Role of 5-HT$_{1B/D}$ receptors in canine gastric accommodation: effect of sumatriptan and 5-HT$_{1B/D}$ receptor antagonists

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De Ponti, Fabrizio, Francesca Crema, Elisabetta Moro, Giuseppe Nardelli, Gianmario Frigo, and Antonio Crema. Role of 5-HT$_{1B/D}$ receptors in canine gastric accommodation: effect of sumatriptan and 5-HT$_{1B/D}$ receptor antagonists. Am J Physiol Gastrointest Liver Physiol 285: G96–G104, 2003. First published March 19, 2003; 10.1152/ajpgi.00280.2002.—The 5-HT$_{1B/D}$ receptor agonist sumatriptan has been proposed to treat dyspeptic symptoms, because it facilitates gastric accommodation. It is unknown whether stimulation of 5-HT$_{1B/D}$ receptors seems to play an important role in gastric accommodation. In addition, sumatriptan was found to relax the colon and to reduce the post-prandial increase in colonic tone and phasic motor activity. Animal models have allowed us to get more insight into the possible mechanism mediating the gastric motor effects of sumatriptan. Coulie et al. (13), using an in vivo cat model, suggested that the sumatriptan-induced increase in gastric volume is due to activation of a nitricergic inhibitory pathway, because the nitric oxide synthase inhibitor N$^\text{G}$-nitro-L-arginine methyl ester (L-NAME) antagonized the effect of sumatriptan. However, they did not investigate the possible involvement of 5-HT$_{1B/D}$ receptors, for which sumatriptan has high affinity (2). Thus we compared the effects of sumatriptan alone and combined with N-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2’-methyl-4’-(5-methyl-1,2,4-oxadiazol-3-yl)-[1,1-biphenyl]-4-carboxamide hydrochloride (GR-127935), 2-[3-[3(dimethylamino)-ethoxy]-4-methoxyphenyl]-2’-[methyl-4’-(5-methyl-1,2,4-oxadiazol-3-yl)][1,1-biphenyl]-4-carboxamide hydrochloride (SB-216641 hydrochloride), or 3-[4-(4-chlorophenyl)piperazin-1-yl]-1,1-diphenyl-2-propanol hydrochloride (BRL-15572 hydrochloride) (respectively, nonselective 5-HT$_{1B/D}$, selective 5-HT$_{1B}$, and selective 5-HT$_{1D}$ receptor antagonists) on gastric accommodation to isobaric distensions performed with a barostat. An exponential and a linear model were used to fit the pressure-volume relationship. An exponential equation fitted the data better than a linear equation. Sumatriptan (800 nmol/kg iv) induced an immediate gastric relaxation ($\Delta$volume: 112 ± 44 ml, $P < 0.05$). After sumatriptan, the pressure-volume curve was shifted toward significantly higher volumes. This effect was fully reversed by GR-127935 or SB-216641 but not by BRL-15572. In conclusion, 5-HT$_{1B}$ receptors seem to play an important role in modulating gastric accommodation to a distending stimulus. An exponential model for pressure-volume curves fits well with the concept of gastric adaptive relaxation.

gastro motility; gastric compliance; barostat; serotonin receptors; dog

SEVERAL STUDIES HAVE NOW DOCUMENTED the effects of the 5-HT$_{1B/D}$ receptor agonist sumatriptan on gastric motility in the same dose range used in migraine. In humans, sumatriptan caused an important delay in gastric emptying of both solids and liquids (12, 24). With the use of an electronic barostat to monitor variations in gastric tone in healthy volunteers, sumatriptan (6 mg sc) was found to relax the gastric fundus, allowing larger intragastric volumes before thresholds for perception of discomfort were reached (36). Because of these actions, sumatriptan has been proposed to treat dyspeptic patients with defective gastric accommodation. In addition, sumatriptan was found to relax the colon (11) and to reduce the post-prandial increase in colonic tone and phasic motor activity (42).

