Effect of Serotoninergic and Cholinergic Nervous Systems and Corresponding Neurotransmitters on Heart Function and Motor Activity of Pelvic Smooth Muscle Organs

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Serotonin potentiated vagal negative chronotropic effect in rabbits and increased the synergistic action of autonomic nervous structures on cardiac function in frogs. Acetylcholine in low doses produced a positive chronotropic effect in frogs, which was related to activation of intracardiac adrenergic neurons. Simultaneous activation of the serotoninergic and cholinergic system suppressed heart function, but increased motor activity of pelvic smooth muscle organs.

Key Words: *serotonin; acetylcholine; heart; urinary bladder; ureter; uterus; seminal duct*

Serotonin (5-hydroxytryptamine, 5-HT) can inhibit or stimulate heart activity [5,6,8]. Acetylcholine in various concentrations has both the inhibitory and stimulatory effect (rarely observed) on heart function [2]. There is no agreement about the mechanism of different effects of neurotransmitters on heart activity. The mechanism of a unidirectional effect of the autonomic nervous system on motor activity of pelvic smooth muscle organs remains unclear [1,3,4,7].

Here we studied the effect of serotoninergic and cholinergic nervous systems and neurotransmitters on heart function and motor activity of pelvic smooth muscle organs.

MATERIALS AND METHODS

Experiments were performed on 42 rabbits narcotized with hexenal. Stimulation of the right stellate ganglion and left vagus nerve (VN) was performed under conditions of β-adrenoceptor blockade with 1-3 mg/kg propranolol. 5-HT in concentrations of 10^{-4}-10^{-7} g/liter (0.1-0.3 ml) not producing hemodynamic effects was administered in three routes: during VN stimulation (n=15); after simultaneous stimulation of the stellate ganglion and VN with little or no synergistic effect (n=15); and 2 h before the study (other animals).

Preliminary experiments showed that simultaneous stimulation of the stellate ganglion and VN potentiates the vagal chronotropic effect (70%). This phenomenon was blocked with ganglionic and peripheral 5-HT receptor antagonists.

The effect of acetylcholine in various concentrations was studied in 33 frogs with completely destroyed CNS. The heart was perfused with Ringer—Lock solution containing various pharmacological agents via a cannula inserted into the heart ventricle through the left aortic arch. Heart activity was recorded using an Elema pressure transducer. Acetylcholine was used in concentrations of 10^{-6}-10^{-22} mmol/liter.

Experiments on pelvic organs were performed on Chinchilla rabbits weighing 3.5-4.0 kg. The animals were narcotized with nembutal. Simultaneous stimulation was applied to the right VN and left sympathetic trunk (ST). Electromotor activity (EMA) of the urinary bladder, ureter, uterus, fallopian tubes (fundus of the uterus), and seminal duct was recorded. Pharmacological study of the studied effects was performed with antagonists of purinergic receptors (theophylline, 20-80 mg/kg), 5-$HT_{3,4}$ receptors (droperidol, 0.5-1.0 mg/kg), and 5-$HT_{1,2}$ receptors (sumatriptan, 0.5-1.0 mg/kg).

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RESULTS

5-HT in a dose of 10^{-4}-10^{-7} g/liter (0.1-0.3 ml) produced no inotropic and chronotropic effects on the heart. Administration of 5-HT during VN stimulation potentiated (55.5% cases, Fig. 1), did not modify (14.4%), or abolished the negative chronotropic effect on heart activity (30.1% cases). 5-HT was administered after simultaneous stimulation of the stellate ganglion and VN. Under these conditions autonomic nervous structures had little or no synergistic inhibitory effect. Further stimulation of the stellate ganglion and VN was followed by the appearance (80 *vs*. 70% in the control) and/or potentiation of the effect (15 *vs*. 3-7% in the control). Experiments with 5-HT administration 2 h before stimulation of the stellate ganglion and VN revealed a significant increase in the degree and incidence of the effect (89%). These results show that 5-HT potentiates the vagal negative chronotropic effect and increases the synergistic action of VN and stellate ganglion on cardiac function.

