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Pharmacological characterization of the 5-HT receptors mediating contraction and relaxation of canine isolated proximal stomach smooth muscle

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1 We aimed to characterize 5-HT receptors mediating contraction and relaxation to 5-HT in dog proximal stomach longitudinal muscle (LM) strips.

2 Of the tryptamine analogues tested, 5-HT was the most potent contractile agent at basal length, while 5-CT was the most potent relaxant of $PGF_{2\alpha}$-induced contraction. Neither the contractions to 5-HT, nor the relaxations to 5-CT were influenced by tetrodotoxin, illustrating that action potential propagation is not involved.

3 The 5-HT-induced contraction was antagonized by mesulergine (0.03 to 0.3 μM) and ketanserin (2–20 nM), but the antagonism was not of a simple competitive nature, indicating multiple receptor involvement. Ketanserin (3 to 30 nM) and mesulergine (30 nM) competitively antagonized the α-Me-5-HT-induced contraction (pK_B: 8.83 ± 0.09 and pA_2: 8.25 ± 0.06 respectively). These affinity values are in line with literature affinities of ketanserin and mesulergine at $5\text{-}HT_{2A}$ receptors in various bioassays.

4 The 5-CT-induced inhibition of $PGF_{2\alpha}$-induced contraction was competitively antagonized by mesulergine (pK_B estimate: 8.52 ± 0.12) and by the selective $5\text{-}HT_7$ receptor antagonist SB-269970 (pK_B estimate: 9.36 ± 0.14). Both pK_B estimates are in line with literature affinities of these compounds for $5\text{-}HT_7$ receptors. Mesulergine (30 nM) and SB-269970 (10 nM) shifted the relaxant curve to 5-HT parallel to the right in the presence of ketanserin (0.3 μM) (pA_2 estimates of 8.08 ± 0.10 and 8.75 ± 0.14 respectively), indicative of $5\text{-}HT_7$ receptor involvement.

5 It is concluded that 5-HT induces dog proximal stomach (LM) contraction *via* smooth muscle $5\text{-}HT_{2A}$ receptors and relaxation *via* smooth muscle $5\text{-}HT_7$ receptors.

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Abbreviations: α-Me-5-HT, α-methyl-5-HT; 5-CT, 5-carboxamidotryptamine; GR113808, [1-[2-[(methylsulphonyl)amino]ethyl]-4-piperidinyl]methyl 1-methyl-1H-indole-3-carboxylate; 5-HT, 5-hydroxytryptamine, KCl, potassium chloride; L-NNA, N^G-nitro-L-Arginine; 2-Me-5-HT, 2-methyl-5-HT; 5-MeOT, 5-methoxytryptamine; NAN-190, 1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl]piperazine HCl; nitroglycerin, glycerol trinitrate 1%; $PGF_{2\alpha}$, prostaglandine F2 alpha; SB-269970, (R)-3-(2-(2-(4-methyl-piperidin-1-yl)ethyl)-pyrrolidine-1-sulphonyl)-phenol; TTX, tetrodotoxin

Introduction

The dog is used as a model to study gastrointestinal motility mechanisms and the way drugs interact with them. With regard to gastric motility, it was shown in conscious dogs that intravenously administered 5-HT induced contraction of a Heidenhain pouch, that was prevented by a $5\text{-}HT_4$ receptor antagonist (Bingham *et al*., 1995) and that $5\text{-}HT_4$ receptor agonists stimulate gastric emptying (Gullikson *et al*., 1993). Recent *in vitro* studies confirmed the presence of $5\text{-}HT_4$ receptors on cholinergic nerves in the canine gastric corpus (Prins *et al*., 2001a). In the canine antrum, also evidence for muscular inhibitory $5\text{-}HT_7$ receptors was obtained (Prins *et al*., 2001b). Careful pharmacological characterization of the receptors mediating contraction by 5-HT in the dog proximal stomach has not been done. In other species it was shown that muscular $5\text{-}HT_{2B}$ receptors mediate contraction in the rat gastric fundus (Baxter *et al*., 1994) and muscular $5\text{-}HT_2$ receptors do so in the guinea-pig gastric fundus (Takemura *et al*., 1999).

The proximal stomach also has a great relaxant capacity to accommodate food without major pressure changes. Different clues towards $5\text{-}HT_1$ receptors mediating proximal gastric relaxation have been published. Kojima *et al*. (1992), Meulemans *et al*. (1993) and Takemura *et al*. (1999) have shown $5\text{-}HT_1$ receptors mediating relaxation in guinea-pig proximal stomach *in vitro*. *In vivo*, sumatriptan (a $5\text{-}HT_1$ receptor agonist) relaxed the proximal stomach of cat (Coulie *et al*., 1999) and of man (Tack *et al*., 2000), a process which has been suggested to involve both nitrergic and cholinergic pathways. Surprisingly, no in-depth characterization of the 5-HT receptors involved in these responses by means of isolated muscle strip studies has been reported.

Therefore the present study was set up to characterize the 5-HT receptors involved in the effects of 5-HT on long-

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itudinal muscle strips of the canine proximal stomach. Strips were studied at basal muscle length or were precontracted. In this manner, the putative presence of contraction-mediating receptors and relaxation-mediating receptors could be identified.

