Regional gastric contractility alterations in a diabetic gastroparesis mouse model: effects of cholinergic and serotoninergic stimulation

Arlene N. James,1 James P. Ryan,2 Michael D. Crowell,3 and Henry P. Parkman1,2

Departments of 1Medicine and 2Physiology, Temple University School of Medicine, Philadelphia, Pennsylvania 19140; and 3Department of Medicine, Mayo Clinic Scottsdale, Scottsdale, Arizona 85259

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James, Arlene N., James P. Ryan, Michael D. Crowell, and Henry P. Parkman. Regional gastric contractility alterations in a diabetic gastroparesis mouse model: effects of cholinergic and serotoninergic stimulation. Am J Physiol Gastrointest Liver Physiol 287: G612–G619, 2004. First published April 23, 2004; 10.1152/ajpgi.00431.2003.—The C57BLKS/J db/db mouse develops hyperglycemia and has delayed gastric emptying that is improved with tegaserod, a partial 5-HT4 agonist. Our aims here were to determine regional gastric contractility alterations in C57BLKS/J db/db mice and to determine the effects of serotonin and tegaserod. The contractile effects of bethanechol, serotonin, and tegaserod in fundic, antral, and pyloric circular muscle were compared in C57BLKS/J db/db mice and normal littermates. The effects of tetrodotoxin, atropine, and 5-HT receptor antagonists were studied. Contractions in response to bethanechol were decreased in the fundus, similar in the antrum, but increased in the pylorus in diabetic mice compared with controls. Serotonin and, to a lesser extent, tegaserod caused contractions that were more pronounced in the fundus than in the antrum and pylorus in both diabetic and normal mice. Serotonin-induced contractions were partially inhibited by atropine, the 5-HT4 antagonist GR113808, and the 5-HT2 antagonist cinanserin but not tetrodotoxin. Regional gastric contractility alterations are present in this diabetic gastroparesis mouse model. Fundic contractility was decreased, but pyloric contractility was increased in the pylorus to cholinergic stimulation in this diabetic mouse model. Fundic contractility alterations were partially inhibited by atropine, the 5-HT4 antagonist GR113808, and the 5-HT2 antagonist cinanserin but not tetrodotoxin. Regional gastric contractility alterations in C57BLKS/J db/db mice and to determine whether there are regional alterations in gastric contractility in C57BLKS/J db/db diabetic mice compared with normal mice. We also investigated the muscarinic subtype mediating cholinergic contractions to determine whether alterations of muscarinic receptor subtypes may explain any alterations in gastric contractility. The second aim was to determine the effects and mechanisms of serotonin on regional gastric contractility in C57BLKS/J db/db diabetic mice compared with normal mice.

MATERIALS AND METHODS

Fundus, antrum, and pyloric muscle ring preparation. The Jackson Laboratory C57BLKS/J db/db transgenic mouse (Jackson Laboratories, Bar Harbor, ME) and its normal control litters (females age 8–12 wk old) were used in this study (13). The phenotype of db/db mice includes early-onset obesity and hyperglycemia (12). This study was approved by the Institutional Animal Care and Use Committee (IACUC) at Temple University School of Medicine. Mice were acclimated at our institution for 5 days before use. On the day of the study, the mouse was weighed. The mouse was killed by CO2 asphyxiation with subsequent removal of the stomach with pylorus and proximal duodenum. A blood sample was obtained for blood glucose concentration (Accu-Check, Roche-Diagnostics, Indianapolis, IN). The stomach was rinsed with Krebs-bicarbonate buffer (composition in mM: 120 NaCl, 4.6 KCl, 2.5 CaCl2, 1.2 MgCl2, 22 NaHCO3, 1.2 NaH2PO4, and 11.5 glucose oxygenated with 95% O2-5% CO2; pH 7.4). Circular muscle rings were prepared from the fundus, antrum, and pylorus in the C57BLKS/J db/db mouse and its normal control litters. In general, from one mouse, two fundic rings, two antral rings, and one pyloric ring were obtained. The rings were attached to isometric force transducers and suspended in 10-mL organ baths containing Krebs-bicarbonate buffer (temperature 37°C). After a 30-min equilibration period, the preparations were stretched until the contractile response to acetylcholine 100 μM was maximal (Lmax).

