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Research Article

Serotonin Receptors Mediate Contractile Activity of Rat's Esophagus *in-vivo*

Abstract

Background: Serotonin (5-hydroxytryptamine, 5-HT) is a regulatory and biologically active neurotransmitter and a hormone in the CNS and many organs, including the esophagus. It is known that serotonin as well as acetylcholine stimulates contractile activity of the esophageal muscles. However, role of different serotonin receptors in the 5-HT contractile activity of the esophagus is insufficiently known.

The aim: To determine which 5-HT receptors mediate serotonin contractile activity of the esophagus.

Subject and methods: This is an electromyography study of esophagus contractile activity of rat under serotonin stimulation of 5-HT3,4 and 5-HT2,1 receptors modulated separately. The role of different serotonin receptors in the 5-HT contractile activity of the esophagus was evaluated by measuring the amplitude and frequency of the slow wave electromyogram (EMG) by the noninvasive microelectrodes imposed on the adventitial layer of the esophagus.

Results: Administration of the 5-HT3,4 receptors inhibitors excluded caused by serotonin the increment of EMG activity of the contractile activity of the esophagus. Administration of the 5-HT1,2 receptors inhibitors blocked the serotonin-induced increment of EMG activity of the contractile activity of the esophagus.

Conclusion: Our results indicate that serotonin plays an important role in the regulation of the rat's esophagus contractility; the enhancing effect of 5-HT on contraction of the esophageal smooth muscles is mediated through the activation of 5-HT1,2 receptors expressed on the smooth muscle cells, and by activation of 5-HT3,4 receptors expressed on the ganglion neurons.

Abbrevations

5-HT: serotonin; 5-HT1-; 5-HT2-; 5-HT3-; 5-HT4: serotonin receptors; EMG: Electromyogram

Introduction

The esophagus is a flattened muscular tube, the main function of the esophagus is to transport food from the mouth to the stomach. The movement of the chyme along the esophagus is provided basically by the peristaltic movement of the muscular membrane of the esophagus, and also the sphincter.

The muscular layer of the esophagus has two types of muscle [1]. The upper third of the esophagus is consist of striated muscle, the lower third is consist of smooth muscle, and the middle third is consist of a mixture of both [2]. The esophageal muscles are arranged in two layers: one in which the muscle fibers run longitudinally to the esophagus, and the other in which the fibers encircle the esophagus. These layers are separated by the myenteric plexus, a tangled network of nerve fibers involved in the secretion of mucus and in peristalsis of the esophageal smooth muscle. The esophagus also has an adventitia, but not a serosa. This makes it distinct from many other structures in the gastrointestinal tract [2].

An important characteristic of the esophagus is its contractile activity. The dominant cell population of the esophagus from that point of view is the muscle cells that contain the contractile apparatus responsible for the generation of the contractile force.

A strict regulation of contractility of the esophagus is essential to avoid gastroesophageal reflux and other dysfunctions of the upper digestive tract. The muscles of the esophagus are controlled by various neuronal, hormonal,

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metabolic, and mechanical factors, including intramural acetylcholine and serotonin (5-hydroxytryptamine, 5-HT).

The esophagus is innervated by the vagus nerve, the cervical and thoracic sympathetic trunk, and Auerbach's plexus in muscular layers. Two sets of nerve fibers travel in the vagus nerve to supply the muscles. The upper striated muscle, and upper esophageal sphincter, are supplied by neurons with bodies in the nucleus ambiguus, whereas fibers that supply the smooth muscle and lower esophageal sphincter have bodies situated in the dorsal motor nucleus.

The sympathetic trunk has a sympathetic function. It may enhance the function of the vagus nerve, increasing peristalsis and glandular activity. It is known that the sympathetic trunk contains serotonergic fiber inside it; activation of these fibers will cause muscle wall constriction [3,4].

The inhibiting neurons are localized mainly in the circular muscle layer and they inhibit constriction through NO or VIP. Muscular fibers that constitute the muscular layer are innervated by the excitatory and inhibitory motor neurons [5].

Serotoninergic system is formed by its bioamines, receptors, SERT transporter (monoamine transporter protein, which is formed by sodium-potassium transporter), enzyme of 5-tryptophan hydroxylase-1 and -2 synthesis and catabolism – monoaminooxidase A (MAO-A).

