

# Esophagus: the effect of large doses of serotonin on motor function

## 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 smooth muscles. However, role of different serotonin receptors in realization of large doses of serotonin in contractile activity of the esophagus is insufficiently known.

**Aim:** To determine receptor mechanisms in realization of large doses of serotonin in contractile activity of the esophagus.

**Subject and methods:** This is an electromyography study of esophagus contractile activity of rat under a large serotonin doses stimulation of 5-HT<sub>3</sub>, 4 and 5-HT<sub>2</sub>, 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 and spikes of electromyogram (EMG) by the noninvasive microelectrodes imposed on the adventitial layer of the esophagus.

**Results:** Administration of the 5-HT<sub>4</sub> receptors inhibitors excluded large dose serotonin induced the increment of EMG activity of the contractile activity of the esophagus. Administration of the 5-HT<sub>3</sub> receptors inhibitors not fully excluded large dose serotonin induced the increment of EMG activity of the contractile activity of the esophagus. Administration of the 5-HT<sub>1,2</sub> 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. Influence of a large doses of 5-HT on contraction of the esophageal smooth muscles is inhibited by blockers of 5-HT<sub>1,2</sub>, 4 receptors but not fully excluded by blocker of 5-HT<sub>3</sub>-receptors.

**Keywords:** esophagus, contraction, large doses serotonin, serotonin receptors

**Abbreviations:** 5-HT, serotonin; 5-HT<sub>1</sub>-, 5-HT<sub>2</sub>-, 5-HT<sub>3</sub>-, and 5-HT<sub>4</sub>, serotonin receptors; EMG, electromyogram

## Introduction

The esophagus is a flattened muscular tube; the main functions of the esophagus are to transport food from the pharynx to the stomach and to prevent the reflux of gastric contents into the esophagus. The movement of the bolus along the esophagus is provided basically by the peristaltic movement of the muscular membrane of the esophagus, and also the sphincter. The mechanisms of peristalsis resides in the esophageal wall.<sup>1</sup> 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, 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, Auerbach's plexus in muscular layers, serotonergic fibres, and neurons.<sup>2</sup>

Serotonergic system is formed by its bioamine, 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). Seven groups (from 5-HT<sub>1</sub> to 5-HT<sub>7</sub>) and several tens 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-HT<sub>3</sub> 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 5-HT<sub>1</sub>-, 5-HT<sub>2</sub>-, 5-HT<sub>3</sub>- and 5-HT<sub>4</sub>-receptors.<sup>3</sup>

Despite the hard work done by researchers on the serotonin receptors in the esophagus, the mechanisms of a large doses serotonin regulation of the esophagus are not fully understood, which was the purpose of this study.

## Aim

To investigate mechanisms of a large doses serotonin regulation of motor function of esophagus.

## Subjects and methods

**Animal experiments:** The experiments were performed on 16 Wistar female rats of 5-6 months old and weigh 220-230 g. The control group consisted of 5 animals. All experiments were carried out in sparing conditions, in accordance with the 22 September 2010 the EU Directive 2010/63/EU on the protection of animals used for

Volume 10 Issue 2 - 2019

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**Received:** January 31, 2018 | **Published:** March 26, 2019

scientific purposes, national ethical guidelines and were approved by the Institutional Animal Use and Care Committee of the Moscow Clinical Research Center.

**Surgery:** After anesthesia the animals were fixed on the operation table, with fur on the neck and anterior abdominal wall removed, and upper-medial laparotomy was performed. In the operating wound, the distal part of esophagus was pulled away and cardiac section of the stomach was isolated. During the experiment, through the catheter, 0.9% NaCl, solution of serotonin and antagonists 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. Infusion of drugs was performed for 10-15 s, which corresponded to 60-80 contraction of the heart of animals. These 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. 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 a large doses of 5-HT to the esophagus contraction, as well as to evaluate its receptor mechanism, serotonin, inhibitors of 5-HT<sub>1</sub>-, 5-HT<sub>2</sub>-, 5-HT<sub>3</sub>- and 5-HT<sub>4</sub>-receptors were administered into the esophageal smooth muscles. Serotonin-adipate was introduced in dose of 500 mkg/kg intravenously.