Strains of rats and mice with spontaneously developing hyperglycemia have been recognized as useful models to study the effects of diabetes. The Jackson Laboratory C57BLKS/J db/db mouse spontaneously develops hyperglycemia (12) and is a model for non-insulin-dependent diabetes mellitus (24). Prior studies in these mouse strain (22) have shown delayed gastric emptying. Tegaserod, a partial 5-HT4 agonist, accelerates gastric emptying in these diabetic mice (21). Gastric muscle dysfunction has not been characterized in this diabetic mouse model, and the effect of tegaserod on gastric contractility is not known.

The aims of these in vitro studies were twofold. The first was to determine whether there are regional alterations in gastric contractility in C57BLKS/J db/db diabetic mice compared with normal mice. We also investigated the muscarinic subtype mediating cholinergic contractions to determine whether alterations of muscarinic receptor subtypes may explain any alterations in gastric contractility. The second aim was to determine the effects and mechanisms of serotonin on regional gastric contractility in C57BLKS/J db/db diabetic mice compared with normal mice.
Experimental protocols. Bethanechol concentration-response curves (CRCs) were performed in doses from 10 nM to 100 μM. Each concentration was added for 3 min with rinsing in between doses and resumption of baseline tone. High potassium (80 mM), which causes muscle cell membrane depolarization, subsequent calcium entry, and muscle contraction, was also used to contact the muscle rings. For this, high-potassium Krebs solution replaced the normal Krebs solution surrounding the muscle ring in the in vitro muscle bath. The potassium-containing Krebs solutions were prepared by increasing the potassium concentration to 80 mM while decreasing the sodium concentration to maintain osmolality (26).

Some studies have suggested that muscle dysfunction in neuropathy and diabetes can be due to alterations in muscarinic receptor subtypes mediating contractions (5). Thus the muscarinic receptor subtypes mediating cholinergic contractions were investigated in this study by determining the inhibitory effects of specific muscarinic receptor subtype antagonists on bethanechol-induced contractions. The M1 muscarinic subtype receptor antagonist pirenzepine, the M2 antagonist methoctramine, and the M3 antagonist 4-diphenylacetoxy-N-methylpiperidine methiodide (4-DAMP) (Sigma, St. Louis, MO) were used (27). After an initial control contractile response to 10 μM bethanechol followed by rinsing, a 10 nM concentration of receptor antagonist was added, followed in 10 min by the addition of another application of 10 μM bethanechol. This was repeated with a 100 nM concentration of the receptor antagonist.

In a separate series of experiments, CRCs to serotonin maleate (Sigma) and tegaserod maleate (Novartis Pharmaceuticals, East Hanover, NJ) were performed in concentrations from 10 nM to 100 μM. Each concentration was added for 3 min with rinsing in between doses and resumption of baseline tone. Tetrodotoxin (Sigma), atropine (Sigma), the 5-HT1A/1B antagonist pindolol (Tocris Cookson, Ballwin, MO) (3), the 5-HT3 antagonist cinanseron hydrochloride (Tocris Cookson) (19), the 5-HT3 antagonist Y25130 hydrochloride (Tocris Cookson) (8), and the 5-HT4 antagonist GR113808 (Tocris Cookson) (9) were used to determine neural and receptor pathways mediating the serotonin contractile responses. For experimental protocols with receptor antagonists, time controls were performed. These control muscle strips were studied with the same protocol but without the addition of antagonists for which similar contractile responses were obtained. There were no changes in contractility over the 3-h in vitro experimental protocols.