Serotonin (5-hydroxytryptamine, 5-HT) is a monoamine neurotransmitter. It is derived from tryptophan [6], and metabolized by liver mainly to 5-HIAA, 90% of the human body's total serotonin is located in the enterochromaffin cells of the small intestine and esophagus (GI tract), where it is taken and stored by the blood platelets, and is used to regulate esophageal, stomach, and intestinal movements [7]. Serotonin was one of the first biological active substance, appearing on the 12th day in cervical and thoracic section of esophagus of chick embryo [8].

Receptors

Seven groups (from 5-HT1 to 5-HT7) and several groups of serotonin receptor subtypes have been classified by International Union of Basic and Clinical Pharmacology Committee on Receptor Nomenclature and Drug Classification on the basis of their structural and signaling features. Except 5-HT3 receptor which is coupled with ion channels and is exclusively expressed in neural components, all 5-HT receptors are G-protein-coupled type. Human and / or rodents esophagus express the following functional serotonin receptors.

Despite the hard work done by researchers on the serotonin receptors in the esophagus, the mechanisms of serotonergic regulation of the esophagus are not fully understood, and, at the same time, the nature of the functional serotonin receptors that mediate the esophageal contraction has not been fully classified yet.

Aim

To investigate the role of ganglionic and effective serotonergic receptors in regulation of motor function of esophagus.

Subjects and Methods

Animal experiments

The experiments were performed on 10 Wistar rats of both sexes, 5-6 months old and weigh 215-230 g. The control group consisted of 5 animals. All experiments were carried out under Nembutal narcosis (40 mg/kg, intraperitoneally), in accordance with the national ethical guidelines and were approved by the Institutional Animal Use and Care Committee of the Russian State Medical University.

Surgery

The rats were under anesthesia, and inferior-medial laparotomy was performed. In the operating wound, the lower third of esophagus was pulled away and cardiac section of the stomach was isolated. The animals were fixed on the operation table, with fur on the neck and anterior abdominal wall removed. The fasciae of the neck, which envelopes right and left neurovascular bundles, was divided with blunt method. Under the neurovascular bundles, ligatures were performed. On the extent of 10-15 mm, right common carotid artery was annulated. That is, acetylcholine can have a stimulating effect on motor function when M-cholinoreceptors are activated in smooth muscle cells. During the experiment, through the catheter, 0.9% NaCl, solution of serotonin and antagonist of serotonin receptors were introduced. The volume of single injection did not exceed 0.25 ml, which corresponded to the ventricular systolic volume of the rats [19]. Infusion of drugs was performed for 10-15 s, which corresponded to 60-80 contraction of the heart of animals. This parameters of injection decreased the risk of damages to semilunar valves and disturbances of hemodynamics, connected with volume load, providing the delivery of drugs to the region of aortic arch. Then, the wound of anterior abdominal wall was covered with occlusive covering for conservation of tissues in natural functional condition. 45 min after the closure of wound of abdominal wall, investigation of motor function of esophagus was conducted.

The duration of experiments constituted from 2 to 9 hours. During the time of experiments, heat of animals was conducted to prevent of hypothermia. On the limb of animals, electrodes were applied for registration of ECG in first standard lead, controlling vital functions. For the maintenance of activity of cardiovascular system, intravenous drip introduction of Ringer Locke solution, rheopolyglucin and lasex were used.

Drugs

To assess the relative contribution of 5-HT to the esophagus contraction, as well as to evaluate its receptor mechanism, serotonin, droperidol, and spiperon were administered into the esophageal smooth muscles. Serotonin was introduced in dose of 50 and 100 mcg/kg intravenously. Serotonin adipinate. Drug was introduced in different doses (50, 100 mcg/kg). 1% solution.

Inhibitors of serotonin receptors used

Mianserin: Is the antagonist of 5HT2A, 5HT2c, 5HT3,

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5HT6, 5HT7, a1-adrenoreceptors, and a2-adrenoreceptors, and it also acts as inhibitor of noradrenaline reuptake [20]. Dosage was 1 mg/kg.

