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Characterization of the contraction to 5-HT in the canine colon longitudinal muscle

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1 Although conscious dogs have often been used for colonic motility studies with 5-hydroxytryptamine (5-HT), the effects of 5-HT on the isolated colon have not been thoroughly characterized yet. The current study was undertaken to characterize the response to 5-HT of the canine isolated colon longitudinal muscle.

2 Longitudinal strips of canine midcolon deprived of (sub)mucosa were prepared for isotonic measurement. 5-HT induced contractions from 3 nM onwards, which were not affected by selective inhibition of 5-HT re-uptake, monoamine oxidase or blockade of α-adrenoceptors. Tetrodotoxin (0.3 μM) did not affect the responses to 5-HT, suggesting that smooth muscle 5-HT receptors are involved. The selective 5-HT_4 receptor antagonist SB 204070 (10 nM) slightly enhanced contractions to 5-HT and therefore it was included in the organ bath solution in all further experiments. The 5-HT_1 and 5-HT_2 receptor antagonist methysergide (0.1 μM) depressed the curve to 5-HT, but the selective 5-HT_3 receptor antagonist granisetron (0.3 μM) had no effect.

3 Besides 5-HT, α-methyl-5-HT (α-Me-5-HT), 5-methoxytryptamine (5-MeOT), 2-methyl-5-HT (2-Me-5-HT) and 5-carboxamidotryptamine (5-CT) also induced contractions, with the following rank order of potency (pEC_{50} values in parentheses): 5-HT (6.9) = α-methyl-5-HT (6.9) > 2-Me-5-HT (5.8) = 5-MeOT (5.7) = 5-CT (5.6), indicative of 5-HT_2 receptor involvement. α-Me-5-HT produced a bell-shaped curve, which was not affected by α-adrenoceptor blockade. 5-HT, 5-MeOT, 2-Me-5-HT and 5-CT produced a monophasic concentration-response curve, consistent with an interaction with a single receptor site. 8-Hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) and tryptamine only induced contractions at a concentration exceeding 1 μM.

4 The selective 5-HT_{2B} receptor antagonist SB 204741 (0.3 μM) did not affect the curve to 5-HT. Ketanserin, cisapride and spiroxatrine behaved as competitive antagonists with pK_b values of, respectively, 8.4, 8.1 and 6.7. Spiroxatrine (1 μM) shifted the curve to 5-MeOT rightward yielding an apparent pA_2 of 7.1. Other antagonists at 5-HT_{2A} receptors also surmountably inhibited the contractions to 5-HT (apparent pA_2 value in parentheses): mesulergine (8.2), cinanserin (8.2), yohimbine (6.2) and mianserin (8.6). However, as well as a rightward shift, methiothepin (8.3), pizotifen (8.6) and spiperone (8.8) also caused a depression of the curve, indicative of 'pseudo-irreversible' antagonism. Taken together, the above mentioned affinity estimates most closely corresponded to literature affinity values for 5-HT_{2A} receptors.

5 It was concluded that 5-HT induces contractions of the canine midcolon longitudinal muscle primarily by stimulation of smooth muscle 5-HT_{2A} receptors. The presence of inhibitory 5-HT_4 receptors cannot be ruled out.

Keywords: 5-Hydroxytryptamine (5-HT); 5-HT_{2A} receptors; colon; smooth muscle

Introduction

5-Hydroxytryptamine (5-HT) is widely present in the gut. It has been found in enterochromaffin cells (Erspamer, 1996) and in neurones of the enteric nervous system (Furness & Costa, 1982). It is currently believed that 5-HT and its receptors play an important role in the regulation of gastrointestinal motility (Gershon *et al*., 1990; Read & Gwee, 1994; Briejer *et al*., 1995c). Recent studies have shown that the guinea-pig colon is not a proper model for the human colon with respect to the distribution of 5-HT receptors. In the guinea-pig colon, 5-HT_3 and 5-HT_4 receptors on the cholinergic nerves and 5-HT_{2A} receptors on the smooth muscle mediate contraction (Elswood *et al*., 1991; Hegde *et al*., 1994; Briejer *et al*., 1995b), whereas a novel 5-HT_2-like receptor mediates relaxation (Briejer *et al*., 1995a). However, in the human colon, 5-HT inhibits motility via 5-HT_1-like receptors on the longitudinal muscle, whereas 5-HT_4 receptors mediate inhibition of spontaneous activity and relaxation of the circular muscle (Tam *et al*., 1994; Hillier *et al*., 1994; Meulemans *et al*., 1995).