Additional studies with electric field stimulation (EFS) were used to activate the intrinsic enteric nerves. These were performed with the muscle strips suspended between platinum electrodes placed adjacent and parallel to the long axis of the muscle strip in the circular muscle direction. These electrodes were connected to an electric stimulator (Grass stimulator model SD9, Grass Instruments, Astro-Med, West Warwick, RI). EFS was performed with 16 Hz, 100 V, 0.5-ms pulse-width duration (square wave), 60-s train duration parameters. After each EFS stimulation, 10⁻⁴ M ACh was added to test overall muscle responsiveness. In initial experiments, 1 μM atropine and 1 μM tetrodotoxin were added to assess the cholinergic neural pathways involved in EFS- and ACh-induced contractions. In subsequent experiments, tegaserod was added in graded concentrations from 10⁻⁷ to 10⁻⁵ M before the performance of EFS.

At the end of each experimental protocol, the muscle length was measured. The muscle ring was removed, blotted dry with filter paper, and weighed (model GA110, Ohaus, Florham Park, NJ). The cross-sectional area was calculated using the relationship area = mass/ (density × length), where the mass was in grams, the length was in centimeters and the density was assumed to be 1.056 g/cm³ (11, 31). The accuracy of this method for determining cross-sectional surface area has been verified using previously optically derived measurements (31, 32). Contractile results were expressed as muscle tension that normalized for differences in tissue strip size by dividing the absolute force of the contraction by the cross-sectional muscle area (kg/cm²).

Data analysis. Contractile responses to bethanechol, potassium, serotonin, and tegaserod were measured as the maximal contractile response above baseline tone after stimulation. Contractile responses are expressed as force (grams per cross-sectional area). For the receptor antagonist studies, each preparation served as its own control, with the amplitude of contraction after incubation with antagonist compared with the amplitude of contraction in Krebs solution immediately preceding the addition of antagonist. Data are expressed as means ± SE of results obtained from 4–10 muscle rings. Two-way analysis of variance was used to determine whether CRCs were different between the diabetic and normal mice. Students t-test was used to determine whether the effects of antagonists on contractile responses were significant. A P value < 0.05 was considered statistically significant.

RESULTS

General observations. Experiments were performed in 46 C57BLKS/J db/db diabetic mice and 48 normal control littersates. In general, the C57BLKS/J db/db diabetic mice were larger than the control littersates. The body weights of the diabetic mice were greater than normal littersates (41 ± 1 vs. 19 ± 1 g; P < 0.001). Blood glucose concentrations were elevated in C57BLKS/J db/db diabetic mice compared with normal control littersates (478 ± 17 vs. 137 ± 4 mg/dl; P < 0.001).

Effect of Bethanechol and Potassium on Regional Mouse Gastric Contractility (in vitro circular muscle rings)

![Fig. 1. Effect of bethanechol and potassium in regional mouse gastric contractility. Bethanechol caused concentration-dependent contractions of the fundus (A), antrum (B), and pylorus (C). These tracings are from a normal mouse using in vitro circular muscle rings.](http://ajpgi.physiology.org/)

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Effects of bethanechol and potassium on fundic, antral, and pyloric muscle. We first investigated the contractile responses in diabetic and control mice using both bethanechol and high potassium, evaluating the responses in three regions of the stomach: the fundus, antrum, and pylorus. Bethanechol elicited dose-dependent contractions of the fundus, antrum, and pylorus (Fig. 1). Fundic contractions were significantly decreased in diabetic mice compared with control mice for bethanechol-induced contractions ($P < 0.026$ by ANOVA II of the CRC; Fig. 2). There were not significant alterations in the $80$ mM potassium-induced contractions ($P > 0.74$; Fig. 3). Antral contractility was not significantly different between the diabetic and control mice to either bethanechol ($P > 0.36$) or potassium ($P > 0.10$) in diabetic mice compared with normal mice (Figs. 2B and 3). Pyloric contractility was significantly increased to both bethanechol ($P < 0.001$) and potassium ($P < 0.005$) in the diabetic mice compared with normal mice (Figs. 2C and 3).

