INHIBITORY EFFECTS OF BOTULINUM TOXIN ON PYLORIC AND ANTRAL SMOOTH MUSCLE

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Running Head: Effect of Botulinum Toxin on the Pylorus

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Botulinum toxin injection into the pylorus is reported to improve gastric emptying in gastroparesis. Classically, botulinum toxin inhibits acetylcholine (ACh) release from cholinergic nerves in skeletal muscle. **Aim:** To determine the effects of botulinum toxin on pyloric smooth muscle. **Methods:** Guinea pig pyloric muscle strips were studied *in vitro*. Botulinum toxin type A was added; electric field stimulation (EFS) was performed every 30 mins for 6 hrs. ACh 100 µM-induced contractile responses were determined prior to and after 6 hrs. **Results:** Botulinum toxin caused a concentration dependent decrease of pyloric contractions to EFS. At a low concentration (2 units/ml), botulinum toxin decreased pyloric contractions to EFS by 43±9% without affecting ACh-induced contractions. At higher concentrations (10 units/ml), botulinum toxin decreased pyloric contraction to EFS by 75±7% and decreased ACh-induced contraction by 79±9%. **Conclusions:** Botulinum toxin inhibits pyloric smooth muscle contractility. At a low concentration, botulinum toxin decreases EFS-induced contractile responses without affecting ACh-induced contractions suggesting inhibition of ACh release from cholinergic nerves. At higher concentrations, botulinum toxin directly inhibits smooth muscle contractility as evidenced by the decreased contractile response to ACh.
Introduction

Treatment of symptomatic gastroparesis is primarily with prokinetic agents that increase antral contractility, improve antroduodenal coordination, and accelerate gastric emptying. Increased gastric outlet resistance due to pyloric sphincter dysfunction or pylorospasm has been described, primarily in diabetic gastroparesis (16). This pylorospasm might also cause a delay in gastric emptying and result in gastroparetic symptoms.

Botulinum toxin injection into the lower esophageal sphincter (LES) decreases LES tone and improves symptoms in achalasia (21). Botulinum toxin injection into the pyloric sphincter is reported to improve gastric emptying and reduce dyspeptic symptoms in patients with gastroparesis (7,14,17).

Classically, in striated muscle, botulinum toxin inhibits acetylcholine release from cholinergic nerves (10). Botulinum toxin A selectively cleaves the synaptic protein SNAP-25, leading to inability of synaptic vesicles containing neurotransmitters to undergo exocytosis and release of neurotransmitters (2). In gastrointestinal smooth muscle, botulinum toxin appears to also reduce cholinergic transmission by inhibiting acetylcholine release, as shown in \textit{in vitro} (1,15) and \textit{in vivo} (20,23) studies. SNAP-25, the substrate for botulinum toxin, is also present in gastrointestinal smooth muscle suggesting an additional site for botulinum toxin (13). The aim of this study was to determine the effects and mechanism of action of botulinum toxin on pyloric and antral smooth muscle.
Materials and Methods

Pyloric and antral muscle strip preparation

Guinea pigs (Covance Research Products, Incorporated. Denver, PA) weighing approximately 400 to 450 grams were used for this study. This study was approved by the IACUC Committee at Temple University School of Medicine. The stomach with pylorus and proximal duodenum were removed after the guinea pig had been sacrificed by CO₂ asphyxiation. The stomach was opened along the longitudinal axis and 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).

The mucosa was removed from the antrum and pylorus. Antral and pyloric circular muscle strips were prepared and suspended in 10 ml organ baths containing Krebs-bicarbonate buffer (temperature 37°C). In general, from each guinea pig, 2 antral muscle strips and 2 pyloric muscle strips were used. Muscle tension was measured along the circular muscle direction using an isometric force transducer (FT .03C, Grass Instruments Co., Astor-Med, W. Warwick, RI). The muscle strips were suspended between platinum electrodes placed adjacent and parallel to the long axis of the muscle strip. The electrodes were connected to an electric stimulator (Model #SD9, Grass Instruments Co., Astor-Med, W. Warwick, RI). After a 30 min equilibration period, the preparations were stretched until the contractile force to acetylcholine 100 μM was maximal (L_max).
Experimental Protocols

