Characterization of 5-HT_7-receptor-mediated gastric relaxation in conscious dogs

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Janssen, Pieter, Nicolaas H. Prins, Benoit Moreaux, Ann L. Meulemans, and Romain A. Lefebvre. Characterization of 5-HT_7-receptor-mediated gastric relaxation in conscious dogs. Am J Physiol Gastrointest Liver Physiol 289: G108–G115, 2005.—We aimed to evaluate the gastric relaxant capacity of the 5-HT_17-receptor agonist 5-carboxamidotryptamine (5-CT) in conscious dogs and to clarify the mechanism of action by use of selective antagonists, vagotomy, and in vitro experiments. A barostat enabled us to monitor the intragastric volume in response to different treatments (intravenously administered) before and after supradiaphragmatic vagotomy [results presented as the maximum volume change after treatment (mean; n = 5–11)]. In vitro experiments were performed with isolated muscle strips cut from four different stomach regions of the vagotomized dogs [results were fitted to the operational model of agonism to determine the efficacy parameter τ (n = 5)]. 5-CT (0.5–10 μg/kg) caused a dose-dependent gastric relaxation (29–267 ml) that was completely blocked by the selective 5-HT_7-receptor antagonist SB-269970 (50 μg/kg). After vagotomy, the relaxation to 10 μg/kg 5-CT was significantly less pronounced (73 vs. 267 ml; P < 0.05) but still blocked by SB-269970, whereas the response to the nitric oxide donor nitroprusside was similar to that before vagotomy (178 vs. 218 ml). In vitro, 5-CT concentration dependently inhibited the PGF_2α-contracted muscle strips before and after vagotomy. Although before and after vagotomy the response in every region was mediated by 5-HT_7 receptors (apparent affinity dissociation constant: SB-269970, 8.2–8.6 vs. 8.3–8.6, respectively), the response after vagotomy was less efficacious (log τ: 1.9 to 0.5 vs. 1.4 to −0.1). The results indicate that the 5-CT-induced proximal stomach relaxation in conscious dogs before and after vagotomy is mediated via 5-HT_7 receptors. The decreased efficacy of 5-CT in vitro after vagotomy is probably related to vagotomy-induced changes in receptor density or coupling efficiency and provides a possible explanation for the decreased in vivo response to 5-CT after vagotomy.

5-HT_7; efficacy distribution; vagotomy; barostat

In between meals, the proximal stomach has a high basal muscle tone. This tone is partially due to the high resting membrane potential of the proximal stomach muscle cells and the vagally mediated cholinergic input (4, 9). The tone of the proximal stomach decreases to accommodate food. This reflex relaxation is believed to be mainly mediated by nitric oxide (NO) release from the nitricergic efferent nerves. Indeed, the NO synthase inhibitor NG-monomethyl-L-arginine impairs gastric accommodation and enhances meal-induced satiety in humans (28).

Different studies have shown the possibility of inducing gastric relaxation by activation of 5-HT receptors. The antimitotane drug sumatriptan, which has affinity at different 5-HT_1-receptor subtypes, was shown to induce feline and human gastric fundus relaxation (27), indicating therapeutic potential in the treatment of patients with functional dyspepsia (FD) (6, 26). In dogs, studied with a barostat, sumatriptan shifted gastric pressure-volume curves toward higher volumes and enhanced gastric accommodation, an effect mediated by 5-HT_1B receptors (8). Recently, we showed that flesinoxan induces proximal gastric relaxation in conscious dogs via 5-HT_1A receptors. This response is mediated through a vagal pathway without involvement of nitrenergic nerves (16).

In vitro, we were able to identify muscular 5-HT_7 receptors mediating relaxation of contracted isolated longitudinal muscle strips of canine proximal stomach by use of the 5-HT_1B and 5-HT_7-receptor agonist 5-carboxamidotryptamine (5-CT) (17). The aim of the present study was to investigate the influence of 5-CT on canine proximal gastric tone in vivo by means of a barostat; 5-CT induced a clear-cut gastric relaxation. Selective antagonists were used to determine the receptors involved. As it was shown before that the vagus nerve is an important mediator of the gastric tone (1, 4), the effect of 5-CT was studied before and after vagotomy to investigate the possible contribution of the vagal pathway in the 5-CT-induced gastric relaxation. The muscular 5-HT_7 receptors in the stomach were further examined in vitro by comparing the response to 5-CT in gastric tissues from vagotomized dogs with those in tissues from nonvagotomized dogs.

METHODS

In Vivo Experiments

Preparation of animals. Experiments were performed on 11 adult female Beagle dogs (9–14 kg body wt), trained to stand quietly in Pavlov frames. The local ethics committee approved the experiments. A gastric cannula was implanted in all dogs, in the ventral gastric wall 2 cm above the nerves of Latarjet, as we described previously (16). Via randomization, 5 of 11 dogs used were assigned to study the influence of drugs before and after supradiaphragmatic vagotomy. The procedure of the vagotomy and the assessment of the completeness by measuring insulin-induced gastric secretion have also been described previously (16). The barostat measurements were performed in a period starting 3 mo after vagotomy and ending ~20 mo after vagotomy.

