Gradients of Serotoninergic Innervation of the Large Intestine

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Experiments were performed on nembutal-narcotized rabbits. To study motor activity of the large intestine stimulation was applied to the right vagus nerve and left sympathetic trunk, as well as to the right pelvic nerve and left sympathetic trunk. We found a decreasing gradient of serotoninergic innervation from the ascending colon to the transverse colon and rectum (45-50, 30-35, and 25-30%, respectively) during simultaneous stimulation of the sympathetic trunk and vagus nerve and an increasing gradient of serotoninergic innervation in the large intestine during simultaneous stimulation of the sympathetic trunk and pelvic nerve. Stimulation of serotoninergic fibers was accompanied by arterial spasm in the microcirculatory bed and venous congestion.

Key Words: *large intestine; gradients of serotoninergic innervation; sympathetic and parasympathetic nervous systems*

The sympathetic and parasympathetic systems can produce similar effects on the function of internal organs. The mechanisms of synergism between various parts of the autonomic nervous system (ANS) regulating activity of the same organ or system are poorly understood.

Apart from catecholamines and acetylcholine, sympathetic and parasympathetic nerve endings can release purines [1], histamine [9], and serotonin [5]. These bioactive substances are involved in the interaction between various parts of ANS. Serotonin was found in the vagus nerves and sympathetic trunk (ST), which suggests the involvement of this bioactive amine in co-directed regulatory effect of various parts in ANS. Published data show that administration of serotonin induces contraction of smooth muscles of the gastrointestinal tract [2-4,6-8].

Here we studied serotoninergic innervation of the large intestine and smooth muscles of the vascular system.

MATERIALS AND METHODS

Acute experiments were performed on 12 rabbits. Electromotor activity (EMA) in the ascending, transverse, and descending colon and rectum was recorded extracellularly with bipolar needle platinum or silver macroelectrodes (diameter 0.3 mm, length 0.5 mm, interelectrode distance 1.5 mm) using a preamplifier.

Each experiment was performed according to the following scheme: narcotization of animals; preparation of vessels, nerves, and organs; baseline recordings; control stimulation of the vagus and pelvic nerves and ST to estimate functional activity of organs and reveal usual effect of nerve stimulation on organs; simultaneous stimulation of the parasympathetic nerve and ST to study the phenomenon underlying similar effects of different parts of ANS; and pharmacological analysis of the mechanisms of this phenomenon (blockade of ganglionic and effector receptors with pharmacological preparations).

The peripheral end of the vagus nerve and ST were stimulated at the level of the fifth cervical vertebrae using bipolar platinum electrodes. Stimulation of the pelvic nerve was applied at the level of L3-S1. During simultaneous stimulation, ST was stimulated 5-10 sec after stimulation of the vagus or pelvic nerve. This period corresponded to permanent increase in the amplitude and frequency of slow waves in EMA of the large intestine. Stimulation was applied to the vagus and pelvic nerves (40-60 sec) and ST (10-20 sec). The interval between simultaneous stimulation of nerves

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was 10-20 min. Control stimulation of the vagus nerve and sympathetic ganglion was applied at an interval of 1-10 min. The nerves were stimulated with rectangular impulses (10-20 Hz frequency, 1.5-3.0 msec pulse duration, and amplitude of 5-15 V for ST and 1-7 V for vagus and pelvic nerves) on a Medicor-St-02 electrical stimulator. EMA was recorded on a Mingograf-82 device.

The neurotransmitter mechanism of the studied effects was estimated using serotonin receptor blockers droperidol (ganglionic receptor blocker) and sumatriptan (peripheral receptor blocker) in a dose of 0.5-1.0 mg/kg.

EMA of the large intestine was induced by stimulation of the peripheral segment in the vagus nerve alone or in combination with ST. EMA of the large intestine was also recorded upon simultaneous stimulation of ST and pelvic nerve.

Baseline EMA of organs was recorded in series I. Control stimulation was applied to the peripheral cervical segment of the vagus nerve. Optimal parameters of electrical stimulation (vagal optimum) were selected so that EMA exceeded baseline activity not more than by 3 times. Besides this, continuous stimulation of the vagus nerve (up to 1.0-1.5 min) was not accompanied by an increase in the frequency of slow waves and spike activity. Control stimulation was applied to cervical ST. In the follow-up period we simultaneously stimulated the vagus nerve and ST.

