On the Problem of Innervation of the Biliary Tracts

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We studied the possibility of co-directed stimulatory effects of the sympathetic and parasympathetic systems on the motility of gallbladder, Oddi sphincter, and adjacent ascending portion of the duodenum. It is established that serotoninergic intramural neurons transmitting excitatory influences to $5HT_{1,2}$-serotonin receptors of the effector tissues participate in this stimulatory effect.

Key Words: *gallbladder; Oddi sphincter; duodenum; serotonin*

Recent papers cast doubt on traditional view on the role of parasympathetic nervous system in the regulation of gallblader motility [2,6,8]. Experiments showed that stimulation of vagus nerve induces contraction of gallbladder similarly to injection of acetylcholine. By contrast, similarly to administration of adrenomimetics, stimulation of sympathetic nerves inhibited contractile activity of gallbladder muscles and simultaneously enhanced activity of the muscles in the Oddi sphincter [2,9].

V. M. Smirnov *et al*. showed that apart from the parasympathetic system, the serotoninergic system participates in activation of duodenal motility [1]. Specifically, stimulation of the thoracic part of the sympathetic trunk, which potentiated contractions of the small intestine in dogs, is mediated via serotoninergic fibers of this trunk.

Our aim was to study the mechanisms of potentiation of electromotor activity (EMA) of the gallbladder, Oddi sphincter, and duodenum, when stimulation of vagus nerve (VN) is accompanied by stimulation of the sympathetic trunk.

MATERIALS AND METHODS

Experiments were performed on 15 Chinchilla rabbits (body weight 3.5-4.0 kg) narcotized with nembutal (40 mg/kg). Mingograph-82 was used to record EMA of the gallbladder, Oddi sphincter, and adjacent part of the duodenum. EMA was assessed by the amplitude (mV), slow wave frequency, and the frequency of fast potentials, which was assessed by the number of spikes per 100 slow waves.

The nerves were stimulated with electrical pulses (amplitude 1.5-15 V, duration 2 msec, and repetition rate 10 Hz) generated by a Medicor-St-02 electrical stimulator.

Four series of experiments were carried out. In series I (control), the baseline EMA of the gallbladder, Oddi sphincter, and duodenum surrounding the sphincter was recorded. The parameters of electrical stimulation of peripheral end of VN were tuned in such a way that EMA amplitude (vagal optimum) no more than 3-fold surpassed the amplitude of baseline EMA (parasympathetic system can control the tone of biliary tract only [4,5]). Then we performed isolated control stimulation of the cervical part of the sympathetic trunk. It was followed by stimulation of VN, which after some time was accompanied by stimulation of sympathetic trunk.

In series II, the pharmacological blockade was used to study possible involvement of purinergic structures in vagal potentiation of EMA in the gallbladder, Oddi sphincter, and ascending part of the duodenum. Theophylline (20-80 mg/kg) was used to block the purinergic systems and first of all, the stimulatory P_1-receptors in smooth muscles.

In series III, we assessed whether ganglionic serotonin receptors ($5HT_{3,4}$ or $S_{3,4}$) and pre- and postgan-

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glionic fibers participate in the synergic effect of vagal and sympathetic branches of ANS on EMA of biliary tract and duodenum. To this end, we blocked simultaneously possible stimulation of enterochromaffin serotoninergic cells, which have $5HT_{3,4}$-receptors on their plasmalemma [3,7]. Droperidol (0.5-1.0 mg/kg) was used as the blocker.

In series IV, possible involvement of peripheral serotonin receptors of effector tissues ($5HT_{1,2}$ or $S_{1,2}$) in stimulation of EMA was assessed using 0.5-1.0 mg/kg sumatriptan (imigran), a specific blocker of these receptor.

The contents of serotonin and acetylcholine were measured by Sadovangvivad and Hestrin methods. The data were processed statistically using Student's *t* test.

RESULTS

In series I (control) we found that baseline EMA of the gallbladder is low. In this case, the frequency and amplitude of slow waves was 7.9±1.5 min^{-1} and 0.11± 0.03 mV, respectively. Transition from relative rest to active phase was accompanied by an increase in the frequency and amplitude of slow EMA waves to 16.1± 1.2 min^{-1} and 0.10±0.02 mV ($p>0.05$), respectively. In addition, the bursts of rapid oscillations of the peak potentials (spikes) occurred in this period. Stimulation of VN increased the frequency of EMA slow waves to 21.0±1.5 min^{-1} or by 30% ($p<0.05$), the amplitude being stable. During combined stimulation of VN and sympathetic trunk the frequency of EMA slow waves increased to 33.0± 1.6 min^{-1} or by 57% ($p<0.01$). However, potentiation of vagal stimulation by sympathetic trunk was observed only in 20% cases, while in other cases EMA did not change.