Recording of gastric volume. Variations in gastric tone were measured with an electronic barostat (model JS 10987, Janssen Scientific Instruments, Beerse, Belgium) maintained at constant pressure (6 mmHg). LabVIEW 2 software, version 5.1, was used for process control and data storage. The barostat had a maximum...
displacement of ~800 ml and maintained a constant pressure in a polyethylene bag that had a capacity of ~1,100 ml.

Dogs were fasted 24 h before the experiment (water was available ad libitum). At the beginning of the experiment, the gastric cannula was opened to remove any gastric juice or food remnants and cleansed with lukewarm water. After calibration of the barostat, the bag was positioned into the proximal stomach through the gastric cannula. A rubber stopper was used to close the space between the tubing and the wall of the cannula. To ensure easy unfolding of the intragastric bag during the experiment, the following protocol, taking ~5 min, was followed before the beginning of the experiment: the intragastric pressure was raised to 20 mmHg in one step, leading to a progressive increase of the volume in the bag; when a volume of 400 ml was reached, pressure was returned to 2 mmHg; and after a short stabilization period at 2 mmHg, the intragastric pressure was raised to 10 mmHg until a volume of 300 ml was reached. Hereafter, the pressure was reduced to 2 mmHg, awaiting the beginning of the experimental protocol.

Due to the loss of vagal control on stomach motility after vagotomy, the mean baseline volume (MBV; calculated as the mean intragastric volume in the last 5 min before treatment) was significantly higher than before vagotomy; e.g., the MBV before administration of 5-CT (10 μg/kg) to the vagotomized and nonvagotomized dogs was 423 (244–602) vs. 152 (73–231) ml, respectively (P < 0.05). Therefore, after vagotomy, all experiments were performed after dosing with the cholinergic agonist bethanechol to increase gastric tone. We have previously shown that this experimental setup is suitable to register gastric relaxation by the barostat method after vagotomy (16).

**Drug administration.** Drugs (one dose of 5-CT with or without antagonist per experimental day) were administered after a baseline period of at least 15 min. The NO donor sodium nitroprusside (SNP), saline, or 5-CT was administered as test compound. Upon 5-CT injection at the doses of 5 and 10 μg/kg, 3 and 4 dogs, respectively, out of 11 withdrew their paw and showed symptoms of discomfort. These symptoms disappeared within 10 s and did not influence the experimental setup. The dogs never showed the symptoms again upon a following administration of 5-CT. To avoid any further discomfort, the dose of 10 μg/kg 5-CT was not exceeded. Saline, the NO synthase inhibitor Nω-nitro-l-arginine methyl ester (L-NAME), the 5-HT1A-receptor antagonist WAY-100635, and the 5-HT3-receptor antagonist SB-269970 were administered 15 min before 5-CT as pretreatment. For each experiment, dogs were randomly assigned to a treatment in such a manner that each dog had received all treatments after completion of the set of experiments. A minimum washout period of 48 h was allowed between two consecutive experiments in the same animal. All drugs were administered intravenously by injection in the cephalic vein (maximum injected volume: 1 ml/5 kg); agonists and antagonists were administered in opposite paws. After vagotomy, bethanechol was intravenously administered as a bolus (15 μg/kg) followed by an infusion (50 μg·kg⁻¹·h⁻¹). Profuse salivation was noted in all bethanechol experiments. Unless otherwise indicated, the intragastric volume was measured during 60 min after administration of the test compound.

**Data analysis.** A Loess regression curve was calculated from the original gastric volume tracing to correct for extreme outliers. Gastric volume changes induced by a given treatment were calculated as the difference between the MBV and the highest (upon volume increase) or lowest (upon volume decrease) volume after treatment, as determined from the Loess regression curve. Results are expressed as means and the 95% confidence interval. For comparison of two groups of data, a paired t-test was performed, unless otherwise indicated. For comparison of more groups, one-way or repeated-measures ANOVA followed by a post-hoc Tukey-Kramer test for multiple comparisons was used. \( P < 0.05 \) was considered significant.

**In Vitro Experiments**

The five dogs that underwent vagotomy were killed after all in vivo experiments were performed (±20 mo after vagotomy). Mucosa-deprived muscle strips were cut in longitudinal direction from four different regions of the ventral corpus stomach (4 muscle strips per region, 16 strips per dog). Regions 1 and 2 are located close to the lesser curvature; regions 3 and 4 are located close to the greater curvature. Regions 1 and 3 are located in the proximal corpus, adjacent to the fundus, whereas regions 2 and 4 are located in the distal corpus, adjacent to the antrum.

