was followed by a period of time necessary to reach the maximal contraction, followed by replacing the organ bath solution twice with fresh Krebs-Henseleit solution.

Preliminary experiments showed that EFS could induce relaxations as well as contractions in both preparations. These relaxations were not sensitive to addition of 5-HT (0.3 μM) or prucalopride (0.3 μM), but they were prevented by incubation of L-NOARG (0.1 mM). Additionally, L-NOARG improved reproducibility of contractions to EFS. Thus, for further experimentation L-NOARG (0.1 mM) was added to the organ bath solution to preclude nitric oxide-induced relaxation to isolate contractions to EFS. In the case where 5-HT was used, methysergide (3 μM) was included in the organ bath solution to prevent interactions of 5-HT with 5-HT$_1$, 5-HT$_2$, 5-ht$_5$, 5-HT$_6$ or 5-HT$_7$ receptors. In the case where 5-HT was tested in human tissue, granisetron (0.3 μM) was present as well to preclude 5-HT$_3$ receptor interactions.

After 30 min of incubation in the presence of L-NOARG and/or methysergide or granisetron, the strips were electrically stimulated by pulses of 1 ms width in trains of 10 s duration at an interval of 3 min with a frequency of 50 Hz and a voltage of 20 V (one pulse train). Then, the frequency was set to 20 Hz (canine tissue) or 12 Hz (human tissue) and the voltage was reduced until approximately 50% of the contraction due to EFS at 50 Hz and 20 V. The submaximal EFS-induced contractions were followed until at least five reproducible contractions (spanning a time period of 15 min) were observed, and then antagonist or solvent was added. Following an incubation period of 15 min, equal to five EFS pulse trains, single doses of agonists were applied and the response to EFS was followed for another five pulse trains (equal to 15 min). Each strip was used to assess the effect of a single treatment on the effect of an agonist. Some strips were used to test the effect of pre-incubation with prucalopride (0.3 μM) against the cumulative concentration–contraction curve to exogenously added acetylcholine (30 nM–1 mM)

Data analysis

For statistical analysis and graphical presentation, the average of the contractions due to five EFS pulse trains observed prior to the addition of antagonist was taken as 100% contraction (called the initial value for graphical presentation) and all contractions (15 in total per muscle strip) were expressed as a percentage of this. Repeated measures over time (longitudinal experimental design) were analysed using PROC MIXED (SAS PC v 6.12) for balanced data with an unstructured covariance matrix, with a level of $P<0.05$ considered to indicate significance. The number of specimens used was denoted by n.

Compounds

The following compounds were used (with their abbreviations, if any, in italics, and respective suppliers given in parentheses): Tetrodotoxin (*TTX*), acetylcholine, 5-hydroxytryptamine creatinine sulphate (*5-HT*; Serva, Germany), atropine sulphate, N^G-nitro-L-arginine (*L-NOARG*, Janssen Chimica, Belgium), [1-[2-[(methylsulphonyl)amino]ethyl]-4-piperidinyl]-methyl 1-methyl-1H-indole-3-carboxylate (GR 113808), granisetron HCl, 4-amino-5-chloro-2,3-dihydro-N-(1-[3-methoxypropyl]-4-piperidinyl)-7-benzofurancarboxamide HCl (prucalopride;

R093877; Janssen Research Foundation), methysergide maleate (Sandoz, Switzerland). All compounds were dissolved and diluted freshly on the day of the experiment in 0.9% NaCl solution, except for GR 113808 and cisapride, which were dissolved in 0.9% NaCl acidified with tartaric acid in the stock solution. The solvents had no effect on the baseline length or the effect of agonists.

Results

Canine large intestine longitudinal muscle

Carbachol (10 μM) produced a stable contraction of ascending, descending and rectum longitudinal muscle strips. In ascending colon, prucalopride (0.3 μM) was without effect on basal muscle length, but in descending colon and rectum, prucalopride induced a small relaxation. Presence of L-NOARG (0.1 mM) did not affect these relaxations.