Animal models have allowed us to get more insight into the possible mechanism mediating the gastric motor effects of sumatriptan. Coulie et al. (13), using an in vivo cat model, suggested that the sumatriptan-induced increase in gastric volume is due to activation of a nitricergic inhibitory pathway, because the nitric oxide synthase inhibitor N$^\text{G}$-nitro-L-arginine methyl ester (L-NAME) antagonized the effect of sumatriptan. However, they did not investigate the possible involvement of 5-HT$_{1B/D}$ receptors, for which sumatriptan has high affinity (2). Thus we compared the effects of sumatriptan alone and combined with N-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2’-methyl-4’-(5-methyl-1,2,4-oxadiazol-3-yl)-[1,1-biphenyl]-4-carboxamide hydrochloride (GR-127935), 2-[3-[3(dimethylamino)-ethoxy]-4-methoxyphenyl]-2’-[methyl-4’-(5-methyl-1,2,4-oxadiazol-3-yl)][1,1-biphenyl]-4-carboxamide hydrochloride (SB-216641 hydrochloride), or 3-[4-(4-chlorophenyl)piperazin-1-yl]-1,1-diphenyl-2-propanol hydrochloride (BRL-15572 hydrochloride) (respectively, nonselective 5-HT$_{1B/D}$, selective 5-HT$_{1B}$, and selective 5-HT$_{1D}$ receptor antagonists) on canine gastric accommodation to isobaric distensions performed with a barostat. Atropine and bethanechol were used as reference drugs because of their well-known relaxing and contracting effect on the stomach, respectively (14, 15). Indeed, canine gastric tone is known to be maintained by a vagally mediated, cholinergic input (6).

MATERIALS AND METHODS

Gastric barostat. Gastric pressure-volume relationships were studied using a gastric barostat (ABS, Licence INRA, Saint-Die, France) (5, 22). The system consists of an ultrathin, flaccid polyethylene bag (1,100-ml capacity, Domopak-Cofresco, Milan, Italy), which, finely folded, can be inserted through a gastric cannula into the proximal stomach and has infinite compliance at the volumes used to distend the canine stomach.
The barostat can operate in two modes. If the barostat mode is used, a constant pressure is maintained in the intragastric bag, so that when the stomach contracts, the system withdraws air and when the stomach relaxes, the system injects air (maximal rate of airflow 50 ml/s). Thus the barostat allows quantitative measurement of variations in gastric tone by recording changes in the volume of air within the intragastric bag. Alternatively, the barostat can be used in the distension mode to assess the ability of the stomach to adapt to a distending stimulus (i.e., its compliance). This mode allows measurement of volume changes induced by a pressure increase (isobaric distensions). The slope of the pressure-volume curve (dV/dP) provides an estimate of gastric compliance.

Experimental model. Experiments were carried out on four female Beagle dogs (12–16 kg body wt) purchased from an authorized local breeder (Green Hill, Montichiari, Italy) and housed in single, air-conditioned boxes. All experiments were performed at the University of Pavia according to European Union Directive 86/609 on the care and use of experimental animals (authorizations of the Italian Ministry of Health 10/96C, 60/99C, 61/99B). The protocol was approved by the Ethics Committee of the Department of Internal Medicine and Therapeutics of the University of Pavia. The animals were fed with dog food in pellets (Stefano Morini, S. Polo d’Enza, Italy). Water was available ad libitum. The dogs were operated on under general thiopental anesthesia (25 mg/kg iv) with aseptic technique and assisted respiration. Through a midline laparotomy, we inserted a modified Thomas cannula into the stomach at the greater curvature opposite to the incisura angularis and exteriorized in the anterior aspect of the abdomen. Antibiotic prophylaxis consisted of intramuscular amoxycillin (0.05 ml/kg Clamoxyl; Pfizer, Rome, Italy) for 3 days starting the day of surgery.

Experimental procedure. Dogs were trained to stand quietly in a sling without sedation. Experiments were performed on conscious dogs after allowing at least 15 days for recovery after surgery. Before each experimental session, the dogs were fasted for at least 18 h; water was available ad libitum. Between consecutive experimental sessions with the same animal, a washout period of at least 72 h was allowed. The dogs were observed throughout the experiment, and any sign of discomfort or anomalous behavior was noted. The gastric cannula was opened, and after verifying that the stomach did not contain any food residues, we introduced the bag of the barostat into the proximal stomach (position of the bag checked fluoroscopically). Before and at the end of the in vivo tests, the bag was checked for air leaks by increasing the pressure to 20 mmHg.