All in vitro experiments were performed in analogy with, and are described in detail in, Janssen et al. (17). After constructing a cumulative concentration-response curve to PGF2α, and washout, a cumulative concentration-response curve to 5-CT (0.1 nM to 0.1 mM) or SNP (1 nM to 1 mM) was performed on a submaximal contraction to PGF2α (50–80% of the maximal effect; 30–300 nM). As it was shown before that high concentrations of 5-CT induced contractions in regions close to the greater curvature, effects of 5-CT were studied in the presence of an antagonist cocktail to avoid interaction with non-5-HT: 5-HT receptors. The antagonist cocktail components were as follows: WAY-100635 (0.1 μM; 5-HT1A-receptor antagonist), GR127935 (0.1 μM; 5-HT1B/1D-receptor antagonist), ketanserin (0.1 μM; 5-HT2A-receptor antagonist), SB-204741 (0.3 μM; 5-HT2B-receptor antagonist), granisetron (0.3 μM; 5-HT3-receptor antagonist), and GR113808 (0.1 μM; 5-HT4-receptor antagonist), as discussed in our laboratory’s previous study (17). Four muscle strips per region were studied, each following a different protocol: protocol A, two successive concentration-response curves to 5-CT were established in the continuous presence of the antagonist cocktail; protocol B, a concentration-response curve to 5-CT was established in the absence of the antagonist cocktail; protocol C, two successive concentration-response curves to 5-CT were established in the continuous presence of the antagonist cocktail, and the second curve was performed in the presence of the selective 5-HT3-receptor antagonist SB-269970 (10 nM, incubated for 20 min); and protocol D, a concentration-response curve to SNP was studied in the continuous presence of the antagonist cocktail. All experiments were conducted in the presence of indomethacin (1 μM) to avoid contractions due to spontaneous synthesis of prostaglandins. Data collection was performed using Chart for Windows (version 4.12, ADInstruments, Chalgrove, UK).

**Data analysis.** All responses to 5-CT and SNP were expressed as percent reduction of the submaximal contraction to PGF2α before administration of 5-CT or SNP. Concentration ([A])-response (E) curves to PGF2α, SNP, and 5-CT were individually fitted to the Hill equation (1) by nonlinear regression obtaining curve parameter estimates for midpoint location (EC50), upper asymptote of the observed maximal effect (Emax), and Hill slope (nH):

\[
E = \frac{[A]^{E\text{max}} \cdot \alpha}{EC_{50}^{nH} + [A]^{nH}}
\]

No correlation was found between the PGF2α and the 5-CT curve parameters, illustrating that the sensitivity of a given tissue to PGF2α did not determine the sensitivity to 5-CT.

The apparent affinity dissociation constant (pA2) for SB-269970 was estimated from the EC50 dose ratio (DR) of the second curves in protocols A (control) and C (in the presence of SB-269970). In every region, the second curve in protocol C was significantly shifted to the right compared with the second curve in protocol A; as no significant difference between the first curves in protocols A and C was observed, a pA2 estimate was determined in every region, as previously described (3):

\[
pA2 = \log (DR - 1) - \log (SB - 269970)
\]
μM), protocols A and C were performed in the presence of the 5-HT-receptor antagonist cocktail. To compensate for the antagonistic properties of the antagonist cocktail, we estimated the corrected pA2 for SB-269970 (pA2') as described before (17, 18):

\[ pA2' = -\log \left( \frac{\text{DR}_{\text{cockt}} \cdot (DR - 1)}{SB - 269970} \right) \]  

(3)

where DR_{cockt} is the DR of the EC50 estimates of the first 5-CT-induced curves in protocols A and B, respectively, in the presence and absence of antagonist cocktail.

For comparison of two groups of data, a paired t-test was performed, unless otherwise indicated. For comparison of more groups, one-way or repeated-measures ANOVA, followed by post hoc Tukey-Kramer’s test for multiple comparisons, was used. Unless otherwise indicated, results are expressed as means with the 95% confidence interval.

The operational model of agonism. To quantify differences in the expression of agonism at the same receptor across the different regions, the operational model of agonism (OMOA) was applied. It has 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 (5, 20). The OMOA describes agonist binding to a receptor population to form an agonist-receptor complex [determined by the agonist-receptor complex dissociation equilibrium constant (K_A)] that, in turn, is transduced into effect [determined by the activated receptor-G-protein complex dissociation constant (K_AR)]. As such, it describes effect E as a function of the agonist concentration A within the following formula:

\[ E = \frac{E_{\text{max}} \cdot \tau^{n} \cdot A^{n}}{(K_A + A)^{n} + \tau^{n} \cdot A^{n}} \]  