In the presence of L-NOARG (0.1 mM), electrical field stimulation resulted in stable (ascending and descending colon) and variable (rectum) contractions. The EFS-induced contractions of ascending and descending colon but not those of rectal muscle strips were enhanced by addition of prucalopride (0.3 μM) or 5-HT (0.3 μM) (Figures 1 and 2). As in dog ascending colon prucalopride did not induce a relaxation, and reproducible contractions to EFS were observed, we used ascending colon to perform further experimentation with.

In the presence of L-NOARG (0.1 mM), the selective 5-HT₄ receptor agonist prucalopride (0.3 μM) enhanced submaximal EFS-induced contractions in ascending colon (Figure 3A). The selective 5-HT₄ receptor antagonist GR 113808 (0.1 μM) did not affect the EFS-induced contractions, but antagonized the effect of prucalopride. Atropine (1 μM) and tetrodotoxin (TTX; 0.3 μM) reduced the EFS-induced contractions to a

level not significantly different from zero and prucalopride (0.3 μM) did not modify this reduction. The effects of prucalopride under the conditions described are summarized
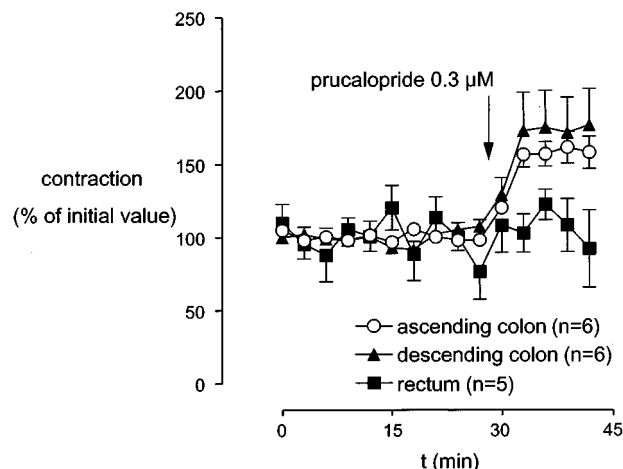
Figure 2 The effect of prucalopride on submaximal EFS-induced contractions in the presence of L-NOARG (0.1 mM) across various regions of the canine isolated large intestine longitudinal muscle. Data points are mean±s.e.mean of EFS-induced contractions.