The role of ganglionic serotonin receptors ($5\text{-}HT_{3,4}$) and pre- and postganglionic fibers in a synergistic effect of the vagus and sympathetic nerves on EMA of effector organs was studied in series II using droperidol. The involvement of peripheral serotonin receptors ($5\text{-}HT_{1,2}$) in the mechanisms of studied effect was evaluated with antagonist sumatriptan.

For histological examination, biopsy specimens from various portions of the large intestine were routinely fixed in 10% neutral formalin, dehydrated in alcohols, and embedded into paraffin. Histological sections were stained with hematoxylin and eosin.

The results were analyzed by Student's *t* test.

RESULTS

The baseline frequency and amplitude of EMA slow waves in the ascending colon were 6.3±0.5 spikes per min and 0.18±0.02 mV, respectively. Stimulation of the vagus nerve was followed by an increase in the frequency and amplitude of EMA slow waves to 7.7± 0.6 spikes per min (22%, $p<0.05$) and 0.25±0.02 mV (38%, $p<0.05$), respectively. Low-amplitude spike activity was present in every third recording (amplitude 0.2 mV, frequency 0.25 spikes per 100 slow waves).

Simultaneous stimulation of ST and vagus nerve increased the frequency and amplitude of EMA slow waves to 8.9±0.7 spikes per min (15.6%) and 0.27± 0.03 mV, respectively. Spike activity had low amplitude (0.2 mV), while the frequency increased to 0.4 spikes per 100 low waves (50%). These data show that simultaneous stimulation of ST and vagus nerve potentiated the vagal stimulatory effect on EMA in the ascending colon of the large intestine.

The baseline frequency and amplitude of EMA slow waves in the transverse colon were 5.5±0.6 spikes per min and 0.18±0.02 mV, respectively. Stimulation of the peripheral segment in the vagus nerve increased the frequency and amplitude of EMA slow waves to 7.3±0.6 spikes per min (32.7%) and 0.23± 0.02 mV (26%, $p<0.05$), respectively. Low-amplitude spike activity was detected in every second recording (amplitude 0.15 mV, frequency 0.28 spikes per 100 slow waves).

Simultaneous stimulation of ST and vagus nerve increased the frequency of EMA slow waves to 8.2± 0.7 spikes per min (12.3%), but had no effect on the amplitude. The frequency and amplitude increased to 0.34 spikes per 100 slow waves (21.4%) and 0.25 mV, respectively. Simultaneous stimulation of ST and vagus nerve potentiated the vagal stimulatory effect on EMA in the transverse colon.

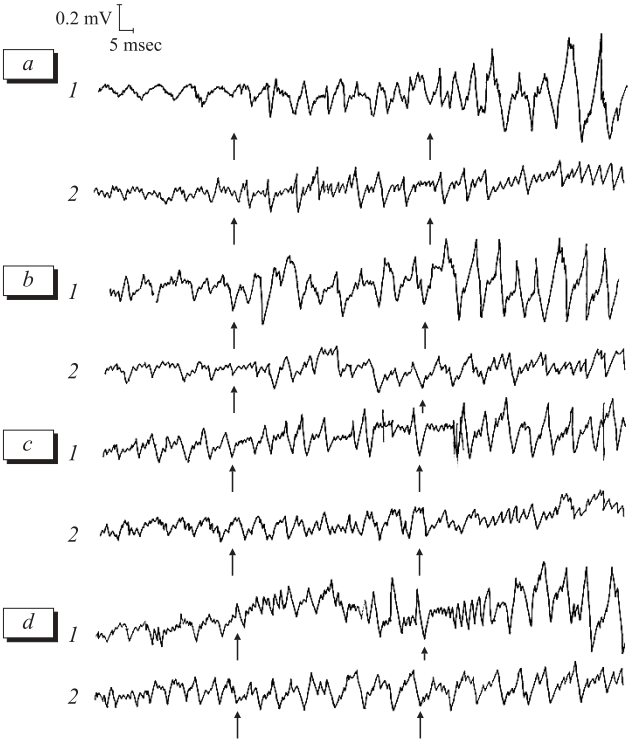

Fig. 1. Ganglionic serotonin receptor antagonist abolishes the sympathetic nerve-mediated potentiation of vagal stimulatory effect on motor activity in various portions of the large intestine. Here and in Fig. 2: ascending colon (*a*), transverse colon (*b*), descending colon (*c*), and rectum (*d*). Left arrow: vagus nerve stimulation. Right arrow: simultaneous stimulation of the sympathetic trunk and vagus nerve.