Methods

Tissue preparation

Beagle dogs of both sexes, weighing between 10 and 15 kg, were sacrificed by decerebration, followed by exsanguination through the carotid artery. The entire stomach was dissected and placed in 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). The stomach was opened by cutting along the lesser curvature and the contents were rinsed out. The muscle strips were derived from the proximal ventral part of the stomach, at approximately 3 cm from the lower oesophageal sphincter. The strips were carefully cleared of mucosa, submucosa and omentum. Longitudinal muscle strips (maximum 16 per dog) of approximately 1.5 cm length and 2–3 mm width were prepared and mounted onto tissue holders. These were placed in an organ bath set-up containing Krebs-Henseleit solution at 37°C, continuously gassed with 95% O_2 and 5% CO_2. The mechanical activity of the preparations was recorded *via* isotonic transducers (2 g load) (Harvard apparatus) on a chart recorder (Model BD 112; Kipp & Sons). All strips were studied on the preparation day.

Experimental protocols

All experiments were conducted in the presence of indomethacin (1 μM) to avoid spontaneous contractions due to prostaglandins. Agonists were added on basal muscle length (protocol 1) or on contracted muscle strips (protocol 2). Both protocols began after a 30 min stabilization period.

Protocol 1 Working on basal muscle length, antagonist or solvent was added to the organ bath and left to incubate for 30 min. After this, a cumulative concentration-response curve to an agonist was established with half log-units ascending concentration increments from 1 nM onwards. Where some agonists, at lower concentrations, induced contraction, they sometimes induced, at higher concentrations, relaxation. To analyse these results, only contraction data were taken into account. (Figure 1A).

Preliminary experiments were carried out to find the optimal pre-contracting agent for relaxation studies with tryptamine analogues (protocol 2). Concentration-contraction curves to KCl, prostaglandin F_2 alpha ($PGF_{2\alpha}$), histamine and carbachol were constructed. After washout, the approximate EC_{50} concentration of each precontracting agent was added to the organ bath, after which a concentration-relaxation curve to 5-CT was established. The $PGF_{2\alpha}$-induced contraction was selected for two reasons. First, $PGF_{2\alpha}$ produced a contraction that was maintained for over 2 h. Second, in comparison with the other precontracting agents, $PGF_{2\alpha}$ allowed 5-CT to induce the relatively most pronounced relaxation.

Protocol 2 Prior to each relaxation curve to any tryptamine analogue, the maximum effect of $PGF_{2\alpha}$ was determined and the within-strip EC_{50} was roughly estimated. After thorough washout, antagonist or solvent was added; after 30 min, the EC_{50} value of $PGF_{2\alpha}$ (ranging from 0.3 to 3 μM) was added to the baths. Upon maintained contraction (usually after 5–10 min), a concentration-relaxation curve to an agonist was established in a cumulative manner. (Figure 1B).

Then, for both protocols, compounds were washed out by replacing the organ bath solution twice. Maximal relaxation to nitroglycerin (10 μM) was achieved after which maximal contraction to KCl (0.16 M) was obtained. In previous experiments 0.16 M KCl was shown to induce maximum contraction in the same tissue. All agonist-induced responses were expressed as percentage of the contraction to KCl (0.16 M) after nitroglycerin relaxation (10 μM). Only one agonist was studied per muscle strip.

Data presentation and statistical analysis

Concentration-response curves to agonists were fitted iteratively to the Hill equation, obtaining curve parameter estimates for mid-point location (pEC_{50}), upper asymptote (α) and Hill slope ($=n_H$). To test the influence of one concentration of a drug on the curve parameters of an agonist, one-way ANOVA was performed. A level of $P<0.05$ was considered to indicate significance. To test the criteria for Schild-analysis (curves must have equal slopes and upper asymptotes), one-way ANOVA was performed, followed by a *post-hoc* Bonferroni's test for multiple comparisons. pEC_{50} values in the absence and presence of different concentrations of antagonists were iteratively fitted to a modified Schild equation, as previously described by Black *et al*., 1985. The pK_B value as true affinity estimate was calculated in case the criteria for Schild analysis were met (no alteration of slope or upper asymptote due to antagonism). If only one concentration of antagonist was tested, and the agonist curve was shifted to the right with no change in upper asymptote or slope, the antagonist affinity was expressed as an apparent dissociation constant, the pA_2 value, calculated using the Schild equation.

All data were expressed as the mean$\pm$s.e.mean, where *n* represents the number of dogs used in one experimental protocol.

Drugs

The following drugs were used (abbreviations and respective suppliers in parentheses): (R)-3-(2-(2-(4-methyl-piperidin-1-yl)ethyl)-pyrrolidine-1-sulphonyl)-phenol (SB-269970), 5-methoxytryptamine (5-MeOT), 2-methyl-5-HT (2-Me-5-HT), granisetron HCl, mesulergine HCl, ketanserin tartrate, 1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl]piperazine HCl (NAN-190), [1-[2-[(methylsulphonyl)amino]ethyl]-4-piperidinyl]methyl 1-methyl-1H-indole-3-carboxylate (GR113808; Janssen Research Foundation, Belgium), atropine sulphate, carbachol, histamine, N^G-nitro-L-Arginine (L-NNA; Acros Chimica, Belgium), 5-hydroxytryptamine creatinine sulphate (5-HT), tetrodotoxin (TTX; Serva, Germany), α-methyl-5-HT (α-Me-5-HT), fluoxetine HCl, 5-carboxamidotryptamine (5-CT; Tocris Cookson, U.K.), potassium chloride (KCl; Sigma, Belgium), methysergide maleate (RBI, U.S.A.), Prostaglandine F2 alpha ($PGF_{2\alpha}$; diluted from Dinolytic® 5 mg ml^{-1};