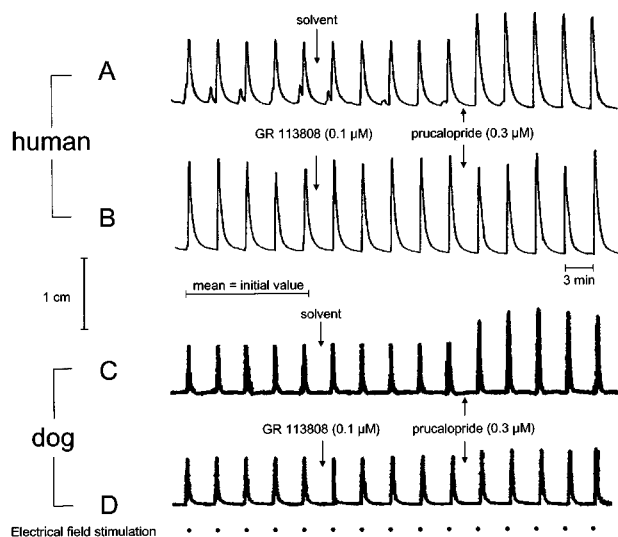
Figure 1 (A, B) Representative chart-recorder tracing of the effect of prucalopride on submaximal electrical field stimulation-induced contractions in the presence of L-NOARG (0.1 mM) of human isolated sigmoid colon (*taeniae coli*) longitudinal muscle and the effect of pre-incubation of the selective 5-HT₄ receptor antagonist GR 113808 on the effect of prucalopride. (C, D) Representative chart-recorder tracing of the effect of prucalopride on submaximal electrical field stimulation-induced contractions in the presence of L-NOARG (0.1 mM) of canine isolated ascending colon longitudinal muscle and the effect of pre-incubation of the selective 5-HT₄ receptor antagonist GR 113808 on the effect of prucalopride.
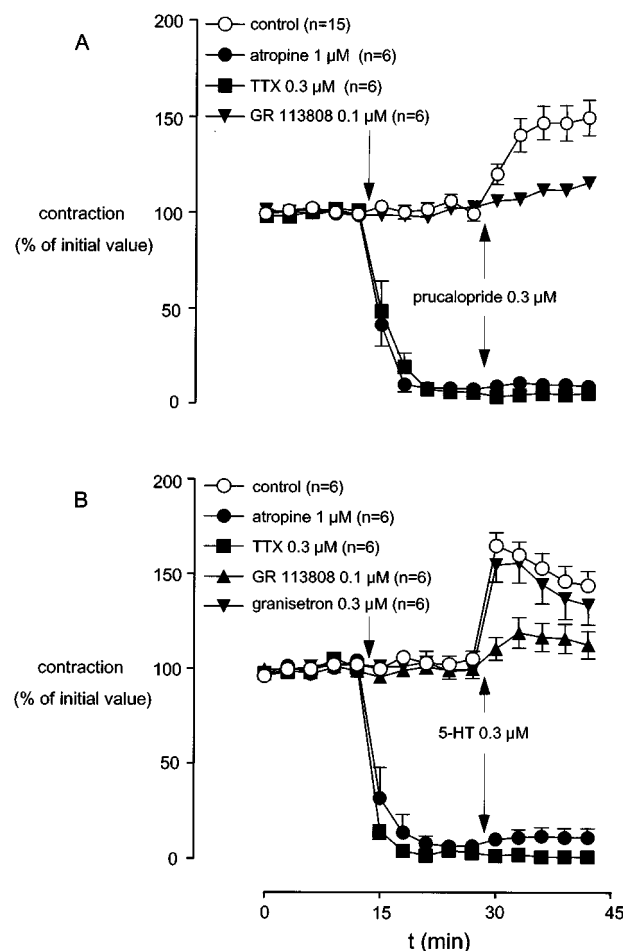
Figure 3 The effect of atropine, tetrodotoxin, granisetron and GR 113808 on the prucalopride-induced (A) or 5-HT-induced (B) enhancement of submaximal electrical field stimulation-induced contractions of canine isolated ascending colon longitudinal muscle. L-NOARG (0.1 mM) was present in the organ bath solution routinely, whereas methysergide (3 μM) was present when 5-HT was used as an agonist. Data points are mean±s.e.mean of EFS-induced contractions.

