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5-HT_7 receptor efficacy distribution throughout the canine stomach

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1 This study aimed to determine, quantify and explain regional differences in the relaxant response to the selective 5-HT_1 and 5-HT_7 receptor agonist 5-carboxamidotryptamine (5-CT) throughout the canine stomach.

2 Longitudinal muscle strips from eight gastric corpus regions and six antrum regions were mounted for isotonic measurement. The 5-CT-induced relaxation was examined on a prostaglandin $F_{2\alpha}$-induced submaximal response, expressed as percentage of this response and fitted to the operational model of agonism (OMOA). 5-HT_7 receptor messenger RNA (mRNA) expression was compared by means of quantitative PCR.

3 5-CT inhibited $PGF_{2\alpha}$-induced tonic contraction (corpus) and increase of phasic contraction amplitude (antrum). The consistent antagonism produced by the selective 5-HT_7 receptor antagonist SB-269970 (10 nM, pA_2 estimates 8.2–8.9) confirmed that in every region, the inhibition by 5-CT was 5-HT_7 receptor mediated. However, variation in the maximum effect (61–108%) and pEC_{50} (6.4–8.6) was observed throughout the different regions. The OMOA explained these differences as differences in the efficacy parameter τ (ratio of receptor density and coupling efficiency; log τ estimates ranging from 0.1 to 2.1). The log τ gradient decreases going from the lesser to the greater curvature. A proportional difference (68%) in the relative expression of 5-HT_7 receptor mRNA between the lesser and the greater curvature indicates that differences in receptor density contribute to the observed functional differences.

4 This study illustrates that 5-HT_7 receptors are present throughout the ventral wall of the canine stomach, but the efficacy (expressed as log τ) is clearly greater close to the lesser curvature. Differences in 5-HT_7 receptor expression at least partially explain the functional differences.
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Keywords: 5-HT7; operational model of agonism; SB-269770; 5-CT; dog; proximal stomach; efficacy distribution; receptor density; quantitative PCR

Abbreviations: 5-CT, 5-carboxamidotryptamine; GR-113808, [1-[2-[(methylsulphonyl) amino]ethyl]-4-piperidinyl]methyl 1-methyl-1H-indole-3-carboxylate; GR-127935, 2-methyl-4-(5-methyl-[1,2,4]oxadiazol-3-yl)-biphenyl-4-carboxylic acid [4-methoxy-3-(4-methyl-piperazin-1-yl)-phenyl]amide; 5-HT, 5-hydroxytryptamine; OMOA, operational model of agonism; PCR, polymerase chain reaction; $PGF_{2\alpha}$, prostaglandin F2 alpha; SB-204741, N-(1-methyl-5-indolyl)-*N*′-(3-methyl-5-isothiazolyl)urea; SB-269970, (R)-3-(2-(2-(4-methyl-piperidin-1-yl)ethyl)-pyrrolidine-1-sulphonyl)-phenol; SNP, sodium nitroprusside; WAY-100635, *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-2-pyridinyl-cyclohexanecarboxamide

Introduction

The stomach is anatomically divided into three regions: an upper fundus part, a middle corpus part and a distal antrum part. With regard to the motor function, we can divide the stomach in two main functional regions: the proximal stomach (consisting of the fundus and the proximal part of the corpus) and the distal stomach (consisting of the distal part of the corpus and the antrum) that have different motility patterns according to their function (Code & Carlson, 1968). In general, the distal stomach is involved in mixing and grinding of solid food, while the proximal stomach is well known as a reservoir for food as it relaxes upon food intake. After relaxation, slow tonic contractions of the proximal stomach will gradually drive its contents to the antrum. By means of a groove-like structure along the lesser curvature of the stomach, liquids and fine-particulate digests can bypass this slow digestive process and decrease directly to the pylorus (Pfeffer, 1987).

Functional dyspepsia is one of the most prevalent functional gastrointestinal disorders (Talley *et al*., 1992). Several studies have shown that impaired meal-induced relaxation of the proximal stomach may play a role in the generation of dyspeptic symptoms in an important subgroup of functional dyspepsia patients (Gilja *et al*., 1996). Pharmacological induction of gastric relaxation and/or evacuation of gastric contents from the proximal stomach might therefore be helpful in these patients.

A number of studies have shown that 5-HT receptor activation can induce stomach relaxation. The antimigraine

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drug sumatriptan that has affinity at different 5-HT$_1$ receptor subtypes, was shown to induce feline and human proximal stomach relaxation (Tack *et al*., 2000). The mechanism by which sumatriptan influences the proximal gastric tone is yet unknown but it has been hypothesized that it activates enteric neuronal 5-HT receptors on intrinsic nitrergic neurons (Coulie *et al*., 1999). In view of the close anatomical and functional resemblance between the human and canine stomach (Kararli, 1995) much research was performed in the canine stomach. We recently showed that the 5-HT$_{1A}$ receptor agonist flesinoxan induced *in vivo* canine proximal stomach relaxation, a response that could be blocked by the selective 5-HT$_{1A}$ receptor antagonist WAY-100635. This response was abolished by vagotomy, but could not be blocked by a NO synthase inhibitor (Janssen *et al*., 2003). We also showed that, in addition to 5-HT$_1$ receptors, activation of 5-HT$_7$ receptors induces stomach relaxation. *In vitro*, the 5-HT$_1$ and 5-HT$_7$ receptor agonist 5-carboxamidotryptamine (5-CT) inhibited electrically induced cholinergic contractions of canine antral longitudinal muscle strips (Prins *et al*., 2001) and induced relaxation of canine proximal stomach longitudinal muscle strips, contracted by prostaglandin F$_{2\alpha}$ (PGF$_{2\alpha}$; Janssen *et al*., 2002b). The latter effect was not influenced by the nerve conduction blocker tetrodotoxin, but was antagonized by the selective 5-HT$_7$ receptor antagonist SB-269970, indicating muscular 5-HT$_7$ receptor involvement.

A detailed analysis of the (functional) distribution of these muscular 5-HT$_7$ receptors in canine proximal stomach has not been performed. This study aimed to determine, quantify and explain regional differences in the response to 5-CT throughout the canine stomach. Concentration–response curves to 5-CT will be constructed in 14 canine corpus and antrum stomach regions and fitted to the operational model of agonism. The operational model of agonism (as originally proposed by Black & Leff, 1983) is built on the premise that an efficacy term emerges from an experimentally observed behaviour of pharmacological systems, namely the saturable relationship between receptor stimulation and the observed response. By fitting our data to the operational model of agonism, we will be able to determine the efficacy parameter τ per region and determine whether regional variation in τ can account for the observed variation in the location, slope and maximal effect of the 5-CT-induced concentration–response curves. By means of quantitative polymerase chain reaction the relative 5-HT$_7$ receptor expression levels in different corpus regions will be quantified to determine whether the differences in τ can be (partially) explained as differences in receptor density.

Methods

Data acquisition

Tissue preparation Beagle dogs of both sexes, weighing between 10 and 15 kg, were killed by intravenous injection of 133 mg kg^{-1} sodium pentobarbital (Kela N.V., Hoogstraten, Belgium). After exsanguination, the entire stomach was dissected and placed in Krebs–Henseleit solution (composition in mM: glucose 11.1, $CaCl_2$ 2.51, $NaHCO_3$ 25, $MgSO_4$ 1.18, KH_2PO_4 1.18, KCl 4.69 and NaCl 118). By cutting along the lesser curvature the stomach was opened and the contents were rinsed out. After removal of the mucosa, longitudinal muscle strips of approximately 1.5 cm length and 2–3 mm width were prepared from different regions of the stomach (two muscle strips per region; maximum 16 strips per dog). Per strip the major part of other muscle layers was removed by macroscopically cutting them apart from the longitudinal muscle layer. Muscle strips cut from regions 1–2, 5–8 and 11–12 originated from the corpus part of the stomach, while muscle strips cut from regions 3–4, 9–10 and 13–14 originated from the antrum part of the stomach (Figure 1a). Regions 11–14 are located on the dorsal side of the stomach; all other regions are located on the ventral side of the stomach. Per experiment, two muscle strips per region were cut and mounted onto tissue holders. The holders 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 length of the muscle strips was recorded *via* isotonic transducers (2 g load). All strips were studied on the day of preparation.

