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Study on Effects of Electrical Stimulation on Rabbit Esophageal Body Motility in vivo

Short Title: Electrical Stimulation on Esophageal Motility

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Summary

Electric stimulation (ES) could induce contraction of intestinal smooth muscle. The aim of this study was to analyze the effects of ES on esophageal motility and the underlying mechanism in vivo. Twenty-eight rabbits were equipped with a pair of subserosa electrodes (connected to an electrical stimulator) in the lower segment of the esophagus. The ES signal consisted of bipolar rectangular pulse trains, lasting for 3.0s, with different amplitudes (1mA, 3mA, 5mA and 10mA), and frequencies (10Hz, 20Hz and 50Hz). The amplitude of the contraction was recognized by High-resolution manometry. The effect of ES was tested under anesthesia and following administration of atropine, phentolamine or L-NAME. ES induced esophageal contraction at the stimulated site. A statistically significant increase in esophageal pressure was observed when the stimulation amplitude was above 3 mA. The increase in esophageal pressure was associated with the amplitude of stimulus as well as the frequency. During stimulation, atropine, phentolamine and L-NAME had no effect on the increase of esophageal pressure induced by ES. These findings implied that ES induced esophageal contraction were not mediated via the NANC, adrenergic or cholinergic pathway. The amplitude of esophageal contraction was current and frequency dependent.

Key words

Electrical stimulation • Esophagus motility • High-resolution manometry

Introduction

Achalasia may be defined as a primary esophageal motility disorder,

characterized by aperistalsis of the esophageal body and incomplete relaxation of the lower esophageal sphincter (LES). Current treatment options for achalasia include pharmacological agents, pneumatic dilation, surgical myotomy and P High-resolution manometry peroral endoscopic myotomy. All these methods are intended to decrease the pressure of LES by relaxing the LES, but they cannot improve esophageal motility, especially the recovery of peristalsis. Many patients still suffer from dysphagia after one or two types of therapy because of abnormal esophageal motility, so there is an urgent need for new treatment modalities that improve esophageal motility and restore esophageal peristalsis.

Recently, there has been increased interest in electrical stimulation (ES) to regulate gut motility. Gastric electrical stimulation and intestinal electrical stimulation can increase gastric emptying and intestinal transit, respectively (Song and Chen 2011, Yin and Chen 2007, Mintchev *et al.* 2000). Gastric electrical stimulation has been used to treat gastroparesis and morbid obesity in clinical practice (Jayanthi *et al.* 2013, Xu *et al.* 2005, Guerci *et al.* 2012). Experiments have confirmed that colonic electrical stimulation with pulse trains has prokinetic effects on colonic contractions and transit, which provide a hope for treatment of constipation with ES (Liu and Chen 2006, Amaris *et al.* 2002, Aellen *et al.* 2009, Martellucci and Valeri 2014). In recent years, more research has indicated that esophageal ES could increase the pressure of LES and provide a good therapeutic effect on gastroesophageal reflux disease (Banerjee *et al.* 2014, Rodriguez *et al.* 2013). However, little study has reported whether esophageal ES can regulate esophageal motility and treat esophageal motility

disorders, such as achalasia.

We hypothesized that ES delivered locally to the esophageal body in vivo would evoke esophageal body contraction and then regulate esophageal motility. Therefore, the aim of this study was to investigate the effects of ES on esophageal motility in a rabbit model and explore the mechanism of this action. We chose stimulus parameters proven to change esophageal pressure or generate smooth muscle contraction in our preliminary experiment.

Materials and Methods

Animal preparation

Twenty-eight adult male New Zealand white rabbits (1.0-2.0 kg, Institute of animal research, Nanjing University, China) were included in this study. After an overnight fast, each animal underwent surgery under general anesthesia (1.5% iso-flurane inhalation). A midline laparotomy was performed and the lower segment of the esophagus was exposed. One pair of custom-designed stainless steel wire electrodes (5×1 mm) were implanted at the subserosa of the lower segment of the esophagus (2 cm above the LES). The two electrodes in the circumferential pair (one active and one ground) were oriented longitudinally and positioned diametrically opposite on the esophageal surface. The electrodes were connected to a multi-channel signal stimulator (A.M.P.I., Israel) by stainless steel wires. The study was approved by the Animal Care and Use Committee of the General Hospital, Tianjin Medical University, Tianjin, China.