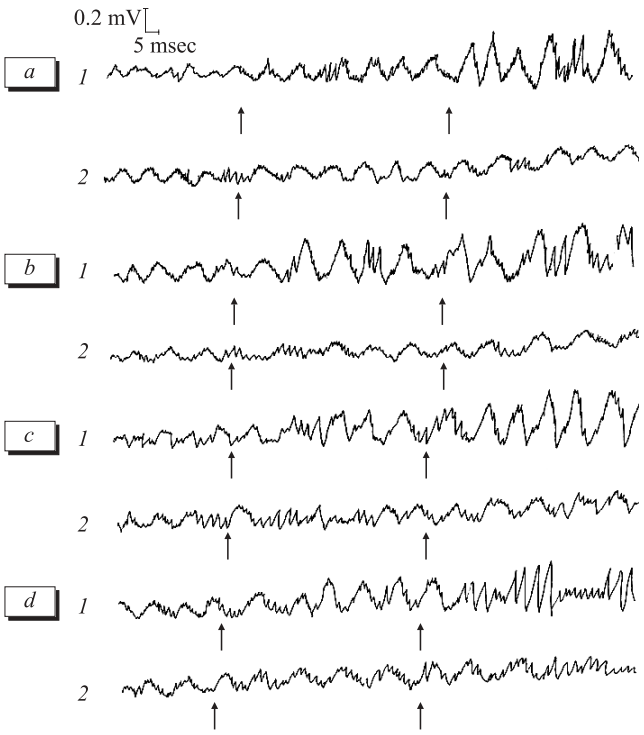

Fig. 2. Antagonist of serotonin receptors in effector tissues abolishes the sympathetic nerve-mediated potentiation of increase in motor activity of various portions in the large intestine produced by the pelvic nerve. Left arrow: parasympathetic nerve stimulation. Right arrow: simultaneous stimulation of the sympathetic trunk and parasympathetic nerve.

The baseline frequency and amplitude of EMA slow waves in the descending colon were 5.8±0.4 spikes per min and 0.25±0.02 mV, respectively. Stimulation of the vagus nerve increased the frequency and amplitude of EMA slow waves to 6.50±0.05 spikes per min (12.1%, $p<0.05$) and 0.30±0.02 mV (20%, $p<0.05$), respectively.

Simultaneous stimulation of ST and vagus nerve increased the frequency of EMA slow waves to 8.0± 0.6 spikes per min (23%, $p<0.05$), but had no effect on the amplitude. Simultaneous stimulation of ST and vagus nerve potentiated the vagal stimulatory effect on EMA in the descending colon.

The baseline frequency and amplitude of EMA slow waves in the rectum were 7.5±0.6 spikes per min and 0.22±0.02 mV, respectively. Low-amplitude spike activity was revealed in every third recording (amplitude 0.1 mV, frequency 0.2 spikes per 100 slow waves). Stimulation of the vagus nerve increased the frequency and amplitude of EMA slow waves to 8.8± 0.6 spikes per min (17.4%) and 0.25±0.03 mV (13%, $p<0.05$), respectively. Low-amplitude spike activity was also present in every third recording (amplitude 0.1 mV, frequency 0.33 spikes per 100 slow waves, 65%, $p<0.05$).

Simultaneous stimulation of ST and vagus nerve increased the frequency of EMA slow waves to 10.9± 0.9 spikes per min (24%, $p<0.05$), but slightly decreased the amplitude (3%). The frequency and amplitude of spike activity were 0.3 spikes per 100 slow waves and 0.3 mV, respectively. Simultaneous stimulation of ST and vagus nerve potentiated the vagal stimulatory effect on EMA in the rectum.

The baseline frequency and amplitude of EMA slow waves in the ascending colon were 6.5±0.5 spikes per min and 0.17±0.02 mV, respectively. Stimulation of the pelvic nerve increased the frequency of EMA slow waves to 8.3±0.6 spikes per min (27.7%, $p<0.05$), but had little effect on the amplitude. We observed moderate-amplitude spike activity (amplitude 0.22±0.20 mV, frequency 0.15 spikes per 100 slow waves).

Simultaneous stimulation of ST and pelvic nerve increased the frequency and amplitude of EMA slow waves to 13.0±0.1 spikes per min (56.6%) and 0.27± 0.03 mV, respectively. Simultaneous stimulation of ST and pelvic nerve potentiated the parasympathetic stimulatory effect on EMA in the ascending colon of the large intestine.