Table 1. Effect of muscarinic receptor subtype antagonists at two concentrations (10 and 100 nM) on the contractile responses of 0.1 $\mu$M bethanechol in normal and diabetic mice

<table>
<thead>
<tr>
<th>Receptor Subtype Antagonist</th>
<th>Normal Fundus</th>
<th>Normal Antrum</th>
<th>Diabetic Fundus</th>
<th>Diabetic Antrum</th>
</tr>
</thead>
<tbody>
<tr>
<td>M$_1$ Receptor Subtype Antagonist</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pirenzepine, 10 nM</td>
<td>$105 \pm 3$</td>
<td>$94 \pm 4$</td>
<td>$113 \pm 4$</td>
<td>$104 \pm 5$</td>
</tr>
<tr>
<td>Pirenzepine, 100 nM</td>
<td>$103 \pm 11$</td>
<td>$85 \pm 7$</td>
<td>$98 \pm 5$</td>
<td>$98 \pm 4$</td>
</tr>
<tr>
<td>M$_2$ Receptor Subtype Antagonist</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methoctramine, 10 nM</td>
<td>$98 \pm 4$</td>
<td>$85 \pm 18$</td>
<td>$105 \pm 3$</td>
<td>$118 \pm 20$</td>
</tr>
<tr>
<td>Methoctramine, 100 nM</td>
<td>$102 \pm 4$</td>
<td>$86 \pm 19$</td>
<td>$111 \pm 10$</td>
<td>$120 \pm 21$</td>
</tr>
<tr>
<td>M$_3$ Receptor Subtype Antagonist</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-DAMP, 10 nM</td>
<td>$25 \pm 5$</td>
<td>$47 \pm 10$</td>
<td>$29 \pm 4$</td>
<td>$57 \pm 10$</td>
</tr>
<tr>
<td>4-DAMP, 100 nM</td>
<td>$5 \pm 3$</td>
<td>$16 \pm 4$</td>
<td>$12 \pm 4$</td>
<td>$26 \pm 6$</td>
</tr>
</tbody>
</table>

Values are means $\pm$ SE of experiments performed in 3–6 muscle rings from different animals. Results expressed as % initial control response without the antagonist. *$P < 0.05$, †$P < 0.01$. 

Fig. 2. Comparison of bethanechol concentration-response curves from diabetic and control mice in the fundus (A), antrum (B), and pylorus (C). A: fundic concentration-response curve to bethanechol was significantly decreased in the diabetic mice compared with the control mice ($P > 0.026$ by ANOVA II). B: antral concentration-response curve to bethanechol was not significantly different between the diabetic and control mice ($P > 0.36$ by ANOVA II). C: pyloric concentration-response curve to bethanechol was significantly greater in the diabetic mice compared with the control mice ($P < 0.001$ by ANOVA II). 

Fig. 3. Comparison of regional contractile responses in diabetic and normal mouse to potassium ($80$ mM). The contractile responses to $80$ mM K$^+$ were not significantly different in the fundus ($P > 0.74$) and antrum ($P > 0.10$) between the diabetic and control animals, whereas they were increased in the pylorus in the diabetic mice ($*P < 0.005$) compared with the control animals.
Effects of muscarinic receptor antagonists on bethanechol-induced contractions. We next investigated the muscarinic subtypes mediating the bethanechol-induced contraction to determine whether there were alterations in the subtypes mediating the cholinergic contractile responses (Table 1 and Fig. 4). In both the normal and diabetic mice, the contractile responses to 10 μM bethanechol were significantly inhibited by 10 nM 4-DAMP in the fundus (75 ± 5 and 71 ± 4% inhibition, respectively) and antrum (53 ± 10 and 43 ± 10% inhibition, respectively). A higher concentration of 4-DAMP (100 nM) produced greater inhibition of the bethanechol-induced contractile responses (Table 1). Pirenzepine and methoctramine did not have a significant effect on bethanechol contractions.  