Esophageal manometry

For the esophageal motility study, esophageal manometry was performed by water-perfusion high-resolution manometry (HRM) with a 22-channel catheter (Medical Measurement Systems, Enschede, the Netherlands) after implantation of electrodes. The esophageal manometry catheter was placed through the mouth of the rabbit into the esophagus and was positioned with the 5 dense sensors straddling the lower segment of the esophagus and the other 17 openings of the catheter located in the esophageal body. Continuous recordings of the pressure of the segment located with electrodes and of esophageal motility were obtained before, after and during each stimulation period.

ES of the lower segment of esophagus

This part of the experiment, performed with 12 rabbits, aimed to assess the effect of ES on esophageal body pressure and esophageal motility. The esophageal ES was controlled by a multi-channel signal stimulator delivering bipolar rectangular pulse trains (output current range ± 10 mA, output current limit 10 mA). According to our preliminary experiments, some stimulation parameters considered optimal for rabbit esophageal smooth muscle were selected: 1 mA, 3 mA, 5 mA and 10 mA current and, 10 Hz, 20 Hz and 50 Hz frequency. To investigate the reaction of the esophageal body to ES and determine the optimal current and frequency to influence esophageal motility, we applied the above currents and frequencies randomly as stimulation parameters. A pulse duration equal to the interval of two pulses was used, according to the frequency. Each rabbit underwent 12 stimulation sessions based on the above stimulation parameters. During each session, a different set of stimulation

parameters was used, as described in Table 1. Each type of stimulation was given randomly and delivered as monophasic pulses. Trains of pulses were delivered for a few seconds every minute (see Table 1) for the duration of the stimulation period. Each session lasted 10 minutes. Tissue impedance between the electrodes was checked at the beginning of each session to ensure that the electrodes were still anchored in the esophageal body muscle. Changes of esophageal pressure and motility induced by ES, as well as the latency period between ES and the contraction of the esophagus, were recorded by HRM synchronously.

Neuromechanism of ES

This part of the experiment, performed with the other 16 rabbits, was designed to explore the mechanisms by which ES has its effects. Continuous esophageal manometry recordings were obtained during 30 min of ES on the lower segment of the esophagus with the parameters of sessions 7, 8, and 9, as described in Table 1. Thirty milliliters of 0.9% saline was intravenously injected before ES as a control. The experiment to investigate the role of adrenergic, cholinergic and non-adrenergic and non-cholinergic (NANC) neurons in mediating the effects of ES on the esophageal body was performed after the above stimulation protocols were completed.

To explore whether the effects of ES on the esophageal pressure were mediated by NANC nerves, the inhibitor of the main neurotransmitter of NANC nerves was utilized. After a pause of 10 min, L-N^G-nitro arginine (L-NNA, Sigma Aldrich, St. Louis, MO) was administered i.v. to 4 rabbits over a few minutes to reach

a total dose of 50 mg/kg. L-NNA is an inhibitor of NO synthase that inhibits endogenous production of NO. Twenty to 30 minutes after the medication was administered, ES was delivered again for 30 min with the above parameters.

To explore whether the cholinergic pathway was involved in the effect of ES on esophageal pressure, atropine, the inhibitor of cholinergic nerves, was utilized. After a pause of 10 min, atropine (0.05 mg/kg, Gensia Sicor Pharmaceuticals, Irvine, CA) was administered to other 4 rabbits as an i.v. bolus and 2 min later ES was delivered for 30 min with the same parameters as above.