Table 1 The effect of various treatments on the 5-HT (0.3 μM) or prucalopride (0.3 μM)-induced effect on electrical field stimulated longitudinal muscle strips of canine ascending colon tissue and human taeniae coli.

Treatment	Agonist	n	Dog pre (%)	P	post (%)	P	Human pre (%)	P	post (%)	P	n
Solvent	Solvent	6	99.6±0.4	>0.05[a]	101±1.3	>0.05[b]	105±1.3	>0.05[a]	106±2.9	>0.05[b]	6
Solvent	5-HT	6	101±2.6	>0.05[a]	165±7.0	<0.01[b]	108±2.4	>0.05[a]	145±5.9	<0.01[b]	6
GR 113808 (0.1 μM)	5-HT	6	102±3.2	>0.05[a]	119±8.0	<0.01[c]	107±1.9	>0.05[a]	120±5.2	<0.01[c]	6
Granisetron (0.3 μM)	5-HT	6	107±4.1	>0.05[a]	1.57±10	>0.05[c]	–		–		–
Atropine (1 μM)	5-HT	6	1.1±1.1	<0.01[a]	2.3±1.4	>0.05[b]	10±4.1	<0.05[a]	19±6.1	>0.05[b]	6
TTX (0.3 μM)	5-HT	6	5.8±2.1	<0.01[a]	14±4.1	>0.05[b]	19±7.5	<0.05[a]	25±8.6	>0.05[b]	6
Solvent	Prucalopride	15	104±1.9	>0.05[a]	158±8.7	<0.01[b]	108±4.4	>0.05[a]	154±8.2	<0.01[b]	12
GR 113808 (0.1 μM)	Prucalopride	6	103±1.1	>0.05[a]	119±8.0	<0.01[c]	104±3.0	>0.05[a]	107±4.0	<0.01[c]	7
Atropine (1 μM)	Prucalopride	6	6.8±3.0	<0.01[a]	11±4.1	>0.05[b]	22±13	<0.01[a]	19±11	>0.05[b]	6
TTX (0.3 μM)	Prucalopride	6	5.5±2.9	<0.01[a]	5.5±2.9	>0.05[b]	17±5.3	<0.01[a]	12±5.9	>0.05[b]	6

Pre-the average maximal EFS-induced contraction during five EFS pulse trains after treatment or solvent addition. Post = the average maximal EFS-induced contraction during five EFS pulse trains after 5-HT or prucalopride addition. Discription of *P* values: [a] = the contractions over time (pre) were analysed versus the initial value. [b] = the contractions over time (post) were analysed versus the contractions over time (pre). [c] = the contractions over time (post) in the treatment group were analysed versus the contractions over time (post) in the solvent/agonist group. *n* denotes the number of dogs or patient specimens used.

in Table 1. In an additional set of experiments, prucalopride (0.3 μM) failed to affect the concentration–response curve to exogenously administered acetylcholine ($n = 3$; data not shown).

In the presence of L-NOARG (0.1 mM) and the mixed 5-HT$_1$, 5-HT$_2$, 5-ht$_5$, 5-HT$_6$ and 5-HT$_7$ receptor antagonist methysergide (3 μM), 5-HT induced an enhancement of EFS-induced contractions, which was unaffected by pre-incubation with the selective 5-HT$_3$ receptor antagonist granisetron (0.3 μM; Figure 3B). Selective 5-HT$_4$ receptor blockade induced by GR 113808 (0.1 μM) did not affect EFS-induced contractions, but this pre-treatment inhibited the enhancement evoked by 5-HT (0.3 μM). Atropine (1 μM) and TTX (0.3 μM) blocked the EFS-induced contractions and 5-HT (0.3 μM) did not modify this reduction. The 5-HT-induced effects under the conditions applied are depicted in Table 1.

Human taeniae coli longitudinal muscle

The selective 5-HT$_4$ receptor agonist prucalopride (0.3 μM) did not affect basal muscle length. In the presence of L-NOARG (0.1 mM), prucalopride (0.3 μM) enhanced submaximal EFS-induced contractions, any effect that was antagonized by the selective 5-HT$_4$ receptor antagonist GR 113808 (0.1 μM; Figure 4A). Atropine (1 μM) and TTX (0.3 μM) reduced the EFS-induced contractions to a level not significantly different from zero and prucalopride (0.3 μM) failed to modify these reductions. The effects of prucalopride under the conditions described are summarized in Table 1. Similar to that observed in canine tissue, prucalopride (0.3 μM) did not modify the concentration–contraction curve to exogenously added acetylcholine ($n = 3$; data not shown). In contrast to that observed in dog tissue, regional differences in response to prucalopride were not observed in human large intestine.

Prucalopride induced in four rectum specimens a maximum effect (expressed as maximal increase of initial value) of 150±6%, in five sigmoid colon specimens 145±5% and in three ascending colon specimens 155±6%. These prucalopride-induced effects were not significantly different.