Muscle strips were studied in vitro for their contractile responses to electric field stimulation (EFS) and acetylcholine (ACh). Electric field stimulation (EFS) with 2-16 Hz, 100 V, 0.5 msec pulse width duration (square wave), 60 sec train duration parameters were used to activate the intrinsic nerves. L-NAME 100 µM was added to inhibit nitric oxide-mediated relaxation. After initial control responses to EFS and ACh 100 µM, botulinum toxin type A (Botox; Allergan, Inc.; Irvine, CA) was added in concentrations of 2, 4, or 10 units/ml; EFS (4 Hz, 60 sec) was performed every 30 minutes for 6 hrs in the continued presence of botulinum toxin. ACh 100 µM-induced contractile responses were also determined prior to and 6 hrs after botulinum toxin incubation. Control muscle strips were also studied with the same protocol but without the addition of botulinum toxin. In additional studies, the contractile responses to substance P (Sigma Chemical Co., St Louis, MO) was performed before and after 6 hours of incubation with botulinum toxin in a similar protocol as described above for ACh and EFS.

Data Analysis

Contractile responses to ACh, substance P, and EFS were measured as the maximal contractile response after stimulation. Data are expressed as mean ± S.E.M. of results obtained from 4 to 15 muscle strips. One and two way analysis of variance with factors of time after botulinum toxin administration and/or concentration of botulinum toxin and Students t test with Bonferroni correction were used to determine if the effects of botulinum toxin on EFS, ACh and substance P-induced contractions were significantly different from the control responses. Each preparation served as its own control with the amplitude of contraction after incubation with
botulinum toxin compared to the amplitude of contraction in Krebs solution immediately preceding the addition of botulinum toxin.
Results

Pylorus

General observations: Effects of EFS, ACh, and substance P

In normal Krebs solution, EFS produced frequency-dependent contractile responses in 28 of 28 pyloric muscle strips. The addition of L-NAME 100 µM increased the EFS-induced contractile response to 4 Hz from 2.3±0.2 gm to 3.1±0.2 gm (p<0.001). Pyloric contractions to EFS were completely inhibited by atropine 1 µM or TTX 1 µM indicating activation of cholinergic nerves (Figure 1).

Acetylcholine caused concentration-dependent contractions of pyloric muscle strips (Figure 2). ACh-induced contractions were completely inhibited by atropine but not TTX indicating a direct smooth muscle effect of ACh on pyloric smooth muscle (Figure 1).

Substance P also caused concentration-dependent contractions of pyloric muscle strips (Figure 2). The contractile effect of substance P was dose dependent with increasing amplitude of contractions from concentrations 10^{-6} M to 3 x 10^{-5} M (Figure 2). The contractile response to substance P 10^{-6} M was not inhibited by tetrodotoxin 1 µM (109±15% of control) or atropine 1 µM (76±15% of control), suggesting primarily a direct contractile effect on smooth muscle. The contractile response to a higher concentration of substance P 10^{-5} M was slightly reduced by tetrodotoxin 1 µM (66±5% of control) or atropine 1 µM (66±16% of control).

Effect of botulinum toxin and atropine

Botulinum toxin caused a time-dependent and concentration-dependent decrease of pyloric contractions to EFS 4 Hz (Figures 3 and 4). The inhibitory effects of botulinum toxin on
EFS-induced contractions were gradual and slowly progressive (Figure 4a). There was a significant concentration-related effect of botulinum toxin to inhibit EFS-induced contractions in the pylorus (two way analysis of variance with factors of concentration and duration of incubation in botulinum toxin: F=20.458; p<0.001). Each of the concentrations (2, 4, and 10 units/ml) of botulinum toxin inhibited EFS-induced contractions over time (2 units/ml: F=3.996; p=0.054, 4 units/ml: F=12.749; p=0.001; 10 units/ml: F=45.35; p=0.001). At a low concentration (2 units/ml), botulinum toxin after 6 hour incubation decreased pyloric contractions to EFS by 43±9% (p<0.01) without affecting ACh 100 µM-induced contractions (2±5% inhibition) (Figures 3a and 4). At higher concentrations (10 units/ml), botulinum toxin decreased pyloric contraction to EFS by 75±7% (p<0.01) and decreased ACh 100 µM -induced contraction by 79±9% (p<0.01) (Figures 3b and 4). For comparison, the muscarinic receptor antagonist, atropine 1 µM, nearly totally abolished the contractile response to EFS and ACh over the entire 6 hour incubation period (Figure 4).