Perfusion of the intact heart with acetylcholine in a concentration of 10^{-18}-10^{-22} mmol/liter produced weak positive inotropic and chronotropic effects (5-13%) in 50% samples. This effect persisted in the presence of atropine, but was abolished during perfusion of the heart with ganglionic blocker benzohexonium and β-adrenoceptor antagonist propranolol. However, acetylcholine in low concentrations sharply inhibited heart function under conditions of simultaneous perfusion with atropine, propranolol, and benzohexonium. Thus, acetylcholine in low concentrations improved heart function due to activation of ganglionic adrenergic neurons transmitting excitation to myocardial β-adrenoceptors. During blockade of intracardiac ganglia and muscarinic cholinergic receptors, the inhibitory effect of acetylcholine in low concentrations could be related to activation of α-adrenoceptors.

Perfusion of the heart with acetylcholine in high concentrations (10^{-6}-10^{-10} mmol/liter) under conditions of atropine treatment produced strong positive inotropic (300%) and chronotropic effects (10-16%). Addition of the ganglionic blocker into the perfusion solution did not abolish and even potentiated the positive inotropic and chronotropic effect. This phenomenon was not observed after administration of β-adrenoceptor antagonist.

These data indicate that the stimulatory effect of acetylcholine on heart activity can be realized via activation of intracardiac adrenergic neurons and stimulation of sympathetic terminals synaptically coupled to β-adrenoceptors.

VN stimulation increased the frequency of EMA slow waves in the urinary bladder. Simultaneous stimulation of ST and VN potentiated the vagal stimulatory effect on EMA of the urinary bladder. We observed an increase in the frequency and amplitude of EMA slow waves to 31 min^{-1} and 0.25 mV, respectively (Fig. 2, *a*; Table 1). The involvement of purinergic systems in the realization of this phenomenon was studied using the purinoceptor antagonist theophylline. Simultaneous stimulation of ST and VN after treatment with theophylline increased the frequency of EMA slow waves in the urinary bladder (Fig. 2, *a*) and ureter (synergistic stimulatory effect). Therefore, purinergic structures did not play a role in the realization of this effect. A possible serotoninergic mechanism of the effect was studied with the ganglionic 5-$HT_{3,4}$ receptor antagonist droperidol. VN stimulation increased the frequency and amplitude of slow waves. Simultaneous stimulation of ST and VN potentiated the vagal stimulatory effect on EMA slow waves. Administration of droperidol was accompanied by a slight decrease in bioelectric activity of smooth muscles in the urinary bladder. VN stimulation after treatment with droperidol produced an activating effect on EMA slow waves in the urinary bladder. EMA remained unchanged after simultaneous stimulation of ST and VN. Therefore, this phenomenon involved 5-HT_3 receptors on intramural neurons. The role of urinary

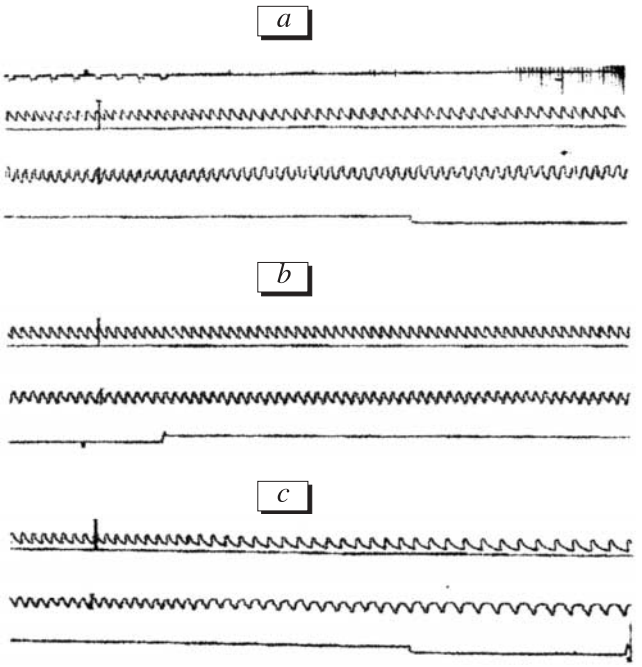

Fig. 1. Stimulation of the right stellate ganglion and left vagus nerve (VN): no effect (*a*); serotonin administration, no inotropic and chronotropic effect (*b*); stimulation of the stellate ganglion and VN, effect (*c*). Indications in each fragment (from top to bottom): time mark (1 sec), VN stimulation; blood pressure in the right carotid artery; zero line; impedance recorded from the anterior wall of the left ventricle; serotonin administration and stimulation of the stellate ganglion. Scale in each fragment (from top to bottom): 1-100 mm Hg; 1-100 Ω.

bladder 5-$HT_{1,2}$ receptors in the realization of this effect was studied using the 5-$HT_{1,2}$ receptor antagonist sumatriptan. After treatment with 5-$HT_{1,2}$ receptor antagonists, VN stimulation increased motor activity of the urinary bladder. Simultaneous stimulation of ST and VN had no effect on motor activity of the urinary bladder and ureter under these conditions. These results indicate that ST potentiates the vagal stimulatory effect on EMA of the urinary bladder via activation of 5-$HT_{1,2}$ receptors in the effector tissues.

