REGIONAL GASTRIC CONTRACTILITY ALTERATIONS
IN A DIABETIC GASTROPARESIS MOUSE MODEL

Effects of Cholinergic and Serotonergic Stimulation

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Running Head: Regional Gastric Contractility in Diabetic Gastroparesis

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Abstract

The C57 BL/Ks/J db/db mouse develops hyperglycemia and has delayed gastric emptying which is improved with tegaserod, a partial 5-HT$_4$ agonist. AIMS: To determine regional gastric contractility alterations in C57 BL/Ks/J db/db mice and to determine the effects of serotonin and tegaserod. METHODS: Contractile effects of bethanechol, serotonin, and tegaserod in fundic, antral, and pyloric circular muscle were compared in C57 BL/Ks/J db/db mice and normal litter mates. Effects of tetrodotoxin, atropine, and 5-HT receptor antagonists were studied. RESULTS: Contractions in response to bethanechol were decreased in the fundus, similar in the antrum, but increased in the pylorus in diabetic mice compared to controls. Serotonin, and to a lesser extent tegaserod, caused contractions which were more pronounced in the fundus than the antrum and pylorus in both diabetic and normal mice. Serotonin-induced contractions were partially inhibited by atropine and the 5-HT$_4$ antagonist GR113808 and the 5-HT$_2$ antagonist cinanseron but not tetrodotoxin. CONCLUSIONS: 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 diabetic mice. Serotonin's contractile effect is mediated in part through muscarinic, 5-HT$_2$ and 5-HT$_4$ receptors. This study suggests that fundic hypomotility and pyloric hypercontractility, rather than antral hypomotility, play important roles for the gastric dysmotility that occurs in diabetes.

Key words: diabetic gastroparesis, cholinergic, serotonin, tegaserod
Introduction

Gastroparesis (delayed gastric emptying) is frequent in diabetic patients. It is a well-recognized complication of long standing diabetes. Although classically gastroparesis occurs in insulin dependent diabetic patients, it also develops in type 2 or non-insulin dependent diabetes mellitus (15). Gastroparesis is associated with antral hypomotility and perhaps pyloric dysfunction (2,23).

Treatment of gastroparesis is with prokinetic agents, which accelerate gastric emptying. Tegaserod, an aminoguanidine indole compound, is a recently developed 5-HT$_4$-partial agonist (1). Activation of 5-HT$_4$ receptors triggers the release of neurotransmitters from the enteric nerves resulting in increased contractility and stimulation of the peristaltic reflex (10). Tegaserod has been shown to accelerate gastric emptying in some (6), but not all studies (28).

Strains of rats and mice with spontaneously developing hyperglycemia have been recognized as useful models to study the effects of diabetes. The Jackson Laboratory C57 BL/Ks/J db/db mouse spontaneously develops hyperglycemia (12) and is a model for non-insulin dependent diabetes mellitus (24). Prior studies in this mouse strain have shown delayed gastric emptying (22). Tegaserod, a partial 5-HT$_4$ 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. First, to determine if there are regional alterations in gastric contractility in C57 BL/Ks/J db/db diabetic mice compared to normal mice. We also investigated the muscarinic subtypes mediating cholinergic contractions to determine if alterations of muscarinic receptor subtypes may explain any alterations in gastric contractility.
Second, to determine the effects and mechanisms of serotonin on regional gastric contractility in C57 BL/Ks/J db/db diabetic mice compared to normal mice.
Materials and Methods

Fundus, antrum, and pyloric muscle ring preparation

The Jackson Laboratory C57 BL/Ks/J db/db transgenic mouse (Jackson Laboratories, Bar Harbor, ME) and its normal control litter mates (females age 8-12 weeks 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 IACUC Committee 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 sacrificed 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 Corporation, Indianapolis, IN). The stomach was rinsed with Krebs-bicarbonate buffer (composition in mM: NaCl, 120; KCl, 4.6; CaCl₂, 2.5; MgCl₂, 1.2; NaHCO₃, 22; NaH₂PO₄, 1.2, and glucose, 11.5; oxygenated with 95% O₂, 5% CO₂; pH 7.4). Circular muscle rings were prepared from the fundus, antrum, and pylorus in the C57 BL/Ks/J db/db mouse and its normal control litter mates. 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 (L_max).

Experimental Protocols

Bethanechol concentration-response curves (CRCs) were performed in doses from 10 nM to 100 µM. Each concentration was added for 3 minutes 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-DAMP (Sigma Chemical Co., St Louis, MO) were used (27). After an initial control contractile response to bethanechol 10 µM followed by rinsing, a receptor antagonist at 10 nM concentration, was added, followed in 10 minutes by addition of another application of bethanechol 10 µM. This was repeated with the receptor antagonist at 100 nM concentration.