In subsequent experiments, the effect of botulinum toxin was determined on EFS and substance P-induced contractions of the pylorus. Botulinum toxin (10 units/ml) decreased the pyloric contractile responses to EFS by 24±11% (p=0.05) after 6 hour incubation in botulinum toxin, but had no effect on substance P (1 µM)-induced contractions (7±13% inhibition) (Figure 5). In additional experiments, the effect of botulinum toxin was tested to a higher concentration of substance P, namely 10 µM. Botulinum toxin 10 units/ml decreased EFS-induced pyloric contractions by 25±2% (p<0.01) after a 6 hour incubation in botulinum toxin, but did not affect the contraction to substance P 10 µM (128±32% of control; p>0.10).
**Antrum**

*General observations*

In normal Krebs solution, EFS produced contractile responses in 13 of 22 (59%) antral muscle strips and a relaxation in 9 of 22 muscle strips (41%) antral smooth muscle strips. The addition of L-NAME 100 µM caused a contractile response in all antral muscle strips averaging $2.4\pm0.3$ gm. Antral contractions to EFS in the presence of L-NAME were completely inhibited by atropine 1 µM or TTX 1 µM indicating activation of cholinergic nerves. ACh-induced contractions were inhibited by atropine but not TTX indicating a direct smooth muscle effect of ACh on antral smooth muscle.

*Effect of botulinum toxin A and atropine*

Botulinum toxin had similar inhibitory effects on antral smooth muscle as in the pylorus (Figures 6 and 7). There was a significant concentration-related effect of botulinum toxin to inhibit EFS-induced contractions in the antrum (two way analysis of variance with factors of concentration and duration of incubation in botulinum toxin: $F=9.318; p<0.001$). The concentrations of botulinum toxin that significantly inhibited EFS-induced contractions over time were with 4 units/ml ($F=16.371; p<0.001$) and 10 units/ml ($F=21.865; p<0.001$), but not with the lowest concentration of 2 units/ml ($F=0.102; p=0.751$). There was no significant inhibition with botulinum toxin 2 units/ml compared to the time dependent control muscle strips (Figure 7a). At 4 units/ml, botulinum toxin decreased antral contractions to EFS by $60\pm14\%$ ($p<0.05$) without affecting ACh-induced contractions (-$6\pm3\%$ inhibition) (Figures 6 and 7). At 10 units/ml, botulinum toxin decreased antral contractions to EFS by $61\pm21\%$ ($p<0.05$) and
decreased ACh-induced contractions by 49±25% (p=0.05) (Figure 8). For comparison, the muscarinic receptor antagonist, atropine 1 µM, nearly totally abolished the contractile response to EFS throughout the 6 hour time period (Figure 7).

The inhibitory effects on the antrum tended to be less pronounced than in the pylorus, but direct statistical comparison of this did not reach statistical significance. At 2 units/ml, botulinum toxin reduced the EFS-induced contractions in the pylorus by 43±9% whereas in the antrum by only 22±27% (p=0.484). Among all the concentrations (0, 2, 4, 10 units/ml), two way ANOVA for the percent inhibition at the end of 6 hours incubation with factors concentration of botulinum toxin and location (antrum vs pylorus) was not significant (F=0.052; p=0.820).
Discussion

Our study has shown that botulinum toxin inhibits pyloric smooth muscle contractility. There appears to be two mechanisms for botulinum toxin to decrease pyloric sphincter contractility: inhibition of ACh release and direct inhibition of smooth muscle contractility. At a low concentration, botulinum toxin decreases EFS-induced contractile responses without affecting ACh-induced contractions suggesting inhibition of ACh release from cholinergic nerves. At higher concentrations, however, botulinum toxin appears to directly inhibit smooth muscle contractility as evidenced by the decreased contractile response to ACh.