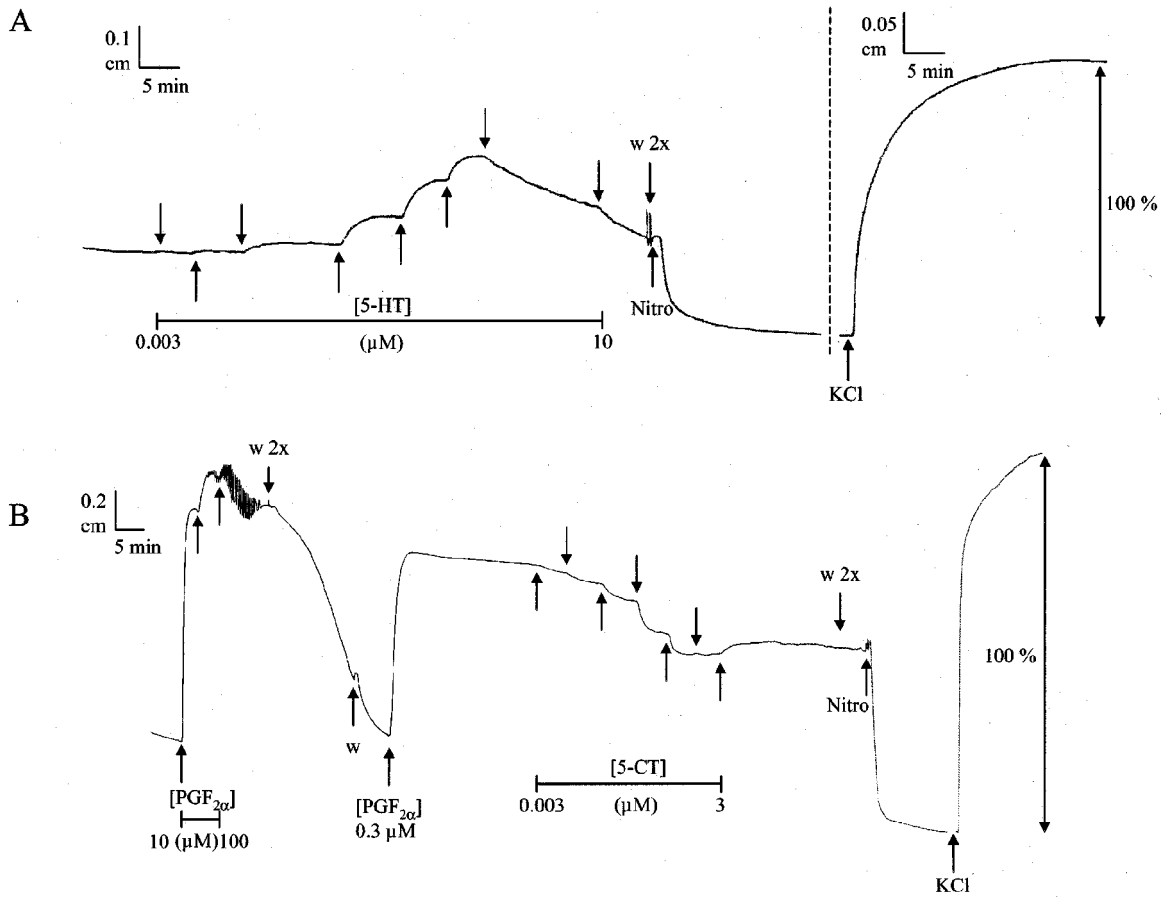

Figure 1 A representative recorder tracing of an example following protocol 1 (A) and protocol 2 (B) of canine proximal stomach longitudinal muscle strips. The dashed line divides areas of different amplification. Nitro = nitroglycerin (10 μM), w = wash, KCl = KCl (0.16 M). In the upper trace, 5-HT was administered with half log unit concentration increments as indicated by the arrows, in the lower trace 5-CT was administered.

Upjohn, Animal Health, Belgium), cocaine HCl, glycerol trinitrate 1% (nitroglycerin; Merck, Germany) and pargyline HCl (Abbott, U.S.A.). All compounds were dissolved in distilled water, except for ketanserin, pargyline and 5-HT. Ketanserin was dissolved in distilled water acidified with tartaric acid in the stock solution; pargyline was dissolved in distilled water with 10% cyclodextrine in the stock solution. 5-HT was prepared with ascorbic acid in the stock solution. The solvents had no effect on the baseline tension or the curves to agonists. All stock solutions were freshly prepared on the experimental day and dilutions were prepared using distilled water.

Results

Most tissue strips showed no phasic contractions. Nevertheless, about 20 per cent of the strips showed a slow and gradual decrease in muscle length shortly after the stabilization period. Since this in general led to maximal tissue contraction, strips could not contract any further. This prevented establishment of reproducible contractile responses to agonists, therefore, these strips were not used. The remaining strips had a stable muscle length on top of which clear-cut agonist-induced effects could be observed.

Response to 5-HT at basal muscle length (protocol 1)

In general, 5-HT induced contraction at lower concentrations (approximately 1 nM to 1 μM), and tended to induce relaxation at higher concentrations (approximately 1 μM to 3 mM). As we were interested in the contractile effect of 5-HT, 5-HT was not further added as soon as relaxation occurred after attainment of maximal contractile response. In about a third of all tissues, 5-HT merely induced a moderate relaxation (Figure 2A).