At the beginning of the experimental session, intrabag pressure was maintained at a baseline level of 2 mmHg, because in all dogs, it represented the minimal distending pressure, i.e., the pressure at which the mean intrabag volume was >25 ml and continuous respiratory fluctuations were first detected. The very small baseline pressure applied by the barostat system allowed accurate recording of gastric volume changes without distortion of the physiological pattern of gut motor activity (4, 5).

After a 15-min equilibration period, we increased intrabag pressure with 2-mmHg increments every 3 min, starting from the baseline value of 2 mmHg up to 12 mmHg (or until an intrabag volume of 1,000 ml was achieved), without deflating the barostat bag at each pressure step. We did not exceed the pressure of 12 mmHg for two reasons: 1) preliminary experiments indicated that dogs started to show the first signs of discomfort (stretching of the legs) at 16–18 mmHg, and 2) a pressure of ~12 mmHg leads to a significant increase in transient lower esophageal sphincter relaxations (TLESRs; at a rate of ~7 every 30 min) in dogs of the same body weight (9). Although TLESRs are physiological events, their occurrence at this rate is considered a sign of excessive distension, and we decided not to exceed this pressure. Although distending pressures between 7 and 12 mmHg are above the normal physiological range, the same pressure range of 2–12 mmHg above minimal distending pressure was used in human volunteers (28).

In each experiment, four pressure-volume curves were obtained, allowing a 40-min interval between the end of a distension cycle and the beginning of the subsequent cycle. During these intervals, pressure was maintained at 2 mmHg. The first distension cycle was used to unfold the intragastric bag, and the values recorded were discarded. Preliminary

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**Fig. 1.** Experimental procedure. In each experiment, 4 pressure-volume curves were obtained. The first distension cycle, which was simply used to unfold the intragastric bag and was discarded from analysis, is not represented. Intrabag pressure was increased by 2-mmHg increments every 3 min, starting from the baseline value of 2 mmHg up to 12 mmHg, allowing a 40-min interval between the end of a distension cycle and the beginning of the subsequent cycle. During these intervals, pressure was maintained at 2 mmHg. A, administration of 5-HT_1B/D receptor antagonists; B, administration of the test drug (bolus injection of sumatriptan, atropine, or N^N-nitro-L-arginine methyl ester (L-NAME)) or the start of the intravenous infusion of bethanechol. See text for further details.
experiments showed that the next three cycles generated reproducible pressure-volume curves (coefficient of variation <10%). Therefore, when studying drug effects, we performed a first distension cycle to unfold the bag. The next two distension cycles were used as controls (Fig. 1). Test drugs (sumatriptan, atropine, or L-NAME) were administered 15 min before starting the fourth distension cycle. 5-HT_{1D} receptor antagonists were injected intravenously 30 min before the fourth distension cycle. Bethanechol was administered by intravenous infusion (15-min infusion, starting 15 min before starting the fourth distension cycle).

In preliminary experiments, it was found that, as in cats and humans, sumatriptan could induce a rapid-onset relaxation (immediately after the end of the intravenous injection). However, this response was short-lasting (the baseline gastric volume recorded at the distending pressure of 2 mmHg invariably returned to the preinjection value within 15 min) and was observed in all dogs only at the doses of 400 and 800 nmol/kg. Because the effect of sumatriptan on intragastric volume may depend on the distending pressure, we devised an experimental procedure that covers the 2- to 12-mmHg pressure range and takes into account both the duration of the acute relaxation (the 15-min period elapsing between sumatriptan administration and the next distension cycle allows recovery to the initial baseline gastric volume) and the pharmacokinetics of sumatriptan (the half-life of sumatriptan in dogs is ~2 h (21)).

In each dog, we carried out three sets of experiments, performing each experiment in duplicate. In the first set of experiments, we tested the effects of intravenous administration of sumatriptan, atropine, bethanechol, and L-NAME. The following doses of sumatriptan were used: 100, 200, 400, 800, 1,600, 2,400 nmol/kg iv (i.e., ~0.5–16 times the subcutaneous dose used in migraine). Atropine was injected intravenously at the doses of 100 and 300 nmol/kg on the basis of a previous dose-response study showing that they provided partial and full blockade of muscarinic transmission, respectively (17). The dose of bethanechol (508 nmol·kg^{-1}·h^{-1} for 15 min) was chosen on the basis of a previous dose-response study showing that this was the minimal dose required to significantly counteract the gastric relaxation induced by vagal blockade in dogs (15). The dose of L-NAME (37.04 μmol/kg iv) was selected from a review of the literature (1, 3).

