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Regulation of Contractile Activity of the Lower Esophageal Sphincter in Rats by Serotonin

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Abstract: *Serotonin (5-hydroxytryptamine, 5-HT) can stimulate the lower esophageal sphincter (LES) by indirect actions on the modulation of reflexes and by a direct action on smooth muscles.*

Aim:*of this study was to investigate the role of 5-HT and selective 5-HT receptor subtypes in the regulation of the lower esophageal sphincter (LES) in the rat by measuring electromotor activity of the LES.*

Methods:*The i.v. administration of 5-HT was carried out, and the LES contractile activity was evaluated by measuring the amplitude and frequency of the slow waveand spikes of electromyogram (EMG). Electromyography is the technique that uses surface microelectrodes (placed on the skin)near the border between the esophagus and cardia of the stomach.*

Results: *Serotonin administration increased the frequency and the amplitude of the EMG ofthe LES. Selective pharmacological inhibition of separate serotonin 5-HT1-, 5-HT2-, 5-HT3-, and 5-HT4-receptors not completely rule outthe serotonin induced increment of EMG activity of theLES .Joint pharmacological inhibition of thepair of 5-HT1- and 5-HT2 receptors completely–but the pair of 5-HT3- and 5-HT4 receptors only partially inhibited the serotonin induced increment of EMG activity of the LES .*

Conclusion:*These data suggest that serotonin enhancement of the LES contractile activity is mediated by activation of 5-HT1,2, 3, and 4 receptor subtypes. The serotonin induced increment of EMG activity of the LES more fully realized by the pair of effector 5-HT1- and 5-HT2-receptors than by the pair of ganglionary5-HT3- and 5-HT4 receptors*.

Keywords: *Lower esophageal sphincter, serotonin,5-HT1,2,3,4-receptors*

Abbreviations:

5-HT– serotonin

5-HT1-, 5-HT2-, 5-HT3-, and 5-HT4- serotonin receptors

EMG - electromyogram

LES – low esophageal sphincter

1. INTRODUCTION

Functionally the lower esophageal sphincter (LES) is a full value sphincter. But the existence of an anatomical substrate of the LES has been controversial [1, 2]. According to modern concepts the LES is an anatomical structure composed by a relatively weak longitudinal circular and spiral smooth muscle fibers. Apaydin N. et al. demonstrated the presence of an anatomical sphincter that is formed by the thickening of circular muscle fibers. The thickest of these muscle rings was located at the middle of the sphincter. This may explain why the middle of the high pressure zone of the LES has been found to be slightly higher than other parts of this zone. These findings suggest the presence of an anatomical sphincter in the lower esophageal segment which may work as an "internal sphincter"(2].

The LES is located in the so-called "gastroesophageal junction", where the epithelium of the esophagus is replaced by the epithelial cells of the stomach. An important characteristic of the LES is its contractile activity. The dominant cell population of the LES consists of smooth muscle cells that contain the contractile apparatus responsible for the generation of the contractile force. A strict

regulation of contractility of the LES is essential to avoid gastroesophageal reflux and other disfunctions of the upper digestive tract. The smooth muscles of the LESare influenced by various neuronal, hormonal, metabolic, and mechanical factors, including intramural acetylcholine and serotonin (5-hydroxytryptamine, 5-HT). Fibers that constitute the LES are innervated by the inhibitory motor neurons located either locally within the LES or in the esophagus [3, 4]. Inhibitory nerves mediate LES relaxation and the excitatory nerves mediate reflex contraction or rebound contraction of the LES. Mediator of excitatory fibers is acetylcholine, inhibitory-VIP and nitric oxide.