Experimental protocols 12 and 8 dogs were used to study the effects of 5-CT in corpus and antrum regions, respectively. To study the effect of 5-CT in corpus regions, tissues obtained from 6 dogs were studied in the absence of 5-HT receptor antagonist cocktail, while tissues from the other 6 dogs were studied in the presence of this cocktail (see below). All experiments were conducted in the presence of indomethacin (1 μM) to avoid contractions due to spontaneous release of prostaglandins. A 30 min stabilization period was respected before the beginning of the experiment. Subsequently, the tissue was challenged with 0.3 μM PGF$_{2\alpha}$. In corpus tissues, this induced a tonic contraction, while in antral tissues PGF$_{2\alpha}$ induced an increase of the amplitude of the spontaneous contractions. At maximum response, the organ bath solution was replaced twice leading to the return of the tissue length to basal length. At basal length a concentration–response curve to PGF$_{2\alpha}$ was established with log-unit ascending concentrations (1 nM–0.1 mM) followed by thorough washout and a 30 min stabilization period. A concentration of PGF$_{2\alpha}$ that induced 50–80% of the maximal response to PGF$_{2\alpha}$ (ranging from 33–300 nM in the corpus regions and 0.01–1 μM PGF$_{2\alpha}$ in the antrum regions) was established in the organ bath,

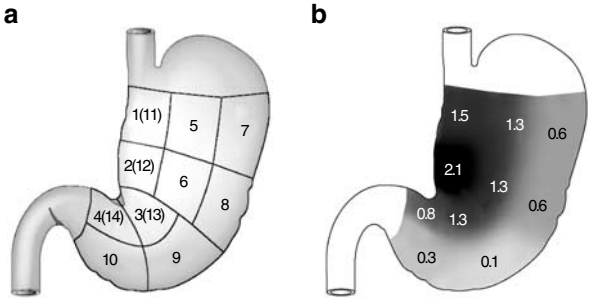


Figure 1 (a) Ventral site of the canine stomach with an indication of the different regions muscle strips were cut from. Numbers between brackets refer to regions located at the dorsal site of the stomach. (b) Ventral site of the canine stomach representing in greyscale the efficacy of the response to 5-CT in the different ventral regions examined whereby a gradual change between the regions is depicted. The intensity of the greyscale represents the population estimate of the log τ-value determined for the respective region (log τ is represented on every respective region). The darkest region (region 2) thus had the largest τ.

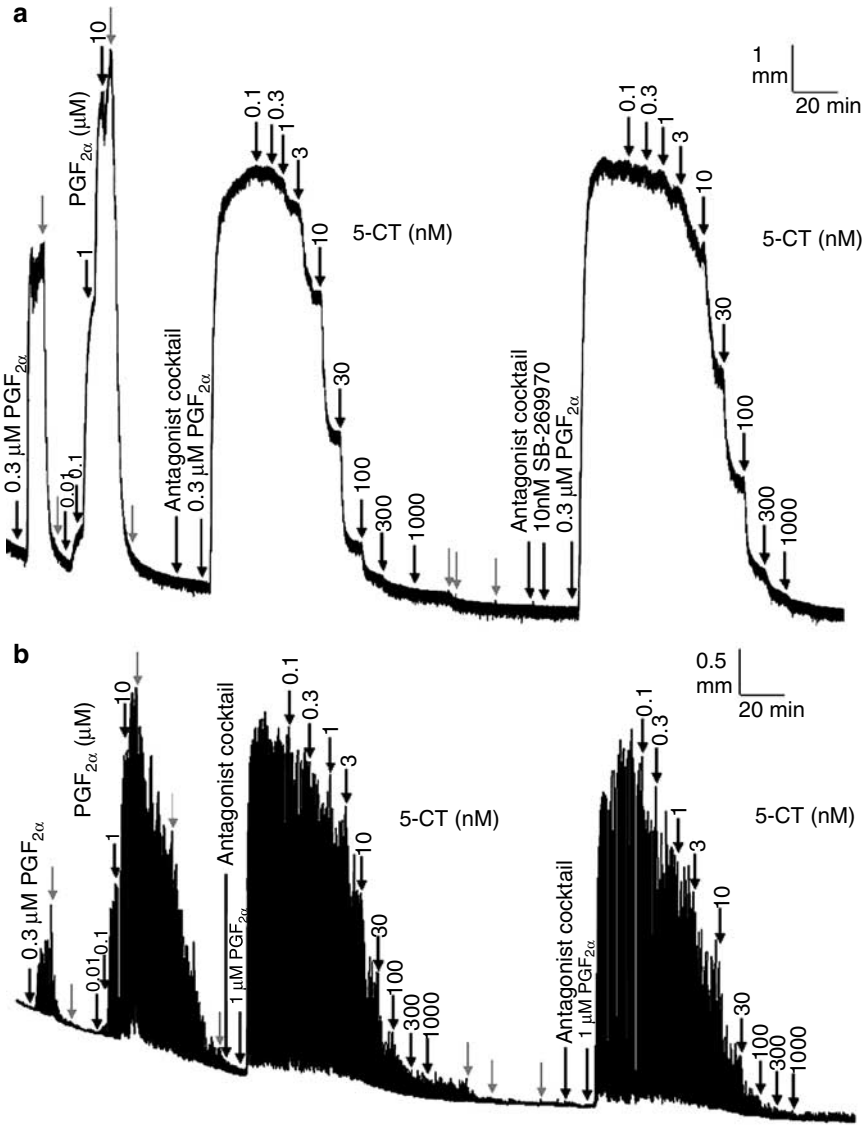


Figure 2 Representative tracings following the protocol in a muscle strip cut from the corpus (region 2: (a)) and the antrum (region 3: (b)). The black arrows indicate drug administration, while the grey arrows indicate replacement of the Krebs solution in the organ bath.

whereupon the relaxant effect of 5-CT was studied. Upon maintained tonic contraction for corpus regions or maintained increase in amplitude of the spontaneous contractions for antrum regions (usually after 5–10 min), a cumulative concentration–response curve for the inhibitory effect of 5-CT was established with half log-unit ascending concentrations (0.1 nM–1 μM; Figure 2). After thorough washout one muscle strip per region was incubated with SB-269970 (10 nM; antagonist tissue), while the other muscle strip served as a control (control tissue). A concentration of $PGF_{2\alpha}$ that induced 50–80% of the maximal response to $PGF_{2\alpha}$ was again established in the organ baths after a 20-min stabilization period. A second cumulative concentration–relaxation curve to 5-CT was established. Similar to 5-CT, 2 successive concentration–response curves to sodium nitroprusside (SNP; 1 nM–1 mM) were established on the $PGF_{2\alpha}$-induced tonic contraction in region 1 and 8.