EMA of resting Oddi sphincter is characterized by slow waves (frequency 13±3 min^{-1}, amplitude 0.20± 0.05 mV) and in 30% cases by the rapid potentials (spikes) appearing at the rate of 0.50±0.13 per 100 slow waves (referred in percentage in the following). Stimulation of peripheral end of the right VN resulted in additional potentiation of muscle activity in Oddi sphincter, which was reflected by increased amplitude and frequency of slow waves to 0.26±0.04 mV (by 30%, $p>0.05$) and to 22.9±2.4 mV (by 76%, $p<0.05$), respectively. In addition, spike activity increased to 0.87±0.12%. Addition of sympathetic stimulation to the vagal one potentiated vagal stimulatory effect: the frequency of slow waves increased to 27.9±2.5 min^{-1} (by 22%, $p<0.05$), while spike activity increased by 13%. The amplitude of slow waves also increased.

At rest, EMA of rabbit duodenum is characterized by slow waves with an amplitude of 0.24±0.03 mV, which oscillate at a frequency of 13.0±1.5 min^{-1}. Stimulation of VN potentiated duodenal EMA: frequency and amplitude of slow waves increased to 17.2±1.8 min^{-1} (by 32.3%, $p<0.05$) and 0.28±0.03 mV (by 17%, $p>0.05$), respectively. In addition, this stimulation triggered spikes (0.64±0.09 per 100 slow EMA waves). Combined stimulation of vagal and sympathetic nerves produced further increase in duodenal motility: the frequency of slow waves increased to 27±2 min^{-1}, although their amplitude remained the unchanged.

Therefore, stimulation of peripheral end of VN led to activation of muscle activity in Oddi sphincter, gallbladder, and ascending part of the duodenum. Stimulation of the sympathetic trunk potentiated vagal stimulatory effect on the biliary tract. However, in the gallbladder this effect was less pronounced than in Oddi sphincter.

The content of acetylcholine in the gallbladder, duodenum, and Oddi sphincter was high (19.8±2.0, 26±3, and 18±2 mg/g, respectively) in comparison with that in the bile (0.50±0.06 mg/ml). The content of serotonin was very low in the bile (2.0±0.3 μg/ml) and in the gallbladder (5.5±0.6 μg/g), but high in the duodenum (16.3±2.0 μg/g) and in Oddi sphincter (23±2 μg/g).

Therefore, combined stimulation of VN and sympathetic trunk resulted in synergistic stimulatory effect on EMA of the duodenum and Oddi sphincter and to a lesser degree on EMA of the gallbladder, which correlates with high content of serotonin in the ascending part of the duodenum and Oddi sphincter.

In series II, purine blocker theophylline increased the baseline frequency of EMA slow waves in the gallbladder from 16.1±1.2 to 21.5±1.4 min^{-1} (by 33.5%, $p<0.05$), but did not change their amplitude. Stimulation of VN potentiated EMA in the gallbladder: the frequency of slow waves increased to 32.2±2.1 min^{-1} (by 53.8%, $p<0.05$), which was accompanied by a minor decrease in the amplitude from 0.17±0.07 to 0.13±0.03 mV. Addition of sympathetic stimulation potentiated vagal stimulatory action: although the amplitude of slow waves was stable, their frequency increased to 38.7±1.7 min^{-1} (by 20%, $p<0.05$).

During application of purine blocker, the frequency of slow waves in Oddi sphincter was 13.0±1.5 min^{-1} and their amplitude was 0.25±0.05 mV. Stimulation of VN activated motility of smooth muscles in Oddi sphincter: the frequency of slow waves increased to 17.3±1.2 min^{-1} (by 33%, $p<0.05$), while their amplitude increased to 0.45±0.02 mV (by 80%, $p<0.05$). The spike rate increased from 0.18±0.03 to 0.77±0.08%. Addition of sympathetic stimulation to vagal excitation potentiated the vagal stimulatory effect: the frequency of slow waves increased to 24.5±1.8 min^{-1} (by 41%, $p<0.05$), and the amplitude of these waves in-

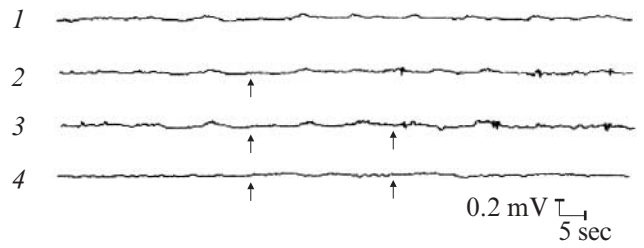

Fig. 1. Electromotor activity of gallbladder before neurostimulation (*1*), during stimulation of vagus nerve (*2*), and during combined vagal and sympathetic stimulation (*3*). Trace *4* was recorded after droperidol injection. Here and in Fig. 2: left and right arrows mark the start of vagal and sympathetic stimulation, respectively.