Serotonin is a regulatory and biologically active neurotransmitter in the CNS and peripheral tissues [5, 6, 7]. It is primarily produced and secreted by enterochromaffin cells of the intestine, and it is also stored into platelets, and in mast cells. The biogenic amine induces different mechanical responses (i.e., contraction, relaxation or both) on vascular and non-vascular smooth muscles (gastrointestinal tract, heart, urethra, prostate, uterus) of several mammalian species. The tissue- and species-related variations in 5-HT-induced mechanical responses are due to the multiple 5-HT receptor subtypes and their heterogeneous expression in the different organs. Seven groups (from 5-HT1 to 5-HT7) and more 16 serotonin receptor subtypes on the basis of their structural and signaling features have been classified by IUPHAR [8]. With the exception of 5-HT3 receptor which is coupled with ion channels and is exclusively expressed in neural components, the other 5-HT subtypes are G-protein associated receptors). Messenger RNA for all seven 5-HT receptor types was identified in the sling and clasp fibers of the LES. At the mRNA level, the expression levels were highest for 5-HT_{3A}R and 5-HT_4R, and lowest for 5-HT_{5A}R, 5-HT_6R and 5-HT_7R. At the protein level, the expression levels at the LES were highest for 5-HT_{3A}R and 5-HT_4R, followed by 5-HT_{1A}R and 5-HT_{2A}R; 5-HT_7R was also detected at a low level [6].

5-HT receptors found in the LES smooth muscle cells has been suggested to be endogenous mediators of the LES contractility. The detection of 5-HT receptors in the LES supports the notion that the serotonergic system is an important modulator of the esophageal motility [6]. A rat experiment showed that serotonin plays an important role in the pathogenesis of gastroesophageal reflux disease because a significant increase in serotonin transporter expression and a reduction in 5-HT_4 receptor expression weaken the effects of serotonin, subsequently causing esophageal mucosal damage and esophagitis [7]. The 5-HT1-, 5-HT2-, 5-HT3-, and 5-HT4-receptors are important for esophageal motility and the transient lowering of esophageal sphincter constrictions in humans [9, 10 11]. Despite these findings, the mechanisms of serotonergic regulation of the LESare not fully understood, and, at the same time, the nature of the serotonin receptors mediating the LES contraction has not yet been definitely classified.

The aim of the article is to achieve more precise evaluation of the receptor mechanisms of serotonin influence on the LES contractile activity in rats in vivo.

To achieve this aim, we used the method electromyography (EMG), because EMG reflects the transmembrane electrical activity associated with contractile function of smooth muscles. The role of specific 5-HT receptor subtypes in the contractile function of the rat LES was also investigated using the selective 5-HT receptor antagonists.

2. MATERIALS AND METHODS

2.1. Animals:

The electrophysiological experiments were performed on48femaleWistar rats, weighing 200-220 g and 4-5months of age**.** Animals were divided into 7 groups according to the number series of experiments (6 animals per group).The control group consisted of 6 rats intact. The animals were provided from the Animal Facility of the Russian National Research Medical University, Moscow, Russian Federation. Experiments were carried out in accordance with national ethical guidelines, and the animals were handled in a manner approved by the Institutional Animal Use and Care Committee of the Russian National Research Medical University.

2.2. Surgery:

Animals were placed under the conditions of the surgical stage of Nembutal narcosis (40 mg/kg, intraperitoneally), and medial laparotomy was performed. Access to low 1/3 of the esophagus and the cardi of stomach was opened. The paired electrodes were superimposed on the surface border between the esophagus and the cardia at the projection zone of "z-line". Contact between the electrode tips and

the contact surface was achieved. Control experiments confirming the absence of instrument-derived artifacts were carried out following standard procedures.

2.3. Drugs:

The drugs used in this study were:

Serotonin adipinate (C16H21N2O4. 1% solution). The drug was administered intravenously at a dose of 0.1 mg / kg). Producer: “Lorr” (Russia).

NAS-181. (C19H26N2O42CH3SO3H). Inhibitor of 5-HT1-receptors. Dose i/v used 0.1 mg / kg. Producer: "Sigma" (USA).

SB 204741. (C14H14N4O). Inhibitor of 5-HT2-receptor. Dose i/v used 0.1 mg / kg.Producer: "Sigma" (USA).