Before adding $PGF_{2\alpha}$ to construct the first and second concentration–response curve to 5-CT or SNP a 5-HT receptor antagonist cocktail was added in corpus tissues of 6 dogs and in all antral tissues studied to avoid interaction with non-5-HT$_7$ receptors: WAY-100635 (0.1 μM; 5-HT$_{1A}$ receptor antagonist), GR127935 (0.1 μM; 5-HT$_{1B}$ and 5-HT$_{1D}$ receptor antagonist), ketanserin (0.1 μM; 5-HT$_{2A}$ receptor antagonist), SB-204741 (0.3 μM; 5-HT$_{2B}$ receptor antagonist), granisetron (0.3 μM; 5-HT$_3$ receptor antagonist) and GR113808 (0.1 μM; 5-HT$_4$ receptor antagonist). Concentrations and antagonists of the cocktail were selected on basis of literature data in different species. No experimentation was performed to investigate whether these receptors were present in the dog stomach in the first place. Data collection was performed using Chart for windows (v4.12, ADInstruments, Oxfordshire, U.K.)

Real-time quantitative PCR

Total RNA extraction and cDNA synthesis Tissues of 6 Beagle dogs were used to quantify the 5-HT$_7$ receptor message RNA (mRNA) expression in regions 2 and 8. After the dogs were killed by intravenous injection of sodium pentobarbital

(see above) the entire stomach was dissected and longitudinal muscle samples deprived from other muscle layers and mucosa were cut from regions 2 and 8 and snap-frozen in liquid nitrogen. By means of a binocular it was assured that no other muscle layer other than the longitudinal was present in the isolated samples. The tissue was stored at −80°C. Total RNA was extracted using RNeasy MiniKit (Qiagen Gmbh, Hilder, Germany). Potential contaminating DNA was removed by treatment with DNase I (RNase-Free DNase Set; Qiagen). A measure of 1 μg of total RNA was subjected to first-strand complementary DNA synthesis using random hexamer primers (Applied Biosystems, Foster City, CA, U.S.A.) and Super Script II (Invitrogen, Carlsbad, CA, U.S.A.).

Polymerase chain reaction (PCR) and sequencing of dog 5-HT$_7$ receptor cDNA Based upon the known human sequence of 5-HT$_7$ receptor (Heidmann *et al*., 1997) and a canine expressed sequence tag (EST; GenBank Accession no.: CF409095) showing 90% identity to the human sequence, primers were designed both near the ATG start codon and the TGA stop codon (Table 1). Touchdown PCR on dog frontal cortex cDNA was performed using the Advantage-GC cDNA PCR Kit (BD Biosciences, Palo Alto, CA, U.S.A.) according to the manufacturer's instructions. After a pre-PCR incubation for 10 min at 95°C the reaction conditions were 30 s at 95°C, 30 s at 60°C and 30 s at 72°C for 40 cycles followed by a final 8-min extension step at 72°C. PCR products were cloned into pCR 2.1-TOPO (Invitrogen). Plasmids were sequenced using the ABI PRISM BigDye Terminators v3.0 Cycling Sequencing Kit according to the instructions of the supplier (Applied Biosystems). Software used for sequence assembly was Sequencher version 4.0.5 from Gene Codes (Ann Arbor, MI, U.S.A.).

Real-time quantitative polymerase chain reaction (qPCR) qPCR was used to measure the expression of 5-HT$_{7(a)}$, 5-HT$_{7(b)}$ splice variants and total 5-HT$_7$ receptor mRNA in longitudinal muscle strips of regions 2 and 8. In order to determine the relative occurrence of 5-HT$_7$ receptor mRNA, the expression of two housekeeping genes was also quantified (β-actin (ACTB) and the smooth muscle myosin heavy chain (MYH11) gene). Primers and FAM-TAMRA probes for quantification of 5-HT$_7$ receptor, β-actin and MYH11 expression were designed on canine sequences using PrimerExpress software (Applied Biosystems; Table 1).

The amplification was carried out on an ABI 7900HT Sequence Detector (Applied Biosystems) using the TaqMan Buffer A Pack (Applied Biosystems). Amplification conditions were 10 min at 95°C, followed by 50 cycles of 15 s at 95°C and 1 min at 60°C. All reactions were performed in quadruple. Results were collected and analysed using SDS 2.2 software (Applied Biosystems).

Drugs The following drugs were used (respective suppliers in parentheses): 5-CT (Tocris Cookson, U.K.); GR-127935 and SB-204741 (kindly donated by GlaxoSmithKline, U.K.); WAY-100635 (Sigma-Aldrich, Belgium); PGF$_{2\alpha}$ (diluted from Dinolytic® 5 mg ml^{-1}; Upjohn, Animal Health, Belgium); SB-269970, granisetron HCl, ketanserin tartrate, GR113808 and SNP (Johnson & Johnson research and development, Beerse, Belgium). Granisetron, ketanserin, SNP and WAY-100635 were dissolved in distilled water. SB-269970 and PGF$_{2\alpha}$ were dissolved in distilled water containing 0.9% NaCl. 5-CT and GR-127935 were dissolved in distilled water containing 10% cyclodextrin + 0.45% NaCl in the stock solution. SB-204741 was dissolved in distilled water containing 20% cyclodextrin +2 equivalents 2,3-dihydroxybutanedioic acid and GR-

Table 1 Primer and probe sequences as used in normal and quantitative polymerase chain reaction

Primers for cloning of canine 5-HT$_7$ receptor sequence	
Conserved primers	
Forward primer:	5′-GCCGCCCGGACCTCTA-3′
Reverse primer:	5′-TCGACTCTGCCGTAGTTGATCTG-3′
Canine primers	
Forward primer:	5′-CCGGCTGTGGGGAACAGATCAA-3′
Reverse primer:	5′-CAACAGATCACCCTGGTTGTTA-3′
Primers and probes for quantitative PCR	
β-*actin*	
Forward primer:	5′-CACTTTCCTGTCTTACCCAATGTTT-3′
Probe:	5′-CCACCCACGGTGTTCTGGCAGG-3′
Reverse primer:	5′-CGTTTTCCAACTTCAAGGCAAT-3′
Myh11	
Forward primer:	5′-CCTGGAGCAAGAGGAGTACCA-3′
Probe:	5′-AGTTCCACTCGATGCCCTCGCG-3′
Reverse primer:	5′-GGTCAAGCCCGAAGTCGAT-3′
5-HT$_7$ (total population)	
Forward primer:	5′-TGATTCTCTCCGTCTGGCTTCT-3′
Probe:	5′-TCGGCCTCCATCACATTACCACCG-3′
Reverse primer:	5′-GTTCTGAGCCCATCCGAAGA-3′
5-HT$_7$ (a-variant)	
Forward primer:	5′-ATGCACGAGGCCCTGAAG-3′
Probe:	5′-GTCTCTCGGGCCTCTCGGCGA-3′
Reverse primer:	5′-CCTACAGTAGTCAGAGTTTTGTAGCACAA-3′
5-HT$_7$ (b-variant)	
Forward primer:	5′-ATGCACGAGGCCCTGAAG-3′
Probe:	5′-TCGGGTCTCTCGGGCCTCTCG-3′
Reverse primer:	5′-CTGTAAGACAAAACTCTGACTACTGTAGGA-3′

113808 was dissolved in distilled water containing two equivalents 2,3-dihydroxybutanedioic acid. The solvents had no effect on the baseline tone or the curves to agonists.

Data analysis

Hill equation curve parameter All responses to 5-CT and SNP in the corpus area were expressed as percentage reduction of the submaximal contraction to $PGF_{2\alpha}$ before administration of 5-CT or SNP. In the antrum, the mean amplitude of the spontaneous contractions after administration of a given concentration of 5-CT or SNP was calculated in the last 3 min before administration of the next concentration and expressed as percentage of the amplitude of the spontaneous contractions in the presence of $PGF_{2\alpha}$ before starting administration of 5-CT or SNP.