NAS-181 is the strong selective blocker of 5-HTlB-receptors. Dosage was 0.1 mg/kg.

SB 204741 is the. Strong selective blockers of 5-HT2B-receptors. Dosage was 0.05 and 0.1 mg/kg.

MDL 72222 is the 5-HT3-receptors blocker. Dosage was 0.1 mg/kg.

RS 39604 hydrochloride is strong and selective 5HT4-antagonist, dosage was 0.1 mg/kg.

Introduction of the listed inhibitors and serotonin was performed intravenously with the use of control injection of analogic amount of physiological solution, which was injected for 2-3 minutes prior to the injection of drugs. Not in any of the cases, introduction of solution changed the amplitude-frequency indicators of slow waves and spike activity.

Experiments was conducted as the following scheme.

Trial introduction of serotonin and assessment of its influence on smooth muscles.

Blockade of 5-HT3 receptors with the subsequent investigation of the influence of serotonin.

Blockade of 5-HT4 receptors with the subsequent introduction of serotonin.

Administration of the inhibitor of 5-HT2 receptors and trial administration of serotonin.

Inhibition of 5HT1 receptors and administration of serotonin for the control of its action. In between the introduction of every subsequent blocker, 40-60 min is passed until the end of action of the previous blocker.

Measurements of the esophagus electromyogram (EMG). Noninvasive electrodes were overlaid on the adventitia for the registration of electromyography of the smooth muscles of lower third of esophagus. The esophagus EMG was measured using bipolar silver electrodes (inter electrode distance 1.5 mm) for the extracellular recordings. The electromyogram recording was performed with a 21-channel electroencephalograph (Neurofax EEG 4400 series, Nihon Kohden, Tokyo, Japan), the amplitude and frequency of slow electrical waves were recorded.

Statistical analysis

Data are expressed as the mean ± standard error. Student's t test was used for statistical comparisons where appropriate, and differences were considered significant at $p < 0.05$.

Results

Serotonin administration led to the increase of frequency-amplitude characteristics of slow waves of the esophagus: frequency was increased from 6.2±0.4 to 7.5±0.5 /min (20.9%, $p<0.05$), amplitude – from 0.25±0.012 to 0.3±0.015 mV (20%, $p<0.05$) (Table 1). That is, serotonin in applied dosage exerts a stimulating influence on the slow wave activity of smooth muscles of the esophagus.

Blockade of 5-HT3 receptors leads to the reduction of slow wave activity of esophagus: frequencies up to 5.8±0.2 / min (6.4%, $p<0.05$), amplitudes – up to 0.22±0.0012 mV (12 %, $p<0.05$) (Table 1). Introduction of serotonin in dosage of 50 mcg/kg on the background of action of blockers of 5-HT3 receptors insignificantly increases the frequency-amplitude characteristic of slow wave EMG: frequencies up to 6.6±0.3 / min (6.4%, $p<0.05$), amplitudes – up to 0.26±0.013 mV (4 %, $p<0.05$). Results of investigation showed that blockade of 5-HT3 receptors leads to partial decrease of the severity of the stimulatory effect of serotonin on smooth muscles of the esophagus. Therefore these receptors are ganglionic.

Introduction of blocker of 5-HT4 receptors is accompanied by an insignificant change of tonal EMG: frequencies from 6.2±0.4 to 6.3±0.35 /min (16.1%, $p>0.05$), amplitudes - from 0.25±0.012 to 0.20±0.018 mV (20%,$p<0.05$). Introduction of serotonin on the background of action of 5-HT4 receptor blockers is accompanied by an increase of frequency-amplitude parameters of slow wave EMG: frequencies – up to 6.6±0.43 / min (6.4%, $p>0.05$), amplitudes – up to 0.23±0.02 mV (15%, $p<0.05$). That is, blockade of 5-HT4 receptors incompletely prevent the stimulatory effect of serotonin, that shows about the location of 5-HT3,4 receptors on the membrane of efferent intramural neurons.