In the presence of L-NOARG (0.1 mM) and a cocktail of methysergide (3 μM) and granisetron (0.3 μM), 5-HT induced an increase in EFS-induced contractions (Figure 4B), without alteration of basal muscle length. Again, GR 113808 (0.1 μM) did not affect EFS-induced contractions, but antagonized the enhancement by 5-HT (0.3 μM). Atropine (1 μM) and TTX
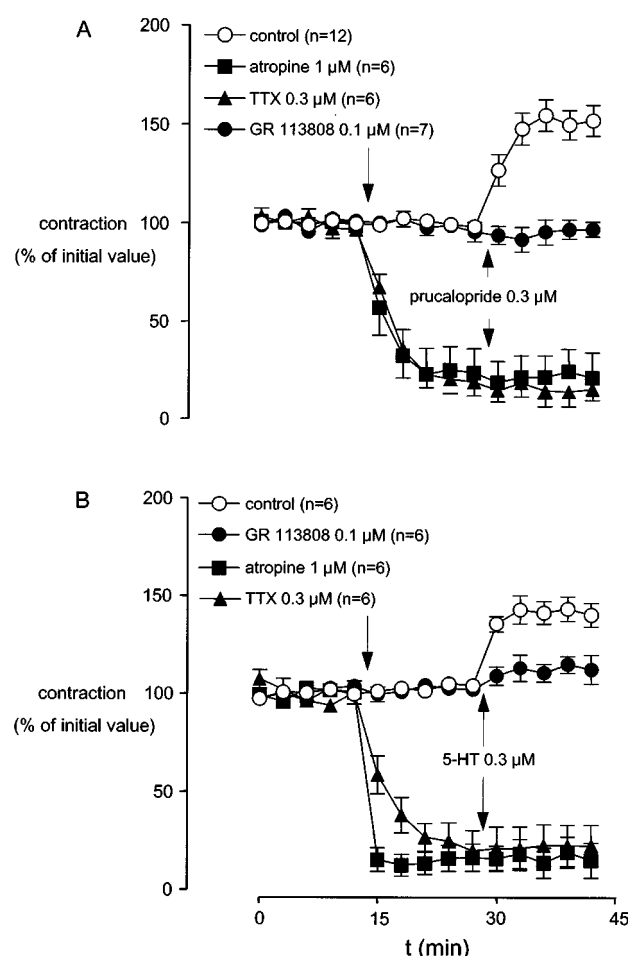

Figure 4 The effect of atropine, tetrodotoxin, and GR 113808 on the prucalopride-induced (A) and 5-HT-induced (B) enhancement of submaximal electrical field stimulation-induced contractions of human isolated large intestine (*taeniae coli*) longitudinal muscle (from ascending and sigmoid colon and rectum). L-NOARG (0.1 mM) was present in the organ bath solution routinely, whereas methysergide (3 μM) and granisetron (0.3 μM) were present as well in the case where 5-HT was used as an agonist. Data points represent mean±s.e.mean of EFS-evoked contractions.

(0.3 μM) blocked the EFS-induced contractions and 5-HT (0.3 μM) could not modify these reductions. The 5-HT-induced effects under the conditions described are given in Figure 4B and Table 1.

Discussion

The data presented in this study clearly demonstrate that in canine and human large intestinal longitudinal muscle, 5-HT$_4$ receptors mediate enhancement of contractions due to electrical field stimulation. These 5-HT$_4$ receptors mediating contractility are most likely located on the cholinergic nerves. This is the first report demonstrating 5-HT$_4$ receptors on cholinergic nerves of the large intestine of higher animal species, a phenomenon that has long been suggested to be absent.

