Role of Serotonin in Systemic Impairment of Motor Function of the Digestive Tract

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We studied the role of serotonin in systemic impairment of motor function of the digestive tract. Administration of a nitric oxide donor methylene blue into the pre-fundal portion of the stomach and application of a loose ligature to the terminal part of the sigmoid colon were performed after pretreatment with serotonin. Serotonin had a stabilizing effect on esophageal antiperistalsis under conditions of dynamic obstruction of the sigmoid colon.

Key Words: *serotonin; motor function; digestive tract*

Nervous regulation of esophageal motor function involves the cholinergic, adrenergic, serotoninergic, and other systems [2-4,7,8,10,11]. In addition to nervous regulation, the esophagus probably exhibits myogenic activity. Isolated preparations of smooth muscles from the esophagus are characterized by slow muscle contractions, which resemble peristalsis. Muscle tissue of the esophagus is highly sensitive to high-frequency stimulation. High-frequency stimulation of the esophageal preparation (*i.e.*, sensitive muscle tissue) is followed by the propagation of muscle contractions in the antegrade and retrograde directions. Proper muscle activity of the esophagus does not necessarily coincide with peristalsis (*e.g.*, during swallowing movements). These properties of the muscle depend on the resting membrane potential, K^+ concentration, and cell membrane permeability for Ca^{2+} [5].

Local regulation of esophageal motor function is activated upon stimulation of the inner surface of the esophagus. This mechanism is directed toward the removal of the stomach contents, fluid, and air from the esophagus. The reflex response is activated in response to dilation of any region of the esophageal wall. The stimulatory (acetylcholine, serotonin, and substance P) and inhibitory factors (catecholamines, ATP, vasoactive intestinal peptide (VIP), and NO) play an important role in activation or inhibition of local regulation.

Inhibitory nitrergic neurons play a role in the regulation of motor function in the distal portion of the stomach, lower esophageal sphincter, and gallbladder. Long-term treatment with an endogenous NO source L-arginine not only inhibits the late postprandial rise in pressure of the lower esophageal sphincter, but also lengthens the relaxation period of this sphincter. These changes are probably mediated by NO [9].

Pharmacological blockade of NO induces peristaltic contraction of the esophagus, increases the basal pressure of the lower esophageal sphincter, and prevents relaxation of this sphincter. The decrease in NO concentration is followed by an increase in permeability of the mucosa, destabilization of mast cells [6], and release of serotonin, histamine, and other biologically active substances.

However, little is known about the role of serotonin in a systemic impairment of motor function of the digestive tract.

Here we studied the role of serotonin in a systemic impairment of motor function of the digestive tract.

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MATERIALS AND METHODS

Experiments were performed on Wistar rats weighing 220-250 g. Physiological saline (0.1-0.2 ml) was administered into the prefundal portion of the stomach in control animals (n=5) during surgical stage of nembutal anesthesia (40 mg/kg). Gastroesophageal reflux (GER) and constipation in animals of treatment group 1 (n=5) were induced by administration of methylene blue (0.1-0.2 ml) into the prefundal portion of the stomach. Serotonin was administered into the prefundal portion of the stomach in rats of the treatment group 2 (n=5) 1-2 min before modeling of GER and constipation. Midline laparotomy was performed. The prefundal portion of the stomach, lower third of the esophagus, and terminal part of the sigmoid colon were isolated. Electromotor activity (EMA) of these organs was recorded with silver electrodes. Methylene blue (0.1-0.2 ml) was administered once or twice into the prefundal portion of the stomach in treated animals. The appearance of this dye in the lower third of the esophagus was monitored. A loose ligature was applied to the terminal part of the sigmoid colon. Relaparotomy was performed in anesthetized rats after 2 weeks. EMA of the esophagus, stomach, and sigmoid colon was recorded. Methylene blue was administered into the prefundal portion of the stomach. The appearance of methylene blue in the esophagus was monitored. Due to progression of macroscopic changes in the sigmoid colon and esophagus, a microscopic study of biopsy specimens from the sigmoid colon tissue and esophagus was performed 2 weeks after application of the loose ligature. Amplitude-frequency characteristics of slow waves were evaluated for studying the motor function of smooth muscle cells in the esophagus, stomach, and sigmoid colon.