An animal species resembling the human better with respect to 5-HT receptor subtype distribution in the colon may be the dog. Dogs are often used as a model for *in vivo* studies of gastrointestinal motility, but it is not known which 5-HT receptor types occur in the canine gastrointestinal tract. In conscious dogs, 5-HT induced contractions of the colon (Pruitt *et al*., 1974), but the receptors involved could not be identified due to a lack of appropriate tools. On the isolated ileocolonic junction and the terminal ileum, 5-HT induced contractions due to stimulation of neuronal 5-HT_3 receptors. Furthermore, it was suggested that 5-HT_1-like receptors, located on the smooth muscle, mediated contraction (Boeckxstaens *et al*., 1990). Very recently, Nagakura and co-workers (1996) studied the effects of intravenously administered 5-HT in dogs equipped with chronically implanted strain gauges. In this model, they found evidence for the involvement of 5-HT_1, 5-HT_2 and 5-HT_4 receptors.

A study on the isolated colon would allow a more definitive analysis of the 5-HT receptors present on the enteric nerves and smooth muscle, but such a study has never been published. In the present study we set out to characterize pharmacologi-

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cally the response to 5-HT of the longitudinal muscle of the canine isolated midcolon.

Methods

Beagle dogs of either sex, weighing between 7 and 14 kg, were used. They were decerebrated by a slaughterhouse gun for cardiovascular studies. A piece of midcolon, approximately 5 cm, was dissected and the luminal contents were removed. The mucosa, submucosa and mesenterium were dissected and strips of longitudinal muscle of ca. 2 cm length and approx. 2–3 mm wide were prepared. The organ bath set-up for isotonic measurement (2 g load) contained Krebs-Henseleit solution (composition in mM: glucose 10.1, $CaCl_2$ 2.51, $NaHCO_3$ 25, $MgSO_4$ 1.18, KH_2PO_4 1.18, KCl 4.69 and NaCl 118) and was gassed continuously with 95% O_2 and 5% CO_2 (37°C). After half an hour stabilization the strips were challenged twice with 3 μM carbachol and consecutively with 3 μM 5-HT until contractions were constant (usually after 3–4 times) and all following contractions were related to the last 5-HT-induced contraction (=100%). After each contraction the chemicals were washed out by replacing the organ bath solution twice. The interval between two subsequent administrations of 5-HT was 10 min. If a strip did not contract at least 0.7 cm after carbachol 3 μM or 0.4 cm after 5-HT 3 μM, it was discarded. About 20% of the strips failed these criteria. For construction of concentration-response curves, 5-HT and its analogues were dosed non-cumulatively in ascending concentrations. Only 1 curve per strip was constructed and always 1 out of 8 prepared strips was used as a control. For construction of concentration-response curves in antagonist studies, antagonists were added 10 min before the construction of the concentration-response curve was started. After each wash-out the antagonists were re-added and the strips were given 10 min stabilization time.

Data analysis

For each individual concentration-response curve, a pEC_{50} value and the slope were estimated by a computerized iterative non-linear curve fitting procedure; a single-receptor site interaction was assumed (see Bowen & Jerman, 1995). For the agonists the maximum response was related to the maximum response of 5-HT, and denoted as the intrinsic activity. For ketanserin, cisapride and spiroxatrine Schild regression analysis was performed in order to determine a pA_2 value and a pK_b value (Schild regression analysis with the slope constrained to unity). For all other antagonists apparent pA_2 values were estimated by use of the Schild equation (Arunlakshana & Schild, 1959).

Statistics

Mean values and s.e.mean were calculated for graphical presentation. For comparison of mean treatment versus control values, analysis of variance (ANOVA), followed by the Dunnett's t test for multiple comparisons was used. A level of $P<0.05$ was considered to indicate a statistically significant difference. The n value denotes the number of animals used.

Chemicals

The following drugs were used (with their abbreviations and respective suppliers in parentheses): 5-methoxytryptamine (5-MeOT), 2-methyl-5-HT (2-Me-5-HT), 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT), (1-butyl-4-piperidinyl)methyl-8-amino-7-chloro-1,4-benzodioxane-5-carboxylate HCl (SB 204070), fluvoxamine, spiroxatrine, spiperone, granisetron HCl, mesulergine HCl, ketanserin tartrate, phentolamine, cisapride (Janssen Research Foundation, Belgium), tryptamine HCl (Janssen Chimica, Belgium), 5-hydroxytryptamine (5-HT) creatinine sulphate, tetrodotoxin (Serva, Germany), α-methyl-5-HT (α-Me-5-HT), 5-carboxamidotryptamine (5-CT; Tocris Cookson, U.K.), methiothepin maleate (Hoffman-La Roche, Switzerland), methysergide maleate, pizotifen HCl (Sandoz, Switzerland), carbachol (Merck, Germany), *N*-(1-methyl-5-indolyl)-N′-(3-methyl-5-isothiazolyl)urea (SB 204741; kindly donated by SmithKline Beecham, U.K.), cinanserin HCl (Bristol-Meyers-Squibb, U.K.), mianserin HCl (Organon, The Netherlands), yohimbine (Sigma, Belgium) and pargyline HCl (Abbott, U.S.A.). All compounds were dissolved in distilled water, except for cisapride, spiperone, spiroxatrine and ketanserin which were dissolved in distilled water acidified with tartaric acid in the stock solution.

