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Research Article

Balance of Activity of Sympathetic, Parasympathetic, and Serotoninergic Divisions of the Autonomic Nervous System in Rabbits Urinary Bladder

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Abstract

Aim

To study the balance of activity of sympathetic, parasympathetic, purinergic, and serotoninergicparts of the autonomic nervous system (ANS) parts in the regulation of the bladder contractile function in rabbits in vivo.

Materials and methods

Neurogenic response of the detrusor smooth muscle, sympathetic trunk, and right vagus nerve was evoked by electrical field stimulation, and the response was composed by a cholinergic, adreneregic, purinergic, and serotoninergic components. Contractile reaction of the bladder was tested by electromyography method. To address receptor mechanisms of serotonergic regulation of the bladder contractile activity selective inhibitors of serotonin 5-HT1,2,3,4 receptors and exogenous serotonin were used.

Conclusion

The parasympathetic system, it bulbar and sacral divisions, have a stimulatory effect on the motility of all parts of the bladder. The sympathetic system have been the synergistic to parasympathetic stimulatory effect on the bladder motility. Purinergic system apparently does not affect the synergistic effect of ANS on the bladder motility. The serotonergic system has a stimulatory effect on the motility of the bladder through the activation of ganglion 5-HT3,4 and effector 5-HT1.2 receptors.

Keywords: Urinary bladder; Autonomous nervous system, Serotonin; Serotonin receptors

Introduction

The storage and periodic elimination of urine depends on the contractile activity of the bladder and an outlet. Contractile activity of the urinary bladder are controlled by adrenergic (sympathetic), cholinergic (parasympathetic) and non-adrenergic – non-cholinergic (purinergic, nitroxidergic and serotonergic) nervous fibers. Maintenance of continence and a normal bladder micturition cycle involves a complex interaction of cholinergic, adrenergic, purinergic, and serotoninergic systems that is currently little understood [1].

Cholinergic control

Excitatory neurotransmission in the rabbit urinary bladder arises predominantly through activation of parasympathetic nerves [2,3]. The cholinergic component of bladder control involves two systems, acetylcholine (ACh) released from parasympathetic nerves and ACh from non-neuronal cells within the urothelium. The actions of ACh on the bladder depend on the presence of muscarinic receptors that are located on the detrusor smooth muscle, where they cause direct (M_3) and indirect (M_2) contraction [1]. At

the same time, excitatory neurotransmission in the urinary bladder is only partially cholinergic. In rabbit [2] as well as guinea-pig [4], and rat [5] the neurogenic response of the detrusor smooth muscle evoked by electrical field stimulation is composed by a cholinergic and even a larger purinergic component [3].

Adrenergic regulation and its interaction with cholinergic component

It is believed that muscarinic receptors and β-adrenoceptors are physiological antagonists for smooth muscle tone in bladder [6]. Excitation of the sympathetic nerves is responsible for bladder filling. Adrenoreceptors mediate urethra constriction, and activation of β-adrenoreceptors results in relaxation of the urethral sphincter muscles [7].

Indeed (M_4) muscarinic receptors expressed on pre-junctional nerve terminals decrease but activated (M_1) receptors increase the release of noradrenaline (NA) [1]. Muscarinic agonism may attenuate β-adrenoceptor-mediated relaxation more than other contractile stimuli. Chronic treatment with one drug class may regulate expression of the target receptor but also that of the opposing receptor. Prejunctional β2-adrenoceptors can enhance neuronal acetylcholine release [6].

Serotoninergic component

The biogenic amine 5-HT provides a variety of regulatory actions on the smooth muscles of the urinary tract, regulating constriction, relaxation, and both effects simultaneously. De Groat WC et al. summarizes anatomical, neurophysiological, pharmacological, and brain imaging studies in humans and animals that have provided insights into the neural circuitry and neurotransmitter mechanisms controlling the lower urinary tract especially on central nervous system level [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 receptor subtype mRNA- 5-hydroxytryptamine (5-HT) receptors 5-HT2A, 5-HT2B, 5-HT3A, 5-HT4, and 5-HT7 within the urothelium and detrusor of normal bladder tissue.5-HT2B receptor protein was distributed in the apical urothelium and among the detrusor smooth muscle layers [9].The smooth muscle cells of the bladder express 5-HT1A, 5-HT2, 5-HT3, and 5-HT4 receptors [10-13]. 5-HT4 receptors are distributed on the smooth muscle membrane of the guinea pig and human bladder. In monkeys, activation of the 5-HT4 receptors located postsynaptically on the smooth muscle cells of the bladder inhibits its neurogenic constrictions [14].

Purinergic signaling and its interaction with cholinergic component

Purinergic signalling is involved in a number of physiological and pathophysiological activities in the lower urinary tract. Purinergic P1 and P2 receptors induce smooth muscle constriction in the mouse bladder [15]. In the bladder of laboratory animals there are parasympathetic excitatory cotransmission with the purinergic (acting via P2X1 receptors) and cholinergic components being approximately equal [16].