Inhibition of re-uptake-1 by cocaine (30 μM), of selective 5-HT re-uptake by fluoxetine (0.3 μM) and of monoamine oxidase by pargyline (0.1 mM) changed neither the potency nor the upper asymptote of the 5-HT-induced concentration contraction curve ($n=4$; data not shown). Tetrodotoxin (TTX; 0.3 μM; $\alpha = 0.55 \pm 0.06$; $pEC_{50} = 6.91 \pm 0.11$), N^G-nitro-L-Arginine (L-NNA; 0.1 mM; $\alpha = 0.37 \pm 0.07$; $pEC_{50} = 6.80 \pm 0.13$) and atropine (1 μM; $\alpha = 0.38 \pm 0.07$; $pEC_{50} = 6.76 \pm 0.15$) did not affect the concentration-contraction curve to 5-HT ($\alpha = 0.57 \pm 0.11$; $pEC_{50} = 6.89 \pm 0.12$; $n=6$; data not shown).

Neither the selective 5-HT_3 receptor antagonist granisetron (Sanger & Nelson, 1989; 0.3 μM), nor the selective 5-HT_4 receptor antagonist GR113808 (Gale *et al.*, 1994; 1 μM), altered the concentration-contraction curve to 5-HT ($n=5$ to 7; Table 1). Although the 5-HT_1, 5-HT_2, 5-ht_5, 5-HT_6 and 5-

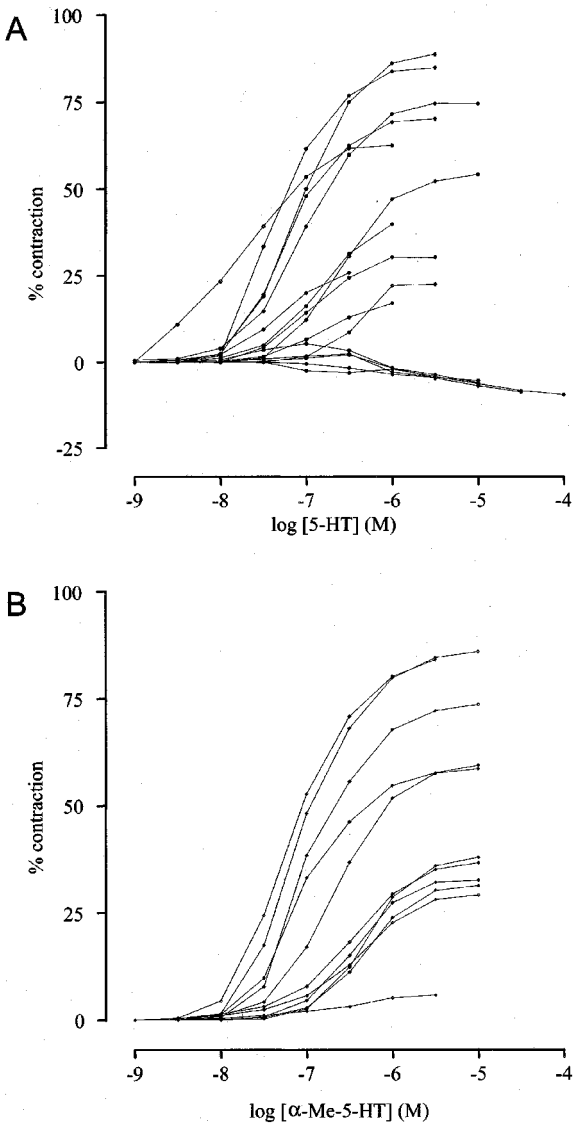

Figure 2 Individual concentration-contraction curves to 5-HT (A; $n=16$) and α-Me-5-HT (B; $n=11$) of canine proximal stomach longitudinal muscle preparations on basal muscle length. The responses are expressed as percentage of muscle strip contraction to KCl (0.16 M).

HT_7 receptor antagonist methysergide (Gommeren *et al.*, 1998; 0.1 μM) displayed a non-significant decrease of the maximal effect, it was feasible to estimate a pA_2 value ($pA_2 = 7.93 \pm 0.35$; $n=6$; Table 1).

The 5-HT_2 and 5-HT_7 receptor antagonist mesulergine (Hoyer *et al.*, 1994; 0.03 and 0.3 μM) produced a parallel shift to the right but this was only significant at 0.1 and 0.3 μM mesulergine (Table 1). At the highest concentration (0.3 μM), mesulergine was not able to shift the curve to 5-HT any further. The 5-HT-induced contraction curve shifted to the right in the presence of the selective 5-HT_{2A} receptor antagonist ketanserin (Hoyer *et al.*, 1994; 2 to 20 nM) but only at the highest concentration (20 nM) this was significant. Although a marked decrease of the upper asymptotes was observed, this is not significant. It was feasible to estimate pA_2 values for both mesulergine (0.1 μM) and ketanserin (20 nM) (8.26 ± 0.25 and 8.56 ± 0.30 respectively; Table 3).

Response to 5-HT receptor agonists at basal muscle length

As described above, relaxation was occasionally seen using 5-HT. α-Me-5-HT on the other hand induced systematically well-defined concentration-contraction curves (Figure 2B). Although 5-MeOT and 2-Me-5-HT never induced relaxation, large variability for all curve parameters was observed in the contraction response. On six experiments 2-Me-5-HT induced no effect twice, 5-MeOT induced no effect once. The preferential 5-HT_1 and 5-HT_7 receptor agonist 5-CT induced, in all but one preparation, a small relaxation ($\alpha = -5 \pm 2\%$). Thus, except for α-Me-5-HT, variability in the response to the different tryptamine analogues was considerable. All concentration-contraction curves were fitted to obtain curve parameters, except for 5-CT, that in all but one preparation induced a small relaxation (see for curve parameters: Table 2). The following rank order of agonist potency was found: 5-HT > α-Me-5-HT > 5-MeOT ⩾ 2-Me-5-HT.