Effects of serotonin on fundic, antral, and pyloric muscle. We next evaluated the effects of serotonin (5-HT) on fundic, antral, and pyloric muscle strips from the diabetic and normal mice. Serotonin elicited concentration-dependent contractile responses of the fundus, antrum, and pylorus (Figs. 5 and 6). The contractions were significantly fewer than those to either acetylcholine or bethanechol. In normal mice, the contractile effect of serotonin was more pronounced in the fundus (44 ± 11 g/cm²) than in the antrum (5 ± 2 g/cm²) or pylorus (7 ± 2 g/cm²) at the concentration for maximal contractile response (ED₉₀) of 30 μM (Fig. 6, A-C). The contractile effects to serotonin seen in diabetic mice (Fig. 6, D-F) were of similar magnitude as in normal mice (Figs. 6, A-C).

Effects of antagonists on serotonin-induced contractions. The effects of neural and receptor inhibitors on the serotonin-induced contractions were studied. The contractile effects of 10 μM serotonin were partially inhibited by atropine in the control mice (43 ± 8% inhibition in the fundus and 61 ± 10% inhibition in the antrum), with similar inhibitory effects in the diabetic mice (Table 2). The 5-HT₁ receptor antagonist pindolol and the 5-HT₃ receptor antagonist Y25130 had no significant effect on serotonin-induced contractions. There was a small, but significant, inhibition seen with the 5-HT₄ receptor antagonist GR113808 (1 μM) in both the fundus and antrum. There was also a small, but significant, inhibitory effect seen with the 5-HT₂ receptor antagonist cinanserin in the antrum, but not the fundus, of diabetic and normal mice (Table 2).

Effects of tegaserod. To further explore the role of 5-HT₄ receptors in mediating the serotonin-induced contractions, the effect of tegaserod on muscle rings was studied (Fig. 6). Tegaserod produced concentration-dependent contractions both in normal (Fig. 6, A-C) and diabetic mice (Fig. 6, D-F), which were also greater in the fundus than in the antrum and pylorus, as seen with serotonin. The tegaserod contractile responses were fewer than the serotonin responses except in the diabetic antrum, where they tended to be greater (Fig. 6).

Effects of EFS. EFS at 16 Hz was used to activate the intrinsic nerves of fundic and antral muscle strips. EFS, at the parameters used, produced contractions of the fundus and antrum. EFS-induced contractions were inhibited by 1 μM tetrodotoxin or 1 μM atropine, whereas ACh-induced contractions were abolished by atropine but were not affected by tetrodotoxin. This indicates that EFS-induced contractions were primarily cholinergically mediated by activation of cholinergic nerves to release ACh, which subsequently acts on muscarinic receptors to cause smooth muscle contraction. Tegaserod increased the EFS-induced contractions of the antrum in both the control and diabetic mice (Table 3). There was no significant effect of tegaserod on EFS-induced contractions of fundic muscle strips in either control or diabetic mice (Table 3). Tegaserod had no effect on contractions induced by exog-
The Jackson Laboratory C57BLKS/J db/db mouse, which spontaneously develops chronic hyperglycemia and obesity, is a model for T2DM (12). This mouse strain has delayed gastric emptying (22), making this animal model useful to investigate abnormalities in diabetic gastroparesis. This study shows that regional differences in gastric contractility are present in this mouse model of diabetic gastroparesis. Specifically, the gastric contractility to bethanechol was significantly decreased in the fundus but increased in the pylorus compared with normal control littermates. In each of the fundus, antrum, and pyloric areas, the cholinergic contractile responses were mediated through muscarinic M3 subtype receptors, as evidenced by the inhibitory effects of the M3 receptor antagonist 4-DAMP on cholinergic contractions.

Prior studies in diabetic gastroparesis (2), particularly human studies in type 1 diabetes mellitus, have shown that gastroparesis is associated with antral hypomotility. Some studies (23) have also shown the presence of pyloric dysfunction, or pylorospasm. Gastroparesis has also been described in T2DM (15). With the use of the C57BLKS/J db/db mouse model of T2DM with delayed gastric emptying (22), this study suggests that the fundic hypomotility and pyloric hypercontractility of diabetic gastroparesis.