**Inhibitors of serotonin receptors used:** Mianserin is the antagonist of 5HT<sub>2A</sub>, 5HT<sub>2c</sub>, 5HT<sub>3</sub>, 5HT<sub>6</sub>, 5HT<sub>7</sub>,  $\alpha$ <sub>1</sub>-adrenoreceptors, and  $\alpha$ <sub>2</sub>-adrenoreceptors, and it also acts as inhibitor of noradrenaline reuptake. Dosage was 1 mg/kg.

NAS-181 is the strong selective blocker of 5-HT<sub>1B</sub>-receptors. Dosage was 0.1 mg/kg.

SB 204741 is the strong selective blocker of 5-HT<sub>2B</sub>-receptors. Dosage was 0.05 and 0.1 mg/kg.

MDL 72222 is the 5-HT<sub>3</sub>-receptors blocker. Dosage was 0.1 mg/kg.

RS 39604 hydrochloride is strong and selective 5HT<sub>4</sub>-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 were conducted according to the following scheme.

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

Blockade of 5-HT<sub>4</sub> receptors with the subsequent investigation of the influence of serotonin.

Blockade of 5-HT<sub>3</sub> receptors with the subsequent introduction of serotonin.

Administration of the inhibitor of 5-HT<sub>2</sub> receptors and trial administration of serotonin.

Inhibition of 5HT<sub>1</sub> receptors and administration of serotonin for the control of its action. After 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 of the smooth muscles of distal part of esophagus for the registration of EMG. The esophagus EMG was measured using bipolar silver electrodes (inter electrode distance 1.5 mm, surface 0.5-0.6 mm<sup>2</sup>) 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 and spikes were recorded.<sup>4,5</sup>

Statistical analysis with programme Statistica-12 was done. 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 in the dose 500 mkg/kg led to the increase of frequency-amplitude characteristics of slow waves of the esophagus: frequency was increased from 6.3±0.2 to 8.0±0.3 /min (26.9%, p<0.05), amplitude – from 0.055±0.003 to 0.55±0.004 mV (900%, p<0.01); spikes frequency was unchanged from 2.0±0.1 to 2.0±0.2 /min (p>0.1), amplitude – from 0.08±0.002 to 0.075±0.0015 mV (6,2% p>0.05). That is, serotonin in massive dosage exerts a stimulatory influence on the slow wave activity of smooth muscles of the esophagus.

Blockade of 5-HT<sub>4</sub> receptors leads to the reduction of slow wave activity of esophagus: frequencies from 6.3±0.2 to 6.0±0.12 /min (4.7%, p>0.05), amplitude – from 0.055±0.003 to 0.55±0.0025 mV (900%, p>0.05), frequency of spikes was changed from 2.0±0.1 to 1.0±0.05 /min (decreased on 50% p<0.05), amplitude – from 0.08±0.002 to 0.05±0.001 mV (decreased on 37.5% p<0.05). Administration of large dose serotonin on the background of action of inhibitors of 5-HT<sub>4</sub> receptors increased the frequency-amplitude characteristic of slow wave EMG: frequency from 6.0±0.12 to 7.9±0.2 /min (31.6%, p<0.05), amplitude from 0.05±0.0025 to 0.1±0.008 mV (99.9%, p<0.05), frequency of spikes was changed from 1.0±0.05 to 1.0±0.06 /min (p>0.1), amplitude – from 0.05±0.001 to 0.05±0.002 mV (p>0.1). Thus, inhibition of 5-HT<sub>4</sub> receptors promoted contractile activity of the esophagus.

Administration of inhibitor of 5-HT<sub>3</sub> receptors is accompanied by an insignificant change of slow waves EMG: frequency from 6.0±0.12 to 7.3±0.15 /min (21.7% p<0.05), amplitude - from 0.05±0.001 to 0.5±0.002 mV (p<0.01), spikes activity not appeared. Ingestion of large dose serotonin on the background of action of 5-HT<sub>3</sub> receptor blockers is accompanied by the increase of frequency-amplitude parameters of slow wave EMG: frequency from 7.3±0.15 to 7.5±0.14 /min (2.7%, p>0.1), amplitude from 0.05±0.001 to 0.05±0.003 mV (p>0.1). That is, blockade of 5-HT<sub>3</sub> receptors almost completely prevent the stimulatory effect of a large doses of serotonin, that

indicate the localization of 5-HT<sub>3,4</sub> receptors on the membrane of efferent intramural neurons.