To explore whether the effects of ES on esophageal pressure were mediated by the adrenergic pathway, the inhibitor of adrenergic nerves phentolamine was utilized. After a pause of 10 min, phentolamine (Sigma Aldrich, St. Louis, MO) was administered IV to the last 4 rabbits over a few minutes to reach a total dose of 1.00 mg/kg, and 2 min later ES was delivered for 30 min with the same parameters as above.

All rabbits underwent euthanasia at the end of the experiment with air injection.

Statistical Analysis

The SPSS 18.0 software package was used for statistical data analyses (SSPS, Inc., Chicago, IL). For each type of stimulation, the mean pressure in a segment of the esophagus was determined over 10 min for each of the following periods: 1) baseline; 2) stimulation; and 3) poststimulation. The latency period between ES and esophageal contraction and the scope of esophageal contraction were

also calculated by averaging the 10-min recording of each type of stimulation. An ANOVA for repeated measures was used to assess differences between periods and types of stimulation. A Spearman correlation was applied for correlation analysis between stimulation amplitude and esophageal pressure, as well as stimulation frequency and esophageal pressure.

For the second experiment, the pressure of the lower segment of the esophagus was measured for the entire ES duration in each period. Changes in the mean esophageal pressure with the same stimulation parameters before and after utilization of the drug (L-NNA, atropine or phentolamine) were recorded in each period. ANOVA for repeated measures was used to assess differences between the changes in pressure.

Data are presented as means \pm SD. $P < 0.05$ was considered statistically significant, otherwise it was nonsignificant (NS).

Results

Effect of esophageal ES on the lower segment of the esophagus

Esophageal manometry was well tolerated by all animals (n=12). As shown in Table 2, a statistically significant increase in lower segment pressure was observed when the stimulation amplitude was 3 mA. The esophageal pressure was 16.2 ± 1.7 mmHg at baseline and increased by 143 percent to 39.5 ± 1.8 mmHg during esophageal ES ($P < 0.01$ vs. baseline) with parameters of 3 mA, 10 Hz, 50 ms, and then immediately recovered to the baseline level after the cessation of ES. Similar to these conditions, the esophageal body pressure increased to 72.2 ± 4.6 mmHg with

stimulation of 5 mA, 10 Hz, and to 131.8 ± 3.5 mmHg with stimulation of 10 mA, 10 Hz ($P < 0.01$) (Table 2). The baseline pressure was consistent and stable in the twelve different sessions on different rabbits (NS). Compared with baseline, pressure during poststimulation was not significantly increased in any session (NS), which indicated that the esophageal body relaxed soon after the end of ES. The increase in esophageal pressure was associated with the amplitude of the stimulus as well as the frequency of the stimulus. A higher increase in esophageal pressure was noted with a higher stimulation amplitude (Fig. 1A) or a higher stimulation frequency (Fig. 1B). The esophageal pressure during stimulation was found to be positively correlated with the stimulation amplitude ($r = 0.970$; $P < 0.001$) and stimulation frequency ($r = 0.942$, $P < 0.001$; Fig. 2). In addition, the latency period between stimulation and esophageal contraction was 53.9 ± 5.2 ms, which was not significantly different among the above stimulation parameters (Fig. 3). Analogously, the contraction scope was not obviously changed among different stimulation parameters and was restricted to the stimulation point (NS).

Effects of ES on esophageal body pressure in presence of L-NNA, atropine and phentolamine

The effect of ES on the rabbits esophageal body pressure was not mediated via the NANC, adrenergic or cholinergic pathway ($n = 16$). Atropine did not block the effect of esophageal ES on the esophageal body pressure; in the presence of atropine, ES still significantly increased the esophageal pressure, and the increase was comparable to that in the control session with saline (Fig. 4). Phentolamine and

L-NNA did not block the effect of ES on the esophageal body pressure. As Figure 2 shows, the esophageal body pressure during stimulation was increased significantly with blockage of phentolamine or L-NNA, and the increase was not obviously different from that in the control session with saline.