In human bladder the purinergic component of parasympathetic cotransmission is less than 3 %, but in pathological conditions, such as interstitial cystitis, obstructed and neuropathic bladder, the purinergic component is increased to 40 % [16]. As we indicated as early as in year 2005, certain balance of different ANS parts on the functions of visceral organs is observed during weak stimulation of the vagus and sympathetic nerves. The balance may be produced by activation of adrenoceptors in nervous terminals and cardiomyocytes with modulation of ganglionic excitability and involvement of serotonin receptors in addition to acetylcholine and catecholamines [17].

Aim

To study the balance of sympathetic, parasympathetic and serotoninergic parts of the ANS in the regulation of the normal bladder contractile function in rabbits in vivo.

Materials and Methods

Animals

The electrophysiological experiments were performed on twenty Chinchilla rabbits, weighing 2.4-3.3 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 was opened. The paired electrodes were superimposed on the surface of the following parts of the urinary bladder: the tip, the body, and the trigone. Contact between the electrode tips and the urinary bladder surface was achieved. Control experiments confirming the absence of instrument-derived artifacts were carried out following standard procedures (Ballaro, 2008).

Drugs

The drugs used in this study (obtained from the sources indicated) were: Droperidol (PubChemCID:3168) (Droleptan), used at the dose of 1.0 mg/kg body weight, was from Gedeon Richter Ltd. Sumatriptansuccinate from Glaxo Group Research, Ware, UK. All drugs were dissolved in physiological 0.9% NaCl solution immediately before use. Spiperone hydrochloride, used at a dose of 2 mg/kg body weight, was from Tocris. Theophylline (Theotard), used at doses of 20-80 mg/kg body weight, was from Krka.

Measurements of urinary bladder EMG

The urinary bladder EMG was measured using surface bipolar silver electrodes (contact area 1.5 - 2.0 mm2, 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 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.

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 and Discussion

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 [17-20]. In this study, we extended this approach to the urinary bladder parts, evaluating the effect of electric stimulation of sympathetic trunk (e.g., serotoninergic fibers in it) on EMG induced by parasympathetic nerves (the vagus and the pelvic nerve) electric stimulation. The presence of 5-HT in the sympathetic trunk has been well ascertained by both direct and indirect (functional) methods [20]. Thus, it appears reasonable to assume that activation of the sympathetic trunk leads to excitation of the serotonergic fibers and subsequent 5-HT release.

The top of the bladder

To avoid the unnecessary doubling results description it was limited to only one part of the rabbit blasser, namely – the top of the bladder (the bladder's top). Electric stimulation of the parasympathetic nerve increased the frequency and amplitude of slow waves of the bladder's top from 8.2±0.5 to 11.7±3.3 /min (42.7 %, $p<0.05$), and from 0.16±0.03 to 0.26±0.04 mV (62.5 %, $p<0.05$), respectively. Thus, irritation of the bulbar part of the parasympathetic system leads to activation of smooth muscle of the bladder's top.

To clarify the possible interaction of sympathetic and parasympathetic systems in the regulation of motility of the bladder the sympathetic trunk and the vagus nerve were simultaneously activated. The significant increase in the frequency and amplitude of slow waves EMA of the bladder's top occured: frequency increased to 16.1 ± 2.8 (37.6%, $p <0.05$), the amplitude - to 0 5 ± 0, 05 mV (92.3%, $p <0.05$). Coupling stimulation of the sympathetic system creates the synergistic phenomenon of enhancement of the parasympathetic stimulation of bladder's top motility.

The role of the purinergic system in the implementation of this synergistic phenomenon was the follow research question. Against the background of the simultaneous activation of the sympathetic and parasympathetic systems the inhibitor of P1,2 receptors teophillin was used. According to the study, the analyzed synergistic phenomenon theophylline did not inhibit. Serotoninergic system role in the implementation of this synergistic phenomenon was the last 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 EMA of the top of the bladder. The frequency increased by 37.6% ($P <.05$) and amplitude by 92.3% ($P <.05$). When 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 EMA of the bladder top. When sumatriptan had been previously administered, stimulation of the para¬sympathetic nerve increased the frequency and amplitude of slow-wave EMAby 66.5% ($P <.05$) and 11% ($P <.05$), respectively. Additional stimulation of the serotoninergic fibers did not change the frequency and amplitude of the slow-wave EMA of the bladder top. Thus, sumatriptan inhibits the 5-HT influence on slow- wave EMA of the bladder top.

In our experiments serotoninergic system was represented by 5-HT, its ganglion 5-HT3, 4-receptors and effector 5-HT1,2-receptors, expressed on membrane of smooth muscles Serotonin. The simultaneous stimulation of the serotoninergic fibers (inside the sympathetic trunk) and parasympathetic nerve enhanced the frequency and amplitude of slow-wave EMA of the bladder's

top. The frequency increased by 37.6% (P <.05) and amplitude by 92.3% (P <.05). Thus the synergistic effect was achieved with endogenous 5-HT participation. It was indirect method administration of serotonin.