Concentration–response ([*A*]–*E*) curves to $PGF_{2\alpha}$, SNP and 5-CT were individually fitted to the Hill equation by nonlinear regression obtaining curve parameter estimates for midpoint location (EC_{50}, estimated as $-\log(EC_{50})$), upper asymptote of the observed maximal effect (α) and Hill slope (n_H):

$$E = \frac{[A]^{n_H}\alpha}{EC_{50}^{n_H} + [A]^{n_H}} \quad (1)$$

To verify whether a relationship is present between the 5-CT curve and the $PGF_{2\alpha}$ curve parameters, all parameters were plotted vs each other. No correlation was found between the $PGF_{2\alpha}$ and the 5-CT curve parameters illustrating that the sensitivity of a given tissue to $PGF_{2\alpha}$ did not determine the sensitivity to 5-CT.

pA_2 and pA_2' estimates for SB-269970 In every region, two cumulative concentration–response curves to 5-CT were established in two parallel muscle strips (control and antagonist tissue). As the second concentration–response curve to 5-CT was shifted to the left compared to the first one in control tissues, the apparent affinity dissociation constant or pA_2 for SB-269970 was estimated from the dose ratio (DR) of the EC_{50} values from the second curves of the parallel antagonist and control tissues. The apparent pA_2 values were only accepted when the pEC_{50} estimates of the concentration–response curve to 5-CT of the first curves of the tissue pairs were not significantly different (this was the case in every region except for region 13 hence no pA_2 was estimated in region 13). A pA_2 for SB-269970 was estimated (according to Arunlakshana & Schild, 1959) in those regions where there was a significant increase of the EC_{50} values in the presence of SB-269970:

$$pA_2 = \log(DR - 1) - \log[SB - 269970] \quad (2)$$

In all corpus regions, experiments with 5-CT were also performed without antagonist cocktail. Without antagonist cocktail 5-CT at lower concentrations induced relaxation but from 0.1 to 1 μM onwards contraction. In the presence of SB-269970, the contractile effect to 5-CT was predominant so that no EC_{50} could be estimated for the relaxation effect. A pA_2 value for SB-269970 in the absence of antagonist cocktail could thus not be determined. Comparing control tissues, 5-CT turned out to be a significantly less potent relaxant in several regions when studied in the presence of antagonist cocktail, illustrating that the antagonist cocktail not only blocks the 5-CT-induced contraction, but also antagonizes the 5-CT-induced relaxation. When determining the pA_2 value for SB-269970, the presence of this antagonistic effect can be taken into account. Therefore, the DR_{Cockt} of the mean EC_{50} estimate from the concentration–response curves to 5-CT in the control tissues obtained in the absence and presence of antagonist cocktail was determined. This enables to estimate a pA_2' for SB-269970 taking into account the antagonistic effect of the antagonist cocktail (Kenakin, 1997):

$$pA_2' = -\log\left(\frac{[SB - 269970]}{DR_{Cockt}(DR - 1)}\right) \quad (3)$$

For comparison of two groups of data a paired or unpaired *t*-test was performed depending on whether results within the same group of tissues or in two different groups were compared. For comparison of more groups one-way or repeated measures ANOVA, followed by a *posthoc* Tukey–Kramer's test for multiple comparisons was used. Unless otherwise indicated results are expressed as the mean with the 95% confidence intervals; $P<0.05$ was considered significant.

The operational model of agonism One theoretical shortcoming of the occupation theory (of which the Hill equation formula (1) is a practical application) is the *ad hoc* nature of the efficacy term (α in formula (1)). In 1983, Black and Leff introduced the operational model of agonism (OMOA) that obviated the need for an empirical constant to account for efficacy. The premise on which the OMOA is built is the fact that the efficacy term emerges from an experimentally observed behaviour of pharmacological systems, namely the saturable relationship between receptor stimulation and the observed response (more information about and practical applications of the OMOA can be found in Leff *et al*., 1990). We applied the OMOA to quantify differences in the expression of agonism at the same receptor across the different regions of the canine stomach. It has indeed been shown that the OMOA is a useful expression for the characterization of the drug–receptor interaction, allowing a separation between drug- and system-related parameters. The OMOA describes agonist binding to a receptor population to form an agonist–receptor complex (determined by K_A) that in turn is transduced into effect (determined by K_{AR}). As such, it describes effect E as a function of the agonist concentration A within the following formula:

$$E = \frac{E_{max}\tau^{n_t}A^{n_t}}{(K_A + A)^{n_t} + \tau^{n_t}A^{n_t}} \quad (4)$$

where E_{max} is the maximum effect achievable in the system, K_A is the agonist–receptor complex dissociation equilibrium constant, n_t is the slope index for the occupancy–effect relation, and τ is the efficacy parameter, which is defined by the ratio of total receptor density (R_0) and the activated receptor – G-protein complex dissociation constant (K_{AR}). A nonlinear mixed-effects (nlme) model fit of the observed data to the operational model of agonism was performed (nlme package as implemented in S-PLUS (v.6.1, Insightful Corporation, Seattle, WA, U.S.A.); S-PLUS code can be obtained from the authors). For every region the interindividual variability on τ was modelled to an exponential equation:

$$\tau_{region,ind.} = \tau_{region}\exp(\eta_{ind.}) \quad (5)$$

where $\tau_{region,\ ind.}$ represents the individual value for τ in a region, τ_{region} is the population value for τ, and $\eta_{ind.}$ is the random deviation of $\tau_{region,ind.}$ from τ_{region}. The values of $\eta_{ind.}$ are assumed to be independently normally distributed with mean zero and variance ω^2. Receptor affinity is generally considered to be constant in different tissues of the same individual and across individuals; moreover, we assume that the maximum achievable contractile force of the muscle strips is constant throughout the regions and individuals examined. We therefore assume that interindividual and inter-regional variation of K_A and E_{max} is insignificant. Furthermore, since n_H and n_t are related (Black *et al*., 1985), and we found no systematic interindividual and inter-regional differences between the Hill slopes it was assumed that interindividual and inter-regional variation of n_t was also insignificant. K_A and τ were estimated as –log (K_A) and log τ respectively, because these parameters are assumed to be log-normally distributed (Leff *et al*., 1990; Van der Graaf & Danhof, 1997). The residual error was characterized by a proportional error model:

$$ym_{ij} = yp_{ij}(1 + \varepsilon_{ij}) \quad (6)$$

where yp_{ij} is the jth prediction for the ith individual predicted by the model, ym_{ij} is the measurement, and ε accounts for the residual deviation of the model predicted value from the observed value. Only data from the first curve of the control tissues were used for the operational model of agonism analysis ($n = 6$ for corpus regions; $n = 8$ for antrum regions).

Quantitative-PCR qPCR data were plotted as the ΔR_n fluorescence signal vs the cycle number C. An arbitrary threshold was set to the midlinear portion of the log ΔR_n cycle plot. The threshold cycle (C_T) is defined as the cycle number at which the ΔR_n crosses this threshold. Standard curves prepared from dilutions of cDNA and 5-HT$_7$ receptor DNA-containing plasmids were used to calculate corresponding mRNA levels. To minimize intra-assay and interassay variability caused by differences in transcriptase efficiency, 5-HT$_7$ receptor mRNA quantities were normalized against the amount of β-actin and Myh11 mRNA in every respective sample and expressed as percentage of the β-actin or Myh11 expression with the 95% confidence intervals. For comparison of relative gene expression in two tissues of the same animal, a Wilcoxon signed-rank test was performed.

Results

Corpus muscle strips

Functional responses Corpus muscle strips (regions 1–2, 5–8 and 11–12) showed no spontaneous contraction during the stabilization period and responded to $PGF_{2\alpha}$ with a sustained tonic contraction (Figure 2a).