(4)

where E_{max} is the maximum effect achievable in the system, n is the slope index for the occupancy-effect relation, and \( \tau \) is the efficacy parameter, which is defined by the ratio of total receptor density (R_0) and K_AR. Increase of \( \tau \) thus implies increase of agonism expression. The observed data were fitted to the OMOA by nonlinear regression. Indeed, in contrast with previously published work (17) in which the model fits were greatly improved by allowing estimation of interindividual variability by a nonlinear mixed-effects procedure, this turned out not to be feasible for this data set and may be attributable to the limited data set available here (4 regions and 5 dogs) compared with the previous work (at least 6 regions and 6 dogs), the variation turned out not to be feasible for this data set and may be attributable to the limited data set available here (4 regions and 5 dogs) compared with the previous work (at least 6 regions and 6 dogs), the variation observed, and the increased amount of parameters to be estimated when mixed-effects modeling is pursued. K_A and \( \tau \) were estimated as \(-\log (K_A)\) and \( \log (\tau) \), respectively, because these parameters are assumed to be log-normally distributed (20, 31).

The nonlinear regression package as implemented in S-PLUS was used (version 6.1, Insightful, Seattle, WA; S-PLUS code can be obtained by the authors). Only data from the first curves of the tissues following protocol A were used for the OMOA analysis (n = 5).

Drugs. The following drugs were used in the in vivo and in vitro experiments (respective suppliers in parentheses): 5-CT (Tocris Cookson); bethanechol (Merck); granisetron HCl, ketanserin tartrate, GR113808, SB-269970, and SNP (Janssen Pharmaceutica, Beerse, Belgium); insulin (Actrapid HM; 100 IU/ml; Novo Nordisk Pharma, Brussels, Belgium); GR-127935 and SB-204741 (kindly donated by GlaxoSmithKline); l-NAME and WAY-100635 (Sigma; Aldrich); and PGF_{2α} (diluted from Dinolytic, 5 mg/ml; Upjohn, Animal Health).

Granisetron, ketanserin, and WAY-100635 were dissolved in distilled water. Bethanechol, l-NAME, SB-269970, SNP, and PGF_{2α} were dissolved in NaCl 0.9% solution. 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 eq 2,3-dihydroxybutanedioic acid, and GR-113808 was dissolved in distilled water containing 2 eq 2,3-dihydroxybutanedioic acid. The solvents had no effect on the baseline volume (in vivo) or tone (in vitro). All drugs for in vivo use were dissolved isotonically, and pH was between 3.5 and 9.

RESULTS

In Vivo Experiments

Influence of agonists and antagonists. The responses to increasing doses of 5-CT (saline and 0.5, 1, 5, and 10 μg/kg 5-CT) were established in 10–11 conscious dogs. The MBV in dogs treated with saline was 196 (142–250) ml; this did not significantly differ from the MBV before addition of any concentration of 5-CT (results not shown). Increasing doses of 5-CT (0.5, 1, 5, and 10 μg/kg) induced 29 (0.17–59), 81 (27–134), 185 (97–273), and 267 (187–347) ml maximal gastric volume increase, respectively. Only the response induced by 5- and 10-μg/kg 5-CT was significantly different vs. the maximum gastric volume increase after administration of saline [23 (1–45) ml; P < 0.01 and P < 0.001, respectively; Fig. 1A]. The gastric relaxant effect of 5 and 10 μg/kg 5-CT reached its maximum at 5.2 (3.5–6.9) and 4.7 (3.1–6.4) min.

![Fig. 1](http://ajpgi.physiology.org/)
(no significant difference was found), and the volume returned to MBV again at 17.6 (9.2–26.1) and 24.0 (14.8–33.1) min, respectively, after 5-CT injection. In the following experiments, volume registration was analyzed up to 24 min, as, even for the highest dose of 5-CT, the maximal effect was clearly reached before 24 min.

The dose of 5 μg/kg 5-CT was selected to study the influence of different pretreatments (n = 6). In a first series of experiments, the effect of pretreatment with different doses of SB-269970 was tested vs. saline pretreatment. Pretreatment with SB-269970 (5, 10, and 50 μg/kg) did not significantly influence the MBV before administration of 5-HT, but dose-dependently decreased the maximal 5-CT-induced gastric volume increase vs. saline pretreatment (Table 1). The relaxant effect of 5-CT was significantly decreased only after pretreatment with 50 μg/kg SB-269970 (P < 0.05).

The effect of pretreatment with WAY-100635 (50 μg/kg) and l-NAME (10 μg/kg) was tested vs. saline pretreatment. Administration of WAY-100635 did not influence the intragastric volume (results not shown). l-NAME, on the other hand, caused a significant gastric contraction compared with saline addition [maximum intragastric volume decrease of 91 (66–117) vs. 30 (−3–63) ml, respectively; P < 0.05]. The maximum effect of l-NAME was reached after 13.1 (9.6–16.7) min. Although l-NAME induced a significant volume decrease vs. saline, the MBV after l-NAME or WAY-100635 addition did not significantly differ from that in the same dogs after treatment with saline (Table 1). Therefore, the effect of 5-CT could be compared between the different pretreatment groups. Pretreatment with l-NAME or WAY-100635 did not influence the 5-CT-induced maximal intragastric volume increase compared with that after saline pretreatment (Table 1).