In a separate series of experiments, concentration response curves to serotonin maleate (Sigma Chemical Co., St Louis, MO) and tegaserod maleate (Novartis Pharmaceuticals Inc., East Hanover, NJ) were performed in concentrations from 10 nM to 100 µM. Each concentration was added for 3 minutes with rinsing in between doses and resumption of baseline tone. Tetrodotoxin (Sigma Chemical Company), atropine (Sigma Chemical Company), the 5-HT_{1A/1B} antagonist pindolol (3, Tocris Cookson Inc., Ballwin, MO), the 5-HT_{2} antagonist cinanseron hydrochloride (19, Tocris Cookson Inc., Ballwin, MO), the 5-HT_{3} antagonist Y25130 hydrochloride (8, Tocris
Cookson Inc., Ballwin, MO), and the 5-HT₄ antagonist GR113808 (9, Tocris Cookson Inc., Ballwin, MO) were used to determine neural and receptor pathways mediating the serotonin contractile responses. After an initial control contractile response to serotonin 10 µM followed by rinsing, the receptor antagonist was added, followed in 10 minutes by addition of another application of serotonin 10 µM.

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 hour 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 Company, Astro-Med, W. Warick, RI). Electric field stimulation (EFS) was performed with 16 Hz, 100 V, 0.5 msec pulse width duration (square wave), 60 sec train duration parameters. After each EFS stimulation, acetylcholine (ACh) 10⁻⁴ M was added to test overall muscle responsiveness. In initial experiments, atropine 1 µM and tetrodotoxin 1 µM 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 two minutes prior to 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 Corp., Florham Park, NJ). The cross sectional area was calculated using the relationship area =
mass/(density x 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 which normalized for differences in tissue strip size by dividing the absolute force of the contraction by the cross-sectional muscle area (kilograms per square centimeter).

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 to the amplitude of contraction in Krebs solution immediately preceding the addition of antagonist. Data are expressed as mean ± S.E.M. of results obtained from 4 to 10 muscle rings. Two way analysis of variance was used to determine if concentration response curves were different between the diabetic and normal mice. Students t test was used to determine if the effects of antagonists on contractile responses were significant. A p <0.05 was considered statistically significant.
Results

General observations

Experiments were performed in 46 C57 BL/Ks/J db/db diabetic mice and 48 normal control litter mates. In general, the C57 BL/Ks/J db/db diabetic mice were larger than the control litter mates. The body weights of the diabetic mice were greater than normal litter mates (41±1 vs 19±1 gm; p<0.001). Blood glucose concentrations were elevated in C57 BL/Ks/J db/db diabetic mice compared to normal control litter mates (478±17 vs 137±4 mg/dl; p<0.001).

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 (Figure 1). Fundic contractions were significantly decreased in diabetic mice compared to control mice for bethanechol-induced contractions (p=0.026 by ANOVA II of the CRC; Figure 2a). Three were not significant alterations in the potassium-80 mM induced contractions (p=0.741) (Figure 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 to normal mice (Figure 2b and 3). Pyloric contractility was significantly increased to both bethanechol (p<0.001) and potassium (p<0.005) in the diabetic mice compared to normal mice (Figure 2c and 3).
Effects of muscarinic receptor antagonists on bethanechol-induced contractions

We next investigated the muscarinic subtypes mediating the bethanechol-induced contraction to determine if there were alterations in the subtypes mediating the cholinergic contractile responses (Table 1 and Figure 4). In both the normal and diabetic mice, the contractile response to bethanechol 10 µM were significantly inhibited by 10 nM of 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-induced contractions at these concentrations (10 nM and 100 nM; Table 1).