The sigmoid colon tissue was fixed with 4% paraformaldehyde in Hanks buffer for 2 h. Postfixation was performed with 1% OsO_4 for 2 h. The tissue was embedded in a mixture of Epon and araldite after dehydration. The sections (1 μ) were stained with methylene blue and examined under a microscope (×240).

RESULTS

Administration of physiological saline into the prefundal portion of the stomach in control animals had little effect on the frequency (7.6±0.7 spikes per min, baseline frequency 7.3±0.9 spikes per min) and amplitude of EMA slow waves in the esophagus (0.24±0.04 mV, baseline amplitude 0.23± 0.03 mV). After administration of physiological saline the frequency and amplitude of EMA slow waves in the pre-fundal portion of the stomach were 4.9±0.4 spikes per min (baseline frequency 4.8±0.6 spikes per min, p>0.1) and 0.25±0.03 mV (baseline amplitude 0.30±0.05 mV, p>0.1), respectively.

Administration of physiological saline into the pre-fundal portion of the stomach had little effect on the frequency (5.5±0.7 spikes per min, baseline frequency 5.0±0.5 spikes per min) and amplitude of EMA slow waves in the sigmoid colon (0.18± 0.03 mV, baseline amplitude 0.20±0.04 mV). These data indicate that EMA of the stomach, esophagus, and sigmoid colon remained unchanged after administration of physiological saline into the prefundal portion of the stomach.

Administration of methylene blue into the prefundal portion of the stomach in animals of treatment group 1 was followed by an increase in the frequency and amplitude of EMA in the esophagus to 10.1±1.2 spikes per min (38.3%, p<0.05) and 0.29±0.03 mV (26%, p<0.05), respectively. After administration of methylene blue, the frequency and amplitude of EMA in the prefundal portion of the stomach were 7.6±0.7 spikes per min (58.3%, p<0.05) and 0.47±0.05 mV (56.6%, p<0.05), respectively. The increase in EMA of the prefundal portion of the stomach preceded the increase in EMA of the esophagus by 3-10 sec. These data indicate that GER is accompanied by retrograde propagation of EMA waves. After application of the loose ligature, the frequency and amplitude of EMA in the sigmoid colon were 6.8±0.4 spikes per min (36%, p<0.05) and 0.30±0.03 mV (50%, p>0.1), respectively. Hence, motor activity of the sigmoid colon increased during experimental constipation.

After 2 weeks of constipation, the frequency and amplitude of EMA slow waves in the esophagus were 5.3±0.4 spikes per min and 0.24±0.04 mV, respectively. The frequency and amplitude of EMA slow waves in the prefundal portion of the stomach were 5.1±0.6 spikes per min and 0.22± 0.04 mV, respectively. The frequency and amplitude of EMA slow waves in the sigmoid colon were 3.5±0.4 spikes per min and 0.20±0.04 mV, respectively. These data indicate that motor activity of the esophagus and sigmoid colon decreases during experimental constipation (compared to the baseline value).

Administration of methylene blue into the prefundal portion of the stomach in animals with severe constipation was followed by an increase in EMA in the upper portions of the digestive tract. The frequency and amplitude of EMA slow waves in the lower third of the esophagus increased to 6.4±0.5 spikes per min (20.8%, p<0.05) and 0.45±

0.05 mV (87.5%, $p<0.05$). After administration of methylene blue, the frequency and amplitude of EMA slow waves in the prefundal portion of the stomach increased to 6.8±0.6 spikes per min (33%, $p<0.05$) and 0.34±0.03 mV (54.5%, $p<0.05$), respectively. The increase in EMA of the prefundal portion of the stomach preceded the increase in EMA of the esophagus by 5-7 sec. The changes were less pronounced compared to those observed after administration of methylene blue alone. After administration of methylene blue to animals with experimental constipation, the frequency and amplitude of EMA in the sigmoid colon were 3.8± 0.4 spikes per min (8%, $p>0.1$) and 0.20±0.03 mV (0%, $p>0.1$), respectively. Therefore, motor activity of the sigmoid colon remained practically unchanged under these conditions.