In a first series of experiments the effect of 5-CT on precontracted muscle strips was tested in the absence of antagonist cocktail. 5-CT induced relaxation in all regions tested. In regions 5–8 however, 5-CT also induced a concentration-dependent contraction from 0.1 to 1 μM onwards. In preliminary experiments it was shown that both contraction and relaxation to 5-CT were not influenced by tetrodotoxin (0.3 μM; results not shown). Antagonist cocktail was added to avoid interference with non-5-HT$_7$ receptors. No contraction to 5-CT was seen in the presence of antagonist cocktail enabling us to focus on the relaxatory component. It was not determined which of the blocked receptors was accountable for the contractile response. Within all corpus regions the concentration–response curves of 5-CT in absence of antagonist cocktail were located to the left and had a decreased maximum observed effect compared to the curves of 5-CT performed in the presence of antagonist cocktail, significance was reached in several regions (results not shown). All following experiments were performed in the presence of antagonist cocktail.

The curve-fit parameters of the first 5-CT-induced concentration–response curves in the control tissues are given in Table 2. pA_2 and pA_2' estimates for SB-269970 are presented in Table 3. EC_{50} values of the second curves in the antagonist and control tissue of region 11 were not significantly different, hence no pA_2 was estimated.

Although the similarity of the pA_2 and pA_2' estimates for SB-269970 between the regions studied suggests that 5-CT

Table 2 Hill equation curve-fit parameters of the first concentration–response curves to 5-CT on $PGF_{2\alpha}$-precontracted control muscle strips in different corpus and antrum regions (in the presence of 5-HT-receptor cocktail)

Region		*pEC$_{50}$*	*α (% relaxation)*	n_H
Corpus	1	7.99 (7.59–8.39)	104.81 (97.93–111.70)	1.30 (1.11–1.48)
	2	8.58 (8.17–9.00)	107.92 (103.93–111.90)	1.47 (1.14–1.80)
	5	7.57 (7.05–8.10)	96.10 (79.57–112.63)	1.03 (0.89–1.17)
	6	7.39 (7.16–7.61)	96.47 (91.70–101.24)	0.92 (0.77–1.07)
	7	7.10 (6.77–7.42)	78.28 (60.51–96.05)	1.15 (0.85–1.46)
	8	6.96 (6.70–7.21)	75.03 (51-85–98.20)	1.21 (1.03–1.39)
	11	7.78 (7.40–8.17)	105.61 (103.35–107.87)	1.25 (0.87–1.64)
	12	8.41 (8.22–8.63)	106.86 (99.96–112.76)	1.29 (0.85–1.73)
Antrum	3	7.16 (6.57–7.75)	94.59 (89.96–99.23)	1.22 (0.94–1.50)
	4	6.94 (6.18–7.70)	96.55 (87.09–106.02)	1.24 (0.80–1.67)
	9	6.39 (5.83–6.94)	64.41 (37.12–94.69)	1.12 (0.46–1.78)
	10	6.78 (6.57–6.98)	61.16 (52.96–69.36)	1.20 (1.01–1.39)
	13	6.91 (6.59–7.23)	86.98 (67.94–106.03)	1.25 (0.87–1.63)
	14	6.97 (6.67–7.27)	99.40 (94.26–104.54)	1.11 (0.77–1.45)

All curve parameters are expressed as mean with the 95% confidence intervals ($n = 6$–8).

Table 3 pA_2 and pA_2' (after correction for the antagonistic properties of the antagonist cocktail for the 5-HT$_7$ receptor) estimates for SB-269970 in different corpus and antrum regions tested

Region		*pA$_2$ estimate*	*pA$_2'$ estimate*
Corpus	1	8.39 (7.54–9.24)	8.84 (8.48–9.21)
	2	8.63 (8.30–8.96)	8.82 (8.36–9.29)
	5	8.57 (7.89–9.25)	8.58 (8.18–8.89)
	6	8.54 (8.05–9.03)	9.11 (7.70–10.51)
	7	8.19 (7.96–8.42)	8.80 (8.46–9.14)
	8	8.24 (7.72–8.75)	9.00 (8.38–9.63)
	12	8.54 (8.25–8.82)	8.53 (7.94–9.12)
Antrum	3	8.63 (8.21–9.06)	n.d.
	4	8.72 (8.03–9.41)	n.d.
	14	8.89 (8.15–9.63)	n.d.

pA_2 or pA_2' estimates are expressed as mean with the 95% confidence intervals ($n=5$–6). No significant difference was found between the pA_2 or pA_2' estimates between the different regions. No pA_2 was estimated in regions 9, 10, 11 and 13; in region 13 there was a significant difference in potency between the first curve in the control and antagonist tissue, in regions 9, 10 and 11 the EC_{50} for 5-CT in the presence of SB-269970 was not significantly different from the EC_{50} for 5-CT in the parallel control tissue, hence no pA_2 was determined; n.d.: not determined.

interacts with the same receptor in the different regions, clear-cut regional variation in the observed maximal effect, location and slope of the 5-CT-induced concentration–response curves was observed throughout the corpus (see Table 2: e.g. pEC_{50} and α ranged from 8.58 and 107.92% in region 2 to 6.96 and 75.03% in region 8). These differences were not related to differences in the contractile response to $PGF_{2\alpha}$. Indeed, all $PGF_{2\alpha}$ and 5-CT-induced curves were fitted to the Hill equation and the curve-fit parameters of the $PGF_{2\alpha}$ and 5-CT-induced curves were plotted vs each other but no correlation was found. To determine whether differences in the response to 5-CT were dependent on the relaxant capacity of the smooth muscle strips in the different regions, the effect of sodium nitroprusside (SNP) was tested in muscle strips from a region close to the lesser curvature (region 1) and close to the greater curvature (region 8). A concentration–response curve to SNP was established ($n=6$) in both regions; fitting to the Hill equation resulted in the following curve parameters: the maximum observed effect (96.22 (94.53–97.92) vs 96.08 (94.71–97.46)), Hill slope (0.91 (0.84–0.98) vs 0.99 (0.94–1.05)) and pEC_{50} estimate value (6.60 (6.18–7.02) and 7.03 (6.66–7.40)) in regions 1 and 8, respectively. Only the pEC_{50} estimate was significantly different ($P<0.05$).

In order to quantify differences in the response to 5-CT between the different regions, the operational model of agonism (OMOA) was applied to the data. E_{max}, n_t and pK_A are considered to be constant throughout the different regions and interindividual variability was only allowed for τ (Table 4). The log τ estimates for the different regions are shown in Figure 1b using a contrast intensity scale. It is clear that the log τ estimates decrease from the lesser to the greater curvature. Population predicted curves from the OMOA fit are given in Figure 3a.

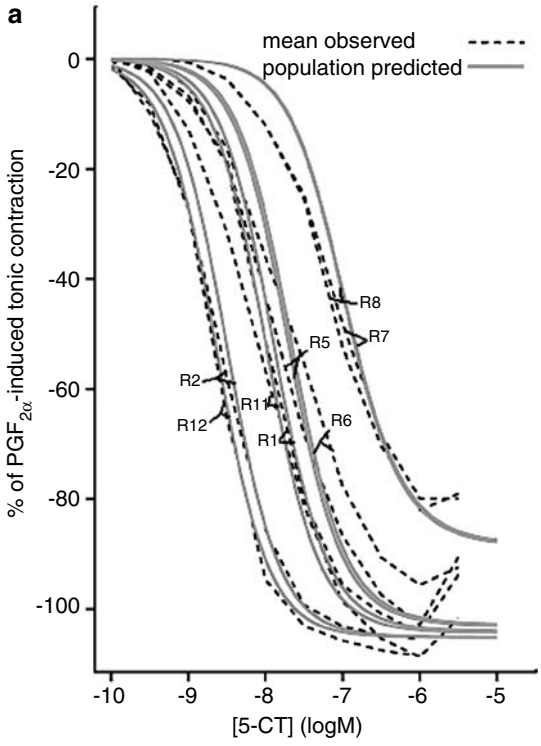
PCR reactions In order to identify canine 5-HT$_7$ receptor sequences, a PCR approach was followed on material from corpus regions 2 and 8. Sequence analysis of different PCR products revealed the presence of two splice variants of the canine 5-HT$_7$ receptor gene. Similar to the human and rat orthologue, we designated these splice variants 5-HT$_{7(a)}$ (EMBL Accession No. AJ628248) and 5-HT$_{7(b)}$ (EMBL Accession No. AJ628249). As in human and rat, the 5-HT$_{7(b)}$ isoform has a 5 bp insert (GTAAG) within the coding sequence introducing a premature termination of the open reading frame.