Effects of serotonin on fundic, antral, and pyloric muscle

We next evaluated the effects of serotonin (5-hydroxytryptamine; 5-HT) on fundic, antral, and pyloric muscle rings from the diabetic and normal mice. Serotonin elicited concentration-dependent contractile responses of the fundus, antrum, and pylorus (Figures 5 and 6). The contractions were significantly less than those to either acetylcholine or bethanechol. In normal mice, the contractile effect of serotonin was more pronounced in the fundus (44±11 gm/cm²) than in the antrum (5±2 gm/cm²) or pylorus (7±2 gm/cm²) at the ED-max of 30 µM (Figure 6a, 6b, 6c). The contractile effects to serotonin seen in diabetic mice (Figures 6d, 6e, 6f) were of similar magnitude as in normal mice (Figures 6a, 6b, 6c).
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 serotonin 10 µM were reduced, but not significantly, by the neural blocker tetrodotoxin (Table 2). The contractile effects of serotonin 10 µM 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-HT$_1$ receptor antagonist pindolol and the 5-HT$_3$ receptor antagonist Y25130 had no significant effect on serotonin-induced contractions. There was a small, but significant, inhibition seen with the 5-HT$_4$ receptor antagonist, GR113808 (1 µM) in both the fundus and antrum. There was also a small, but significant, inhibitory effect seen with the 5-HT$_2$ receptor antagonist cinanseron in the antrum, but not the fundus, of diabetic and normal mice (Table 2).

Effects of tegaserod

To further explore the role of 5-HT$_4$ receptors in mediating the serotonin-induced contractions, the effect of tegaserod on muscle rings were studied (Figure 6). Tegaserod produced concentration-dependant contractions both in normal (figures 6a, 6b, 6c) and diabetic mice (figures 6d, 6e, 6f), which were also greater in the fundus than the antrum and pylorus as seen with serotonin. The tegaserod contractile responses were less than the serotonin responses except in the diabetic antrum, where they tended to be greater (Figure 6).

Effects of electric field stimulation (EFS)

Electric field stimulation (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 tetrodotoxin 1 µM or atropine 1 µM whereas ACh-induced contractions were abolished by atropine but 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 exogenous application of ACh 10^{-4} M in either the fundus or antrum in the control and diabetic mice (data not shown).
Discussion

The Jackson Laboratory C57 BL/Ks/J db/db mouse, which spontaneously develops chronic hyperglycemia and obesity, is a model for Type 2 diabetes mellitus (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 to 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, particularly human studies in type 1 diabetes mellitus, have shown the gastroparesis is associated with antral hypomotility (2). Some studies have also shown the presence of pyloric dysfunction, or pylorospasm (23). Gastroparesis has also been described in type 2 diabetes mellitus (T2DM) (15). Using the C57 BL/Ks/J db/db mouse model of T2DM with delayed gastric emptying (22), this study suggests that the fundic hypomotility and pyloric hypercontractility, rather than antral hypomotility, play important roles for the delayed gastric emptying in diabetes. Our study primarily concentrated on muscle function in this diabetic model, and suggests smooth muscle dysfunction is present in diabetic gastroparesis as shown by the alterations in the contractile response to bethanechol and potassium. It is recognized that diabetic gastroparesis is classically thought to be due to impaired neural control of gastric motor function, possibly resulting from a vagal neuropathy. Abnormal
neural innervation and neurotransmission of both inhibitory and excitatory neurotransmitters, interstitial cells of Cajal, and muscle function have been described (25,30,36). Smooth muscle dysfunction has been previously suggested in streptozotocin-induced diabetic models (16,33).

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 the two groups. A prior study has shown alterations of contractile responses to serotonin in gastric fundic smooth muscle from streptozotocin-induced diabetic rat (39).

We investigated the mechanism of the serotonin eliciting contractions of the gastric muscle. Our studies used receptor antagonists at each of the 5-HT<sub>1</sub>, 5-HT<sub>2</sub> 5-HT<sub>3</sub>, 5-HT<sub>4</sub> receptors. Using these serotonin receptor antagonists, serotonin's contractile effect appears to be mediated through 5-HT<sub>2</sub> and 5-HT<sub>4</sub> receptors. The 5-HT<sub>4</sub> receptor effects are evidenced by the inhibitory effects of the 5-HT<sub>4</sub> antagonist (GR 113808) on serotonin-induced contractions. A 5-HT<sub>4</sub> response was also shown by the contractile effects of 5-HT<sub>4</sub> partial agonist, tegaserod. The contractile effect of tegaserod was less than that for serotonin. Since tegaserod is a partial 5-HT<sub>4</sub> agonist (1), full stimulation similar to serotonin is not expected. The inhibitory effect of the 5-HT<sub>4</sub> antagonist on serotonin contractions was small although significant suggesting that 5-HT<sub>4</sub> receptor mediates part of the serotonin-induced contraction.

