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Research Article

Serotonergic Regulation of the Rabbit Bladder and The Distal Colon Contractions *in vivo*

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Abstract

The structure and physiology of the urinary bladder and the distal part of colon rabbits are presented. A smooth muscle of the colon and the bladder distal parts contraction electromyogram was recorded by the multichannel Nihon-Kohden encelograph. The effect of serotonin enhancement of cholinergic effects on smooth muscle contractions was studied by electric field stimulation of the vagus and the serotonergic (as part of the sympathetic trunk) nerve fibers. The serotonin influence on contractile activities of both organs through the 5-HT1-5-HT4 receptors by the relevant inhibitor introduction was studied. It was found that in the bladder as in the colon serotonin contribution to cholinergic influence on contractile activity mediated by the same method: serotonin excited the ganglionary 5-HT3,4-receptors which transmitted excitation on the effector 5-HT1,2 receptors.

Keywords: 5-HT1-5-HT4 Receptors; Bladder and Colon; Cholinergic System; Contraction; Serotonin

Introduction

The urinary bladder and the distal part of colon (the distal colon) belong to the pelvic organs. In recent years, interest in the problems of combined dysfunction of the pelvic organs has increased. In connection with the widespread introduction of new research methods into practice, it became possible to study in depth the etiology and pathogenesis of this pathology. However, despite the progress achieved, this area remains insufficiently studied. Urinary bladder and the distal part colon are controlled by adrenergic (sympathetic), cholinergic (parasympathetic), and non-adrenergic-non-cholinergic (serotonergic, including) nervous fibers. Comorbid dysfunction of the pelvic organs is quite common among the child population [1] and in adulthood [2]. Although the data are scant, some evidence suggests that adult women with Lower Urinary Tract (LUT) symptoms suffered from similar symptoms as children, suggesting a continuation or recurrence of abnormal voiding patterns.

The parasympathetic innervation of the pelvic organs is represented by the vagus nerve (proximal organs), the pelvic nerve (distal organs) and the mixed parasympathetic innervation by the vagus and pelvic nerves, predominating in the median region of the pelvic organs [3]. In most cases, the pathology of the pelvic organs is a functional, caused by dysfunction of various parts of the Autonomic Nervous System (ANS), which leads to combined disorders of the bladder and rectum, which must be examined and treated simultaneously [4,5]. The ANS include of the sympathetic (adrenergic) and parasympathetic (cholinergic) divisions. In our opinion [6], another ANS division is the serotonergic one. Excitation of the sympathetic nerves is responsible for bladder filling. A-adrenoceptors mediate urethra constriction, and activation of β-adrenoceptors results in relaxation of the urethral sphincter muscles [7]. The cholinergic component of bladder control involves two systems, Acetylcholine (ACh) released from parasympathetic nerves and ACh from non-neuronal cells within the urothelium. It is believed that muscarinic receptors and β-adrenoceptors are physiological antagonists for smooth muscle tone in bladder. It was shown that the serotonergic system has a synergistic to parasympathetic one control of the pelvic organs contractility [8]. In human bladder specimens, expressions of 5-HT2B and 5-HT7 receptor mRNAs in the urothelium, detrusor, and whole mucosa were greater than the average expression for all other receptors - 5-hydroxytryptamine (5-HT) receptors 5-HT2A, 5- 5-HT3A, and 5-HT4. 5-HT2B receptor protein was distributed in the apical urothelium and among the detrusor smooth muscle layers. The smooth muscle cells of the bladder express 5-HT1A, 5-HT2, 5-HT3, and 5-HT4 receptors [9,10].

Aim

To study the balance of parasympathetic and serotoninergic parts of the ANS in the regulation of the normal bladder contractile function and contractile function of the distal colon in rabbits *in vivo*.

Materials and Methods

Animals

The electrophysiological experiments were performed on sixteen Chinchilla rabbits, weighing 2.4-3.5 kg, and 5-6 months of age. 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.

Surgery

Animals were placed under the conditions of the surgical stage of Nembutal narcosis (40 mg/kg, intraperitoneally), and inferior-medial laparotomy was performed. Access to the urinary bladder and the colon distal part was opened. The paired electrodes were superimposed on the surface of the urinary bladder and the colon distal part. Contact between the electrode tips and the urinary bladder and colon surface was achieved. Control experiments confirming the absence of instrument-derived artifacts were carried out following standard procedures [11].