The effect of the selective 5-HT$_4$ receptor agonist prucalopride in both preparations pointed clearly to interactions with 5-HT$_4$ receptors. Prucalopride expresses nanomolar affinity for 5-HT$_4$ receptors and possesses approximately 1000 fold selectivity for 5-HT$_4$ receptors over other 5-HT receptors (Briejer *et al*., 1998c; Janssen Research Foundation; data on file). Thus, the concentration used in this study (0.3 μM) producing an effect, strongly suggested 5-HT$_4$ receptor involvement.

In canine ascending colon, involvement of 5-HT receptors other than 5-HT$_4$ receptors in the increase in contractions due to 5-HT was ruled out by the blockade of 5-HT$_1$, 5-HT$_2$, 5-ht$_5$, 5-HT$_6$ and 5-HT$_7$ receptors by methysergide and the lack of effect of the selective 5-HT$_3$ receptor antagonist granisetron on the 5-HT-evoked effect. In experiments using human *taeniae coli*, the organ bath solution already contained a combination of methysergide and granisetron at concentrations expected to block every 5-HT receptor with the exception of 5-HT$_4$ receptors (for which both methysergide and granisetron, at the concentrations used, do not express significant affinity). Therefore, it was expected that the 5-HT-induced amplification of contractions due to EFS was due to activation of 5-HT$_4$ receptors. Indeed, the observation that the selective 5-HT$_4$ receptor antagonist GR 113808 (Gale *et al*., 1994) was able to antagonize the enhancement induced by 5-HT in both preparations, strongly suggested involvement of 5-HT$_4$ receptors, as was also corroborated by the blockade of the effect of prucalopride by GR 113808. GR 113808 expresses 5-HT$_4$ receptor antagonist affinity at nanomolar concentrations (pK$_B$ estimates in human and canine tissues ranging from 8.8 to 9.4; Gale *et al*., 1994; Kaumann, 1993; Prins *et al*., 1999; 2000) and does not produce significant binding to all other 5-HT receptors up to 0.3 μM (Gale *et al*., 1994). Thus, the GR 113808-induced antagonism of the 5-HT- and prucalopride-induced responses indicates involvement of 5-HT$_4$ receptors.

Due to the positive coupling of 5-HT$_4$ receptors to adenylate cyclase smooth muscle relaxation would have been expected if these receptors were located on the smooth muscle. In contrast, the 5-HT$_4$ receptor-mediated responses in the current study were contractile, which pointed to an indirect 5-HT$_4$ receptor-mediated effect *via* depolarization of nerves releasing a contractile neurotransmitter. Indeed, involvement of nerves was demonstrated by the blockade of EFS-induced contractions in both preparations by TTX. The contractile neurotransmitter was shown to be acetylcholine, as indicated by the blockade induced by the non-selective muscarinic antagonist atropine, thus suggesting 5-HT$_4$ receptors are located on the cholinergic nerves. Furthermore, the observation that prucalopride (0.3 μM) failed to affect the curve to exogenously administered acetylcholine ruled out the possibility of a postjunctional 5-HT$_4$ receptor-mediated potentiation mechanism of acetylcholine-induced contraction or an acetylcholine esterase inhibition property of prucalopride at this concentration. Thus, in canine and human large intestine longitudinal muscle, prejunctional 5-HT$_4$ receptors located on cholinergic nerves

mediated enhanced contractility provided these cholinergic nerves were operational. Earlier, 5-HT$_4$ receptors on cholinergic nerves were found to mediate contraction of guinea-pig colonic segments (Briejer & Schuurkes, 1996). This study indicates that also non-rodent animal species including humans are endowed with 5-HT$_4$ receptors on cholinergic nerves of large intestine longitudinal muscle.