Results

The strips showed only minor spontaneous rhythmical activity. After first administration of carbachol (3 μM), the initial spontaneous activity of the strips decreased further and in many strips was not evident. Contractions to the challenger concentration of 5-HT (3 μM) were usually only small after the first application of 5-HT, but when given repeatedly the contractions increased in magnitude. Contractions to 5-HT were maintained and mostly became stable after 3 to 4 applications. Since the baseline length tended to increase during the course of the experiment, the maximum response to 5-HT (assessed at the end of the experiment) was often greater than 100% relative to the priming 3 μM 5-HT-induced contraction.

Tetrodotoxin (0.3 μM) did not affect to curve to 5-HT ($n=4$; results not shown). Selective inhibition of 5-HT reuptake by fluvoxamine (0.3 μM), inhibition of monoamine-oxidase by pargyline (0.1 μM) and blockade of α-adrenoceptors by phentolamine (0.3 μM) also did not modify the curve to 5-HT ($n=4-5$; results not shown). The selective 5-HT_3 receptor antagonist granisetron (0.3 μM) did not affect the curve to 5-HT ($n=5$), whereas the non-selective 5-HT_1 and 5-HT_2 receptor antagonist methysergide (0.1 μM) depressed the curve to 5-HT (by about 50%) and furthermore shifted it rightward. Despite the depression, an apparent pA_2 of 8.6 was estimated ($n=5$; results not shown). The selective 5-HT_4 receptor antagonist, SB 204070 (10 nM) enhanced the contractions to 5-HT by about 10% ($n=5$; results not shown). Based on these results, SB 204070 (10 nM) was included in the Krebs solution in all further experiments. A recorder tracing of contractions to ascending non-cumulative concentrations to 5-HT in the presence of SB 204070 (10 nM) is presented in Figure 1. Under these conditions, the curve to 5-HT had a sigmoid shape (Figure 2) and the pEC_{50} value was 6.9 ± 0.2 ($n=12$).

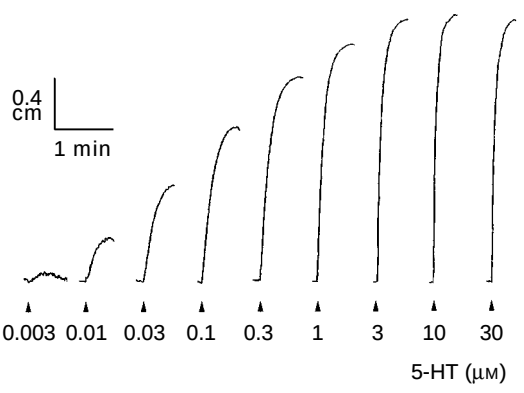

Figure 1 A representative recorder tracing of the contractions induced by ascending concentrations to 5-HT applied in a non-cumulative manner.

Agonists

In the presence of SB 204070 (10 nM), 5-HT, some of its analogues and 8-OH-DPAT induced contractions with the following rank order of potency: 5-HT = α-Me-5-HT > 2-Me-5-HT = 5-MeOT = 5-CT (Figure 2, Table 1). Contractions to agonists were stable and maintained. 8-OH-DPAT and tryptamine were almost ineffective up to 1 μM.

The concentration-response curve to α-Me-5-HT was bell-shaped. At higher concentrations, α-Me-5-HT induced a biphasic response, consisting of a contraction followed by a relaxation. The latter effect and the resulting bell-shaped curve was not seen with any of the other agonists. The specificity of the 5-HT receptor analogues has not been studied extensively. Zondag *et al.* (1994) showed that 2-Me-5-HT was an agonist at α-adrenoceptors on the guinea-pig colon and caused relaxation. We, therefore, hypothesized that the depression phase of the concentration-response curve to α-Me-5-HT could be mediated by inhibitory α-adrenoceptors as well, but the curve to α-Me-5-HT was not affected by the presence of the α-adrenoceptor antagonist phentolamine 0.3 μM ($n=3$; results not shown). The identification of the receptor mediating the relaxation was not further pursued.