Ketanserin (3 – 30 nM) produced a parallel rightward displacement of the concentration-contraction curve to α-Me-5-HT (Figure 3), yielding a Schild slope (1.16 ± 0.14) that was not significantly different from unity. After constraining the Schild slope to unity, a pK_B estimate was obtained (8.83 ± 0.09; $n=11$; Table 3). Mesulergine (30 nM) produced a parallel rightward displacement of the concentration-contraction curve to α-Me-5-HT, yielding a pA_2 estimate (8.25 ± 0.06; $n=6$; Tables 1 and 3).

Responses to 5-HT and 5-HT receptor agonists on pre-contracted strips (protocol 2)

Since the experiments with 5-CT on basal muscle length indicated the putative presence of relaxatory 5-HT receptors, we decided to study the relaxant effect of 5-HT and 5-HT receptor agonists more in depth on strips contracted by the EC_{50} value (ranging from 0.3 to 3 μM) of $PGF_{2\alpha}$ ($53 \pm 9\%$ of the KCl contraction ($n=6$)).

5-MeOT systematically induced pronounced relaxations. 5-CT induced concentration-dependent relaxations but at higher concentrations (from 3 μM onwards) a small contraction occurred ($9 \pm 2\%$ of KCl contraction, $n=6$). α-Me-5-HT always induced contractions on top of the $PGF_{2\alpha}$-induced contraction, although sometimes, at higher concentrations (from 10 μM onwards), a relaxation of variable amplitude occurred. The relaxant responses to 5-CT and 5-MeOT and the contractile responses to α-Me-5-HT are shown in Figure 4; the curve parameters are given in Table 2. The response to 5-HT and 2-Me-5-HT was very variable. Sometimes 5-HT and 2-Me-5-HT induced relaxation only ($n=3$) while in other strips contraction was seen using lower concentrations (1 nM to 1 μM) of the agonist and relaxation was seen using higher agonist concentrations (from 10 μM onwards; $n=3$). The mean effect in all strips can be seen in Figure 4.

Influence of antagonists on the 5-CT-induced relaxation

Neither TTX (0.3 μM) nor L-NNA (0.1 mM) affected the concentration-relaxation curve to 5-CT ($n=6$). NAN-190 (5-HT_{1A} receptor antagonist; Cao & Rodgers, 1997; 30 nM; $pEC_{50} = 7.42 \pm 0.10$; $\alpha = -39.00 \pm 7.74$; $n_H = 1.09 \pm 0.04$) and

Table 1 Curve parameters for the concentration-contraction curves to 5-HT and α-Me-5-HT on basal muscle length in control conditions and in the presence of the antagonists indicated

Agonist/Antagonist	*pEC_{50}*	α	*n_H*
5-HT-induced contraction			
Control ($n=7$)	6.95±0.12	60.76±9.83	1.57±0.17
Granisetron (0.3 μM; $n=5$)	6.96±0.12	56.07±7.90	1.32±0.04
GR113808 (1 μM; $n=7$)	6.84±0.07	59.75±10.25	1.51±0.13
Methysergide (0.1 μM; $n=6$)	5.89±0.40(*)	42.23±8.96	1.39±0.17
Control ($n=7$)	7.16±0.22	38.61±9.37	1.44±0.17
Mesulergine (30 nM; $n=7$)	6.49±0.23	41.16±7.95	1.47±0.11
Mesulergine (0.1 μM; $n=6$)	5.93±0.27(*)	41.03±6.65	1.47±0.19
Mesulergine (0.3 μM; $n=5$)	5.97±0.40(*)	45.67±8.27	1.27±0.24
Control ($n=7$)	7.16±0.12	61.75±8.22	1.41±0.09
Ketanserin (2 nM; $n=6$)	6.97±0.20	52.68±9.38	1.40±0.12
Ketanserin (6 nM; $n=6$)	6.57±0.17	39.90±10.67	1.43±0.14
Ketanserin (20 nM; $n=6$)	6.06±0.31(*)	39.90±8.10	1.12±0.10
α-Me-5-HT-induced contraction			
Control ($n=6$)	6.65±0.08	52.19±12.40	1.26±0.12
Mesulergine (30 nM; $n=6$)	5.90±0.12(*)	46.57±12.14	1.39±0.10

The parameters α, pEC_{50} and n_H were obtained from the iterative fitting procedure to the Hill equation. (*) indicates significant difference as compared to control conditions (one-way ANOVA followed by *post-hoc* Bonferroni's test for multiple comparisons). Values are expressed as mean±s.e.mean.