In a second set of experiments, we tested the effects of intravenous administration of 5-HT_{1D} receptor antagonists alone (GR-127935, SB-216641, or BRL-15572 at doses of 54, 559, and 676 nmol/kg, respectively).

In a third set of experiments, we combined a 5-HT_{1D} receptor antagonist (GR-127935, SB-216641, or BRL-15572 at doses of 54, 559, and 676 nmol/kg, respectively) with sumatriptan (800 nmol/kg iv). The doses of antagonists were chosen on the basis of a review of the literature (18, 23, 43).

**Drugs.** Atropine sulfate, bethanechol chloride, and L-NAME hydrochloride were purchased from Sigma (St. Louis, MO). Sumatriptan succinate and GR-127935 were kindly donated by GlaxoSmithKline (Stevenage, Hertfordshire, UK). SB-216641 hydrochloride and BRL-15572 hydrochloride were purchased from Tocris Cookson (Bristol, UK). All drugs were dissolved in distilled water.

**Data analysis.** Baseline gastric tone was defined as the intragastric bag volume when pressure was maintained at 2 mmHg and was calculated by averaging the barostat volume tracing over 30-s periods. A change in gastric tone was defined as a change in intragastric volume >30 ml lasting >10 s. Changes in intragastric volume occurring after administration of sumatriptan, atropine, bethanechol, or L-NAME were measured at the peak of the response. During stepwise distensions, the maximal volume achieved at each pressure level was calculated by averaging the intrabag volume for 30 s at the peak of the response. Data obtained in the same animal with duplicate experiments were averaged to calculate grand means (±SE) for the four dogs and perform the statistical analysis.

![Graph](image.png)

**Fig. 2.** Pressure-volume relationship in the canine proximal stomach in control conditions (●) and after intravenous infusion of bethanechol (●; 508 nmol/kg). The equation that best fitted the experimental data was exponential: \( V = V_0 e^{aP} \) (see Table 1). Note that bethanechol shifted the pressure-volume curve towards lower volumes (\( P < 0.01 \)). Values are means ± SE (\( n = 4 \)).

### Table 1. Parameters calculated for the volume-pressure relationship in control conditions and after bethanechol, atropine, or L-NAME

<table>
<thead>
<tr>
<th></th>
<th>Exponential Model</th>
<th>Linear Model</th>
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<tbody>
<tr>
<td></td>
<td>( r^2 )</td>
<td>( V_0, \text{ml} )</td>
</tr>
<tr>
<td>Control</td>
<td>0.9979</td>
<td>48 ± 9</td>
</tr>
<tr>
<td>Bethanechol (508 nmol·kg^{-1}·h^{-1})</td>
<td>0.9995</td>
<td>6 ± 1*</td>
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<tr>
<td>Control</td>
<td>0.9919</td>
<td>117 ± 19</td>
</tr>
<tr>
<td>Atropine (100 nmol/kg)</td>
<td>0.9797</td>
<td>262 ± 43*</td>
</tr>
<tr>
<td>Control</td>
<td>0.8965</td>
<td>47 ± 9</td>
</tr>
<tr>
<td>Atropine (300 nmol/kg)</td>
<td>0.9802</td>
<td>228 ± 40*</td>
</tr>
<tr>
<td>Control</td>
<td>0.8776</td>
<td>57 ± 15</td>
</tr>
<tr>
<td>L-NAME (37.04 μmol/kg)</td>
<td>0.8554</td>
<td>13 ± 6*</td>
</tr>
</tbody>
</table>

Values are means ± SE (except in the column reporting slopes at \( \frac{1}{2} P_{\text{max}} \)), where means and 95% confidence intervals are indicated; \(* P < 0.05\) vs. its control; \( \dagger P < 0.01 \) vs. its control; slope at \( \frac{1}{2} P_{\text{max}} \) calculated as follows: \( y' = V_0 k_2 e^{aP} \), with \( P = 6 \) mmHg. L-NAME, N^\circ-\text{nitro-arginine methyl ester}; \( V_0 \), theoretical volume when \( P = 0 \).