5-HT$_2$ receptor antagonists

The selective 5-HT$_{2B}$ receptor antagonist SB 204741 (1 μM) did not affect the curve to 5-HT ($n=6$; results not shown). Ketanserin (10, 30 and 100 nM), spiroxatrine (1, 3 and 10 μM) and cisapride (30, 60 and 300 nM) surmountably antagonized the contractions to 5-HT (Figures 3, 4 and 5). The slopes of the concentration-response curves in the presence and absence of ketanserin, spiroxatrine and cisapride were not significantly different from unity. Schild regression analysis of the inhibition due to ketanserin, spiroxatrine and cisapride yielded slopes and pA$_2$ values of, respectively, 1.2±0.2 (not different from unity), pA$_2$ = 8.3±0.2 (pK_b = 8.4±0.1); 0.9±0.2 (not different from unity) and a pA$_2$ of 6.8±0.3 (pK_b = 6.7±0.1); 0.7±0.2 (not different from unity) and a pA$_2$ of 8.6±0.5 (pK_b = 8.1±0.4) (Figures 3, 4 and 5). Mianserin (0.1 μM) surmountably antagonized the curve to 5-HT and an apparent pA$_2$ value of 8.6 was estimated. Likewise, for cinanserin (10 nM and 30 nM) the apparent pA$_2$ values were 8.2 and 8.2, for mesulergine (10 nM) 8.2 and yohimbine (1 μM) 6.2 (Figure 6; see Table 2). Methiothepin (10 nM), spiperone (10 nM) and pizotifen (10 nM) caused, as well as a rightward shift, a depression of the curve to 5-HT, indicative of a 'pseudo-irreversible' interaction (Figure 6). Despite the depression, apparent pA$_2$ values were estimated of 8.3, 8.8 and 8.6, respectively (Table 2). Spiroxatrine (1 μM) produced a parallel rightward shift of the curve to 5-MeOT, and an apparent pA$_2$ of 7.1±0.3 was calculated (Figure 7).

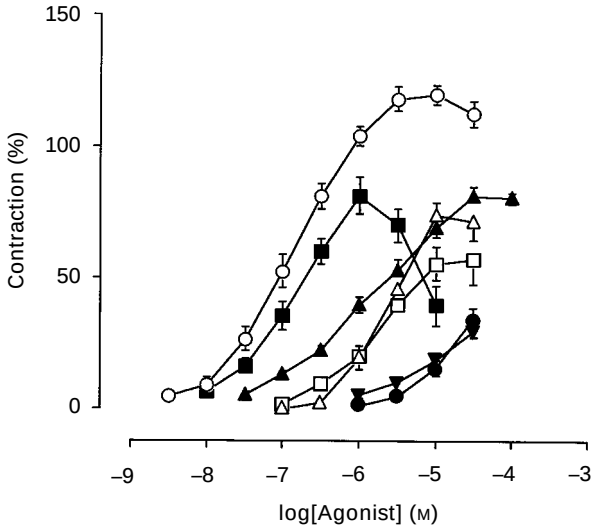

Figure 2 Concentration-response curves to 5-HT (○), 5-MeOT (▲), α-Me-5-HT (■), 5-CT (△), 2-Me-5-HT (□), 8-OH-DPAT (●) and tryptamine (▼). Responses are expressed as a percentage of contractions to 5-HT 3 μM and presented as mean with vertical lines showing s.e.mean ($n=6-12$).

Table 1 Estimated affinities (pEC$_{50}$), slopes of the corresponding concentration-response curves of several agonists and their intrinsic activity

Agonist	*pEC$_{50}$*	*Slope*	*Intrinsic activity*
5-HT	6.9±0.1	0.9±0.2	1.00
α-Me-5-HT	6.9±0.1	1.3±0.2	0.68
5-CT	5.6±0.2	2.0±0.3	0.62
2-Me-5-HT	5.8±0.1	1.2±0.1	0.46
5-MeOT	5.7±0.2	0.7±0.2	0.68
8-OH-DPAT	ND	ND	0.29
Tryptamine	ND	ND	0.25

pEC$_{50}$ values and slopes shown as mean±s.e.mean. ND = not determined.