Fig. 6. Concentration-response curves of serotonin and tegaserod in gastric fundus, antrum, and pylorus of normal (A-C) and diabetic mice (D-F). Both serotonin and tegaserod caused concentration-dependent contractions that were more pronounced in the fundus than in the antrum or pylorus both in diabetic and in normal mice. The tegaserod contractile responses were generally significantly fewer than the serotonin responses except in the diabetic antrum, which tended to be greater.
response was also shown by the contractile effects of 5-HT4 serotonin contractions was small, although significant. The inhibitory effect of the 5-HT4 antagonist on serotonin receptors mediating GI contractile responses, as suggested in our study, 5-HT2A smooth muscle receptors have been reported on porcine gastric smooth muscle mediating contraction (14).

Whether serotonin is acting primarily at the muscle level and/or neural level is not entirely clear from these experiments. Tetrodotoxin did not abolish the effect of serotonin, implying that neural conduction is not needed for the serotonin contractile effect. However, atropine reduced the serotonin-induced contractions, implying a cholinergic pathway for serotonin-induced contraction. One possibility is that serotonin activates serotonin receptors on cholinergic nerve terminals that lead to the release of acetylcholine leading to muscle contraction. Further experiments evaluating combinations of 5-HT2, 5-HT4, and muscarinic receptor antagonists may help evaluate the neural circuitry of the serotonin response. We were able to show that the 5-HT4 receptor agonist tegaserod was able to increase the EFS-induced contractions in the antrum without an effect on ACh contractions, suggesting that 5-HT4 receptors may facilitate release of ACh from activated cholinergic nerves. Consistent with our results, the literature would also support the presence of 5-HT4 receptors on cholinergic nerves and 5-HT2 receptors on the smooth muscle (14, 17, 29).

Several studies have reported presynaptic 5-HT4 receptors on cholinergic neurons innervating the circular muscle in the canine human proximal stomach, whose activation leads to release of ACh from the cholinergic nerves. Consistent with our results, the literature would also support the presence of 5-HT4 receptors on cholinergic nerves and 5-HT2 receptors on the smooth muscle (14, 17, 29). Our study has also shown that serotonin (5-HT) has contractile effects in normal and diabetic mouse stomach. The contractile effects of serotonin were more prominent in the fundus than in the antrum or pylorus in both the diabetic and the normal mice. Interestingly, in contrast to the bethanechol contractions, the serotonin-induced contractions were similar in both groups. A prior study (39) has shown alterations of contractile responses to serotonin in gastric fundic smooth muscle from streptozotocin-induced diabetic rats.

We investigated the mechanism of the serotonin-eliciting contractions of the gastric muscle. Our studies used receptor antagonists at each of the 5-HT1, 5-HT2, 5-HT3, and 5-HT4 receptors. With the use of these serotonin receptor antagonists, serotonin’s contractile effect appears to be mediated through 5-HT2 and 5-HT4 receptors. The 5-HT4 receptor effects are evidenced by the inhibitory effects of the 5-HT4 antagonist (GR113808) on serotonin-induced contractions. A 5-HT4 response was also shown by the contractile effects of 5-HT4 partial agonist tegaserod. The contractile effect of tegaserod was less than that for serotonin. Because tegaserod is a partial 5-HT4 agonist (1), full stimulation similar to serotonin is not expected. The inhibitory effect of the 5-HT4 antagonist on serotonin contractions was small, although significant, suggesting that 5-HT4 receptor mediates part of the serotonin-induced contraction.