To quantify and compare the expression of total 5-HT$_7$ receptors, and of 5-HT$_{7(a)}$ and 5-HT$_{7(b)}$ receptors in regions 2 and 8, quantitative PCR was used. The total 5-HT$_7$ receptor expression level was significantly lower in region 8 compared to region 2 ($P<0.05$), this both relative to Myh11 (68 (42–94)%) and β-actin (61 (30–92)%; Figure 4). The 5-HT$_{7(a)}$ receptor expression was also significantly lower in region 8 compared to region 2 ($P<0.05$), relative to Myh11 (62 (46–78)%) and β-actin (62 (49–74)%). Relative to the total 5-HT$_7$ receptor expression, the expression of 5-HT$_{7(a)}$ receptor was 50 (−4–104)% in region 2 and 50 (25–76)% in region 8. No significant difference was found between the expression of 5-HT$_{7(b)}$ receptor in regions 2 and 8 (results not shown), the

Table 4 Population and individual parameters (expressed as a 95% confidence interval (95% CI) around the population value) of the operational model of agonism model fit of 5-CT-induced relaxation of corpus and antrum muscle strips using a nonlinear mixed effects modelling approach

	Corpus			*Antrum*	
Parameter	*Population prediction mean (s.e.)*	*Individual variability 95% CI*	*Parameter*	*Population prediction mean (s.e.)*	*Individual variability 95% CI*
E_{max}	105.31 (0.97)	n.d.	E_{max}	101.56 (2.55)	n.d.
log τ_1	1.49 (0.17)	1.22–1.77	log τ_3	1.30 (0.13)	1.21–1.40
log τ_2	2.09 (0.19)	1.88–2.31	log τ_4	0.82 (0.23)	0.26–1.38
log τ_5	1.31 (0.25)	0.85–1.77	log τ_9	0.07 (0.05)	n.d.[a]
log τ_6	1.27 (0.14)	1.01–1.52	log τ_{10}	0.27 (0.06)	0.27–0.27
log τ_7	0.56 (0.21)	−0.35–1.46	log τ_{13}	0.88 (0.14)	0.63–1.13
log τ_8	0.57 (0.17)	−0.16–1.29	log τ_{14}	1.01 (0.13)	0.84–1.13
log τ_{11}	1.59 (0.20)	1.28–1.90	pK_A	6.89 (0.11)	n.d.
log τ_{12}	2.24 (0.10)	2.15–2.33	n_t	1.08 (0.09)	n.d.
pK_A	6.40 (0.06)	n.d.			
n_t	1.28 (0.03)	n.d.			

The overall estimated residual was 0.47 and 0.52 for the modelling of the corpus and antrum data, respectively.
[a]Interindividual variability in log τ of region 9 was infinitely small and therefore constraint to 0 during the modelling. n.d.: not determined.



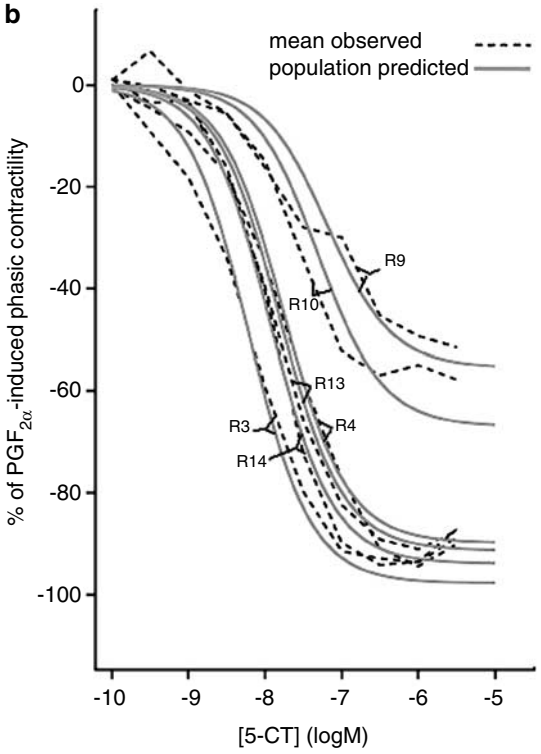


Figure 3 Prediction of the 5-CT induced effect in different regions of corpus (a) and antrum (b) based on population parameter estimates from the nonlinear mixed effect model fit of the observed data to the operational model of agonism (continued lines). Discontinued lines connect the mean observed data points from the different regions indicated. R1–R14 indicate the respective regions (regions 1–14) from which the data are obtained.

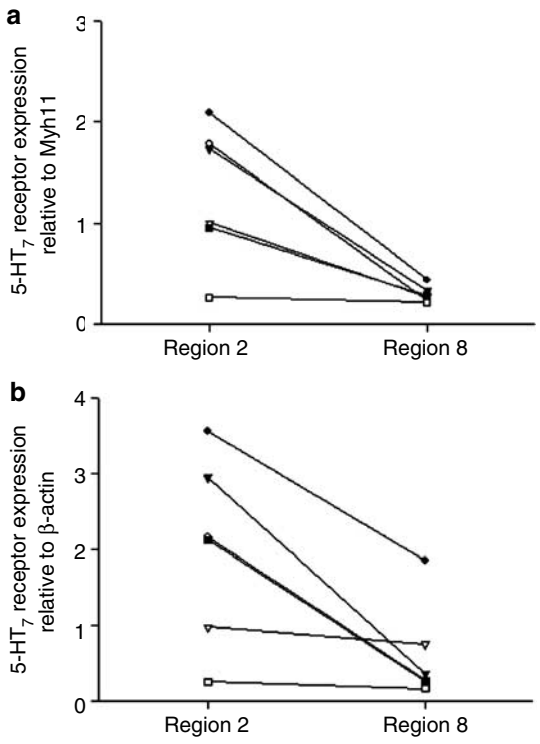


Figure 4 Total 5-HT$_7$ receptor mRNA expression, relative to the Myh11 (a) and β-actin (b) expression in regions 2 and 8.

expression of this splice variant was however insignificant relative to the expression of total 5-HT$_7$ receptor mRNA (0.28 (0.01–0.55)% in region 2 and 1.24 (0.32–2.15)% in region 8.

Antrum muscle strips No experiments without antagonist cocktail were performed in the antrum regions. In concordance with the findings of the corpus region however, we decided to perform the antrum muscle strip experiments in the presence of antagonist cocktail to avoid interference with non-5-HT$_7$ receptors.

During the stabilization period, muscle strips cut from antrum regions (regions 3–4, 9–10 and 13–14) showed spontaneous contractions. In response to PGF$_{2\alpha}$, muscle strips from the antrum regions responded with a sustained increase in amplitude of the spontaneous contractions (Figure 2b). Hill curve parameters of the PGF$_{2\alpha}$-induced curves in all tested regions were plotted vs the parameters of the 5-CT-induced curves, but no correlation was found (parameters of the first 5-CT-induced curves in the control tissues are shown in Table 2). In two out of six muscle strips from region 14, no increase in amplitude of the spontaneous contractions could be induced by PGF$_{2\alpha}$; nevertheless, the spontaneous contractions present allowed to examine the effects of 5-CT.