The baseline frequency and amplitude of EMA slow waves in the transverse colon were 6.3±0.4 spikes per min and 0.15±0.01 mV, respectively. Stimulation of the pelvic nerve increased the frequency and amplitude of EMA slow waves to 7.9±0.6 spikes per min (25.4%, $p<0.05$) and 0.25± 0.02 mV (66.6%, $p<0.05$), respectively. Simultaneous stimulation of ST and pelvic nerve increased the frequency and amplitude of EMA slow waves to 9.0±0.7 spikes per min (14%) and 0.4±0.4 mV (37.5%), respectively. Simultaneous stimulation of ST and pelvic nerve potentiated the parasympathetic stimulatory effect on motor activity in the transverse colon.

The baseline frequency and amplitude of EMA slow waves in the descending colon were 5.8±0.4 spikes per min and 0.25±0.02 mV, respectively. Stimulation of the pelvic nerve increased the frequency and amplitude of EMA slow waves to 6.50±0.05 spikes per min (12.1%) and 0.30±0.02 mV (20%, $p<0.05$), respectively.

Simultaneous stimulation of ST and pelvic nerve increased the frequency of EMA slow waves to 8.0± 0.6 spikes per min (23%, $p<0.05$), but had little effect on the amplitude. Simultaneous stimulation of ST and pelvic nerve potentiated the parasympathetic stimulatory effect on EMA in the descending colon of the large intestine.

The baseline frequency and amplitude of EMA slow waves in the descending colon were 6.3±0.4 spikes per min and 0.25±0.02 mV, respectively. Stimulation of the pelvic nerve increased the frequency of

EMA slow waves to 9.3±0.5 spikes per min (47.6%, $p<0.05$), but had no effect on the amplitude.

Simultaneous stimulation of ST and pelvic nerve increased the frequency and amplitude of EMA slow waves to 10.3±0.7 spikes per min (17.5%) and 0.40± 0.04 mV (60%, $p<0.05$), respectively. Simultaneous stimulation of ST and pelvic nerve potentiated the parasympathetic stimulatory effect on motor activity in the descending colon of the large intestine.

The baseline frequency and amplitude of EMA slow waves in the rectum were 5.9±0.6 spikes per min and 0.28±0.02 mV, respectively. Stimulation of the pelvic nerve increased the frequency and amplitude of EMA slow waves to 7.5±0.6 spikes per min (37.4%, $p<0.05$) and 0.31±0.03 mV (10.7%, $p<0.05$), respectively.

Simultaneous stimulation of ST and pelvic nerve increased the frequency of EMA slow waves to 8.40± 0.07 spikes per min (12%, $p<0.05$). Simultaneous stimulation of ST and pelvic nerve potentiated the parasympathetic stimulatory effect on EMA in the rectum.

Pretreatment with a 5-$HT_{3,4}$ receptor antagonist droperidol completely abolished the stimulatory effect in different portions of the large intestine (Fig. 1). These data show that 5-$HT_{3,4}$ receptors on ganglionic neurons are involved in the ST-mediated potentiation of the parasympathetic stimulatory effect on motor activity in different portions of the large intestine.

We evaluated whether 5-$HT_{1,2}$ receptors in effector tissues play a role in the ST-mediated potentiation of a parasympathetic stimulatory effect on motor activity. A 5-$HT_{1,2}$ receptor antagonist sumatriptan completely abolished the stimulatory effect (Fig. 2). The sympathetic nerve potentiates a parasympathetic stimulatory effect on smooth muscles in the large intestine via preganglionic serotoninergic fibers transmitting impulses to 5-$HT_{1,2}$ receptors in the large intestine.

Histological examination of different portions of the large intestine confirmed the important role of serotoninergic structures in the development of this phenomenon. Stimulation of serotoninergic structures was followed by arterial spasm in the microcirculatory bed and venous congestion.

Our results indicate that serotoninergic structures are involved in similar effect of the sympathetic and parasympathetic systems. A decreasing serotoninergic gradient from the ascending colon to the rectum was revealed during simultaneous stimulation of the vagus nerve and ST. An increasing gradient of serotoninergic innervation in the large intestine was observed during simultaneous stimulation of the pelvic nerve and ST. Hence, stimulation of ST in combination with stimulation of the vagus and pelvic nerve potentiates the stimulatory effect of the serotoninergic system. The stimulatory effect remains practically unchanged during stimulation of the following structures: pelvic nerve and ST; and vagus nerve and ST. These data should be taken into account under conditions of associated with reduced functional activity of the parasympathetic system (*e.g.*, age-related changes).

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