The experimental protocol used in this study involved incubation of the muscle strips to botulinum toxin for a prolonged period (6 hours). The inhibitory effects of botulinum toxin on EFS-induced contractions were not immediate; the onset of the inhibitory effect was gradual and slowly progressive. This time dependent effect may be related to either the mechanism of action of botulinum toxin or diffusion of the toxin into the tissue. The inhibitory effects were also concentration dependent in the concentration range of 2 to 10 units/ml. At the end of 6 hours, the contractile effect to EFS was inhibited by 43% with 2 units/ml botulinum toxin and by 75% with 10 units/ml. By comparison, atropine which blocks muscarinic cholinergic receptors nearly completely abolished EFS induced contractions. Studies in hippocampal slices have also shown that the inhibitory effect of botulinum toxin A is time and concentration-dependent (19).

At a low concentration, botulinum toxin decreased EFS-induced contractile responses of the pylorus without affecting ACh-induced contractions. This suggests botulinum toxin inhibits ACh release from cholinergic nerves. Classically, in striated muscle, botulinum toxin inhibits
acetylcholine release from cholinergic nerves (10). Neurotransmitter release, most notably acetylcholine, involves fusion of the synaptic vesicles with the plasma membrane and extrusion of the acetylcholine out of the neuron into the synapse. SNAP-25 is a protein implicated in the fusion of neuronal presynaptic vesicles with the plasma membrane (6). Botulinum toxin is the neurotoxin produced by the bacteria Clostridium botulinum (26). It consists of a heavy protein chain linked by a disulfide bond to a lighter protein chain. The heavy chain can bind to the synaptic membrane and the entire molecule is then taken up into the synaptic terminal by receptor-mediated endocytosis. The light chain interferes with SNAP-25. When botulinum toxin cleaves SNAP-25, the ACh-containing vesicles can no longer fuse with the plasma membrane and exocytosis of ACh is inhibited (9,14). In gastrointestinal smooth muscle, it is also thought that botulinum toxin reduces cholinergic transmission by inhibiting acetylcholine release. This has been previously suggested in in vitro studies with the lower esophageal sphincter (20) and the sphincter of Oddi (23) and now in the present in vitro study with the pyloric sphincter. Other studies have suggested botulinum toxin may have effects on other nerves besides cholinergic nerves (15,24). In rabbit hippocampal slices, although the release of ACh is most sensitive, botulinum A toxin incubation also inhibited noradrenaline and 5-HT release (19). In guinea pig uterine artery, botulinum toxin appears to affect EFS-induced contractility by several mechanisms including nearly abolishing contractions due to ACh release, reducing neuropeptide-mediated contractions, but without an affect on nitric oxide mediated relaxations (18). Other studies in gastrointestinal smooth muscle have suggested that botulinum toxin has no effect on nonadrenergic, noncholinergic inhibitory response of the guinea pig fundus (22) and the lower esophageal sphincter (4).
Our studies showed that higher concentrations of botulinum toxin (10 units/ml) directly inhibits cholinergic smooth muscle contractility as evidenced by the decreased contractile response to ACh. Acetylcholine, in this study, acts primarily on smooth muscle muscarinic receptors to directly contract the muscle, since the contractions to ACh were inhibited by atropine, but not by tetrodotoxin. This suggests high concentrations of botulinum toxin may have a direct inhibitory effect on smooth muscle contractility. Our further studies showed that botulinum toxin decreased contractile responses to ACh but not to substance P. Substance P contracts the pylorus predominantly by a direct effect on smooth muscle. This differential effect of botulinum toxin on ACh but not substance P-induced contractions may potentially offer some insight into the mechanism of botulinum toxin as it appears not to be a nonspecific effect on muscle contractility which would decrease contractility to a variety of agonists. High concentrations of botulinum toxin affects smooth muscle contractility to cholinergic muscarinic muscular transmission, possibly at the receptor level or on intracellular pathways. The exact cellular mechanism of botulinum toxin inhibiting acetylcholine-induced contractions was not detailed in this study. Interestingly, SNAP-25, the substrate for botulinum toxin, has also been found to be present in gastrointestinal smooth muscle (12). This suggests an additional site for botulinum toxin in regulating muscle contractility, besides the classical effect of acting on presynaptic neurons to reduce acetylcholine release (12). Botulinum toxin light chain administered intracellularly in smooth muscle cells has been shown to increase potassium channel currents (12). This would tend to hyperpolarize the muscle membrane and result in a decrease in muscle tone. SNAP-25 may have an additional role in inhibiting calcium channels (11,12,25). Both of these would decrease ACh-induced contractions. It is not known whether
smooth muscle cells have the membrane pathways to internalize the toxin to act on intracellular SNAP-25, as are present on neurons (13). Further studies are needed to reconcile the apparent neurotransmitter (ACh) specific effect of botulinum toxin on the pylorus as shown in this study with the putative effects on potassium and calcium channels shown in other studies.