Discussion

This study has shown that esophageal ES increased the esophageal body pressure in the healthy rabbits and that the effect was stimulation amplitude and frequency dependent. The antagonists of NANC nerves, cholinergic nerves and adrenergic nerves, L-NNA, atropine and phentolamine, respectively, all did not block the effect of ES on the esophageal body.

Although there have been many studies regarding esophageal electric stimulation, the methods used in the current study are fundamentally different from those reported in the literature (Rodriguez *et al.* 2013, Sanmiguel *et al.* 2008, Christensen and Lund 1969). In the previous studies, ES used to treat gastroesophageal reflux disease (GERD) was delivered at 20 Hz, , 3–8 mA, 215 μ s in multiple 30-minute sessions (Rodriguez *et al.* 2013). In that method, a train of short pulses with a pulse width of 215 μ s and a long duration of 30 minutes was given repeatedly. In the current study, esophageal ES was performed using a train of pulses approximately 10-50 ms, with a short total duration of 3 s. That is, the width of pulses used in the current study was approximately 100 times that used in the previous study, whereas the duration of ES was 10 percent of that used in the previous study. The selection of different stimulation parameters was based on the different aims of the

two studies. The aim of the previous study was to increase LES pressure chronically to treat GERD, whereas the current study aimed to induce esophageal contraction immediately to promote esophageal peristalsis. Based on the aim and our preliminary study, the current study chose a longer pulse and a shorter duration.

The effects of ES on the gastrointestinal tract are inconsistent and even conflicting (Yang *et al.* 2004, Yao *et al.* 2005), but one consistent result is that ES increases the pressure of the stimulated segment (Nie *et al.* 2006, Rashev *et al.* 2002). Esophageal ES was always studied to stimulate the LES and then used to treat GERD, which has achieved greatly success clinically (Rodríguez *et al.* 2013, Rodríguez *et al.* 2012). It has been reported that short pulses stimulation caused a sustained increase in LESP in canine model: 32.1 ± 12.9 (prestimulation) vs. 43.2 ± 18.0 (stimulation) vs. 50.1 ± 23.8 (poststimulation) (Sanmiguel *et al.* 2008). The LESP during poststimulation was still increased significantly compared to that during prestimulation. In contrast, the esophageal body pressure decreased to baseline during poststimulation in this study, and the difference between them may owe to different stimulation durations and parameters used in the train of pulses. Consistent with our study, Sallam HS *et al.*'s experiment showed that electrical stimulation with a train of pulses could induce colonic contraction and increase colonic pressure (Sallam and Chen 2013). In addition, numerous previous studies with gastric or intestinal electric stimulation have reported that electric stimulation was capable of entraining gastric or intestinal slow waves (Lin and Chen 2002, Hocking *et al.* 1992, Lin *et al.* 1998), altering gastric or intestinal contractions (Eagon and Kelly 1995), and accelerating gastric emptying

(Bellahsène *et al.* 1992, Lin *et al.* 2011, McCallum *et al.* 1998, Ouyang *et al.* 2003), which was consistent with our results as well as our ultimate goal of accelerating esophageal emptying. In our preliminary research, the threshold for inducing an increase in esophageal body pressure was found to be a stimulation frequency of 10 Hz, a pulse width of 50 ms, and an amplitude of 3 mA. Further experiments and analyses revealed a correlation between the increased esophageal pressure and the stimulation amplitude, frequency or stimulation energy.

Mechanisms involved in the effect of ES on the esophageal body pressure were not clarified before. In this study, we found that the prokinetic effect of esophageal ES appeared to be not mediated via the NANC, cholinergic or adrenergic pathways. It is possible that pulse-train esophageal ES acted directly on some ionic channel of esophageal smooth muscle cells to induce esophageal contraction. This would not agree with the previous study in which both cholinergic and nitrergic pathways were involved in mediating the excitatory effect of colonic ES (Sallam and Chen 2013). The reason for this discrepancy may be associated with difference in the anatomical structure and nerve distribution between the esophagus and colon. Sanmiguel *et al.* reported that the effect of ES on LES was mediated via cholinergic nerves (Sanmiguel *et al.* 2008). The nitrergic pathway-mediated mechanism was also previously reported (Forster *et al.* 2001). These findings are not consistent, and more research is needed to elucidate the mechanism of ES.