Introduction of blockers of 5-HT2 receptors lead to insignificant change of frequency of slow wave EMG: frequency is decreased from 6.0±0.33 to 5.5±0.23 /min (8.3%, $p>0.05$), amplitude is remained stable (table 1). Introduction of serotonin in dosage of 50 mcg/kg on the background of action of 5-HT2 receptors leads to a decrease of frequency index from 5.5±0.23 to 5.0±0.2 /min (-10%, $p>0.05$) during stable amplitude. In the result of investigation, it is shown that blockade of 5-HT2 receptors completely turns off the investigated effect – increase of motor function of esophagus with introduction of serotonin.

Deactivation of 5-HT1 receptors of serotonin decreases the frequency of slow wave EMG from 5.0±0.2 to 4.±0.14 /min (-4%, $p>0.05$), amplitude is remained unchanged. Introduction of serotonin on the background of action of blocker of

Table 1: Effect of 5-HT1,2,3,4 receptors on slow wave activity EMG of esophagus of rats.

	Before administration of inhibitors		After administration of inhibitors	
Inhibitors of	frequency	amplitude	frequency	amplitude
5-HT2 receptors	6.0±0.33	0.20±0.018*	5.5±0.23	0.20±0.02
5-HT1 receptors	5.0±0.22*	0.20±0.016*	4.8±0.14*	0.2±0.021
5-HT3 receptors	6.2±0.2	0.25±0.03	5.8±0.2	0.22±0.0012
5-HT4 receptors	6.2±0.4	0.25±0.02	6.3±0.35	0.2±0.18

Frequency, /min, amplitude – mV
*p<0.05

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5-HT1 receptors excludes the identification of investigated phenomenon: frequency of the slow wave EMG is decreased from 4.8±0.14 to 4.5±0.11 /min (-4.2%, p>0.05), amplitude is remained unchanged. Blockade of 5HT1 receptors completely excludes the investigated effect.

Given the complete exclusion of the investigated phenomenon with blockade of 5-HT2 and 5-HT1 receptors, we believe that 5-HT1 and 5-HT2 receptors are receptors of effector tissues.

Discussion

Currently, in gastroenterology, a large attention is given to the study of motor function of gastrointestinal tract, particularly of esophagus. This is connected with the investigation demonstrated in the recent years, that certain disorders of esophageal motor function can be the leading pathogenic factor, which contributes to the development of many widespread gastroenterological diseases. To this group of disease, we relate gastroesophageal reflux disease and different dyskinesia of esophagus with the primary disturbance of motor function of esophagus (diffuse and segmental esophagospasm, cardiospasm),

The results obtained from this study show that: 1) 5-HT is the important player in the regulation of the rat's esophagus contractility; 2) Serotonin enhances of the esophageal smooth muscles; 3) 5-HT enhancing effect on contraction of the esophageal smooth muscles is mediated through the activation of 5-HT1,2 receptors expressed on the smooth muscle cells, and by activation of 5-HT3,4 receptors expressed on the ganglion neurons.

Conclusion

We conclude that the regulation of esophageal smooth muscles by various parts of the autonomic nervous system involves ganglionic serotoninergic neurons, which expressed 5-HT3,4 receptors transmitting excitation to 5-$HT_{1,2}$ receptors. Activation of these receptors contributes to serotonin stimulation of EMG of the esophageal smooth muscles.

There is an opinion that serotonin can release acetylcholine from the Auerbach plexus. According to this opinion, serotonin stimulates ganglion cells, which in fact leads to firing and, consequently, to an increase of acetylcholine release from the nerve terminals [11]. Synaptic serotonin release is similar to release of acetylcholine in the neuromuscular junction [12]. That is, acetylcholine stimulate motor function by M-cholinoreceptors activated of smooth muscle cells. However, we does not confirm this opinion. As was shown in this study, the action of serotonin on the motor function of the esophagocardiac zone is completely turned off by inhibitors of effector serotonin receptors. The partial exclusion of the effect of serotonin on the motor function of the esophagocardiac zones by ganglionic serotonin receptors blockers is due to the fact that serotonin continues to exert its influence on effector serotonin receptors upon exclusion of ganglionic receptors.

The relevance of this article is that the received results will contribute to the further development of notion of vegetative nervous system. The data presented in this article on the stimulatory effect of serotonin on esophagus motility and inhibitory – blocators of its receptors can be used in the development of new pharmacological drugs.

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