The same was true if administration of 5-HT was made directly. Exogenous direct 5-HT administration enhanced the parasympathetic stimulatory influence on the EMA of the bladder's top. The EMA slow-wave frequency of the bladder's top smooth muscles increased from 11.3 ± 2.4 /min with vagus stimulation to 14.2 ± 2.8 /min after 5-HT administration. The corresponding increase in the amplitude was from 0.23 ± 0.02 to 0.46 ± 0.03 mV (100%, P <.05). Thus the synergistic effect of serotonin is significant and proved itself independently from method of 5-HT administration.

5-HT3,4-receptors. Droperidol is inhibitor of 5-HT3, 4 receptors. When droperidol had been previously administered, the synergistic effect from following simultaneous stimulation of the serotoninergic fibers of the sympathetic trunk and parasympathetic nerve was disappeared. Thus 5-HT3, 4 receptors participate in the realization of the synergistic effect of serotonergic and cholinergic systems interaction. 5-HT1, 2 receptors. Sumatriptan is inhibitor of 5-HT1,2 receptors. When sumatriptan had been previously administered, the synergistic effect from following simultaneous stimulation of the serotoninergic fibers of the sympathetic trunk and parasympathetic nerve was disappeared. Thus 5-HT1, 2 receptors participate in the realization of the synergistic effect of serotonergic and cholinergic systems interaction.

5-HT2 receptors. Spiperone is inhibitor of 5-HT2 receptors. When spiperone had been previously administered, the synergistic effect from following simultaneous stimulation of the serotoninergic fibers of the sympathetic trunk and parasympathetic nerve was absent. Thus 5-HT2 receptors participate in the realization of the synergistic effect of serotonergic and cholinergic systems interaction. Similar results were obtained in the bladder's body as in the bladder's trigone (Table 1).

Bladder parts			parasympathetic (vagus. pelvic) nerve		sympathetic trunk and parasympathetic (vagus. pelvic) nerves	
	Frequency. min-1	Amplitude. mV	Frequency. min-1	Amplitude. mV	Frequency. min-1	Amplitude. mV
Before to the administration of droperidol. sumatriptan. and spiperone						
Top	8.2±0.5	0.16±0.03	11.7±3.3*	0.26±0.04*	16.1±2.8*	0.5±0.05*
Body	7.8±0.6	0.14±0.03	9.2±0.7*	0.25±0.03*	13.0±2.7*	0.3±0.04*
Trigone	8.8±0.9	0.12±0.02	11.7±1.7*	0.25±0.04*	12.7±1.8*.	0.25±0.04*
On the background of droperidol						
Top	10.4±1.9	0.19±0.03	13.3±2.1*	0.28±0.03*	12.3±2.9*	0.23±0.03*
Body	7.0±0.6	0.11±0.02	12.5±0.8*	0.20±0.08*	12.5±0.8*	0.3±0.09*
Trigone	7.3±0.6	0.2±0.02	10.3±0.9*	0.25±0.03*	10.1±0.8*	0.2±0.03*
On the background of sumatriptan						
Top	7.5±0.8	0.18±0.02	12.5±3.0*	0.2±0.02*	13.0±3.5*	0.21±0.02*
Body	7.1±0.8	0.15±0.02	8.5±0.8*	0.22±0.02*	12.3±2.4*	0.21±0.02*
Trigone	6.5±0.5	0.2±0.02	12.5±3.0*	0.25±0.03*	10.2±1.0*	0.23±0.02*
On the background of spiperone						
Top	8.0±0.3	0.13±0.01	13.3±0.9*	0.22±0.03*	-	-
Body	8.1±0.7	0.12±0.02	9.5±0.8*	0.21±0.02*	-	-
Trigone	8.8±0.5	0.15±0.01	10.3±0.4*	0.22±0.02*	-	-

Table 1: Electromotor activity of bladder departments. Before to stimulation of nerves. during stimulation of the vagus (pelvic) nerve and jointly stimulation of the sympathetic trunk and the vagus (pelvic) nerves

Conclusion

The parasympathetic system, it bulbar and sacral divisions, have a stimulatory effect on the motility of all parts of the bladder. The sympathetic system has the synergistic to parasympathetic stimulatory effect on the bladder motility. Purinergic system apparently does not affect the synergistic effect of ANS on the bladder motility. The serotonergic system has a stimulatory effect on the motility of the bladder through the activation of ganglion 5-HT3, 4 and effector 5-HT1.2 receptors.

Note: Stimulation of the vagus nerve has been used in the study of

electromotive activity of the bladder's top and body and stimulation of the pelvic nerve in the study of electromotive activity of bladder's trigone. *p<0.05

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