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Pressure-volume curves were fitted by regression analysis (GraphPad Prism version 3.00 for Windows, GraphPad Software, San Diego, CA) using a linear \((V = V_0 + k_2P)\) or an exponential \((V = V_0e^{k_1P})\) equation, where \(V\) is volume (in ml), \(V_0\) is theoretical volume when \(P = 0\) mmHg, \(P\) is pressure (in mmHg), \(k_1\) is the rate constant in the exponential equation, and \(k_2\) is the slope in the linear model. The overall fit for each curve was summarized as an \(r^2\) value. The first derivative of the pressure-volume curve \((dV/dP)\) is a measure of gastric compliance. If a linear model is used, the slope of the regression line is a constant \((k_2)\), whereas if an exponential model is used, the first derivative is \(dy = V_{0}\cdot ke^{k_1P}\) and increases with pressure. Therefore, to compare compliance values obtained with the two models, we calculated the slope at \(\frac{1}{2} P_{\text{max}}\) (see Table 1). Note that \(V_0\) and \(k_1\) (rate constant) are reported in Table 1.

Effect of bethanechol, atropine, and L-NAME on the pressure-volume relationship. The baseline gastric volume recorded at 2 mmHg was 45 ± 18 and 25 ± 5 ml before and after bethanechol administration, respectively \((P > 0.05)\). After bethanechol, the pressure-volume curve was significantly \((P < 0.01)\) shifted toward significantly lower volumes and the curve that best fitted the experimental data was still exponential \((P < 0.01)\). Table 1 reports the values obtained with the exponential and the linear model. With the use of the exponential model, \(V_0\) and slope at \(\frac{1}{2} P_{\text{max}}\) calculated after bethanechol were significantly lower with respect to controls, whereas \(P_{\text{half}}\) was significantly higher \((P < 0.05)\).

Both doses of atropine decreased baseline gastric tone \((\Delta V\) at 2 mmHg = 165 ± 43 and 145 ± 44 ml, respectively, for the lower and higher dose; both \(P < 0.05)\) and significantly \((P < 0.01)\) shifted the pressure-volume curve toward higher volumes \((P < 0.01)\). After atropine, the linear model provided a better fit of the experimental data \((P < 0.05)\). Table 1 reports the values obtained with the exponential and the linear model. Atropine significantly \((P < 0.05)\) increased \(V_0\) and tended to decrease \(k_1\) (rate constant), although the latter effect did not achieve statistical significance and no significant change in the slope at \(\frac{1}{2} P_{\text{max}}\) was observed. The dependent variable was the delta between the values obtained before and after sumatriptan at each dose level, using the lowest dose of sumatriptan as baseline. The mixed procedure from the SAS package (SAS/STAT Software Release 6.11, SAS Institute, Cary, NC) was used.

RESULTS
The pressure-volume relationship before drug administration. Baseline gastric volume measured at 2 mmHg was 53 ± 7 ml. Stepwise distensions increased intragastric volume \((P < 0.01)\), and the pressure-volume curve toward higher volumes \((P < 0.01)\) and \(P < 0.001\) for the lower and higher dose, respectively). After 300 nmol/kg atropine, distensions at 12 mmHg were not performed because the intrabag volume exceeded 1,000 ml. Values are means ± SE \((n = 4)\).
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Fig. 5. Volume-pressure relationship in the canine proximal stomach in control conditions (A) and after administration of sumatriptan: •, 100 nmol/kg (A); †, 200 nmol/kg (B); ●, 400 nmol/kg (C); □, 800 nmol/kg (D); ○, 1,600 nmol/kg (E); △, 2,400 nmol/kg (F). Note that sumatriptan shifted the pressure-volume curve toward higher volumes (P < 0.01 at 200 nmol/kg, and P < 0.001 at higher doses). Values are means ± SE (n = 4).