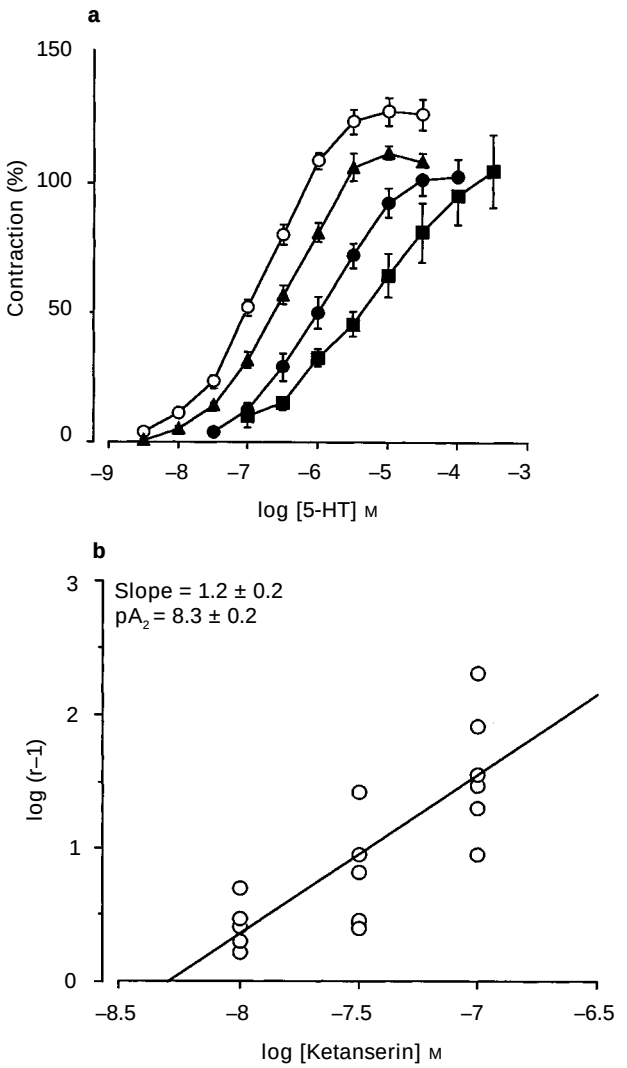

Figure 3 Surmountable antagonism by ketanserin 10 nM (▲), 30 nM (●) and 0.1 μM (■) (a), as compared to control (○), of the concentration-response curves for 5-HT. The concomitant Schild regression plot is depicted in (b) (r represents the concentration-ratio). Responses are expressed as a percentage of contractions to 5-HT 3 μM and presented as mean with vertical lines showing s.e.mean ($n=5-11$).

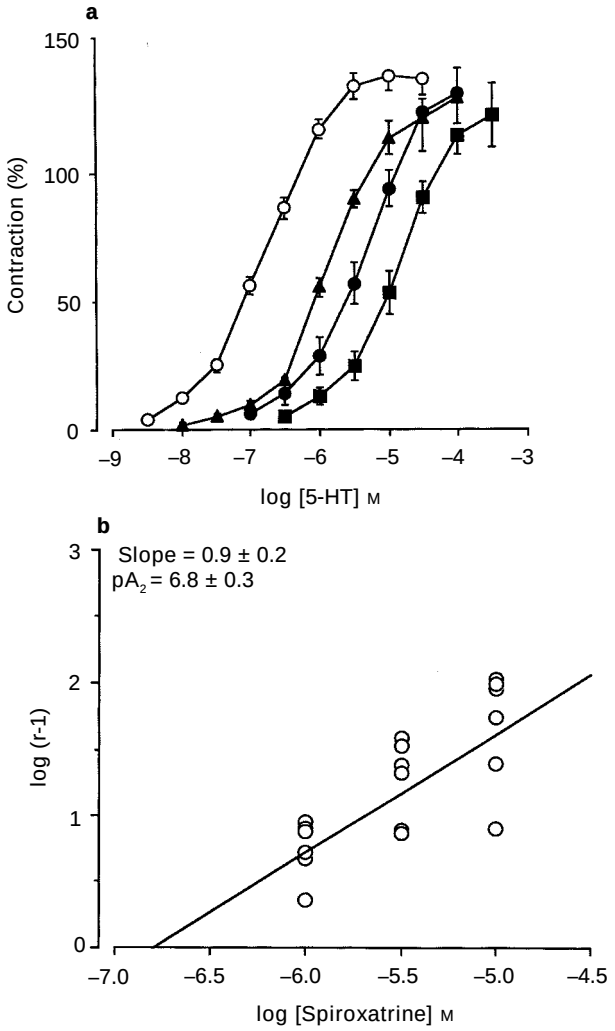

Figure 4 Surmountable antagonism by spiroxatrine 1 μM (▲), 3 μM (●) and 10 μM (■) (a) on the concentration-response curve to 5-HT (○) in the presence of SB 204070 (10 nM). The concomitant Schild regression plot is depicted in (b) (r represents the concentration-ratio). Responses are expressed as a percentage of contractions to 5-HT 3 μM and presented as mean with vertical lines showing s.e.mean ($n=6$).