Previously, it was assumed that the 5-HT$_4$ receptor-mediated effects in the large intestine of these animal species were limited to circular muscle, where smooth muscle 5-HT$_4$ receptors mediate relaxation. It was even suggested that a 5-HT$_4$ receptor-mediated mechanism of release of acetylcholine was absent in the human colon, on basis of the observation that the 5-HT$_4$ receptor agonist cisapride failed to modify unstimulated or EFS-stimulated release of ^{3}H-acetylcholine from human colonic taenial muscle (Burleigh & Trout, 1985). Interestingly, the endogenous ligand 5-HT was not tested in that study. In comparison with 5-HT, cisapride has been reported a weak partial 5-HT$_4$ receptor agonist in the guinea-pig colon (5-HT$_4$ receptors on cholinergic nerves; Elswood *et al*., 1991) but also on smooth muscle 5-HT$_4$ receptors in human colon (Tam *et al*., 1994). Thus, the 5-HT$_4$ receptor agonism produced by cisapride might be insufficient to evoke release of acetylcholine from human colonic cholinergic nerves. In agreement with Burleigh & Trout's study, cisapride (1 μM) failed to induce an effect on EFS-induced contractions in the bioassay with human colon specimens as reported here ($n = 5$; unpublished results). Assessment of the efficacy of 5-HT, prucalopride and cisapride in ^{3}H-acetylcholine release experiments with human colonic tissue could further clarify this issue.

In conscious dogs chronically equipped with circularly orientated force transducers on the serosal side of the large intestine, prucalopride enhanced proximal and inhibited distal large intestinal contractility (Briejer *et al*., 1998a) through a 5-HT$_4$ receptor-mediated mechanism (Briejer *et al*., 1998b). The current *in vitro* study demonstrates the presence of 5-HT$_4$ receptors mediating contractility in the proximal to mid, but not the distal region of the canine large intestine longitudinal muscle. Additionally, we conducted some EFS experiments with circular muscle strips of canine ascending colon, and found that GR 113808 (0.1 μM) prevented the prucalopride (0.3 μM)-induced increase in EFS-induced contractions ($n = 6$, unpublished results). Thus, the enhanced contractility due to 5-HT$_4$ receptor activation is most likely not limited to the longitudinal muscle portion of dog large intestine but also affects the circular muscle. In this manner, the proximal enhancement of contractility in conscious dogs might correspond to the enhanced contractility observed in ascending colon (both muscle layers) *in vitro*. Further, our previous study with canine large intestine demonstrated smooth muscle 5-HT$_4$ receptors mediating relaxation of circular muscle, which was almost absent in the ascending colon, but increased towards the rectum (Prins *et al*., 1999). As such, 5-HT$_4$ receptors may influence canine large intestinal contractility by combination of proximal enhanced contractility and distal circular muscle inhibited contractility. Interestingly, in human tissue, regional differences in amplitude of responses after 5-HT$_4$ receptor stimulation have not been found, neither in circular muscle relaxation (previous studies) nor in longitudinal muscle enhanced contractility (this study). Although the number of specimens from each region of human large intestine available to us was limited and unequally distributed along the large intestine, 5-HT$_4$ receptor-mediated effects, indistinguishable in amplitude, were observed in specimens spanning from the proximal (ascending colon) to the distal (rectum) region. Thus, it seems unlikely that the regional differences in 5-HT$_4$

receptor-mediated contractility in large intestine longitudinal muscle are similar in dogs and humans.

We conclude that in human large intestinal taenial longitudinal muscle strips, pre-junctional 5-HT$_4$ receptors on cholinergic neurones facilitate contractility, presumably through release of acetylcholine. Regional differences in 5-HT$_4$ receptor-mediated responses in human longitudinal muscle were not found in this study. In canine ascending colon and descending colon, but not in rectum longitudinal muscle strips pre-junctional 5-HT$_4$ receptors on cholinergic nerves mediate enhancement of contractility. The stimulating properties of selective 5-HT$_4$ receptor agonists on colonic motor function *in vivo* may be explained by inhibition of

circular muscle resistance (previous studies) and stimulation of longitudinal muscle contractility (this study). Furthermore, the canine large intestine, which is frequently used in *in vivo* models to study motility, was found to have appreciable predictive value for the human large intestine with respect to 5-HT$_4$ receptor function.

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