Table 2. Parameters calculated for the volume-pressure relationship in control conditions and after sumatriptan according to the equation \( V = V_0 e^{k_P P} \)

<table>
<thead>
<tr>
<th></th>
<th>( r^2 )</th>
<th>( V_0, \text{ ml} )</th>
<th>( k_1, \text{ mmHg}^{-1} )</th>
<th>Slope at ( \frac{1}{2} P_{\text{max}}, \text{ ml/mmHg} )</th>
<th>( P_{\text{half}}, \text{ mmHg} )</th>
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</thead>
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<tr>
<td>Control</td>
<td>0.9921</td>
<td>80 ± 21</td>
<td>0.21 ± 0.02</td>
<td>59 (21–91)</td>
<td>8.28 ± 1.19</td>
</tr>
<tr>
<td>Sumatriptan (100 nmol/kg)</td>
<td>0.9923</td>
<td>86 ± 22</td>
<td>0.20 ± 0.02</td>
<td>57 (24–92)</td>
<td>7.87 ± 1.02</td>
</tr>
<tr>
<td>Control</td>
<td>0.9814</td>
<td>72 ± 29</td>
<td>0.19 ± 0.04</td>
<td>48 (16–92)</td>
<td>9.32 ± 0.81</td>
</tr>
<tr>
<td>Sumatriptan (200 nmol/kg)</td>
<td>0.9955</td>
<td>109 ± 16</td>
<td>0.17 ± 0.01</td>
<td>51 (39–66)</td>
<td>8.21 ± 1.01</td>
</tr>
<tr>
<td>Control</td>
<td>0.9858</td>
<td>64 ± 24</td>
<td>0.21 ± 0.04</td>
<td>47 (19–99)</td>
<td>9.46 ± 1.23</td>
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<tr>
<td>Sumatriptan (400 nmol/kg)</td>
<td>0.9908</td>
<td>116 ± 26</td>
<td>0.17 ± 0.02</td>
<td>55 (33–84)</td>
<td>7.74 ± 1.50*a</td>
</tr>
<tr>
<td>Control</td>
<td>0.9896</td>
<td>49 ± 17</td>
<td>0.22 ± 0.03</td>
<td>49 (19–74)</td>
<td>9.91 ± 1.03</td>
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<tr>
<td>Sumatriptan (800 nmol/kg)</td>
<td>0.9878</td>
<td>104 ± 27</td>
<td>0.18 ± 0.03</td>
<td>55 (28–87)</td>
<td>8.20 ± 1.16*a</td>
</tr>
<tr>
<td>Control</td>
<td>0.9882</td>
<td>60 ± 21</td>
<td>0.21 ± 0.03</td>
<td>44 (23–82)</td>
<td>9.45 ± 0.79</td>
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<tr>
<td>Sumatriptan (1,600 nmol/kg)</td>
<td>0.9911</td>
<td>93 ± 23</td>
<td>0.19 ± 0.02</td>
<td>55 (33–86)</td>
<td>8.12 ± 1.28</td>
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<tr>
<td>Control</td>
<td>0.9881</td>
<td>66 ± 21</td>
<td>0.20 ± 0.03</td>
<td>44 (21–80)</td>
<td>9.11 ± 0.72</td>
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<tr>
<td>Sumatriptan (2,400 nmol/kg)</td>
<td>0.9895</td>
<td>109 ± 26</td>
<td>0.17 ± 0.02</td>
<td>51 (31–80)</td>
<td>7.89 ± 0.97*a</td>
</tr>
</tbody>
</table>

Values are means ± SE (except in the column reporting slope at \( \frac{1}{2} P_{\text{max}} \), where means and 95% confidence intervals are indicated); *P < 0.05 vs its control; slope at \( \frac{1}{2} P_{\text{max}} \) was calculated as follows: \( y = V_0 e^{k_1 P} \), with \( P = 6 \) mmHg.

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measures analysis of variance, used to detect differences among doses of sumatriptan, revealed that only with the dose of 800 nmol/kg was the delta obtained before and after sumatriptan significantly higher than that observed with the lowest dose (100 nmol/kg) in at least four of six pressure levels. Therefore, the dose of 800 nmol/kg was selected for the experiments with 5-HT<sub>1B/D</sub> receptor antagonists.