The findings of the present study suggest the therapeutic potential of esophageal ES for the esophageal dysmotility diseases. Esophageal ES induced

esophageal contraction temporarily at the stimulated site in vivo, and this effect was not mediated by neurotransmitters, which made it easier to stimulate esophageal body sequentially with successive electrodes and then motivate esophageal peristalsis in the future. Further studies are warranted to explore the effects of sequentially electrical stimulation on esophageal body peristalsis and its feasibility.

Conflict of Interest

There is no conflict of interest.

Acknowledgments

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Table 1. Types of stimulation

ES Type	Pulse Frequency	Train Duration, s	Pulse Duration, ms	Pulse amplitude	Trains/min
Session 1	10 Hz	3	100	1 mA	1
Session 2	20 Hz	3	50	1 mA	1
Session 3	50 Hz	3	20	1 mA	1
Session 4	10 Hz	3	100	3 mA	1
Session 5	20 Hz	3	50	3 mA	1
Session 6	50 Hz	3	20	3 mA	1
Session 7	10 Hz	3	100	5 mA	1
Session 8	20 Hz	3	50	5 mA	1
Session 9	50 Hz	3	20	5 mA	1
Session 10	10 Hz	3	100	10 mA	1
Session 11	20 Hz	3	50	10 mA	1
Session 12	50 Hz	3	20	10 mA	1

This table depicts the parameters used in each type of electrical stimulation (ES) applied to the lower segment of rabbits esophagus (n=12). Each type of stimulation was applied at random and for 10 min.

Table 2. Effect of ES on the lower segment of rabbits esophagus (n=12)

Stimulation parameter	Lower segment of esophageal body pressure, mmHg		
	Baseline	Stimulation	Poststimulation
1 mA, 10 Hz	16.2±2.4	16.3±3.1	16.2±4.5
1 mA, 20 Hz	16.1±2.8	15.9±3.7	16.1±3.8
1 mA, 50 Hz	16.0±3.4	16.1±2.6	16.1±2.9
3 mA, 10 Hz	16.2±1.7	39.5±1.8*	16.2±3.5
3 mA, 20 Hz	16.2±3.7	46.2±5.3*	16.2±5.1
3 mA, 50 Hz	16.1±4.5	67.0±5.3*	16.0±4.1
5 mA, 10 Hz	15.9±4.6	72.2±4.6*	15.8±5.3
5 mA, 20 Hz	15.9±3.6	101.2±5.7*	15.8±4.7
5 mA, 50 Hz	16.2±4.2	110.2±2.7*	16.2±3.9
10 mA, 10 Hz	16.2±5.5	131.8±3.5*	16.1±4.2
10 mA, 20 Hz	16.0±4.1	141.3±3.7*	16.1±5.2
10 mA, 50 Hz	16.1±3.4	155.6±3.6*	16.1±4.5