Influence of vagotomy. The vagotomized dogs were studied at a constant intragastric bag pressure of 6 mmHg to mimic the experimental condition, as was used before vagotomy. At this pressure after vagotomy, the MBV in the 5-CT (10 μg/kg) treatment session was significantly greater then before vagotomy [423 (244–602) vs. 152 (73–231) ml, respectively; P < 0.05]. To compensate the loss of gastric tone after vagotomy, the cholinergic agonist bethanechol was administered as a bolus (15 μg/kg) followed by infusion (50 μg·kg⁻¹·h⁻¹). Bethanechol infusion induced an intragastric volume decrease of 214 (92–335) ml (n = 5). The MBV after bethanechol administration was 177 (90–263), 163 (132–195), and 188 (100–277) ml in the saline, nitroprusside, and 5-CT treatment sessions, respectively, and did not significantly differ from the MBV of the saline treatment group before vagotomy. Both nitroprusside and 5-CT were able to induce a significant intragastric volume increase compared with saline in these conditions after vagotomy (Table 2; Fig. 1B).

The nitroprusside-induced relaxation was not significantly different from the relaxation induced in nonvagotomized dogs. The 5-CT-induced relaxation, on the other hand, was significantly less pronounced then before vagotomy (P < 0.05). Pretreatment with 50 μg/kg SB-269970 abolished the 5-CT-induced relaxation after vagotomy vs. saline pretreatment [2 (−46–50) vs. 73 (37–109) ml; P < 0.01].

In Vitro Experiments

In vitro experiments with muscle strips from the five vagotomized dogs were performed on four corpus stomach regions. The muscle strips showed no spontaneous contraction during the stabilization period and responded to PGF₂α with a sustained tonic contraction (Fig. 2). 5-CT and SNP induced a concentration-dependent relaxation of the PGF₂α-induced contraction. The responses were fitted to the Hill equation; the curve fit parameters are described in Table 3.

In Table 3, the curve fit parameters for muscle strips from the same regions in nonvagotomized dogs, obtained in our laboratory’s previous study (17), are also given. In general, 5-CT was less potent and induced a smaller observed maximal effect in vagotomized dogs, although significance was only reached for the observed maximal effects. No significant difference between the curve parameters of the SNP-induced concentration-response curves was observed (Table 3).

In the muscle strips from vagotomized dogs, SB-269970 induced a significant rightward shift of the curve to 5-CT compared with the curve in the parallel control muscle strip in all regions (pA₂ ranging from 8.29 in region 1 to 8.59 in region 4). To compensate for the antagonistic effect of the antagonist cocktail, a pA₂* was estimated that corrects for the presence of a second antagonistic effect on the 5-HT₁ receptors (ranging from 8.34 in region 1 to 8.96 in region 4).

Although the antagonistic effect of SB-269970 suggests that 5-CT interacts with the same receptor in the different regions, regional variation in the amplitude, location, and slope of the 5-CT-induced concentration-response curves was observed between the regions (Table 3). These differences were not related to differences in the contractile response to PGF₂α. Indeed, all PGF₂α and 5-CT-induced curves were fitted to the Hill equation. The curve fit parameters of the PGF₂α and 5-CT-induced

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Table 1. Mean baseline volume after administration of different drugs, and maximal intragastric volume increase by 5-CT (5 μg/kg) after pretreatment with these drugs

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose</th>
<th>Mean Baseline Volume, ml</th>
<th>Maximal Intragastric Volume Increase by 5-CT, ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td></td>
<td>266 (213–319)</td>
<td>177 (55–298)</td>
</tr>
<tr>
<td>SB-269970</td>
<td>5 μg/kg</td>
<td>234 (124–344)</td>
<td>134 (−11–273)</td>
</tr>
<tr>
<td></td>
<td>10 μg/kg</td>
<td>198 (80–345)</td>
<td>98 (−18–215)</td>
</tr>
<tr>
<td>SB-269970</td>
<td>50 μg/kg</td>
<td>252 (137–368)</td>
<td>22 (−0.38–44)*</td>
</tr>
<tr>
<td>WAY-100635</td>
<td>50 μg/kg</td>
<td>225 (137–312)</td>
<td>170 (61–279)</td>
</tr>
<tr>
<td>l-NAME</td>
<td>10 μg/kg</td>
<td>194 (118–270)</td>
<td>182 (45–319)</td>
</tr>
</tbody>
</table>

Intragastric volumes are shown as means ± 95% confidence intervals (in parentheses) (n = 6). 5-CT, 5-carboxamidotryptamine; l-NAME, N⁶-nitro-l-arginine methyl ester. *P < 0.05 vs. saline pretreatment.