Part of the serotonin's effect also appears to be mediated through 5-HT<sub>2</sub> receptors. The 5-HT<sub>2</sub> receptor effects are evidenced by the inhibitory effect of cinanseron on serotonin-induced contractions. The inhibitory effect of cinanseron was seen in the antrum, but not the fundus, in both the diabetic and normal mice. Early studies had not shown an effect of serotonin on 5-HT<sub>2</sub>
receptors in the GI tract (4). More recent studies have suggested a role for 5-HT$_{2A}$ receptors mediating GI contractile responses (17), as suggested in our study. 5-HT$_{2A}$ 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 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-HT$_2$, 5-HT$_4$ and muscarinic receptor antagonists may help evaluate the neural circuitry of the serotonin response. We were able to show that the 5-HT$_4$ receptor agonist, tegaserod, was able to increase the EFS-induced contractions in the antrum without an effect on ACh contractions suggesting 5-HT$_4$ receptors may facilitate release of ACh from activated cholinergic nerves. Consistent with our results, the literature would also support the presence of 5-HT$_4$ receptors on cholinergic nerves and 5-HT$_2$ receptors on the smooth muscle (14,17,29). Several studies have reported presynaptic 5-HT$_4$ receptors on cholinergic neurons innervating the circular muscle in the canine human proximal stomach, whose activation lead to release of acetylcholine from the cholinergic neurons (29). Recent studies have shown that serotonin affects fundic murine muscle by acting through prejunctional 5-HT$_3$ and 5-HT$_4$ receptors and postjunctional excitatory 5-HT$_{1D}$ and inhibitory 5-HT$_{1A}$ receptors (38). Using electrophysiologic recordings, a 5-HT$_4$ receptor antagonist partially inhibited the EFS-induced IJP suggesting 5-HT$_4$ receptor may also mediate inhibitory effects in the fundus. Interestingly, in our studies, tegaserod tended to reduce the EFS contractile response
in the fundus of the diabetic mouse. Human studies have shown an effect of 5-HT₄ receptor activation, with either cisapride or tegaserod, enhancing gastric accommodation (35). Sumatriptan, a 5-HT₁ agonist, also induces relaxation of gastric fundus in man (34).

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 this dose-specific effects is unclear. The physiologic effects of tegaserod in the gastrointestinal 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 antrum. In addition to a cholinergic pathway, part of serotonin's contractile effect may be mediated through 5-HT₂ and 5-HT₄ receptors.
Acknowledgements

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References


13. Jackson Laboratory. JAX Mice Data Sheet - C57 BL/Ks/J db/db mice


Table 1. Effect of muscarinic receptor subtype antagonists at two concentrations (10 nM and 100 nM) on the contractile responses of bethanechol 0.1 µM 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>M1 Receptor Subtype Antagonist</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pirenzepine 10 nM</td>
<td>105±3</td>
<td>94±4</td>
<td>113±4</td>
<td>104±5</td>
</tr>
<tr>
<td>Pirenzepine 100 nM</td>
<td>103±11</td>
<td>85±7</td>
<td>98±5</td>
<td>98±4</td>
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<tr>
<td>M2 Receptor Subtype Antagonist</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methoctramine 10 nM</td>
<td>98±4</td>
<td>85±18</td>
<td>105±3</td>
<td>118±20</td>
</tr>
<tr>
<td>Methoctramine 100 nM</td>
<td>102±4</td>
<td>86±19</td>
<td>111±10</td>
<td>120±21</td>
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<tr>
<td>M3 Receptor Subtype Antagonist</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-DAMP 10 nM</td>
<td>25±5**</td>
<td>47±10**</td>
<td>29±4**</td>
<td>57±10*</td>
</tr>
<tr>
<td>4-DAMP 100 nM</td>
<td>5±3**</td>
<td>16±4**</td>
<td>12±4**</td>
<td>26±6**</td>
</tr>
</tbody>
</table>

Results expressed as percent of initial control response without the antagonist. Shown are mean±SEM of experiments performed in 3-6 muscle rings from different animals. *; p<0.05, **; p<0.01.
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>
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<tbody>
<tr>
<td>Neural Blocker</td>
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<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>
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<tr>
<td>Cholinergic muscarinic receptor antagonist</td>
<td></td>
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</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-HT(_1) 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-HT(_2) receptor antagonist</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-HT(_3) receptor antagonist</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>
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<tr>
<td>5-HT(_4) receptor antagonist</td>
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<tr>
<td>GR113808 1 µM</td>
<td>93±6</td>
<td>57±21*</td>
<td>81±7*</td>
<td>69±21</td>
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</table>