What is the clinical corollary of these in vitro studies? Delayed gastric emptying in diabetic gastroparesis is associated with antral hypomotility, increased pyloric tone and pylorospasm. Increased gastric outlet resistance due to pyloric sphincter dysfunction or pylorospasm has been described, primarily in diabetic gastroparesis (5,16) and may be in part responsible for the delay in gastric emptying and resultant symptoms. Botulinum toxin injected into the pylorus in patients with gastroparesis might relax the pylorus and facilitate gastric emptying. Several small open label studies have shown this in diabetic gastroparesis (7,14) and in idiopathic gastroparesis (17). Intrasphincteric injection of botulinum toxin has been shown to reduce pyloric pressure waves and pylorospasm in diabetic gastroparesis, but had no effect on antral contractility (5,8).

Our study showed that botulinum toxin, in addition to decreasing pyloric contractility, also decreased antral smooth muscle contractility in vitro. Diffusion of botulinum toxin into the antrum or a misplaced injection might occur when attempting to inject the pylorus using an endoscopic sclerotherapy needle. The inhibitory effect of botulinum toxin on EFS-induced antral contractility extrapolated to the in vivo condition might cause antral hypomotility and worsen gastric emptying. Postprandial antral contractility, as measured by antral manometry, correlates with gastric emptying of a solid meal (3). In our in vitro study, the pylorus tended to be more
sensitive than the antrum to the inhibitory effect of botulinum toxin, however, direct comparison of this failed to reach statistical significance.

We studied the effects of botulinum toxin on both antral and pyloric muscle strips. It is also possible that the observed direct smooth muscle effect of the toxin may be peculiar to an in vitro system. Our experiments used time controls with Krebs incubation but did not use a protein control. Furthermore, we did not investigate if the effect of botulinum toxin is a temperature dependent. A natural extension of our in vitro studies is to investigate if these inhibitory effects of botulinum toxin occur in vivo by injecting the pylorus, in a similar manner to that performed clinically, and measuring changes in pyloric sphincter contractility.

In summary, botulinum toxin inhibits pyloric and antral smooth muscle contractility. At a low concentration, botulinum toxin decreases EFS-induced contractile responses without affecting ACh-induced contractions suggesting inhibition of ACh release from cholinergic nerves. At higher concentrations, botulinum toxin directly inhibits smooth muscle contractility as evidenced by the decreased contractile response to ACh.
Acknowledgements