Table 2. Effect of receptor antagonists on the contractile responses of serotonin maleate in normal and diabetic mice

<table>
<thead>
<tr>
<th>Antagonist</th>
<th>Normal Fundus</th>
<th>Normal Antrum</th>
<th>Diabetic Fundus</th>
<th>Diabetic Antrum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neural Blocker</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tetrodotoxin, 1 μM</td>
<td>107±5</td>
<td>85±21</td>
<td>83±10</td>
<td>80±10</td>
</tr>
<tr>
<td>Cholinergic muscarinic receptor antagonist</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atropine, 1 μM</td>
<td>57±8*</td>
<td>39±10†</td>
<td>63±14*</td>
<td>54±13*</td>
</tr>
<tr>
<td>5-HT1 receptor antagonist</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pindolol, 1 μM</td>
<td>97±3</td>
<td>67±33</td>
<td>71±11</td>
<td>83±17</td>
</tr>
<tr>
<td>5-HT2 receptor antagonist</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Cinanseron, 1 μM</td>
<td>70±18</td>
<td>36±18*</td>
<td>85±11</td>
<td>47±3†</td>
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<tr>
<td>5-HT3 receptor antagonist</td>
<td></td>
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<tr>
<td>Y25130, 1 μM</td>
<td>100±1</td>
<td>88±12</td>
<td>91±6</td>
<td>100±10</td>
</tr>
<tr>
<td>5-HT4 receptor antagonist</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GR113808, 1 μM</td>
<td>93±6</td>
<td>57±21*</td>
<td>81±7*</td>
<td>69±21</td>
</tr>
</tbody>
</table>

Values are means ± SE of experiments performed in 3–6 muscle rings from different animals. Results expressed as % initial control response without the antagonist. Shown are *P < 0.05, †P < 0.01.

Table 3. Effect of tegaserod on the EFS-induced contractile responses in fundic and antral muscle of normal and diabetic mice

<table>
<thead>
<tr>
<th>Tegaserod Concentration</th>
<th>Baseline EFS Response</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fundus</td>
</tr>
<tr>
<td>Normal control mice</td>
<td></td>
</tr>
<tr>
<td>10⁻⁷ M</td>
<td>0.40±0.30</td>
</tr>
<tr>
<td>10⁻⁶ M</td>
<td>0.13±0.08</td>
</tr>
<tr>
<td>Diabetic mice</td>
<td></td>
</tr>
<tr>
<td>Fundus</td>
<td>0.83±0.54</td>
</tr>
<tr>
<td>Antrum</td>
<td>0.08±0.04</td>
</tr>
</tbody>
</table>

Values are means ± SE of experiments performed in 3–4 muscle rings from different animals. Results expressed as the contractile response to electrical field stimulation (EFS) at 16 Hz, expressed as grams of tension. *P < 0.05.
REGIONAL GASTRIC CONTRACTILITY IN DIABETIC GASTROPARESIS

The 5-HT4 partial agonist, tegaserod, accelerates gastric emptying in these diabetic mice (21). This is analogous to the contractile effects of tegaserod seen in this in vitro study. Interestingly, this acceleration of gastric emptying seen in vivo reportedly occurred at low doses but not at high doses in diabetic mice and in normal mice (20, 21). The reason for these dose-specific effects is unclear. The physiological effects of tegaserod in the GI tract have been reported to have a bell-shaped curve (21). Such diminished response at higher doses may be related to the desensitization of the 5-HT4 receptor or to the recruitment of other 5-HT receptors with opposing action. In healthy volunteers, tegaserod stimulates interdigestive small intestinal motility and postprandial antral and intestinal motility (7). Tegaserod has also been shown to increase gastric emptying in normal subjects and patients with gastroparesis (6, 37).

In summary, this study shows that regional differences in gastric contractility are present in this mouse model of diabetic gastroparesis. Specifically, the gastric contractility to bethanechol was significantly decreased in the fundus but increased in the pylorus with similar responses in the antrum. In each area, the cholinergic contractile responses appear to be mediated through M3 subtype muscarinic receptors. This study suggests that the fundic hypomotility and pyloric hypercontractility, rather than antral hypomotility, may play important roles for the delayed gastric emptying in diabetes. Serotonin was shown to have gastric contractile effects in normal and diabetic mice, being more prominent in the fundus than in the antrum. In addition to a cholinergic pathway, part of serotonin’s contractile effect may be mediated through 5-HT2 and 5-HT4 receptors.

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