MDL 72222. (C15H17C12NO2).Inhibitor of 5-HT3-receptor. Dose i/v used 0.1 mg / kg.Producer: "Sigma" (USA).

RS 39604 hydrochloride.(C26H36CIN3O6S • HC1).Inhibitor of5-HT4-receptor. Dose i/v used 0.1 mg / kg. Producer: "Roche Bioscience" (France).

All drugs were dissolved in physiological 0,9% NaCl solution immediately before use.

2.4. Measurements of The Lower Esophageal Sphincter EMG:

The lower esophageal sphincter EMG was measured using surface bipolar silver electrodes (contact area 1.5-2.0 mm^2, distance between electrodes 1.5 mm) for extracellular recordings.

EMG recording was performed with a 21-channel electroencephalograph (Nihon-Kohden, Neurofax, EEG 4400 series, Washington, DC).

2.5. Statistical Analysis:

The data were analyzed statistically using Statistica-6 software, χ2-test, and Student’s t test at $p<0.05$. The results are summarized as m±SEM.

RESULTS AND DISCUSSION

Serotonin.Serotonin administration enhanced EMA of thelower esophageal sphincter: slow waves frequency increased from 5.8±0.3 to 8.0±0.4 in min (37.9%, p <0.05), the amplitude - from 0.27±0.015 to 0.28±0.013 mV (3.7%, p> 0.05); spike activity also increased: the frequency - increased from 1.0±0.12 to 1.1±0.15 (10.0%, p<0.05), the amplitude - with 0.01±0.001 to 0.10±0.002 mV (900.0%, p <0.01) (table 1).

Table1.*Motor function of the lower esophageal sphincter by the action of serotonin receptor inhibitors*

		фон	serotonin	Inhibitor of 5-HT4	serotonin	Inhibitor of 5-HT3	serotonin
Slow waves	Amplitude	5,8±0,3	8,0±0,4	4,3±0,15	6±0,4	4,7±0,2	6,2±0,3
	Frequency	0,27±0,015	0,28±0,0013	0,25±0,012	0,25±0,013	0,25±0,01	0,27±0,002
Spikes	Amplitude	1,0±0,012	1,1±0,15	2,5±0,5	2,5±0,11	1±0,11	1,7±0,1
	Frequency	0,01±0,002	0,1±0,002	0,01±0,003	0,08±0,002	0,05±0,0003	0,1±0,003

		Inhibitor of 5-HT2	serotonin	Inhibitor of 5-HT1	serotonin
Slow waves	Amplitude	4,8±0,2	4,5±0,22	3,3±0,2	3,5±0,23
	Frequency	0,25±0,0021	0,25±0,01	0,3±0,0012	0,25±0,0018
Spikes	Amplitude	1,5±0,1	1,5±0,017	2±0,21	1,7±0,2
	Frequency	0,08±0,0012	0,09±0,001	0,1±0,02	0,07±0,0025

Serotonin receptors.The next step was selective inhibition of5-HT4-, 5-HT3-, 5-HT2-, and 5-HT1-receptors.There receptors are important for esophageal motility and the transient lowering of esophageal sphincter constrictions in humans [9, 10, 11].Smooth muscles of LES are under control of autonomic nerves and myenteric plexus[12].

5-HT-receptor. In this series of experiments firstpossible involvement of the 5-HT4 receptor of autonomic ganglia in the serotonin regulation ofthe LES contractile activitywas studied. An inhibitor 5-HT4 receptors administration reduced serotonin administration effect on the LES contractile activity. Serotonin administration in this case increased the frequency of slow waves of the LES by 39% (from 4.3±0.15 to 6.0±0.4 min; p <0.05), amplitudeof slow waves the LES remain stable (0.25±0.012 mV, table. 1). The frequency of spikes after serotonin administration on the background of the inhibitorof 5-HT4 receptors introduction also remained stable (2.5±0.1; p<0.05), the amplitude increased from 0.01±0.03to 0.08±0.02 mV (700%, p <0.01). Hence, inhibition of 5-HT4 receptors does not prevent serotonin induced stimulation of the LES contractile activity, but reduces its expression. That is, 5-HT4 receptors of the LESganglionary neurons mediate astimulatory effect of serotonin on the lower esophageal sphincter contractile activity.