Table 2. Maximal intragastric volume increase after administration of different drugs before vagotomy and after vagotomy

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose</th>
<th>Nonvagotomized, ml</th>
<th>Vagotomized, ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>7 (−9–24)</td>
<td>0.3 (−7.2–7.9)</td>
<td></td>
</tr>
<tr>
<td>Nitroprusside</td>
<td>10 μg/kg</td>
<td>218 (134–301)*</td>
<td>178 (96–260)‡</td>
</tr>
<tr>
<td>5-CT</td>
<td>10 μg/kg</td>
<td>267 (187–347)†</td>
<td>73 (37–109)‡</td>
</tr>
</tbody>
</table>

Intragastric volumes are shown as means ± 95% confidence intervals (in parentheses) (n = 5). Experiments in vagotomized dogs were done under bethanechol infusion. *Response to nitroprusside before vagotomy was determined in another set of dogs, significantly different vs. saline [24.8 (1.7–51.3) ml; P < 0.001; n = 6]. ‡P < 0.01 and §P < 0.001 vs. saline addition in the same condition.
curves were plotted vs. each other, but no correlation was found (results not shown). 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 SNP was tested in all four regions. However, no significant differences between the SNP curve fit parameters in the regions tested were observed.

To quantify differences in the responses to 5-CT between the different regions, the data were simultaneously fitted to the OMOA. Using nonlinear regression, \( E_{\text{max}}, n_t, \) and \( pK_A \) are considered to be constant throughout the different regions, and interregional variability was only allowed for \( \log \tau \). From Table 4, it is clear that the \( \log \tau \) estimates decrease from the lesser to the greater curvature. Figure 3 shows the predictions of the OMOA model fit superimposed on the mean experimental data.

**DISCUSSION**

**Effect of 5-CT on Conscious Dogs Before and After Vagotomy**

In this study, we showed that the 5-HT1- and 5-HT7-receptor agonist 5-CT induces a dose-dependent stomach relaxation in...
consecutive dogs. Because of the observed side effects, we could not increase our dose >10 μg/kg to determine the maximal-inducible effect. Side effects to the administration of 5-CT in conscious animals have been reported before but are mainly cardiovascular (36); no reports were found describing symptoms of discomfort. To our knowledge, no experiments with 5-CT have been performed in conscious dogs; we, therefore, concluded that the observed side effects were related to intravenous injection of high doses of 5-CT in conscious dogs.

5-CT has been used in other similar dose in vivo studies, in similar doses as in our experiments, showing 5-HT7 receptor-mediated feline tachycardia (0.01–30 μg/kg) (35) and rabbit saphenous vein dilatation (5 μg/kg) (33). The interaction of 5-CT with 5-HT7 receptors in the present dog study was confirmed by the effect of SB-269970. SB-269970 is a highly selective and high-affinity antagonist at 5-HT7 receptors in vivo (12, 13, 25). In our experiments, SB-269970 administration did not influence the gastric tone. Pretreatment with SB-269970 dose-dependently inhibited the 5-CT-induced gastric relaxation from 5 μg/kg onwards. In the presence of 50 μg/kg SB-269970, the 5-CT-induced relaxation was completely blocked. A similar or even higher dose was used to show 5-HT7-receptor involvement in the control of micturition (3–300 μg/kg) (25) and the 5-CT-induced body temperature decrease in mice (10 mg/kg) (12).

In a previous study, we showed that the flesinoxan-induced canine stomach relaxation was mediated by 5-HT1A receptors (16). As 5-CT is a 5-HT1A and 5-HT7-receptor agonist, we, therefore, tested whether the 5-CT-induced effect was blocked by the highly selective and high-affinity 5-HT1A-receptor antagonist WAY-100635 (10). In a dose higher than required to block the gastric relaxant effect of flesinoxan in the conscious dog (a response mediated by 5-HT1A receptors), WAY-100635 did not influence the 5-CT-induced stomach relaxation; hence we can conclude that 5-HT1A receptors are most probably not involved in the 5-CT-induced stomach relaxation.

A possible nervous pathway by which 5-CT might induce gastric relaxation comprises fibers ending on intrinsic nitrergic neurons in the stomach. Indeed, activation of nitrergic neurons was shown to be involved in the gastric accommodation reflex in humans (28) and dogs (24). To assess this possibility, the influence of the NO synthase inhibitor L-NAME was investigated. A dose of L-NAME was chosen that has been previously shown to affect intestinal motility in dogs (2). The administration of L-NAME caused a decrease in gastric volume, reflecting a stomach contraction. This illustrates that there is constant nitrergic input toward the stomach, as shown before (24). However, L-NAME did not influence the relaxant effect of 5-CT, illustrating that this is not mediated via nitrergic nerves.