In general, the second curve in the control tissue was located to the left compared to the first curve (significance was reached in regions 9, 10, 13 and 14). No pA_2 was estimated in regions 9, 10 and 13: in region 13 there was a significant difference in potency between the first curve in the control and antagonist tissue, while the EC$_{50}$ values between the second curves in the antagonist and control tissue of regions 9 and 10 were not significantly different (Table 3).

Throughout the antrum, regional variation in the observed maximal effect, location and slope of the 5-CT-induced concentration–response curves was observed. In order to quantify these differences, the observed data were fitted to

the OMOA. The nlme modeling resulted in a population E_{max}, n_t and pK_A for all regions while different τ estimates were found for the different regions tested (Table 4; Figure 1b). The τ estimates again decreased from the lesser to the greater curvature. Population predicted curves from the OMOA fit are given in Figure 3b. Interindividual variability in region 9 was infinitely small and therefore constraint to 0 during the modelling.

Discussion

This study aimed to determine, quantify and explain regional differences in the response to the selective 5-HT$_1$ and 5-HT$_7$ receptor agonist 5-CT throughout the canine stomach. The experiments were performed on longitudinal muscle strips, as this layer is easily distinguishable from the other muscle layers in the stomach. In the fundus, no clear longitudinal muscle orientation was present hence only experiments with corpus and antrum muscle tissues were performed. 5-CT inhibited PGF$_{2\alpha}$-induced tonic contraction in corpus regions while in antrum regions 5-CT inhibited PGF$_{2\alpha}$-induced phasic contractions. A concentration–response curve to PGF$_{2\alpha}$ was established in every muscle strip. Differences between the PGF$_{2\alpha}$ curve parameters were shown between regions, both in corpus and antrum. To verify whether a relationship is present between the 5-CT and the PGF$_{2\alpha}$ curve parameters, all parameters were plotted vs each other but no correlation was found. This indicates that the different responses to 5-CT between regions are not related to variability in the PGF$_{2\alpha}$-induced effect. To assure a comparable degree of PGF$_{2\alpha}$-induced effect on which the responses to 5-CT were studied, a concentration of PGF$_{2\alpha}$ was chosen that induced 50–80% of the maximal effect to PGF$_{2\alpha}$.

Interaction of 5-CT with 5-HT$_7$ receptors

In corpus regions, the effect of 5-CT on the PGF$_{2\alpha}$-induced submaximal contraction was tested in the presence and absence of 5-HT receptor antagonist cocktail. In absence of cocktail high concentrations of 5-CT induced TTX-resistant contraction in regions close to the greater curvature. In a previous study, we showed that smooth muscle 5-HT$_{2A}$ receptors mediated longitudinal muscle strip contraction in the canine proximal stomach (Janssen *et al*., 2002b). Although 5-CT has a very low binding affinity to 5-HT$_{2A}$ receptors (pK_i = 4.7; Centurion *et al*., 2002) it was shown before in functional experiments on canine longitudinal colon muscle strips that 5-CT could indeed induce contraction *via* activation of smooth muscle 5-HT$_{2A}$ receptors (pEC$_{50}$ = 5.6; Prins *et al*., 1997). This indicates that, also in our experiments, the contractions to high concentrations of 5-CT might be mediated by 5-HT$_{2A}$ receptors. It was however not the aim of this study to characterize the receptor(s) mediating contraction in response to 5-CT in regions close to the greater curvature. In order to focus on the responses induced by 5-HT$_7$ receptor activation, we decided to perform all experiments in the presence of a 5-HT receptor antagonist cocktail blocking most 5-HT receptors except the 5-HT$_7$ receptor. The antagonists used and their concentration were selected on the basis of literature data and (Hoyer *et al*., 1994; 2002; Watts & Cohen, 1999; Vanhoenacker *et al*., 2000). In the presence of this cocktail, no contraction to 5-CT was seen indicating that the receptor(s) mediating contraction to 5-CT was/were most probably 5-HT receptor(s) blocked by the antagonists used. In the corpus regions it was observed that, in the presence of the antagonist cocktail, the concentration–relaxation curve to 5-CT was shifted to the right compared to the curve in absence of antagonist cocktail. It is unlikely that this is caused by antagonism of non-5-HT$_7$ relaxant 5-HT receptors because, in the concentration range used to observe this relaxation, 5-CT has little affinity to other 5-HT receptors than 5-HT$_1$ and 5-HT$_7$ (Boess & Martin, 1994). Moreover, the 5-CT-induced relaxation on PGF$_{2\alpha}$-induced contraction in canine proximal stomach is TTX insensitive indicating that muscular 5-HT receptors are involved (Janssen *et al*., 2002b) and muscular 5-HT$_1$ receptor activation is known to induce contraction (Hoyer *et al*., 2002). The potency shift after addition of the antagonist cocktail can thus best be explained if one or more antagonists used in the antagonist cocktail have antagonistic properties towards the 5-HT$_7$ receptor in their concentration used.

In general, the selective 5-HT$_7$ receptor antagonist SB-269970 (Lovell *et al*., 2000) antagonized the effect of 5-CT; however, the antagonistic effect was not significant in regions 9, 10 and 11, most probably due to the concentration of SB-269970 being too low. In the corpus, the pA_2 values in the different regions ranged from 8.2 to 8.6. This is somewhat smaller than expected based on our previous study, where we characterized smooth muscle 5-HT$_7$ receptors mediating relaxation of canine proximal stomach muscle strips (pK_B estimate: 9.4; Janssen *et al*., 2002b). The estimated affinity parameter for an antagonist can, however, be influenced by the presence of a second antagonistic effect, leading to an underestimation of the affinity parameter for the tested antagonist (Kenakin, 1997). As, apart from SB-269970, the antagonist cocktail was also shown to have antagonistic properties, the apparent affinity estimate was corrected as described in the methods (pA_2') similar to the correction Trist *et al*. (1987) applied in 1987 in their study on the effect of cimetidine in a cardiac adenylate cyclase assay. As expected the estimated pA_2' values in the corpus regions (8.5–9.1) were in general greater compared to the pA_2 values. Still, from the comparison of pA_2 and pA_2' estimates it is clear that the antagonistic effect of the antagonist cocktail differs between the regions as no difference between the pA_2 and pA_2' values was found in regions 5 and 12; no clear explanation for this observation can be given. As no experiments without cocktail were performed in the antrum, the affinity estimate for SB-269970 could not be corrected for the antagonistic properties of the antagonist cocktail. In line with our findings of the corpus, however, it is to be expected that in the antrum the pA_2 estimate for SB-269970 would also slightly increase when corrected for the antagonistic properties of the cocktail. The pA_2' estimates are well in line with the previously reported pK_B; moreover, in the concentration used, the selective 5-HT$_7$ receptor antagonist SB-269970 is not expected to have affinity to other 5-HT receptors (Lovell *et al*., 2000), allowing to conclude that the responses to 5-CT seen in both corpus and antrum are mediated by the 5-HT$_7$ receptor.

Regional differences in efficacy of 5-CT

Although in every region the response to 5-CT is mediated by the same receptor, large variability in the response was

observed between regions (Table 2). In general, 5-CT was more efficacious in regions close to the lesser curvature compared to regions close to the greater curvature. Dorsal and ventral regions from the same area close to the lesser curvature have similar efficacies. To compare the relaxant properties of muscle strips from a region close to the lesser curvature and a region close to the greater curvature a concentration–response curve to SNP was established in regions 1 and 8. As SNP was more potent in the region close to the greater curvature where 5-CT was least efficacious, it was concluded that the differential responses to 5-CT were not related to the relaxant capacities of the muscle strips.