* $P < 0.01$ vs control

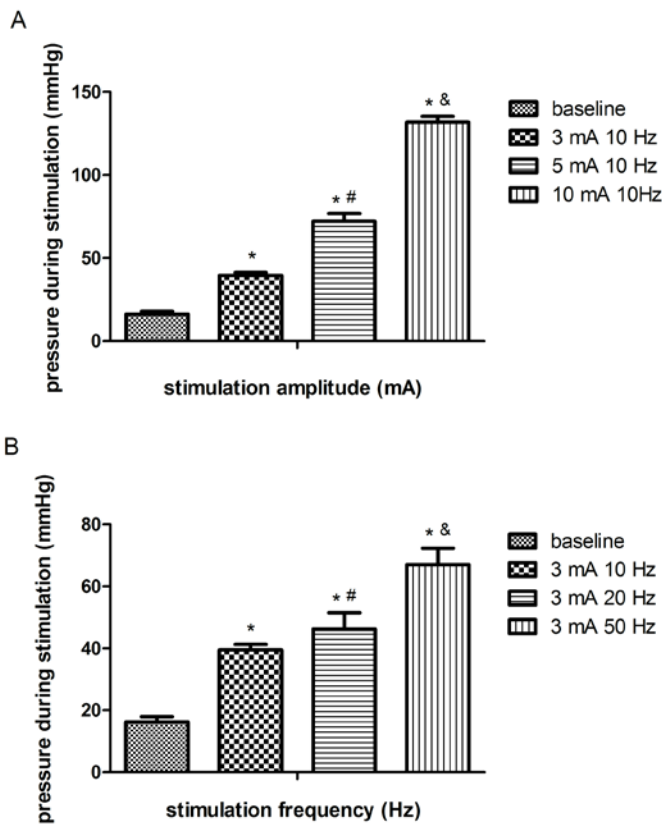


Figure 1. The esophageal body pressure during baseline and stimulation with different parameters (n=12). A. Esophageal pressure during ES with different stimulation amplitudes (3mA, 5mA and 10mA) at a frequency (10Hz) and a pulse (50 ms). * $P < 0.01$ vs. baseline; # $P < 0.05$ vs. 3 mA; & $P < 0.01$ vs. 3 mA or 5 mA. B. The esophageal body pressure during ES with different stimulation frequencies (10Hz, 20Hz and 50Hz) at a pulse amplitude (3 mA). * $P < 0.05$ vs. baseline; # $P < 0.01$ vs. 10 Hz; & $P < 0.01$ vs. 10 Hz or 20 Hz.

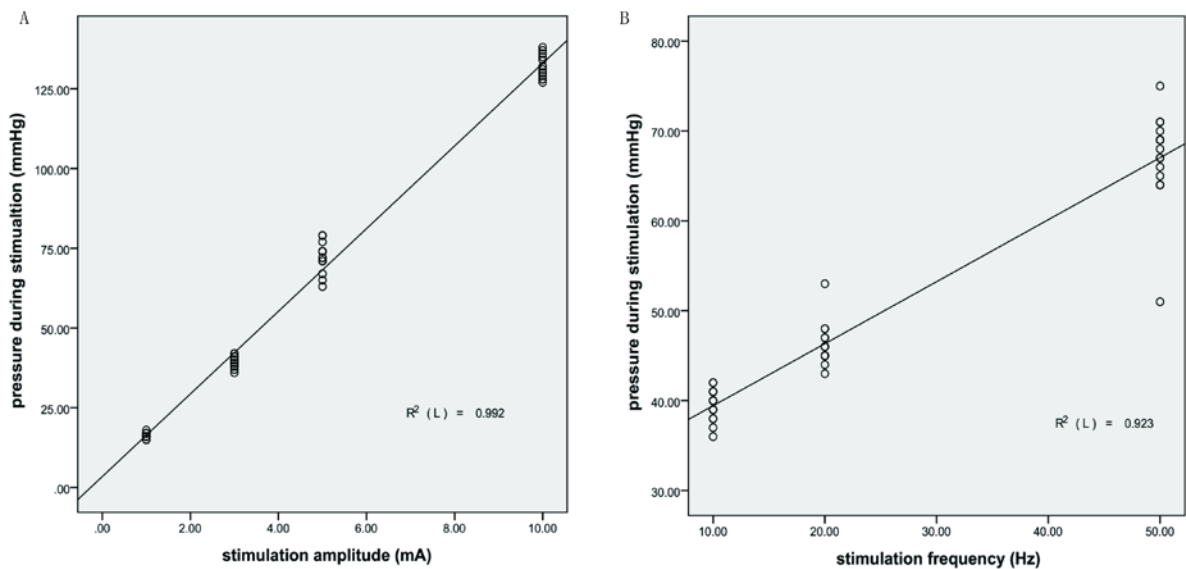


Figure 2. Correlation between electrical stimulation parameter and pressure of stimulation segment in esophageal body. The results suggested that the esophageal pressure during stimulation was positively correlated with the stimulation amplitude ($r=0.970$; $P<0.001$) and stimulation frequency ($r=0.942$, $P<0.001$).