The effect of 5-CT was compared before and after vagotomy to investigate the involvement of the vagus nerve. It is thoroughly established that the vagal pathway is a key pathway in the regulation and maintenance of canine gastric tone during fasting (1) and the decrease of gastric tone during meal ingestion (1, 15, 24). We previously showed that the gastric relaxant effect of flesinoxan in the conscious dog was also not influenced by L-NAME, but it was influenced by vagotomy (16), illustrating that vagal nonnitrergic pathways can be involved in serotoninergic gastric relaxation in the conscious dog. After vagotomy, the MBV at an intragastric balloon pressure equal to that used before vagotomy is significantly higher compared with the MBV before vagotomy, illustrating a relaxed status. The difference in MBV before and after vagotomy makes it difficult to compare drug effects. The main component of the vagal drive maintaining the gastric tone during fasting is cholinergic (22), and the gastric relaxation seen after vagotomy may, therefore, be due to the loss of vagal cholinergic drive. The investigation of drugs after vagotomy was, therefore, performed during administration of the cholinergic agonist bethanechol. Intravenous infusion of bethanechol is a classic method to maintain the gastric tone after vagal cooling or vagotomy (7); infusion of bethanechol restored the MBV to the prevagotomy value. After vagotomy with bethanechol infusion, SNP induced a similar degree of gastric relaxation as observed in nonvagotomized dogs. This suggests that SNP induces gastric relaxation by a direct effect on the stomach and illustrates that prevagotomy degrees of gastric relaxation can be obtained in the postvagotomy condition with bethanechol infusion. 5-CT still induced a gastric relaxation in the postvagotomy condition, illustrating that 5-CT-induced stomach relaxation is not mediated via nitrergic nerves.

Table 4. Operational model of agonism model fit parameters of 5-CT-induced concentration-response curves in 4 different regions of the corpus stomach before and after vagotomy

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>Nonvagotomized</th>
<th>Vagotomized</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emax</td>
<td>108.40±2.30</td>
<td>98.25±4.22</td>
<td></td>
</tr>
<tr>
<td>log t1</td>
<td>1.29±0.14</td>
<td>0.68±0.13</td>
<td></td>
</tr>
<tr>
<td>log t2</td>
<td>1.88±0.15</td>
<td>1.44±0.18</td>
<td></td>
</tr>
<tr>
<td>log t3</td>
<td>0.46±0.09</td>
<td>0.04±0.08</td>
<td></td>
</tr>
<tr>
<td>log t4</td>
<td>0.45±0.08</td>
<td>-0.11±0.07</td>
<td></td>
</tr>
<tr>
<td>pKd</td>
<td>6.67±0.13</td>
<td>6.76±0.17</td>
<td></td>
</tr>
<tr>
<td>nS</td>
<td>1.05±0.09</td>
<td>1.02±0.16</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. The operational model of agonism (OMOA) parameters after vagotomy were modeled to the data using nonlinear regression, as described in METHODS. The OMOA parameters in nonvagotomized regions 1–4, regions 1–4, Kd, agonist-receptor complex dissociation equilibrium constant; nS, slope index for occupancy-effect relation.
siveness to PGF2

These differences were not related to differences in response compared with regions 3

5-HT7 receptors. However, the 5-CT-induced intragastric volume increase was significantly smaller then before vagotomy.

Effect of 5-CT in Gastric Corpus Muscle Strips of Vagotomized Dogs

Our laboratory has previously shown that 5-CT interacts in vitro with muscular 5-HT7 receptors in the canine gastric corpus (17). The observation that 5-CT still induces gastric relaxation after vagotomy illustrates that these receptors might be involved in the in vivo effect of 5-CT. To assess whether changes in the direct gastric effect of 5-CT might contribute to the decreased response to 5-CT in vivo after vagotomy, we studied the in vitro effect of 5-CT in gastric smooth muscle strips of the vagotomized dogs and compared the results to those obtained before in nonvagotomized dogs. In the same experimental setup as used before in tissues of nonvagotomized dogs, we now examined the effect of 5-CT on PGF2α-precontracted longitudinal smooth muscle strips from four different corpus stomach regions. Two regions were located at the lesser curvature, and two at the greater curvature (as it was previously demonstrated that, between regions from the greater and the lesser curvature, a difference in efficacy of 5-CT was present (17)). After vagotomy, 5-CT induced a concentration-dependent relaxation of the PGF2α-precontracted smooth muscle strips in every region examined, and the pA2 for SB-269970 (8.3–9.0) was not found to be significantly different from the pA2 for SB-269970 determined in the same regions in nonvagotomized dogs (8.8–9.0). It is, therefore, reasonable to assume that the 5-CT-induced in vitro relaxation of muscle strips from nonvagotomized and vagotomized dogs is mediated by the same receptor: the 5-HT7 receptor. Indeed, the affinity estimates for SB-269970 are well in line with the expected affinity for 5-HT7 receptors (real affinity dissociation constant, or pKd: 9.4) (17, 23).