To quantify differences in the response to 5-CT, the OMOA was applied. It has been shown that the OMOA is a useful expression for the characterization of drug–receptor interaction, allowing a separation between drug- and system-related parameters (Leff *et al*., 1990; Vivas *et al*., 1997; Van der Graaf & Stam, 1999). Corpus and antrum data were fitted separately as the experimental set-up for 5-CT was different (inhibition of tonic contraction vs inhibition of phasic activity). Population-predicted curves from the OMOA fit described the observed data well (Figure 3). Regional differences in the response to 5-CT can thus be explained by inter-regional variation in log τ. Figure 1b illustrates the log τ gradient present in the canine stomach, with a clear decrease in log τ going from the lesser curvature to the greater curvature. As τ is the ratio of receptor density (R_0) and coupling efficiency (K_{AR}), differences in τ can thus be explained by differences in R_0 or K_{AR} or both. In order to assess whether changes in R_0 contribute to the observed changes in log τ, qPCR was performed on mRNA of longitudinal muscle strips from region 2 (close to the lesser curvature) and region 8 (close to the greater curvature) to determine the relative expression of 5-HT$_7$ receptor mRNA.

Although rat, mouse, guinea pig, porcine and human 5-HT$_7$ receptors were cloned by several groups, the canine 5-HT$_7$ receptor was not cloned yet (Vanhoenacker *et al*., 2000). Cloning of the canine 5-HT$_7$ receptor revealed two splice variants, which we designated 5-HT$_{7(a)}$ and 5-HT$_{7(b)}$ by analogy with the human and rat splice variants. Although a third and fourth isoform was identified in rats (5-HT$_{7(c)}$) and humans (5-HT$_{7(d)}$) their expression is relatively low compared to the expression of 5-HT$_{7(a)}$ and 5-HT$_{7(b)}$ mRNA, that in different organs of humans and rats add up to more than 90% of the total 5-HT$_7$ mRNA (Heidmann *et al*., 1997). By means of qPCR in dog tissue, we confirmed that the 5-HT$_{7(a)}$ receptor splice variant contributes for about 50% to the total 5-HT$_7$ receptor expression, similar to other species. The 5-HT$_{7(b)}$ receptor expression on the other hand only contributes for about 1% to the total 5-HT$_7$ receptor expression indicating that one or more splice variants are additionally present in the dog. Up till now, no functional differences between the splice variants of the 5-HT$_7$ receptor were shown. Indeed, Krobert *et al*. (2001) showed that the human 5-HT$_7$ receptor splice variants are pharmacologically indistinguishable and that modifications of the carboxyl tail do not influence coupling to adenylyl cyclase; moreover, they display similar constitutive activity and inverse agonist properties (Krobert & Levy, 2002).

In our experiments, we compared the amount of 5-HT$_7$ receptor mRNA in longitudinal muscle strips from regions 2 and 8. Although the strips were deprived from other muscle layers and mucosa, we cannot exclude that nerves and small blood vessels were present in the examined tissue. We found, however, no evidence for the presence of 5-HT$_7$ receptors in other stomach tissue than the longitudinal muscles; moreover, the amount of nonmuscular tissue in the muscle bundles of the intestine is relatively low (Gabella, 1981). A significant difference in total 5-HT$_7$ receptor mRNA expression was observed between regions 2 and 8 (61–68% less expression was observed in region 8 vs region 2; Figure 4). When this difference in 5-HT$_7$ receptor mRNA expression level leads to a corresponding difference in muscular 5-HT$_7$ receptor density between the two regions, this illustrates that the lower log τ value at the greater curvature is at least partially due to a lower density of 5-HT$_7$ receptors.

Possible physiological relevance

In this study, we demonstrated that 5-CT is more efficacious in regions close to the lesser curvature than regions close to the greater curvature, while regions located on the dorsal and ventral site of the stomach display a similar efficacy to 5-CT (Table 4, Figure 1b). As we have shown that this regional variation in the response to 5-CT is at least partially due to a proportional difference in receptor density, it is reasonable to accept that similar functional differences will occur in response to 5-HT, the natural ligand of 5-HT$_7$ receptors. In physiological conditions, 5-HT in the gut might originate from 5-HT-containing neurons or enterochromaffin cells (EC cells). There is, however, no evidence that 5-HT released from EC cells affects the longitudinal muscle cells of the bowel. Serotonin-containing neurons on the other hand have been found in the myenteric and submucous plexus of the gut (Kurian *et al*., 1983). Furthermore, 5-HT is well established as an enteric neurotransmitter (Gershon, 1999; Kunze & Furness, 1999). Different studies have indicated the involvement of 5-HT and 5-HT receptors in canine stomach motility. It was for example shown that, in a perfused canine stomach setting, the neurotoxin 5,6-dihydroxytryptamine decreased 5-HT content in the muscle layer and abolished motilin-induced contractions, indicating that endogenous 5-HT, probably in serotoninergic neurons, plays an important role in the canine gastric motility (Haga *et al*., 1996). Muscular 5-HT receptors might therefore be activated by serotonin-containing neurons of the myenteric or submucous plexus of the stomach.

In view of our findings it is therefore reasonable to suppose that in physiological conditions, release of 5-HT to the longitudinal smooth muscle cells will result in a more pronounced relaxation of smooth muscles in the regions close to the lesser curvature compared to the greater curvature. Although one has to be cautious since we have no information from the oblique and circular muscle layers, our findings could implicate that 5-HT$_7$ receptor activation might be involved in the establishment of a preferential pathway of less resistance for food passage along the lesser curvature and is well in line with the physiology of the gastric or reticular groove. The gastric groove has been described in several mammalian species and represents a region inside the stomach cavity along the lesser curvature, where the mucosa folds are strongly fixed to the muscle layer and have a marked longitudinal orientation (Langer, 1993). Although the gastric groove was mainly proven to have an important function in young ruminants (known as reticular groove and assuring that, for example, milk will be carried past the forestomach into the further aboral parts of the digestive tract in order to minimize

the microbial fermentation (Black, 1970)) it is also present in nonruminants, where it directs liquids and fine particulate digests along the lesser curvature directly to the pylorus (Pfeffer, 1987). Relaxation of the gastric groove might thus stimulate evacuation of food from the proximal stomach.

Stomach-relaxing drugs as glyceryl trinitrate, glucagon and the 5-HT$_1$ receptor agonist sumatriptan were already proven to be efficient in the treatment of FD patients (Notivol *et al*., 1995; Gilja *et al*., 1997; Tack *et al*., 2000). Indeed, it was shown that in an important subgroup of FD patients impaired meal-induced relaxation of the proximal stomach might play a role in the generation of symptoms (Gilja *et al*., 1996). We have already demonstrated that 5-HT$_7$ receptor activation induced stomach relaxation in conscious dogs (Janssen *et al*., 2002a); moreover, we have now indications that 5-HT$_7$ receptor activation might stimulate evacuation of the proximal stomach by creating passage of lesser resistance through the gastric groove. If these findings can be confirmed *in vivo* in humans, selective 5-HT$_7$ receptor agonists might be useful for the treatment of functional dyspepsia. One should however be cautious when developing 5-HT$_7$ agonists or antagonists for this purpose as 5-HT$_7$ receptors were shown to mediate different vascular and central nervous effects (Vanhoenacker *et al*., 2000).

Conclusion

In this study, we have confirmed the presence of muscular 5-HT$_7$ receptors mediating relaxation to 5-CT in longitudinal muscle of canine stomach. It was shown that the most efficacious response to 5-CT (in regions yielding the highest log τ) is situated close to the lesser curvature, while efficacy decreased going to the greater curvature. By means of qPCR, we illustrated that the observed log τ decrease going from the lesser to the greater curvature can, at least partially, be explained by a proportional decrease in 5-HT$_7$ receptor mRNA expression. Our present results suggest that 5-HT$_7$ receptors might play a role in the physiology of the gastric groove along the lesser curvature.

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