The nonpregnant uterus is characterized by slow waves of EMA with a frequency and amplitude of 8.6±1.1 min^{-1} and 0.16±0.04 mV, respectively. VN stimulation increased the frequency and amplitude of EMA slow waves in the uterus to 11.2±1.5 min^{-1} (by 30%, $p<0.01$) and 0.26±0.09 mV (by 61%, $p<0.05$), respectively. Simultaneous stimulation of ST and VN potentiated the vagal stimulatory effect on EMA of the uterus. The frequency and amplitude of slow waves increased by 44 (16.1±2.4 min^{-1}, $p<0.05$) and 8% (from 0.26±0.09 to 0.28±0.01 mV, Fig. 2, *b*), respectively. Similar results were obtained in studying the right and left fallopian tubes (Table 2). Therefore, simultaneous stimulation of ST and VN increases EMA of female smooth muscle sex organs.

Treatment with the purinoceptor antagonist theophylline in a dose of 30-80 mg/kg did not block and even potentiated this effect in the uterus. Under these conditions the amplitude of fast waves increased by 22%. The amplitude and frequency of slow waves increased by 22 (from 0.18±0.05 to 0.22±0.04 mV, $p<0.05$) and 74% (from 15.0±2.6 to 26.1±4.5 min^{-1}, $p<0.05$), respectively.

Administration of the 5-$HT_{3,4}$ receptor antagonist droperidol and 5-$HT_{1,2}$ receptor antagonist sumatriptan abolished this effect (Fig. 2, *b*). VN stimulation after treatment with droperidol increased the frequency of EMA in the uterus from 9.9±1.6 to 14.8±2.5 min^{-1}. Simultaneous stimulation of ST and VN decreased the frequency and amplitude of EMA to 12.0±3.8 min^{-1} and 0.12±0.04 mV, respectively. Thus, droperidol blocked this effect.

Before treatment with sumatriptan, simultaneous stimulation of ST and VN increased the frequency and amplitude of EMA to 18.4±2.2 min^{-1} and 0.25±0.05 mV, respectively. However, simultaneous stimulation