Macroscopically, the terminal part of the large intestine in animals of treatment group 1 was filled with a dense content. The volume of this portion was elevated by 3-4 times. The stomach was empty in the majority of animals.

Amplitude-frequency characteristics of slow waves increased in the lower third of the esophagus and stomach in animals of treatment group 2. EMA of the sigmoid colon remained practically unchanged in these rats (Table 1). Administration of serotonin was followed by a greater increase in EMA of the esophagus and stomach (compared to animals of the GER group).

After administration of methylene blue, the development of GER and constipation in animals of treatment group 2 was characterized by a relative decrease in the frequency of EMA slow waves in the stomach and esophagus (as compared to group 1 rats with constipation, Table 1). It was probably related to the inhibitory cologastric reflex, which occurs under normal conditions and inflammation of the ileocecal region in the large intestine and rectum.

Application of a loose ligature to the sigmoid colon was accompanied by a decrease in EMA. These changes were followed by the development of constipation for 2 weeks. Administration of a NO donor methylene blue into the pre-fundal portion of the stomach was followed by relaxation of the lower esophageal sphincter and induction of antiperistaltic slow-wave activity in the esophagus and pre-fundal portion of the stomach in animals of the control and treatment groups.

After administration of serotonin to animals with constipation (2 weeks), the slow-wave activity of the stomach, esophagus, and sigmoid colon did not differ from that in control rats (Table 1). Administration of methylene blue to serotonin-treated animals with severe constipation was followed by a greater increase in EMA of the esophagus and stomach. EMA of the sigmoid colon remained unchanged under these conditions (Table 1).

The serotonin-induced inhibitory cologastric reflex was impaired with an increase in the severity of constipation. Administration of serotonin into the prefundal portion of the stomach in animals with bowel obstruction was accompanied by an increase in the degree of GER. These data are consistent with the results of clinical observations [1].

Microscopic study showed that the mucosa of the sigmoid colon retains a normal structure. The surface epithelium and crypt epithelium included a considerable number of goblet cells at various stages of secretory activity. Cell nuclei were located in the basal part. Nearly all area of the cytoplasm was filled with homogenous or granular mucus.

TABLE 1. EMA of the Esophagus, Stomach, and Sigmoid Colon in Animals with Experimental GER and Constipation under Various Experimental Conditions

Treatment	Esophagus		Stomach		Sigmoid colon	
	frequency	amplitude	frequency	amplitude	frequency	amplitude
Baseline	5.9±0.8	0.16±0.03	6.5±0.9	0.13±0.02	5.1±0.6	0.15±0.03
Administration of methylene blue after pretreatment with serotonin	9.3±1.4* (57.6%)	0.3±0.05* (87.4%)	10.0±1.6* (53.8%)	0.28±0.04* (115%)	5.3±1.4 (3.9%)	0.18±0.03 (20%)
Administration of methylene blue after application of a loose ligature and treatment with serotonin	7.1±0.8 (20%)	0.36±0.08* (125%)	7.3±0.6 (12.3%)	0.3±0.05* (130.8%)	4.7±0.7 (-7.9%)	0.18±0.04 (20%)
Baseline value after 2 weeks of experimental constipation	6.3±0.8	0.17±0.03	6.2±0.7	0.15±0.03	5.1±0.7	0.14±0.02
Administration of methylene blue to serotonin-treated animals with severe constipation	10.1±1.2* (60.3%)	0.31±0.05* (82.3%)	9.4±1.3* (51.6%)	0.26±0.05* (42.3%)	5.2±0.6+ (2%)	0.15±0.03 (7%)

Note. *$p<0.05$ compared to administration of methylene blue after pretreatment with serotonin.

The peripheral region of some granules was weakly stained. A colorless content of goblet cells was often found in the lumen of crypts. The vascular network was well developed. The veins were slightly widened. Small groups of erythrocytes were found in veins.

The intercellular matrix was irregularly condensed, had a loose structure, and contained the non-oriented collagen fibers. Some regions of dense connective tissue were characterized by the presence of polysaccharides. Fibrous structures were located between the crypts and formed a fine-fibrous reticulum. Mucus with small dense granules was revealed in the lumen of some crypts.