Table 2 reports the values obtained with the exponential model to fit the data obtained before and after sumatriptan administration. Again, the exponential model provided a better fit of the data points (data not shown). After sumatriptan, P<sub>half</sub> was significantly decreased (Table 2). Although V<sub>0</sub> and the slope at ½ P<sub>max</sub> tended to be higher after sumatriptan at doses ≥200 nmol/kg (Table 2), this trend did not achieve statistical significance.

Effect of 5-HT<sub>1B/D</sub> receptor antagonists alone. GR-127935, SB-216641, and BRL-15572 per se had no effect on baseline gastric volume and on the volume-pressure relationship in the stomach (33, 41). A significant methodological difference from studies investigating gastric barosensitivity to assess visceroresponses [see, for example Toulouse et al. (41)] is that we applied a maximal distending pressure of 12 mmHg to study gastric adaptation to a more physiological level of distension. By contrast, studies on visceroresponses usually investigate pain thresholds to gastric distension using higher pressure levels. In our control conditions (i.e., before drug administration), an exponential model provided a better fit of the experimental data than a linear model. In other words, minimal changes in pressure were accompanied by huge variations in volume.

Although investigators often resort to a simpler, linear equation to fit pressure-volume data (26–28, 33–37), an exponential equation fits well with the concept of adaptive accommodation to a distending stimulus, at least within a narrow pressure range. Indeed, the proximal stomach can rapidly accept relatively large meals with only a minimal increase in pressure (adaptive relaxation). When, unlike in the present study, higher pressure levels are achieved, discomfort and pain are induced and the pressure-volume relationship does not fit an exponential model, probably because of the influence of the elastic properties of the gut wall (10). An advantage of the exponential with respect to the linear model is that the former does not provide negative values for V<sub>0</sub>, which have no biological meaning.

The exponential model proposed in this manuscript is simpler than the power exponential model, proposed by the Mayo group (7, 8, 39). The latter model has certainly the advantage of providing excellent fit for the sigmoid pressure-volume curves that tend to reach a plateau at the highest pressure levels (8). On the other hand, it is less straightforward than the simple

Fig. 6. Volume-pressure relationship in the canine proximal stomach before (●) and after intravenous N-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2’-methyl-4’-(5-methyl-1,2,4-oxadiazol-3-yl)-[1,1-biphenyl]-4-carboxamide hydrochloride (GR-127935; 54 nmol/kg; ▲; A), N’-[3-(3-dimethylamino)-ethoxy]-4-methoxyphenyl]-2’-[methyl-4’-[(5-methyl-1,2,4-oxadiazol-3-yl)]-1,1-biphenyl]-4-carboxamide hydrochloride (SB-216641; 559 nmol/kg; ○; B), and 3-[4-(4-chlorophenyl)piperazin-1-yl]-1,1-diphenyl-2-propanol hydrochloride (BRL-15572; 676 nmol/kg; ▼; C). No significant change was observed. Values are means ± SE (n = 4).
exponential model, because it requires transformation of data (proportionate volume is expressed as a function of reciprocal pressure). In this study, we propose the simple exponential model for the following reasons: first, in our experiments, for both ethical and technical reasons (see MATERIALS AND METHODS), we investigated the pressure-volume curves in the 2- to 12-mmHg pressure range and, within this range, the curves were not sigmoid. Second, because the simple exponential model provided very good fit of the experimental data, it was unnecessary to resort to the more sophisticated power exponential model. However, we acknowledge that we investigated a limited pressure range that may not fully reflect the biomechanical properties of the stomach.

The shape of the exponential curve is consistent with a progressive activation of reflex mechanisms, which tend to prevent pressure increases in response to increasing gastric volumes and are mediated by neural pathways having nitric oxide as an important neurotransmitter (20, 40). Several studies have documented the important role played by nitric oxide in mediating vagally induced gastric relaxations in different species, including guinea pigs (32) and dogs (30). Notably, the intravenous administration of the nitric oxide synthase inhibitor N⁵-nitro-L-arginine (L-NNA) shifted the pressure-volume curve toward lower volumes and decreased gastric compliance in dogs, suggesting the presence of a continuous nitrergic inhibitory tone (33). Our results with L-NAME confirm this hypothesis. The fact that the L-NAME per se affected the pressure-volume curve prevented us from formally testing the effect of L-NAME against sumatriptan. In any case, the sumatriptan-induced shift of the pressure-volume curve toward higher volumes is consistent with the hypothesis put forward by Coulie et al. (13) that 5-HT₁ receptors activate a nitrergic inhibitory pathway.