Although in every region the response to 5-CT is mediated by the same receptor, large variability in the response was observed between the regions (Table 3). 5-CT was more efficacious in regions 1 and 2 close to the lesser curvature compared with regions 3 and 4 close to the greater curvature. These differences were not related to differences in responsiveness to PGF2α or responsiveness to SNP, as demonstrated by the absence of correlation between PGF2α and 5-CT curve parameters and the absence of differences in response to SNP throughout all regions. It is thus more likely that the variation in the response to 5-CT is intrinsic to the 5-CT-5-HT7-receptor interaction being different across regions, and the OMA, as proposed by Black and Leff in 1983 (5), provides a useful model to quantify these differences. In the model, only log τ was allowed to vary between the regions. The resulting model fit described the data well (Fig. 3), thus indicating that the observed differences in the response to 5-CT can be explained by differences in the efficacy parameter log τ. Log τ decreases going from the lesser to the greater curvature. Although similar differences between the regions were present in strips from nonvagotomized dogs, it is obvious from Table 4 that the log τ values are lower in all regions after vagotomy (while pKd, nτ, and Emax are similar). As log τ is decreased after vagotomy, the response to 5-CT will be less efficacious. Indeed, from Table 3 it can be observed that, compared between the same regions, both potency and maximal effect are decreased after vagotomy, with significance being reached for the decrease in maximal effect. On the other hand, no difference between the responses to SNP can be observed between muscle strips from regions in nonvagotomized dogs and vagotomized dogs, indicating that the relaxant capacity of the smooth muscle strips is not altered. As τ represents the ratio of R0 and the coupling efficiency (KAR), differences in τ can thus be explained by differences in R0 or KAR or both. Downregulation of mRNA (e.g., stomach ghrelin mRNA levels were significantly decreased after truncal vagotomy) and receptor expression (e.g., muscarinic acetylcholine receptors of the small intestine and pancreas of the rat after vagotomy) in peripheral cells after vagotomy are well described and could indeed explain the less efficacious response to 5-CT after vagotomy (14, 19, 21).

In Vitro-In Vivo Correlation

Our in vitro findings correlate very well to our in vivo findings. Indeed, both in vivo and in vitro, we have shown that, after vagotomy, the gastric relaxation to 5-CT is less pronounced, whereas the relaxation to SNP is not different. In view of the similarities between our in vivo and in vitro findings, we find it reasonable to suggest that 5-CT exerts its effects in vivo by activating smooth muscle 5-HT7 receptors. The decrease in τ that we observed after vagotomy in vitro might very well explain why the response to 5-CT in vivo is less pronounced after vagotomy. 5-CT in vivo was already shown to act at a peripheral level in canine arteries: Villalon et al. (34) showed in 1997 that 5-CT in vivo mediates carotid vasodilatation in vagosympathectomized anesthetized dogs. This vascular response was later confirmed in vitro, where it was shown that 5-HT7 receptors mediate smooth muscle relaxation in canine cerebral arteries deprived of endothelium (30).

For the moment, it is unknown in what (patho)physiological conditions the muscular 5-HT7 receptors could be involved. We previously suggested that they could be involved in the relaxation of the gastric groove and thus could be stimulating evacuation of food from the proximal stomach (17). Still, a clearly characterized, peripherally located mechanism for stomach relaxation might be an interesting target for the development of a better treatment for a subset of functional dyspeptic patients. Indeed, impaired gastric accommodation was shown to be an important factor in the pathogenesis of FD (29). In this subset of FD patients, restoring gastric accommodation with a fundus-relaxing drug such as glyceryl trinitrate (11) or sumatriptan (26) has already proven to be an efficient treatment. Important undesirable side effects, however, have dampened their clinical use. It still needs to be confirmed, however, that the 5-HT7-receptor-mediated stomach relaxation is also present in humans; moreover, one should be cautious when developing 5-HT7 agonists or antagonists for this purpose, as 5-HT7 receptors were shown to mediate different vascular and central nervous effects (32).

In conclusion, 5-CT induces 5-HT7-receptor-mediated canine stomach relaxation in vivo and relaxation of PGF2α-precontracted proximal gastric smooth muscle strips in vitro. After vagotomy, the response to 5-CT remained but was less
pronounced. In vitro, we showed that the decreased efficacy of 5-CT on tissue from vagotomized dogs was related to a decrease in log $\tau$. Whether this is related to a decrease in the receptor density and/or the coupling efficiency still needs to be confirmed. The similarities between the in vivo and in vitro findings indicate that the 5-CT-induced stomach relaxation in vivo is probably mediated by the same mechanism that we assessed in vitro. If this mechanism of action can be confirmed in humans, the gastric relaxant effect of 5-HT$\gamma$-receptor agonists might be a possible therapeutic application in FD.

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GRANTS

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