Table 2 Curve parameters for the concentration-response curves to 5-HT and tryptamine analogues on basal muscle length and on $PGF_{2\alpha}$-induced contraction

Agonist	*pEC_{50}*	α	*n_H*
On basal tone			
5-HT ($n=12$)	7.04±0.10	55.55±7.67	1.48±0.12
5-CT	nd	nd	nd
α-Me-5-HT ($n=11$)	6.68±0.10	48.57±7.45	1.28±0.07
2-Me-5-HT ($n=4$)	6.17±0.41	21.93±12.20	1.37±0.14
5-MeOT ($n=5$)	6.19±0.28	20.66±11.68	1.10±0.12
On $PGF_{2\alpha}$-induced contraction			
5-HT	nd	nd	nd
5-CT ($n=6$)	7.43±0.12	−66.80±10.68	1.09±0.06
α-Me-5-HT ($n=5$)	6.62±0.15	34.24±10.33	0.99±0.12
2-Me-5-HT	nd	nd	nd
5-MeOT ($n=6$)	4.99±0.20	−47.26±15.86	1.04±0.24

The parameters α (positive values refer to contraction, negative values to relaxation), pEC_{50} and n_H were obtained from the iterative fitting procedure to the Hill equation. Values are expressed as mean±s.e.mean; nd=not determined.

ketanserin (0.1 μM; $pEC_{50}=7.60\pm0.14$; $\alpha=-42.25\pm6.01$; $n_H=0.88\pm0.05$) did not alter the concentration-relaxation curve to 5-CT as compared with control conditions ($pEC_{50}=7.52\pm0.08$; $\alpha=-34.62\pm4.74$; $n_H=0.97\pm0.04$) ($n=5$ to 8). Mesulergine (10–100 nM) produced a parallel rightward displacement of the concentration-relaxation curve to 5-CT (Figure 5A), yielding a Schild slope (0.84±0.16) that was not significantly different from unity. After constraining the Schild slope to unity, a pK_B estimate was obtained (8.52±0.12; $n=10$; Table 3). The selective 5-HT_7 receptor antagonist SB-269970 (Hagan *et al*., 2000; 1–10 nM) also produced a parallel rightward displacement of the concentration-relaxation curve to 5-CT (Figure 5B), yielding a Schild slope (1.15±0.17) that was not significantly different from unity. After constraining the Schild slope to unity, a pK_B estimate was obtained (9.36±0.14; $n=8$; Table 3).

5-HT-induced relaxation–pA_2-estimates

In the presence of ketanserin (0.3 μM), 5-HT induced a relaxation on top of the $PGF_{2\alpha}$-induced contraction with a corresponding pEC_{50} of 5.96±0.17. Mesulergine (30 nM) shifted the curve to 5-HT in parallel to the right, yielding a pA_2 estimate (8.08±0.10; $n=6$; Table 3). SB-269970 (10 nM) also produced a dextral shift of the 5-HT curve, without change in Hill slope or upper asymptote, yielding a pA_2 estimate (8.75±0.14; $n=5$; Table 3).

5-HT-induced contraction–pA_2-estimates

After blocking the 5-HT_7 receptor induced relaxation with SB-269970 (0.1 μM), 5-HT induced contraction only (pEC_{50}: 6.92±0.05). In the presence of SB-269970, ketanserin (10–

Table 3 Estimated pA_2/pK_B values of tested antagonists against different agonists on basal muscle length and on $PFG_{2\alpha}$-induced contraction

Agonist (in the presence of)	*Antagonist*	*pK_B*	*pA_2*
On basal tone			
5-HT	ketanserin	nd	8.56±0.30
	mesulergine	nd	8.26±0.25
α-Me-5-HT	ketanserin	8.83±0.09	nd
	mesulergine	nd	8.25±0.06
5-HT (SB-269970)	ketanserin	8.75±0.11	nd
	mesulgerine	nd	8.46±0.15
On $PGF_{2\alpha}$-induced contraction			
5-CT	SB-269970	9.36±0.14	nd
	mesulergine	8.52±0.12	nd
5-HT (ketanserin)	SB-269970	nd	8.75±0.14
	mesulergine	nd	8.08±0.10

pA_2/pK_B values shown as mean±s.e.mean; nd = not determined.

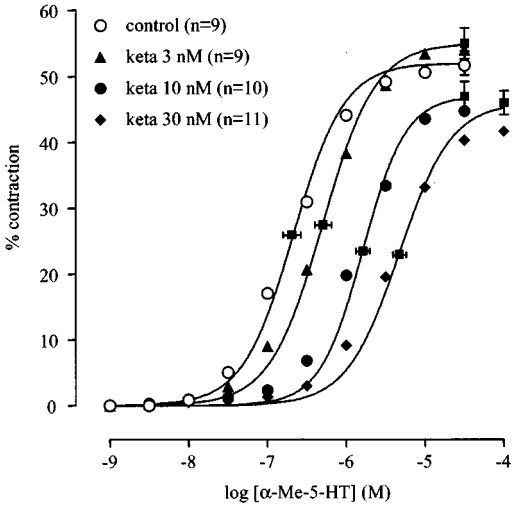

Figure 3 Influence of increasing concentrations of ketanserin (keta) on the α-Me-5-HT-induced contractions of canine proximal stomach longitudinal muscle preparation. Contractions are expressed as percentage of muscle strip contraction to KCl (0.16 M). The curves represent simulations using the Hill equation; the parameters for α (with vertical error bars) and EC_{50} (shown with horizontal error bars) were obtained from the iterative fitting procedure.

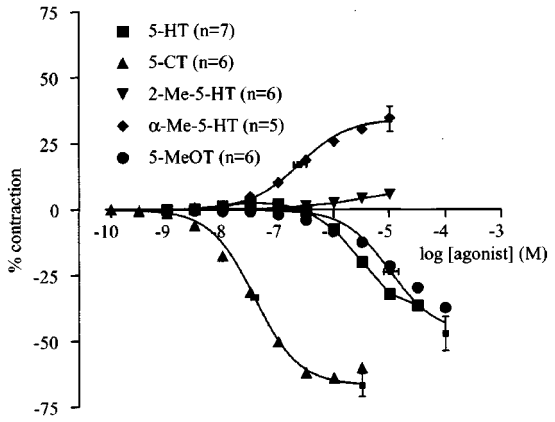

Figure 4 Effect of 5-HT, 5-CT, 2-Me-5-HT, 5-MeOT and α-Me-5-HT on the $PGF_{2\alpha}$-induced contraction of canine proximal stomach longitudinal muscle preparation. The curves to 5-CT, α-Me-5-HT and 5-MeOT represent simulations using the Hill equation; the parameters for α (shown in vertical error bars) and EC_{50} (shown with horizontal error bars) were obtained from the iterative fitting procedure. The vertically averaged data points of 5-HT and 2-Me-5-HT are connected. The responses are expressed as percentage of muscle strip contraction to KCl (0.16 M).