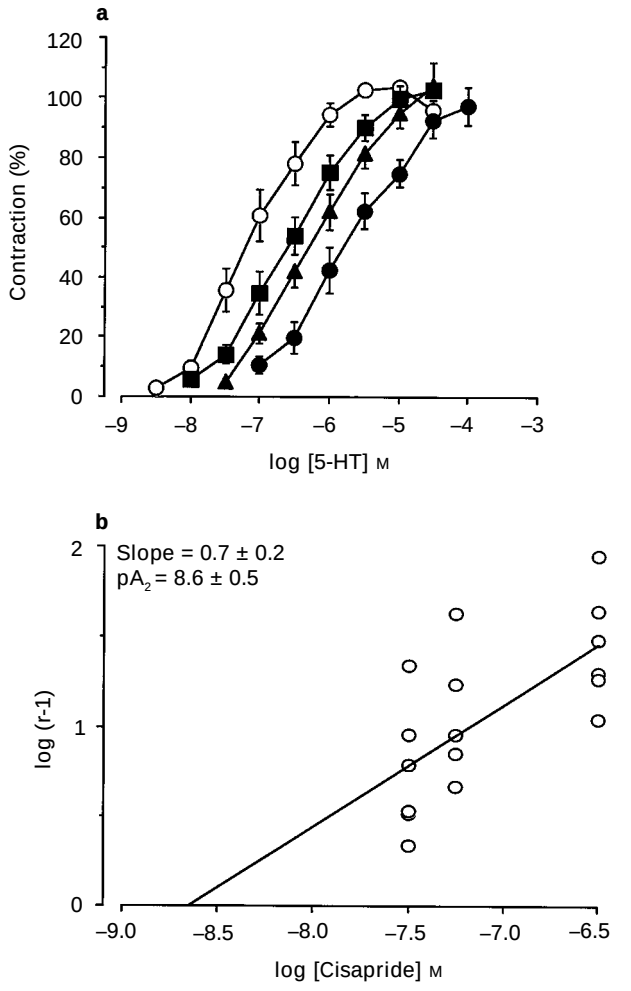

Figure 5 Surmountable antagonism of cisapride 30 nM (■), 60 nM (▲) and 0.3 μM (●) (a) on the concentration-response curve to 5-HT (○) in the presence of SB 204070 (10 nM). The concomitant Schild regression plot is depicted in (b) (r represents the concentration-ratio). Responses are expressed as a percentage of contractions to 5-HT 3 μM and presented as mean with vertical lines showing s.e.mean ($n=6$).

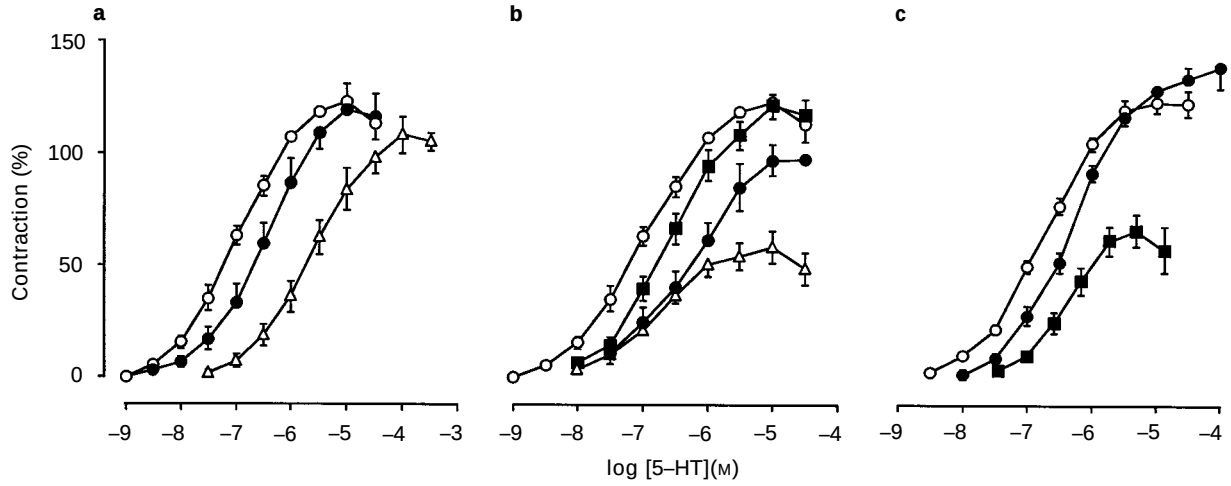

Figure 6 Effects of (a) mianserin 100 nM (△) and cinanserin 10 nM (●), (b) spiperone 10 nM (●), methiothepin 10 nM (△) and mesulergine 10 nM (■), and (c) yohimbine 1 μM (●) and pizotifen 10 nM (■) on the concentration-response curve to 5-HT (○) in the continuous presence of SB 204070 10 nM. Responses are expressed as a percentage of contractions to 5-HT 3 μM and presented as mean with vertical lines showing s.e.mean ($n=6$).