Drugs

The drugs used in this study (obtained from the sources indicated) were: Droperidol (PubChemCID:3168) (Droleptan), the blockator of 5-HT3,4-receptors used at the dose of 1.0 mg/kg body weight, was from Gedeon Richter Ltd. Sumatriptan succinate, the inhibitor of 5-HT1-receptors, was from Glaxo Group Research, Ware, UK. Spiperone hydrochloride, blockator of 5-HT2-receptors, was used at a dose of 2 mg/kg body weight, was from Tocris. All drugs were dissolved in physiological 0.9% NaCl solution immediately before use.

Measurements of Urinary Bladder and Distal Colon Electromyograms (EMG)

The urinary bladder and the distal colon EMG were 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).

Electrical Stimulation of Nerves

The EM-42 Medicor (Hungary) electro stimulator was used to stimulate cholinergic and serotonergic nerve fibers (as part of the sympathetic trunk). Electric field stimulation was applied to the peripheral segment of the right vagus nerve. The level of parasympathetic nerve stimulation was sufficiently low (2 msec, 1.5-7.0 V, 10 Hz,), so that the urinary bladder and colon contraction rate remained stable during 60-90 s in each experiment. Electrical stimulation was also applied to the serotonergic fibers contained in the peripheral segment of the left sympathetic trunk.

Methodological Approach

We had previously described and used an electrophysiological approach to test *in vivo* the action of peripheral 5-HT and some other neuromediators on contractile activity of smooth muscles of different organs and tissues. To achieve comparability of research results the same type of experimental animal, the same set of equipment, one set of receptor blockers; electric irritation t was carried out on the same nerve fibres set of the ANS; the subject of the study in both cases - were the smooth muscles of the urinary bladder and the distal colon.

Statistical Analysis

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

Results

The Urinary Bladder

Electric stimulation of the parasympathetic nerve increased the frequency and amplitude of slow waves of the bladder from 7.8±0.5 to 9.1±0.6 /min (16.7 %, $p<0.05$), and from 0.14±0.03 to 0.25±0.04 mV (78.5 %, $p<0.05$), respectively. Thus, irritation of the bulbar part of the parasympathetic system leads to activation of smooth muscle of the bladder. To clarify the possible interaction of serotonergic and parasympathetic systems in the regulation of motility of the bladder the sympathetic trunk and the vagus nerve were simultaneously electric field activated. The significant increase in the frequency and amplitude of slow waves EMG of the bladder occured: frequency increased to 12.9 ± 0.9 (41.7%, $p <0.05$), the amplitude - to 0.3 ± 0,02 mV (20%, $p <0.05$). Thus, coupling stimulation of the serotonergic system creates the synergistic phenomenon of enhancement of the parasympathetic stimulation of bladder motility. Serotoninergic system role in the implementation of this synergistic phenomenon was the next research question that ought to respond. The simultaneous stimulation of the serotoninergic fibers and parasympathetic nerve led to additional strengthening of the frequency and amplitude of slow-wave EMG of the bladder. The frequency increased by 41.7% ($p <0.05$) and amplitude by 20% ($P <.05$). When the blockator of 5-HT3,4 ganglionary receptor droperidol had been previously administered, simultaneous stimulation of the serotoninergic fibers

of the sympathetic trunk and parasympathetic nerve did not change the frequency and amplitude of the slow-wave the bladder EMG.

When blockator of 5-HT1 receptors sumatriptan and blockator of 5-HT2 receptors spiperon had been previously together administered, stimulation of the parasympathetic nerve increased the frequency and amplitude of slow-wave EMG by 19.7% ($p < 0.05$) and 57,1% ($p<0.05$), respectively. Additional stimulation of the serotoninergic fibers did not change the frequency and amplitude of the slow-wave the bladder EMG. Thus, sumatriptan and spiperon inhibited the 5-HT influence on slow- wave EMG of the bladder effector cells. Our experiments indicated that serotoninergic system in the bladder is represented by 5-HT, its ganglionary 5-HT3, 4-receptors and effector 5-HT1,2-receptors, expressed on membrane of smooth muscles. The simultaneous stimulation of the serotoninergic fibers (inside the sympathetic trunk) and parasympathetic nerve enhanced the frequency and amplitude of slow-wave EMG of the bladder.