100 nM) produced a parallel rightward displacement of the concentration-contraction curve to 5-HT. This yielded a Schild slope (0.94±0.13) that was not significantly different from unity. After constraining the Schild slope to unity, a pK_B estimate was obtained (8.75±0.11; $n=7$; Table 3).

In the presence of SB-269970 (100 nM), mesulergine (30 nM) produced a parallel rightward displacement of the concentration-contraction curve to 5-HT. A pA_2 estimate was obtained (8.46±0.15; $n=6$; Table 3).

Discussion

This study aimed to characterize the 5-HT-receptors involved in the 5-HT-induced length changes of dog proximal stomach longitudinal muscle. The experiments with pargyline, fluoxetine and cocaine imply that neither breakdown nor re-uptake of 5-HT impacts the interaction of 5-HT with its receptors. Furthermore, the inability of TTX to affect the 5-HT-induced contractions and the 5-CT-induced relaxations indicates that the receptors involved are located on smooth muscle.

Tryptamine analogues

The rank order of tryptamine analogues potency can be used to roughly identify the 5-HT receptor subtype involved in a particular response (Baxter *et al*., 1994). Except for α-Me-5-HT, that always induced contraction, tryptamine analogues induced contraction as well as relaxation, expressing varying efficacy at both phenomena. Since α-Me-5-HT preferentially stimulates 5-HT_2 receptors, the presence of a 5-HT_2 receptor population mediating contraction is likely, especially in view of the observation that 5-HT and α-Me-5-HT are nearly equipotent. The 5-HT_1 and 5-HT_7 receptor agonist 5-CT and the 5-HT_1, 5-HT_4 and 5-HT_7 receptor agonist 5-MeOT

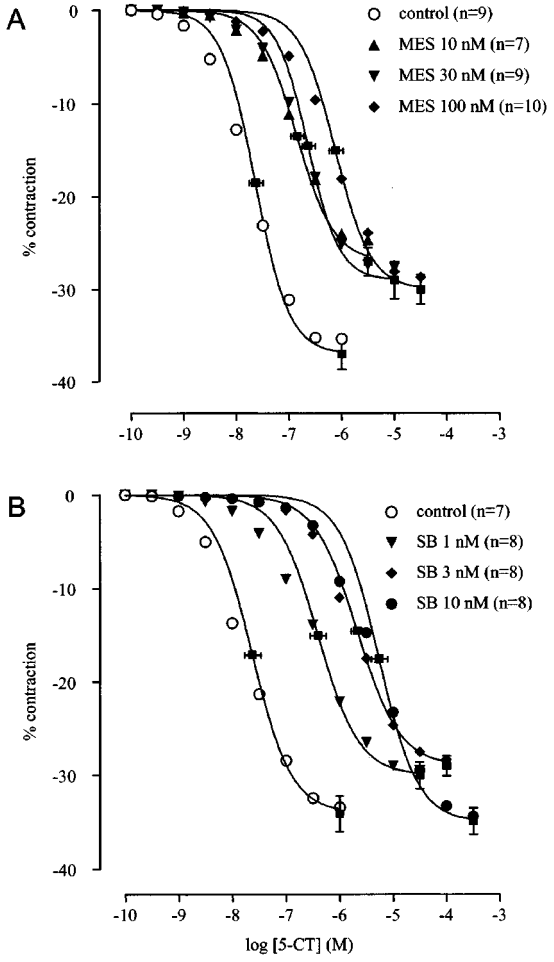

Figure 5 Antagonism by mesulergine (MES; A) and by SB-269970 (SB; B) of the 5-CT-induced relaxation of $PGF_{2\alpha}$-contracted longitudinal muscle preparations of dog proximal stomach. The curves represent simulations using the Hill equation and the parameters for α (shown with vertical error bars) and EC_{50} (shown with horizontal error bars) that were obtained from the iterative fitting procedure. The responses are expressed as percentage of muscle strip contraction to KCl (0.16 M).

induced relaxation of the $PGF_{2\alpha}$-induced contraction, raising the possibility of 5-HT_1, 5-HT_4 and 5-HT_7 receptors mediating relaxation. As 5-CT, that is not active at 5-HT_4 receptors was the most potent relaxant agonist, the involvement of 5-HT_4 receptors is unlikely. Involvement of 5-HT_7 receptors is more likely than involvement of 5-HT_1 receptors, due to the inability of TTX to affect the relaxation to 5-HT. 5-HT_1 receptors located on smooth muscle would be expected to mediate contraction rather than relaxation, since they are negatively coupled to adenylate cyclase. In contrast, 5-HT_7 receptors are positively coupled to this enzyme (Bard *et al.*, 1993), thus when located on smooth muscle, are likely candidates to mediate relaxation. Thus, the experiments with agonists point to the presence of contractile 5-HT_2 receptors and relaxatory 5-HT_7 receptors.