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References


Figure Legends

Figure 1. Contractile effects of electric field stimulation (EFS) and acetylcholine (ACh) on guinea pig pyloric muscle strips. Figure 1a: Effect of L-NAME and tetrodotoxin. In normal Krebs solution (top tracing), EFS produced a frequency-dependent contractile response with frequencies of 2, 4, 8, and 16 Hz; the response to ACh 100 µM is shown on the right. Note the change of scale after the 4 Hz EFS stimulation. The addition of L-NAME 100 µM (middle tracing) increased the contractile response to EFS. The addition of tetrodotoxin to L-NAME (bottom tracing) abolished the contractile response to EFS, without abolishing the contractile response to ACh. Figure 1b: Effect of atropine on pyloric contractions to EFS and ACh. The addition of atropine to L-NAME inhibited the contractile response to both EFS and ACh. Small relaxations are seen during EFS in the presence of atropine and L-NAME (bottom tracing).

Figure 2. Concentration-response curves for the contractile effects of ACh and substance P on the guinea pig pylorus. Both ACh and substance P caused concentration-dependent contractions. The contractile response to substance P 10^{-6} M was 0.082±0.055 kg/cm^{2} compared to that of substance P 10^{-5} M, 0.120±0.068 kg/cm^{2}, and to that of ACh 10^{-4} M, 0.206±0.067 kg/cm^{2}.

Figure 3. Effect of botulinum toxin type A on pyloric contractions to EFS and ACh. Figure 3a: Addition of 2 units/ml of botulinum toxin A (Botox) caused a progressive decrease in EFS-induced contractions. At the end of the 6 hour botulinum toxin incubation, the ACh-induced contractile response is similar to the control ACh response at the beginning of the tracing.
Figure 3b: The addition of 10 units/ml of botulinum toxin type A (Botox) caused a progressive decrease in EFS-induced contractions. At this concentration of botulinum toxin, after 6 hour incubation, there was also a marked decrease in the contractile response to ACh 100 µM.

Figure 4. Effect of incubation of botulinum toxin on pyloric muscle strip contractility.

Figure 4a: There was time dependent decrease in the pyloric contractile response to EFS. This inhibitory effect was also concentration dependent with increasing inhibition from 2 to 10 units/ml. Shown for comparison is the near total inhibitory response seen with atropine. There was a significant concentration-related effect of botulinum toxin to inhibit EFS-induced contractions in the pylorus using two way analysis of variance with factors time and concentration of botulinum toxin (F=20.458; p<0.001). Each of the concentrations of botulinum toxin inhibited EFS-induced contractions over time: 2 units/ml: F=3.996; p=0.054, 4 units/ml: F=12.749; p=0.001; 10 units/ml: F=45.35; p=0.001). Figure 4b: The effect of botulinum toxin on ACh-induced pyloric contractions are shown. There was no effect on 2 and 4 units/ml botulinum toxin on the contractile response to ACh. In contrast, the highest concentration, 10 units/ml, caused a 79±9% inhibitory effect on ACh-induced contractions. Shown also is the near complete inhibition seen with atropine. There was a significant concentration-related effect of botulinum toxin in inhibit ACh-induced contractions in the pylorus using one way analysis of variance with factor of concentration of botulinum toxin (F=4.578; p=0.013). Only the concentration of botulinum toxin 10 units/ml was significantly different from control (F=7.57; p=0.019).
Figure 5. Effect of botulinum toxin type A on pyloric contractions to EFS and substance P 1 µM. The addition of 10 units/ml of botulinum toxin A (Botox) caused a progressive decrease in EFS-induced contractions. At the end of the 6 hour botulinum toxin incubation, the substance P (1 µM)-induced contractile response is similar to the control substance P response at the beginning of the tracing. Note that for the EFS contractions, there is a scale change.

Figure 6. Effect of botulinum toxin type A on antral contractions to EFS and ACh. The addition of 4 units/ml of botulinum toxin A (Botox), caused a progressive inhibitory effect on EFS-induced contractions. At the end of the 6 hour incubation, ACh is given and the response is similar to the control ACh response at the beginning of the tracing.

Figure 7. Effect of incubation of botulinum toxin on antral muscle strip contractility.