5-HT3-receptor. Another receptor of the LES autonomic ganglia is 5-HT3-receptor. An inhibitor 5-HT3-receptors administration reduced serotonineffect on the LES contractile activity. Serotonin administration on the background of the inhibitorof 5-HT3 receptors introduction increased the frequency of slow waves of the LES by 31.9% (from 4.7±0.2 to 6.2±0.3 in min; p<0.05), the amplitude of the slow waves EMA - from 0.25±0.01 to 0.27±0.02 mV (8%, p> 0.05). The frequency of spikes prior to the serotonin administration was 1.0±0.11, amplitude - 0.05±0.003 mV; after the introduction of serotonin frequency of spikes was 1.7±0.1 (70%, p <0.05), the amplitude increased to 0.1±0.03 mV (100%, p <0.01) (table. 1). Hence, blockade of 5-HT3-receptor does not prevent serotonin induced enhancement of thelower esophageal sphincter contractile activity, but only reducedits expression (table. 1). Thus, 5-HT3-receptors mediate stimulatory effect of serotonin on the LES contractile activity.

5-HT2-receptor.The LES contractions depends on smooth muscle properties that lead to opening of L-type Ca(2+) channels; however it can be modulated by enteric motor neurons, the parasympathetic and sympathetic extrinsic nervous system and several neurohumoral substances [13], serotonin including.One of important smooth muscle properties of the LES is expression of 5-HT1,2-receptors. .On isolated strips of canine was shown that the LES contain a contractile 5-HT2 receptors, which isimportant for contractile activity of this esophageal sphincter [9].These 5-HT1- and 5-HT2-receptors may be named as “effector” receptors of the LES.Serotonin directly activates 5HT1, 2-receptorsand induce enhancement of the LES activity.

Serotonin administration on the background of the inhibition of 5-HT2-receptors led to a reduction of slow waves frequency from 4,8±0,2 to 4,5±0,22 in min (6,3%, p> 0,05) at a stable amplitude 0,25 ± 0,021 mV (table 1). The frequency of spikes at the same time did not change and amounted to 1, 5 ± 0, 1, spike amplitude increased from 0, 08 ± 0, 0012 to 0,09 ± 0,001 mV (12.5%, p <0.05). Hence, inhibition of 5-HT2 receptors does not completely exclude the serotonin stimulation of the lower esophageal sphincter, but reduces the degree of its expression. Thus, 5-HT2 receptors are involved in realization of the stimulatory effect of serotonin on the LES.

5-HT1-receptor is another oneof “effector” receptors of the LES. Serotonin administration on the background of the inhibition of 5-HT1-receptors increased frequency and reduced amplitude of slow waves of the LES:frequency - with 3.3±0,2 to 3.5±0.23 min (6.1%, p> 0.05), the amplitude - with 0.3±0.012 to 0.25±0.02 mV (-16.7%, p <0.05). The frequency of spikes decreased from 2.0±0.21 to 1.7±0.2 (-15%, p <0.05), the amplitude - with 0.1±0.02 to 0.07±0.0025 mV (-30%, p <0.05). That isinhibition of 5-HT1 receptors although partially prevented the stimulatory effect of serotonin on the LES contractile activity, andmediate this effect, but not fully control it.

Thus, each of the 5-HT4-, 5-HT3-, 5-HT2- and 5-HT1-receptorsparticipates in realization of serotonin stimulatory effect on motor function of the lower esophageal sphincter but in various degrees. It was important to compare the role of ganglionary and effector serotonin receptors in the implementation of the serotonin stimulatory effect. For this the respective serotonin receptors - 5-HT-3, 4 and 5-HT1, 2 - were inhibited in pairs.