In animals of treatment group 1, the mucosa of the esophagus was composed of coarse fibrous connective-tissue structures spread in the epithelium. Clusters of fibroblast cells were revealed in the subepithelial layer. Striated muscle fibers were densely located. The endomysium was well-developed in some regions. The perimysium was well-developed and contained arterial vessels.

Exfoliated regions were found in the sigmoid colon epithelium of animals from treatment group 2. Lymphoid infiltrates were present in the lamina propria of the mucosa. The crypt epithelium was characterized by the prevalence of goblet cells. The submucosa layer had coarse bundles of collagen fibers, which spread in the intermuscular space. Groups of atrophic myocytes were revealed in various regions of the muscle layer.

Some goblet cells were in the stage of secretion. The secretion of the apical region of goblet cells, as well as the released secretion, were weakly stained with methylene blue. Small osmotic vacuoles (probably osmotic vacuoles) were found in the dilated space between enterocytes. Some goblet cells were destructed. The globular structure of the secretion was impaired. Goblet cells of a normal or abnormal structure were located close to each other. Coarse collagen fibers of the connective tissue were densely located. Fibroblast cells were present between these structures.

Administration of NO donor methylene blue into the prefundal portion of the stomach was accompanied by an increase in retrograde slow-wave activity of the stomach. These changes were followed by retrograde activation of EMA in the esophagus.

Application of a loose ligature to the terminal part of the sigmoid colon contributed to constipation. It was accompanied by a decrease in EMA of the sigmoid colon and activation of mucosa production by goblet cells. These changes can prevent the development of constipation upon a decrease in EMA of smooth muscles in the terminal part of the large intestine. Administration of methylene blue to animals with severe constipation was followed by a decrease in the severity of GER (compared to treatment with NO donor alone).

Pretreatment with serotonin promotes an increase in the degree of GER. Administration of serotonin after application of a loose ligature reduces the severity of esophageal antiperistalsis. This effect is probably associated with the inhibitory cologastric reflex, which prevents the progression of antidromic peristaltic activity. The development of constipation with symptoms of bowel obstruction is accompanied by changes in the stabilizing effect of serotonin on GER. Serotonin has an activating effect on GER, which is manifested in an increase in the degree of reflux of the stomach contents into the esophagus (*i.e.*, suppression of the inhibitory cologastric reflux). Morphologically, these changes in the sigmoid colon coincide with the appearance of coarse bundles of the connective tissue and atrophy of smooth muscle cells. Our findings indicate that serotonin is involved in a systemic impairment of motor function of the digestive tract.

REFERENCES

1. P. Ya. Grigor'ev and E. P. Yakovenko, *Formed Compendium of Gastroenterology and Hepatology* [in Russian], Moscow (2003).
2. *Collected Lectures on Gastroenterology* [in Russian], Eds. V. T. Ivashkina and A. A. Sheptulina, Moscow (2002).
3. I. V. Maev, Yu. A. Kucheryavyi, and M. S. Danilin, *Eksper. Klin. Gastroenterol.*, No. 5, 10-12 (2003).
4. *Basics of Physiology* [in Russian], Ed. A. D. Nozdrachev, St. Petersburg (2004).
5. J. M. Henderson, *Pathophysiology of Digestive Organs* [in Russian], Moscow, St. Petersburg (1997).
6. M. J. Beyak, P. I. Collman, S. Xue, *et al.*, *Neurogastroenterol. Motil.*, **15**, No. 4, 401-407 (2003).
7. W. J. Hogan, *Curr. Gastroenterol. Rep.*, **5**, No. 3, 206-212 (2003).
8. K. P. Kerr, F. Mitchelson, and I. M. Coupar, *Acta Physiol. Scand.*, **154**, No. 2, 213-220 (1995).
9. Y. C. Luiking, B. L. Weusten, P. Portincasa, *et al.*, *Am. J. Physiol.*, **274**, No. 6, Pt. 1, G984-G991 (1998).
10. S. J. Spechler, *Digestion*, **51**, Suppl. 1, 24-29 (1992).
11. M. Storr, F. Geisler, W. L. Neuhuber, *et al.*, *Auton. Neurosci.*, **91**, Nos. 1-2, 1-9 (2001).