In this study, sumatriptan facilitated gastric accommodation to a distending stimulus with a peak effect at 800 nmol/kg. Interestingly, the effect of sumatriptan was statistically significant already at the dose of 200 nmol/kg, which approximately corresponds to the single subcutaneous dose used in humans for the relief of a migraine attack (6 mg). Sumatriptan exerted its action on gastric accommodation through 5-HT₁B receptors, because both GR-127935 and SB-216641 fully reversed the gastric motor effects of sumatriptan, whereas the lack of effect of BRL-15572 suggests that 5-HT₁D receptors are not involved. Whether the location of the 5-HT₁B receptors mediating the effect of sumatriptan is central or peripheral remains to be determined. The fact that sumatriptan penetrates poorly the blood-brain barrier (19) and can relax the guinea pig isolated stomach (31) would argue against a central site of action. We tentatively suggest a neuronal location of these 5-HT₁B receptors, because the effect of sumatriptan in vitro was blocked by tetrodotoxin (31). We should also consider 5-HT₁P receptors, because their presence is reported in enteric neurons (29), and some authors have suggested that sumatriptan might act via this receptor subtype (36). However, 5-HT₁P receptors are not included in the official International Union of Pharmacology classification of serotonin receptors [they are still considered “orphan” receptors (2, 25)], and to the best of our knowledge, none of the authors reporting the gastric motor effects of sumatriptan in vivo has ever tested the effect of 5-HT₁P receptor antagonists because of the lack of selective agents suitable for in vivo use. The fact that, in our hands, the effect of sumatriptan was fully...
reversed by GR-127935 and SB-216641 supports the involvement of 5-HT1B receptors.

The fact that we studied drug effects in the 2- to 12-mmHg pressure range instead of working at a fixed pressure level [a fixed pressure level of 2 mmHg above the minimal distending pressure was used by Couli et al. (13)], allowed us to appreciate some qualitative differences among the effects of sumatriptan, atropine, and bethanechol. Indeed, both the lower and higher dose of atropine induced a significant gastric relaxation (see also Ref. 16) at the baseline pressure of 2 mmHg and shifted the pressure-volume curve toward higher volumes with a change in the best-fit equation, which became linear. These observations confirm the important role played by vagal cholinergic pathways in maintaining gastric tone in the fasting dog (6, 16) and are in line with recent findings in humans (28). We interpret the change in the shape of the pressure-volume curve (i.e., from exponential to linear) after atropine as a phenomenon due to the significant gastric relaxation induced by muscarinic receptor blockade at baseline pressure, which masks the ability of the stomach to undergo the process of “adaptive accommodation.” Notably, $k_1$ values (rate constant) were lower after atropine, although this trend did not reach statistical significance.

By contrast, bethanechol did not significantly affect the shape of the curve (the exponential equation provided the best fit) but made the stomach less susceptible to undergo distension, as indicated by the shift of the pressure-volume curve to the right, the lower gastric compliance, and $V_0$ values after bethanechol. The lack of a statistically significant difference in baseline gastric volume at 2 mmHg distending pressure after bethanechol is probably due to a type II error.

Finally, sumatriptan significantly shifted the pressure-volume curve toward higher volumes and enhanced gastric accommodation, an action that has potential application in the treatment of functional dyspepsia (36, 38). Indeed, contrary to the view that most dyspeptic patients have delayed gastric emptying and a relaxed stomach (hence, the use of prokinetic agents to relieve symptoms), a significant proportion of these subjects display impaired fundic relaxation to a meal or altered gastric sensitivity to distension (38). In these patients, prokinetics are contraindicated, whereas a gastric relaxing drug could decrease early satiety, a cardinal symptom of dysmotility-like dyspepsia.

In conclusion, sumatriptan facilitates gastric accommodation to a distending stimulus, and 5-HT1B receptors play an important role in mediating this effect. The finding that an exponential model fits gastric pressure-volume data better than a linear model well describes the concept of gastric adaptive relaxation.

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