Results expressed as percent of initial control response without the antagonist. Shown are mean±SEM of experiments performed in 3-6 muscle rings from different animals. *; 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></th>
<th>Baseline EFS Response</th>
<th>Tegaserod Concentration</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>10^{-7} M</td>
<td>10^{-6} M</td>
</tr>
<tr>
<td><strong>Normal Control Mice</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Fundus</td>
<td>0.40±0.30</td>
<td>0.45±0.25</td>
<td>0.43±0.27</td>
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<tr>
<td>Antrum</td>
<td>0.13±0.08</td>
<td>0.12±0.05</td>
<td>0.27±0.12</td>
</tr>
<tr>
<td><strong>Diabetic Mice</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fundus</td>
<td>0.83±0.54</td>
<td>0.75±0.48</td>
<td>0.53±0.48</td>
</tr>
<tr>
<td>Antrum</td>
<td>0.08±0.04</td>
<td>0.12±0.06</td>
<td>0.16±0.07*</td>
</tr>
</tbody>
</table>

Results expressed as the contractile response to EFS at 16 Hz, expressed as grams of tension. Shown are mean±SEM of experiments performed in 3-4 muscle rings from different animals. *: p<0.05.
Figure 1. Effect of bethanechol and potassium in regional mouse gastric contractility. Bethanechol caused concentration dependent contractions of the fundus, antrum, and pylorus. These tracings are from a normal mouse using in vitro circular muscle rings.

Figure 2. Comparison of bethanechol concentration response curves from diabetic and control mice in the fundus (Figure 2a), antrum (Figure 2b), and pylorus (Figure 2c). Figure 2a: The fundic concentration response curve to bethanechol was significantly decreased in the diabetic mice compared to the control mice (p=0.026 by ANOVA II). Figure 2b: The antral concentration response curve to bethanechol was not significantly different between the diabetic and control mice (p=0.363 by ANOVA II). Figure 2c: The pyloric concentration response curve to bethanechol was significantly greater in the diabetic mice compared to the control mice (p<0.001 by ANOVA II).

Figure 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 to the control animals.
Figure 4. Effect of the M3 muscarinic subtype antagonist 4-DAMP on bethanechol 10 µM contractions of normal mouse fundus. Increasing concentration of 4-DAMP caused increasing inhibition of bethanechol contractions.

Figure 5. Contractile effect of serotonin on normal mouse fundus and antral muscle rings.

Figure 6. Concentration response curves of serotonin and tegaserod in gastric fundus, antrum, and pylorus of normal (Figure 6a, 6b, 6c) and diabetic mice (Figure 6d, 6e, 6f). Both serotonin and tegaserod caused concentration-dependent contractions which were more pronounced in the fundus than in the antrum or pylorus in both diabetic and normal mice. The tegaserod contractile responses were generally significantly less than the serotonin responses except in the diabetic antrum, which tended to be greater.
Effect of Bethanechol and Potassium on Regional Mouse Gastric Contractility (in vitro circular muscle rings)

A. Fundus

B. Antrum

C. Pylorus

B 10^{-6} M  B 10^{-5} M  B 10^{-4} M  KCl 80 mM

B 10^{-6} M  B 10^{-5} M  B 10^{-4} M  KCl 80 mM

B 10^{-6} M  B 10^{-5} M  B 10^{-4} M  KCl 80 mM

2 gm

2 gm

1 gm

3 min
B. Antrum

Contractile Response (kg/sq cm)

- Diabetic Mice
- Control Mice

Bethanechol Concentration (log M)
Regional Contractile Response to Potassium (80 mM) in Mouse Stomach

Contractile Response (kg/sq cm)

- Diabetic Fundus
- Control Fundus
- Diabetic Antrum
- Control Antrum
- Diabetic Pylorus
- Control Pylorus

* indicates significant difference.
Effect of the M3 Muscarinic Subtype Antagonist 4-DAMP on Bethanechol $10^{-5}$ M Contractions of Normal Mouse Fundus
Effect of Serotonin on Normal Mouse Gastric Smooth Muscle

A. Fundus

B. Antrum
A. Normal Mouse Fundus

Muscle Contraction Tension (kg/cm sq)

Concentration (log M)

- Serotonin
- Tegaserod
B. Normal Mouse Antrum

- **Serotonin**
- **Tegaserod**

**Muscle Contraction Tension (kg/sq cm)**

**Concentration (log M)**

-8 -7 -6 -5 -4
C. Normal Mouse Pylorus

- **Serotonin**
- **Tegaserod**

Muscle Contraction Tension (kg/sq cm)

Concentration (log M)
D. Diabetic Mouse Fundus

- Solid line: Serotonin
- Dashed line: Tegaserod

Muscle Contraction Tension (kg/sq cm)

Concentration (log M)
F. Diabetic Mouse Pylorus

Serotonin
Tegaserod

Muscle Contraction Tension (kg/sq cm)

Concentration (log M)