Inhibition of 5-HT<sub>2</sub> receptors increased frequency of slow wave EMG from 7.3±0.15 to 11.0±0.3 /min (50.6%, p<0.05), amplitude – from 0.05±0.001 to 0.18±0.007 (260% p<0.01). Introduction of large dose serotonin on the background of inhibition of 5-HT<sub>2</sub> receptors lead to a decrease of EMG frequency from 11.0±0.3 to 7.6±0.2 /min (-30,9%, p<0.05), amplitude – from 0.18±0.07 to 0.20±0.009 mV (11,1% p<0.05). I.e. blockade of 5-HT<sub>2</sub> receptors completely turns off the investigated effect – increase of motor function of esophagus with introduction of serotonin.

Deactivation of 5-HT<sub>1</sub> receptors of serotonin decreases the frequency of slow wave EMG from 11.0±0.3 to 6.5±0.18 /min (40%, p>0.05), amplitude is remained unchanged. Administration large dose serotonin on the background of inhibition of 5-HT<sub>1</sub> receptors almost excluded the investigated phenomenon: frequency of the slow wave EMG is slightly increased from 6.5±0.18 to 6.7±0.18 /min (3.1%, p>0.1), amplitude was remained unchanged. Blockade of 5HT<sub>1</sub> receptors completely excludes the investigated effect. Given the complete exclusion of the investigated phenomenon with blockade of 5-HT<sub>2</sub> and 5-HT<sub>1</sub> receptors, we believe that 5-HT<sub>1</sub> and 5-HT<sub>2</sub> receptors are receptors of effector tissues.

## Discussion

In the regulation of motor activity of the esophagus involved the central nervous system and a number of hormones, neurotransmitters, among the latter a significant role is assigned to serotonin and its receptor apparatus.<sup>6,7</sup> It is known that an elevated serotonin level accompanies gastroesophageal reflux.<sup>8</sup>

Serotonin has a stimulatory effect on the motor function of the esophagus. Activation of serotonin receptors can be performed by various doses of the neurotransmitter.<sup>2</sup> As we showed earlier, small and medium doses of serotonin, and, in this study, also large doses of serotonin have a stimulatory effect on the neurons of the intermuscular plexus by activating 5-HT<sub>3,4</sub> receptors and on effector (smooth muscle cells) by activating 5-HT<sub>1, 2</sub> receptors. Blockade of 5-HT<sub>3,4</sub> receptors does not completely turn off the effect of large doses of serotonin due to the reverse capture of the excess of the neurotransmitter. In a number of pathologies of the upper parts of the digestive tract, both increased (gastroesophageal reflux) and decreased serotonin level can be observed. Thus, an inverse correlation between the level of serotonin and the total duration of gastroesophageal reflux ( $\rho = -0.380$ ,  $p = 0.002$ ) was revealed in all groups of patients, regardless of the clinical form of the disease.<sup>9</sup>

Hence the importance of study the dose-dependent effects of serotonin in the esophagus. Given that 5-HT<sub>3,4</sub>-receptor blockers

do not completely disable the effect of large doses of serotonin, we believe that these receptors are ganglionic, and excess serotonin interacts either with presynaptic receptors or with effector 5-HT<sub>1,2</sub> receptors

## Conclusion

We conclude that the regulation of esophageal smooth muscles by a large doses of serotonin involves receptors of ganglionic serotonergic neurons, which expressed 5-HT<sub>3,4</sub> receptors transmitting excitation to 5-HT<sub>1,2</sub> receptors. Activation of these receptors contributes to serotonin stimulation of EMG of the esophageal smooth muscles.

## Acknowledgments

None.

## Conflicts of interest

The authors declare no conflict of any kind.

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