Figure 7a: There was time dependent decrease in the antral contractile response to EFS. This inhibitory effect was seen with the concentrations of 4 and 10 units/ml, but not with the lower concentration of 2 units/ml. Shown for comparison is the near total inhibitory response seen with atropine. There was a significant concentration-related effect of botulinum toxin to inhibit EFS-induced contractions in the antrum using two way analysis of variance with factors time and concentration of botulinum toxin (F=9.318; p<0.001). The concentrations of botulinum toxin that significantly inhibited EFS-induced contractions over time were: 4 units/ml: F=16.371; p<0.001; 10 units/ml: F=21.865; p<0.001, but not the lowest concentration of 2 units/ml (F=0.102; p=0.751). Figure 7b: The effect of botulinum toxin on ACh-induced antral contractions are shown. There was a trend for botulinum toxin to cause a concentration
dependent decrease in ACh-induced antral contractions; F=2.991; p=0.053 using one way analysis of variance with factor of concentration of botulinum toxin. The concentration of botulinum toxin 10 units/ml was significantly different from control (F=11.094; p=0.005). Shown also is the near complete inhibition of ACh-induced contractions seen with atropine.
Figure 1a.

Effect of L-NAME and Tetrodotoxin on Pyloric Contractions to Electric Field Stimulation (EFS) and Acetylcholine (ACH)

A. Krebs

B. L-NAME 100 µM

C. TTX 1 µM and L-NAME 100 µM
Figure 1b.

Effect of Atropine on Pyloric Contractions to EFS and ACh

A. L-NAME 100 μM

EFS 8 Hz  EFS 16 Hz  ACh 100 μM

5 gm

B. Atropine 1 μM and L-NAME 100 μM

EFS 8 Hz  EFS 16 Hz  ACh 100 μM

3 min 5 gm
Figure 2.

Concentration Response Curves of Acetylcholine and Substance P for the Guinea Pig Pyloric Smooth Muscle

Muscle Contraction Tension (kg/sq cm)

Acetylcholine
Substance P

Concentration (log M)
Figure 3a.

**Effect of Botulinum Toxin Type A (Botox; 2 units/ml) on Pyloric Contractions to EFS 4 Hz and ACh 100 μM**

- ACh 100 μM
  - 3 min

- EFS 4 Hz
- EFS
- EFS 1 hour
- EFS 3 hour
- EFS 6 hour
- ACh 100 μM

- Botox 2 u/ml
- L-NAME 100 μM
Figure 3b.

Effect of Botulinum Toxin Type A (Botox; 10 units/ml) on Pyloric Contractions to EFS 4 Hz and ACh 100 µM
Figure 4a.

Effect of Incubation with Botulinum Toxin on Pyloric Muscle Strip Contractility

- Control 4 Hz EFS
- Botox 2 u/ml
- Botox 4 u/ml
- Botox 10 u/ml
- Atropine 1 μM

Mean ± SEM
n = 3-10
Figure 4b.

Effect of Six Hour Incubation with Botulinum Toxin on Pyloric Contractile Response to Acetylcholine 100 μM

Percent of Control Contractile Response to ACh 100 μM

Mean ± SEM
n = 3-11
** p<0.01
Figure 5.

Effect of Botulinum Toxin Type A (Botox; 10 units/ml) on Pyloric Contractions to EFS 4 Hz and Substance P 1 μM.
Figure 6.

**Effect of Botulinum Toxin Type A (Botox; 4 units/ml) on Antral Contractions to EFS 4 Hz and Ach 100 μM**
Figure 7a.

Effect of Incubation with Botulinum Toxin on Antral Muscle Strip Contractility

- Control 4 Hz EFS
- Botox 2 u/ml
- Botox 4 u/ml
- Botox 10 u/ml
- Atropine 1 μM

Contractile Response to EFS 4Hz (% of Control)

Time (hours)
Figure 7b.

Effect of Six Hour Incubation with Botulinum Toxin on Antral Contractile Response to Acetylcholine 100 µM

Mean±SEM
n = 3-11
**; p<0.01
*; p<0.05

Percent of Control Contractile Response to ACh 100 µM