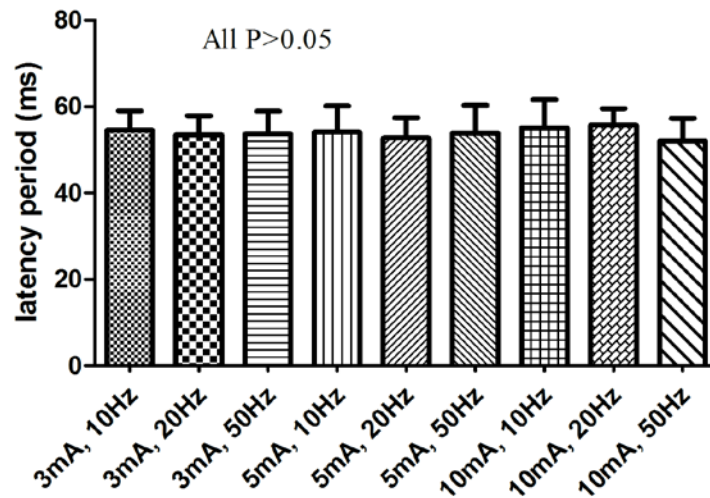


Figure 3. The latency period between electrical stimulation with different parameters and esophageal contraction. There was no significantly different on latency period among the above stimulation parameters. All were nonsignificant ($P>0.05$).

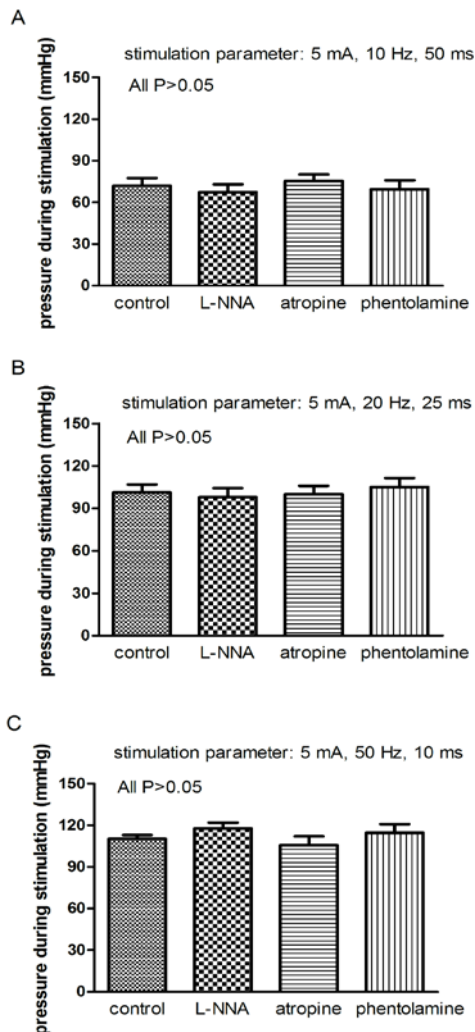


Figure 4. Possible mechanism of esophageal ES involving NANC, cholinergic and adrenergic pathways (n=16). A. Esophageal body pressure during stimulation with parameter 5mA 10Hz 50ms, in the condition of control, L-NNA, atropine or phentolamine. All were nonsignificant ($P>0.05$). B. Esophageal pressure during stimulation at a pulse amplitude (5 mA), a frequency (20 Hz) and a width (25 ms) in the condition of control, L-NNA, atropine or phentolamine. All were nonsignificant ($P>0.05$). C. Esophageal pressure during stimulation with 5 mA, 50 Hz and 10 ms at present of control, L-NNA, atropine or phentolamine. All were nonsignificant ($P>0.05$).