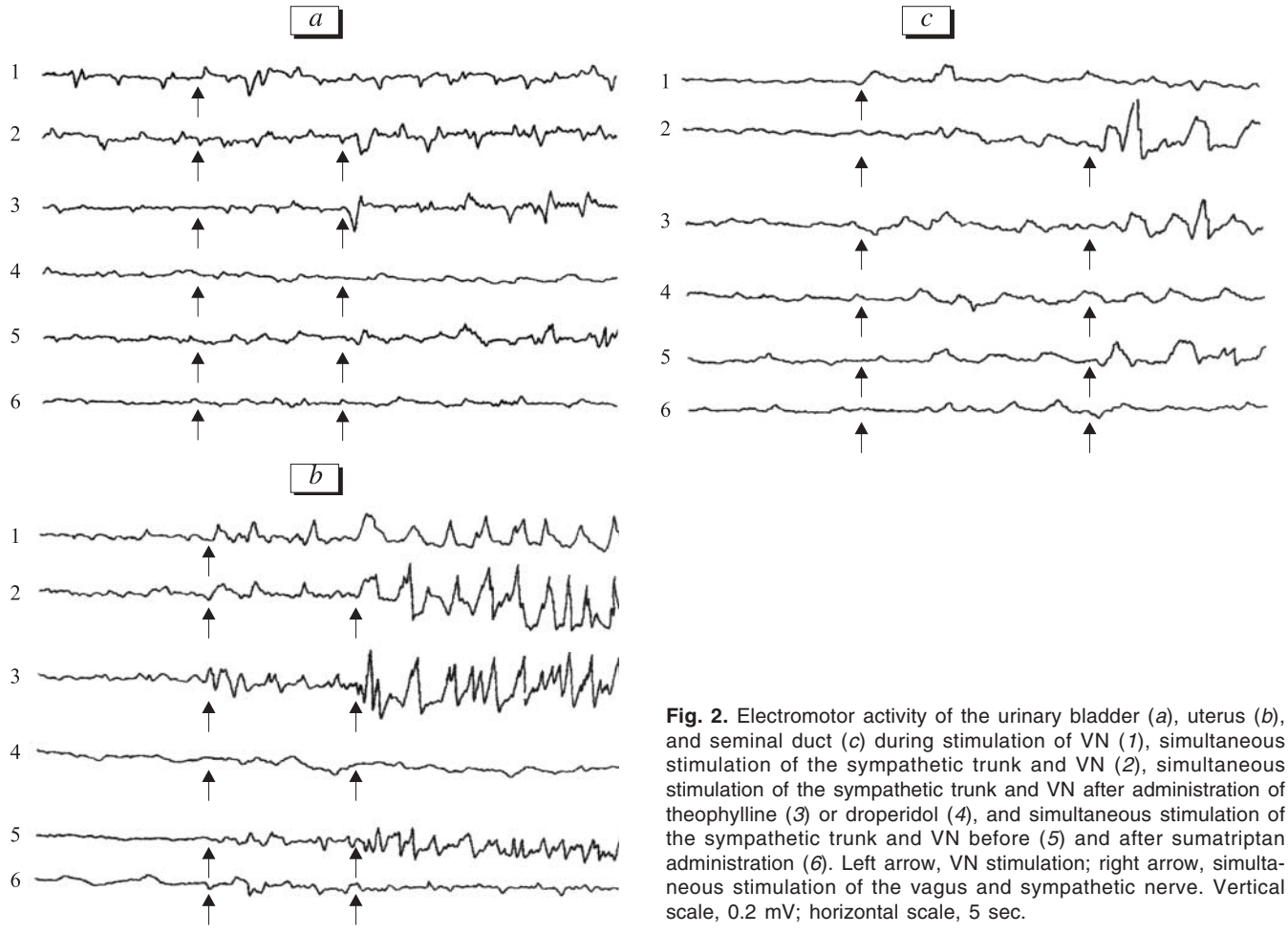

Fig. 2. Electromotor activity of the urinary bladder (*a*), uterus (*b*), and seminal duct (*c*) during stimulation of VN (*1*), simultaneous stimulation of the sympathetic trunk and VN (*2*), simultaneous stimulation of the sympathetic trunk and VN after administration of theophylline (*3*) or droperidol (*4*), and simultaneous stimulation of the sympathetic trunk and VN before (*5*) and after sumatriptan administration (*6*). Left arrow, VN stimulation; right arrow, simultaneous stimulation of the vagus and sympathetic nerve. Vertical scale, 0.2 mV; horizontal scale, 5 sec.

TABLE 1. EMA of the Right and Left Ureter during Stimulation of VN Alone or in Combination with ST (*M*±*m*)

Ureter	Baseline EMA, min^{-1}		VN stimulation		Stimulation of VN and ST	
	frequency	%	frequency	%	frequency	%
Right	17.5±1.6	36.6	24.0±1.9	37.1	40.5±3.8*	68.8
Left	12.8±1.3		19.0±1.8	48.4	28.4±2.3*	49.5

Note. Here and in Table 2: *$p<0.05$ compared to baseline EMA.

TABLE 2. EMA of the Right and Left Fallopian Tubes before and after Stimulation of VN Alone or in Combination with ST (*M*±*m*)

Fallopian tube	Baseline EMA, min^{-1}		VN stimulation		Stimulation of VN and ST	
	frequency	%	frequency	%	frequency	%
Right	31.4±4.2	57.0	45.6±4.4	45.2	54.7±4.6*	19.8
Left	20.0±2.8		26.2±2.3	31.0	31.5±2.7*	20.0

of ST and VN after sumatriptan administration did not increase and even decreased the frequency of EMA slow waves in the uterus to 10.0±1.2 min^{-1}. Therefore, the 5-$HT_{1,2}$ receptor antagonist abolished the synergistic effect in the uterus. These data indicate that the sympathetic nerve potentiates vagal stimulation of EMA in the uterus, which is realized via blockade of presynaptic serotoninergic fibers transmitting excitation to intramural serotoninergic neurons and 5-$HT_{1,2}$ receptors in the effector organs.