The Distal Colon

Control irritation of the vagus nerve leads to the development of vagal increase in the slow-wave activity of EMG of the distal colon from 8.6 ± 0.8 to 13.4 ± 0.9 per minute (55.8%, $p < 0.05$) with a stable amplitude - 0.17 ± 0.02 mV. The subsequent connection of sympathetic trunk irritation to irritation of the vagus nerve increases the frequency of EMG slow waves: up to 16.5 ± 2.0 per minute (23.1%, $p < 0.05$) and amplitude - up to 0.31 ± 0.04 mV (82.3%, $p < 0.05$). That is, the connection of stimulation of the sympathetic trunk to irritation of the vagus nerve leads to an increase in the vagal stimulatory effect on the EMG of the distal colon. The study of the possible involvement of ganglionic 5-HT3,4 receptors in the implementation of the studied stimulatory phenomenon was performed using the blocker 5-HT3,4-receptors of droperidol. Irritation of the vagus nerve causes an increase in the frequency-amplitude characteristics of the EMG: frequencies from 8.4 ± 1.0 to 12.1 ± 1.3 per minute (44.0%, $p < 0.05$), amplitudes - 0.13 ± 0.03 to 0.17 ± 0.04 mV (30.7%, $p < 0.05$). The blockade of 5-HT3,4-receptors eliminates the identification of the investigated effect. Subsequent connection of the sympathetic trunk irritation to irritation of the vagus nerve leads to a change in the frequency of slow waves to 10.7 ± 1.0 per minute (-10.8%, $p < 0.05$), and amplitudes up to 0.18 ± 0.04 ($p > 0.1$). Thus, the studied stimulatory phenomenon is carried out by preganglionic serotonergic fibers that transmit excitation to the 5-HT3,4 receptors of ganglionic serotonergic neurons in the distal large intestine. Confirmation of a possible serotonergic mechanism for the implementation of the phenomenon under study was the results of an examination in which 5-HT1,2-receptors were switched off using sumatriptan and spiperone. Against the background of the action of both simultaneously introduced blockers, irritation of the vagus nerve leads to the activation of slow EMG waves from 10.2 ± 1.5 to 14.3 ± 2.0 per minute (42%, $p < 0.05$) with a stable amplitude of 0.15 ± 0.05 mV. Subsequent stimulation of the sympathetic trunk against the background of irritation of the vagus nerve with the 5-HT1,2 receptors turned off does not lead to the development of the studied effect of enhancing vagal activation of the EMG: the frequency of slow waves was 10.1 ± 1.4 per minute, the amplitude was 0.15 ± 0, 04 mV. Thus, the sympathetic nerve amplification effect of the vagal activation of EMA of the distal large intestine is carried out by preganglionic serotonergic fibers, transmitting excitation to 5-HT3,4 ganglion receptors, which in turn activate 5-HT1,2 receptors of effector cells.

Discussion

We have previously shown that serotonin is contained in the vagus nerve and the sympathetic trunk [8]. We believe that the apex of the bladder and the distal colon (sigmoid and rectum) are innervated by the vagus nerve. These data suggest that serotonergic regulation of the pelvic organs can be carried out by serotonergic fibers passing through the vagus nerve and the sympathetic trunk. Isolated irritation of the vagus nerve and the sympathetic trunk does not lead to pronounced serotonergic effects. Only simultaneous stimulation of the sympathetic trunk and the vagus nerve leads to a pronounced serotonergic effect. The serotonergic system - serotonin and its receptors-presents in the bladder and the distal colon. More important, serotonin enhance cholinergic stimulation of contractile activity of the smooth muscles of these organs. The vagus nerve, as was confirmed in this study, innervates the bladder and the distal colon [3,12,13]. At the same time, there is a gradual decrease in the density of vagal innervation in the distal direction in the small pelvis.

Conclusion

The parasympathetic system, its bulbar part, have a stimulatory effect on the motility of the bladder and the distal colon the serotonergic system enhanced the parasympathetic stimulatory effect on the bladder motility as well on the distal colon motility. But the investigated phenomenon magnitude in the distal colon is higher than in the bladder. In both cases the serotonergic system enhances motility of the bladder and the distal colon through the activation of ganglionary 5-HT3, 4 and effector 5-HT1.2 receptors.

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