5-HT3- and 5-HT4-receptorsin pair.Simultaneous inhibitionin pair of 5-HT3- and 5-HT4 receptors increased serotonin effect on the LES. Serotonin administration on the background of the inhibition inpair of 5-HT3- and 5-HT4-receptors increased the frequency of slow waves of the LES: from 4.7±0.3 to 6.2±0.34 in min (31.9%, p<0.05) and the amplitude of the slow waves EMA with 0.28±0.012 to 0.33±0.02 mV (17.5%, p<0.05) (table 2). Frequency of spikes increased to 2.2±0.11

(10%, p <0.05), the amplitude - to 0.08±0.021 mV (166.7%, p <0.01). Therefore, the ganglionary 5-HT3- and 5-HT4- receptorsmediated serotonin induced enhancement of the LES contractile activity.

5-HT1- and 5-HT2- receptorsin pair.Further studies were carried out on the involvement the pair of effector 5-HT1- and 5-HT2- receptors in the realization of the investigated phenomenon. Serotonin administration on the background of the inhibition of 5-HT1- and 5-HT2 receptors reduced the frequency of the LES slow waves from 4,7±0,25 to 4,5±0,22 in min (-4.3%, p <0.05), the amplitude - from 0,29±0,02 to 0,27±0,012 mV (-6.9%, p> 0.05). The frequency of spikes prior to the introduction of serotonin was 1,7±0,16, amplitude - 0,04±0,001 mV; after the introduction of serotonin frequency of spikes was 1,5±0,12 (-11,8%, p <0.05), the amplitude of up 0,01±0,002 mV (-75%, p <0.01) (table. 2). Hence, inhibition of 5-HT1- and 5-HT2-receptors eliminates serotonin induced stimulation of the lower esophageal sphincter contractile activity (table 2).

Table2.*Motor function of the lower esophageal sphincter by the action of inhibitors and ganglion serotonin receptors of effector.*

		фон	serotonin	Inhibitors of 5-HT3 and 5-HT4	serotonin	Inhibitors of 5-HT1 and 5-HT2	serotonin
Slow waves	Amplitude	5,8±0,3	8,0±0,4	4,3±0,15	6±0,4	4,7±0,2	6,2±0,3
	frequency	0,27±0,015	0,28±0,0013	0,25±0,012	0,25±0,013	0,25±0,01	0,27±0,002
Spikes	amplitude	1,0±0,012	1,1±0,15	2,5±0,5	2,5±0,11	1±0,11	1,7±0,1
	frequency	0,01±0,002	0,1±0,002	0,01±0,003	0,08±0,002	0,05±0,0003	0,1±0,003

Thus, the pair of 5-HT1- and 5-HT2-receptors completely realized serotonin stimulatory effect on the lower esophageal sphincter contractile activity.

3. CONCLUSION

Lower esophageal sphincter isa thickening of the circular muscle layer of the gastro-esophageal region [2].We had previously used an electrophysiological approach to map the presence and role of functional serotonin in peripheral organs and tissues[14, 15, 16].In this study, we extended this approach to the LES, evaluating its contractile reaction on serotonin administration. We confirm previous studies results that have shown that serotonin can stimulate the lower esophageal sphincter (LES) by a receptor mediated action on smooth muscles.To the best of our knowledge it is first systematic study of 5-HT-receptor mechanisms of the LES contractile activity stimulation. All studied serotonin 5-HT1-, 5-HT2-, 5-HT3-, and 5-HT4-receptors mediatedthe serotonin induced increment of EMG activity of the LES. The serotonin induced increment of EMG activity of theLES morefully realized by the pair of effector 5-HT1- and 5-HT2-receptors than by the pair of 5-HT3- and 5-HT4 receptors. Accordingly pair of effector 5-HT1- and 5-HT2-receptors may be preferable target of therapeutic regulation of lower esophageal contractile activity.

Competing interests: The authors declare that they have no competing interests.

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