EMA of right-sided smooth muscle organs (right ureter and right fallopian tube) was higher than that of left-sided organs (left ureter and left fallopian tube) by 36.6 and 57.0%, respectively (Tables 1 and 2). The synergistic action of autonomic nervous structures increased EMA of the right ureter, left ureter, right fallopian tube, and left fallopian tube by 131.4, 121.9, 74.2, and 57.5%, respectively.

EMA of the right ureter and right fallopian tube surpassed that of the urinary bladder and uterus. The frequency of EMA slow waves in these organs was 12.5±4.8 and 8.6±1.1 min^{-1}, respectively. It can be hypothesized that EMA of the right ureter and right fallopian tube contacting with the urinary bladder and uterus, respectively, provides the baseline biological rhythm of organs under rest conditions and during the synergistic action of autonomic nervous structures.

EMA of the seminal duct was characterized by slow waves with a frequency and amplitude of 9.3±3.6 min^{-1} and 0.20±0.02 mV, respectively. VN stimulation increased the frequency and amplitude of EMA to 13.2±3.9 min^{-1} (42%, $p<0.05$) and 0.23±0.05 mV (15%, $p<0.01$), respectively. Simultaneous stimulation of ST and VN increased the frequency and amplitude of EMA to 18.5±4.5 min^{-1} (40%, $p<0.05$) and 0.27±0.05 mV (17%, $p<0.01$), respectively.

We studied a possible purinergic mechanism of this phenomenon. VN stimulation after administration of theophylline increased the frequency and amplitude of EMA slow waves to 11.0±1.3 min^{-1} (77%, $p<0.01$) and 0.23±0.04 mV (15%, $p<0.01$), respectively. Simultaneous stimulation of ST and VN potentiated the vagal stimulatory effect on EMA. Under these conditions we observed an increase in the frequency and amplitude of EMA to 17.6±3.8 min^{-1} (60%, $p<0.05$) and 0.23±0.04 mV (15%, $p<0.01$), respectively. The data indicate that purinergic structures are not involved in the realization of this effect (Fig. 2, *c*).

We studied the role of 5-$HT_{3,4}$ receptors in the realization of this effect. Simultaneous stimulation of ST and VN after treatment with droperidol was not accompanied by changes in EMA of the seminal duct. Under these conditions the frequency and amplitude of EMA slow waves were 12.0±4.0 min^{-1} and 0.20±0.02 mV, respectively. Therefore, this effect was realized via 5-$HT_{3,4}$ receptors (Fig. 2, *c*).

Simultaneous stimulation of ST and VN before sumatriptan administration increased the frequency of EMA slow waves to 23.5±3.5 min^{-1} (47%, $p<0.05$). The frequency of slow waves observed during VN stimulation was 16.0±2.0 min^{-1}. The 5-$HT_{1,2}$ receptor antagonist sumatriptan had no effect on vagal stimulation of EMA, but completely blocked this effect (Fig. 2, *c*). The frequency and amplitude of slow waves in bioelectric activity of the seminal duct were 9.0±1.5 min^{-1} and 0.18±0.03 mV, respectively. After VN stimulation the frequency of slow waves reached 13.5±1.6 min^{-1} (150%, $p<0.05$). It should be emphasized that the frequency and amplitude of EMA slow waves decreased

to 11.0±1.0 min^{-1} and 0.15±0.05 mV, respectively, during simultaneous stimulation of ST and VN.

These results indicate that the sympathetic nerve potentiates vagal stimulation of EMA in the seminal duct, which is realized via preganglionic serotoninergic fibers transmitting excitation to intramural serotoninergic neurons and 5-$HT_{1,2}$ receptors in the effector organs.

Our study showed that 5-HT potentiates the vagal negative chronotropic effect and increases the synergistic action of autonomic nervous structures on cardiac function. Acetylcholine in low doses improves heart function due to activation of intracardiac adrenergic neurons. Simultaneous stimulation of serotoninergic and cholinergic systems suppresses heart function and decreases motor activity of pelvic smooth muscle organs.

Structures of the autonomic nervous system produce a synergistic stimulatory effect on motor activity of the uterus and seminal duct. These data indicate that the serotoninergic nervous system plays a role in reproductive processes.

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