The contractile 5-HT receptor

The inability of GR113808 and granisetron to alter the 5-HT-induced concentration-contraction curves suggests that 5-HT_4 and 5-HT_3 receptors are not involved (Table 1). Used at higher concentrations, the antagonism produced by mesulergine and ketanserin to 5-HT was not competitive, thus the real dissociation constant, the pK_B value, could not be estimated. Nevertheless it was possible to estimate the apparent affinity, the pA_2 value using the lowest effective concentration of the antagonists (Table 3). The true affinity estimate pK_B can be used as a valuable tool to characterize receptors. Although pA_2 estimates in general do not provide conclusive evidence, it can be used as suggestive for the presence of a given receptor subtype. As the pharmacological characterization of the relaxant 5-HT receptor (see below) suggested that the relaxation to 5-HT is due to an interaction with 5-HT_7 receptors, mesulergine and ketanserin were also tested versus 5-HT in the presence of the selective 5-HT_7 receptor antagonist SB-269970. In the presence of SB-269970 (100 nM), ketanserin and mesulergine competitively antagonized the 5-HT-induced contraction curve, yielding a pK_B estimate for ketanserin and a pA_2 estimate for mesulergine. Ketanserin also competitively antagonized the contraction to α-Me-5-HT (Table 3).

Ketanserin has an agonist-independent affinity for the contractile 5-HT receptor, as both pK_B estimates (to 5-HT and to α-Me-5-HT) are not significantly different. The ketanserin affinity for the 5-HT receptor involved agrees well with literature affinities for the 5-HT_{2A} receptor (dog colon: pK_B of 8.4; Prins *et al.* (1997), rat caudal artery: pA_2 of 8.4; Blackburn *et al.* (1988)). This confirms the presence of contractile 5-HT_{2A} receptors in dog proximal stomach muscle.

Also mesulergine shows agonist-independent affinity for the contractile 5-HT receptor as the pA_2 estimates to 5-HT (with (8.46 ± 0.15) and without (8.26 ± 0.25) SB-269970) and α-Me-5-HT (8.25 ± 0.06) are not significantly different. For mesulergine, literature affinity estimates for 5-HT_{2A} receptors vary from 7.4 (Jerman *et al.*, 2001) over 8.2 (Prins *et al.*, 1997) to 9.1 (Briejer *et al.*, 1997). This might well be due to differences in affinity values between animal species, as described for mesulergine by Johnson *et al.* (1993). So, our findings for mesulergine (pA_2 estimate 8.25–8.46) do not contradict 5-HT_{2A} receptor involvement.

The relaxatory 5-HT receptor

Of all the tryptamine analogues tested on $PGF_{2\alpha}$-induced contraction, 5-CT was the most potent and most efficacious relaxant agonist. This pointed to the involvement of 5-HT_1 or 5-HT_7 receptors. 5-HT_1 receptor involvement is highly unlikely for reasons indicated above. This is further stressed by the experiments with NAN-190 (30 nM), that did not antagonize the 5-CT-induced relaxations indicating that 5-HT_{1A} receptors are not involved. Both mesulergine and the selective 5-HT_7 receptor antagonist SB-269970 competitively antagonized the 5-CT-induced relaxation (Table 3). SB-269970 has been described as a selective and potent 5-HT_7 receptor antagonist (pKi of 8.9; Lovell *et al.*, 2000). It can be used as a selective radioligand for 5-HT_7 receptors (Thomas *et al.*, 2000) but few functional models have been tested with SB-269970. SB-269970 antagonized the 5-CT-induced stimulation of adenylyl cyclase activity in human 5-HT_7/HEK293 membranes (pA_2 of 8.5) and in guinea-pig hippocampal membranes (pK_B of 8.3; Hagan *et al.*, 2000). It is conceivable

that the dog 5-HT_7 receptor expresses higher affinity for SB-269970 than the human or guinea-pig 5-HT_7 receptor. Overall, the obtained affinities strongly point to 5-HT_7 receptor involvement.

The pK_B for mesulergine against 5-CT agrees well with the affinity estimates obtained in 5-HT_7 receptor bioassays of the guinea-pig ileum (7.8; Carter *et al*., 1995), rat jejunum (8.1; McLean & Coupar, 1996) and human colon (8.3; Prins *et al*., 1999). Thus, the antagonism by mesulergine emphasizes that 5-HT_7 receptors mediate relaxation in this bioassay of dog stomach.

In the presence of ketanserin (0.3 μM), the 5-HT-induced relaxation could be competitively antagonized by SB-269970 and mesulergine. The estimates obtained are in good agreement with literature affinities at 5-HT_7 receptors and thus strongly point to 5-HT_7 receptors mediating relaxation in this bioassay of canine proximal stomach. This finding is not surprising since we recently reported smooth muscle 5-HT_7 receptors mediating inhibition of electrically induced contraction in the canine distal stomach (antropyloric tissue) (Prins *et al*., 2001b). This firmly establishes the pharmacological impact of 5-HT_7 receptor stimulation in the dog stomach *in vitro*. It remains to be determined as to whether this is translated into a significant role of 5-HT_7 receptor stimulation as a trigger for dog gastric relaxation *in vivo*.

Conclusion

5-HT elicits both contraction and relaxation in canine proximal stomach longitudinal muscle strips *via* 5-HT receptors located on the smooth muscle cells. The receptor mediating contraction has the characteristics of a 5-HT_{2A} receptor, while the receptor mediating relaxation is a 5-HT_7 receptor.

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