Table 2 Estimated pA_2/pK_b values of the tested antagonists versus affinity pA_2/pK_i values in the literature

Drug	*Concentration(s)*	*Affinity with 5-HT*	*Affinity with 5-MeOT*	*Literature affinity* 5-HT$_{2A}$	5-HT$_{2B}$	5-HT$_{2C}$
Methiothepin	10 nM	8.3±0.1		9.0	–	7.6
Methysergide	300 nM	8.2±0.2		8.6	7.1–8.2	8.6
Mianserin	100 nM	8.6±0.2		10.1	6.7–7.3	8.0
Cisapride	30, 60, 300 nM	8.1±0.4		8.1–8.6	–	5.6–5.8
Pizotifen	10 nM	8.6±0.2		8.9	–	–
Spiperone	10 nM	8.8±0.3		9.1	<4–5.5	5.9
Cinanserin	10 nM	8.2±0.2		9.2	–	–
Cinanserin	30 nM	8.2±0.3				
Mesulergine	10 nM	8.2±0.2		9.1	7.4	9.1
Yohimbine	1 μM	6.2±0.2		6.0	7.9	4.4
SB 204741	1 μM	<6		<5.2	8.0	5.8
Ketanserin	10, 30, 100 nM	8.4±0.1		9.3	5.4	6.6
Spiroxatrine	0.1, 0.3, 1 μM	6.7±0.1	7.1±0.3*	6.2–6.9	<5	5.1

Data shown are mean±s.e.mean. All experiments were done in the presence of SB 204070 10 nM, with the exception of the experiment with methysergide. *Apparent pA_2 for spiroxatrine 1 μM. References: Briejer *et al.* (1995c), Hoyer (1988), Hoyer & Schoeffer (1991), Nelson & Taylor (1986), Cohen & Fludzinski (1987), Wainscott *et al.* (1993), Foguet *et al.* (1992), Kalkman & Fozard (1991), Forbes *et al.* (1995) and Rizzi *et al.* (1994).

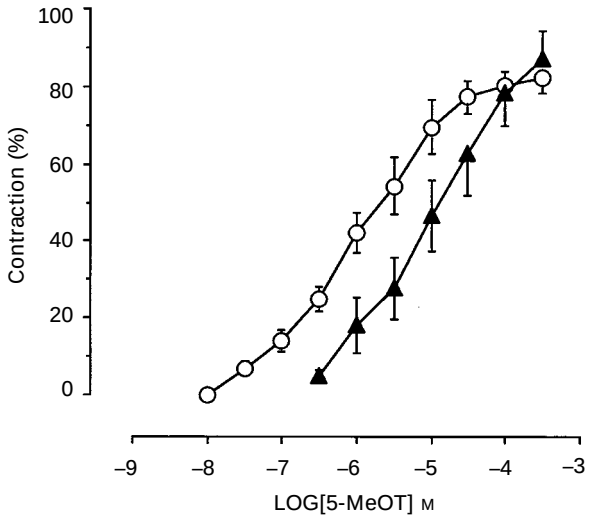

Figure 7 Effect of spiroxatrine 1 μM (▲) on the concentration-response curve to 5-MeOT (○) in the presence of SB 204070 (10 nM). Responses are expressed as a percentage of contractions to 5-HT 3 μM and presented as mean with vertical lines showing s.e.mean (n=5–12).

Discussion

Our data clearly indicate that under the applied conditions, mainly 5-HT$_{2A}$ receptors mediate the contractions to 5-HT of the canine colon longitudinal muscle. These 5-HT$_{2A}$ receptors seem to be located on the smooth muscle, as suggested by the absence of effect of tetrodotoxin on the responses to 5-HT.

The rank order of potency, derived from experiments with the 5-HT analogues (5-HT = α-Me-5-HT > 2-Me-5-HT = 5-MeOT = 5-CT) most closely resembles the order which Leff & Martin (1988) have identified for 5-HT$_2$-like receptors (5-HT = α-Me-5-HT > 5-CT). This rank order of potency excludes 5-HT$_1$-like and 5-ht$_7$ receptors (rank order of potency 5-CT > 5-HT) (Eglen *et al.*, 1992; Carter *et al.*, 1995). The absence of effect of granisetron, a selective and potent 5-HT$_3$ receptor antagonist (Sanger & Nelson, 1989), on the curve to 5-HT and the approximately 10% enhancement of contractions to 5-HT by SB 204070, a selective and potent 5-HT$_4$ antagonist (Wardle *et al.*, 1994), suggest that 5-HT$_3$ receptors and 5-HT$_4$ receptors do not mediate the contraction to 5-HT. However, a small population of 5-HT$_4$ receptors mediating relaxation could be present on the canine colon, but data from pilot experiments exploring this possibility were not conclusive. Thus, 5-HT$_2$ receptors are the most likely candidates to mediate the contraction to 5-HT.

SB 204741 (Forbes *et al.*, 1995) and yohimbine are selective 5-HT$_{2B}$ receptor antagonists (see Table 2). SB 204741 (1 μM) was ineffective and yohimbine displayed inhibition with an estimated affinity about 100 fold lower than would be expected for 5-HT$_{2B}$ receptors (see Table 2). Thus, it is highly unlikely that 5-HT$_{2B}$ receptors are involved. Although only the affinity estimate of mianserin was in accordance with its affinity for 5-HT$_{2C}$ receptors (Table 2), the affinities of ketanserin, cisapride, spiperone and to a lesser extent spiroxatrine and yohimbine clearly indicate that 5-HT$_{2A}$ rather than 5-HT$_{2C}$ receptors are involved (see Table 2).

The non-selective 5-HT$_1$ and 5-HT$_2$ receptor antagonists pizotifen, methysergide and methiothepin behaved as non-surmountable antagonists. For the latter two compounds, both non-surmountable as well as surmountable antagonism at 5-HT$_1$ and 5-HT$_2$ receptors has been described, depending on the tissue (Leff & Martin, 1988). It may be due to a very slow dissociation rate of the antagonist-receptor complex, which could vary between tissues. Slow dissociation kinetics ('pseudo-irreversible' antagonism) can yield a combination of depression and rightward shift, as seen in the current experiments. However, the observation of non-surmountable antagonism as such does not help in the identification of the receptor involved.

For some of the antagonists tested, particularly for ketanserin, cinanserin, mesulergine and mianserin, affinity estimates were up to 10 fold lower than those in the literature for 5-HT$_{2A}$ receptors (Table 2). Most of these published affinity data were obtained with rat tissues. It is now well known that there are differences in affinity values between animal species and also in man. Such variations may result from relatively small amino acid sequence differences between receptors (sometimes even only one amino acid, like in human and rat 5-HT$_{1B}$ receptors: Oksenberg *et al.*, 1992; Metcalf *et al.*, 1992). Such species differences have also been described for the rat and human 5-HT$_{2A}$ receptors by Johnson *et al.* (1993). In their study, they found that, for example, mesulergine displayed 10 fold higher affinity for rat than for human 5-HT$_{2A}$ receptors. Thus, it is likely that canine 5-HT$_{2A}$ receptors also have a slightly distinct pharmacology.

To date, not much is known as to the physiological role of 5-HT$_{2A}$ receptors in the gut. In the guinea-pig, 5-HT$_{2A}$ receptors have been identified along the entire gut on the longitudinal smooth muscle and been shown to mediate contraction (Engel *et al.*, 1984; Briejer *et al.*, 1995b). However, in the guinea-pig ileum, at least, 5-hydroxytryptaminergic neurones are known to be exclusively interneurones, thus

lacking direct projections to the smooth muscle (Furness & Costa, 1982; Gershon *et al*., 1990). Similar data on the neuronal localization of 5-HT in the dog is lacking. 5-HT is also present in the enterochromaffin cells in large quantities. These cells are found in the lining of the mucosa, which is quite distant from the muscularis, on a molecular scale. It is therefore unlikely that 5-HT from the enterochromaffin cells can reach the muscularis to stimulate 5-HT$_{2A}$ receptors. Still, *in vitro* both in the guinea-pig and dog colon a considerable part of the contraction to exogenously administered 5-HT is mediated by this receptor subtype. It is difficult to imagine that these receptors are expressed in the smooth muscle to serve no purpose, but it is as yet not understood where the 5-HT necessary to stimulate these receptors is released from. In man 5-HT$_{2A}$ receptors mediating contraction have only been identified so far on the longitudinal and circular muscle of the jejunum (Kuemmerle *et al*., 1995), but on the colon 5-HT$_{2A}$ receptors have not yet been found. In clinical trials testing ketanserin for the treatment of hypertension, no significant effect on the patients bowel habits was noted as a side effect, although some patients complained of hard stools (data on file). The present study revealed the presence of contractile 5-HT$_{2A}$ receptors on the colon of the dog. This is in good accordance with the observations of Nagakura and co-workers (1996). They showed that in the conscious dog, intravenously administered 5-HT stimulates colonic contractile patterns. This effect could be blocked by ketanserin in the distal colon and inhibited in the midcolon, suggesting that 5-HT$_{2A}$ receptors mediate this effect to 5-HT *in vivo*. Thus, although smooth muscle 5-HT$_{2A}$ receptors seem present in a variety of gut tissues, their role and importance in human and animal gastrointestinal motility remains unclear. Further *in vivo* studies in which the effects of selective 5-HT$_{2A}$ receptor antagonists are evaluated on the different motility patterns and segmental transit time may prove interesting.

It is concluded that, under the applied conditions, 5-HT contracts the longitudinal muscle of the dog mainly by stimulation of smooth muscle 5-HT$_{2A}$ receptors. A population of 5-HT$_4$ receptors mediating relaxation may be present as well.

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