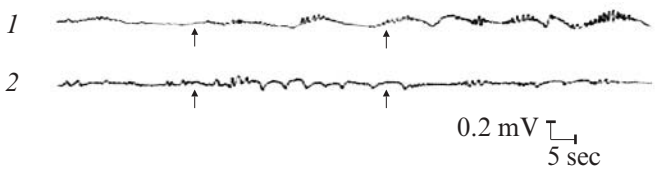

Fig. 2. Electromotor activity of Oddi sphincter before (*1*) and after (*2*) droperidol injection.

creased to 0.60±0.07 mV (by 33%, $p<0.05$). At the same time, the spike rate was 0.93%.

Injection of purine blocker had no effect on the baseline EMA in the duodenum: the frequency of slow waves was 12.8±1.7 min^{-1} and their amplitude was 0.25±0.05 mV. Stimulation of VN increased the frequency of slow waves and their amplitude to 18±1 min^{-1} (by 29%, $p<0.05$) and 0.27±0.09 mV ($p>0.05$), respectively. The following stimulation of the sympathetic trunk against the background of vagal stimulation potentiated vagal activation of muscle motility of the duodenum: the frequency of slow waves and their amplitude increased to 23±2 min^{-1} (by 28%, $p<0.05$) and 0.43±0.04 mV (by 59%, $p<0.05$), respectively. Therefore, the purinergic systems do not mediate the effect of neurostimulation on EMA in the gallbladder, Oddi sphincter, and duodenum.

In series III, we studied possible participation of $5HT_{3,4}$-serotoninergic ganglia in the observed effects of neurostimulation. EMA in the gallbladder (Fig. 1, Table 1) and Oddi sphincter (Fig. 2, Table 1) was recorded before (upper curve) and after (lower curve) injection of droperidol. Similar experiments were carried out on the duodenum (Table 1).

In series IV, the amplitude of slow waves in the gallbladder was 0.12±0.03 mV. Stimulation of VN increased frequency and amplitude of slow waves to 32.1±1.8 min^{-1} (by 79%, $p<0.05$) and 0.15±0.02 mV ($p<0.01$), respectively. Combined sympathetic and vagal stimulation increased the frequency of slow waves to 40±2 min^{-1} (by 25%, $p<0.05$), although the amplitude of these waves remained unchanged. Sumatriptan induced a minor decrease in the frequency of background slow waves to 15.5±0.5 min^{-1} (by 14%, $p<0.05$), but did not change the amplitude of these waves. Vagal stimulation increased the frequency of slow waves to 29.8±0.4 min^{-1} (by 89%, $p<0.05$). However, addition of sympathetic stimulation to the vagal one produced no significant effect on the frequency of slow waves in the gallbladder (27.5±1.4 min^{-1}, $p>0.1$). Therefore, sumatryptane completely blocked the potentiating effect of sympathetic stimulation on EMA in gallbladder.

Before injection of sumatriptan, the frequency and amplitude of slow waves in Oddi sphincter were 12.0±0.7 min^{-1} and 0.13±0.01 mV, respectively. Vagal stimulation increased the frequency to 1.9±1.3 min^{-1} (by 59%, $p<0.05$) and triggered spike activity (0.11±0.01 spikes per 100 slow waves). Addition of sympathetic stimulation potentiated the vagal effect and enhanced the frequency and amplitude of slow waves to 23.3± 2.2 min^{-1} (by 21%, $p<0.05$) and 0.23+0.02 mV (by 70%, $p<0.05$), respectively.

After injection of sumatriptan, the baseline frequency and amplitude of slow waves in Oddi sphincter

TABLE 1. Potentiation of Vagal-Induced EMA of Oddi Sphincter by Electrical Stimulation of Sympathetic Trunk Before and After Injection of $5HT_{3,4}$-Blocker Droperidol (*M*±*m*)

Experimental conditions	Gallbladder	Oddi sphincter	Duodenum
Before droperidol			
baseline	23.3±1.2	14.7±1.2	12.9±1.5
vagal stimulation	32.0±1.1*	18±2*	18.3±1.3*
vagal+sympathetic stimulation	38.3±1.3+	26.0±3.3+	26.6±3.0+
After droperidol			
baseline	11.5±0.6	13.5±1.0	12.3±1.2
vagal stimulation	18.5±0.4*	17.0±1.3*	22.3±1.2*
vagal+sympathetic stimulation	16.5±0.6°	17.0±1.1°	22.3±2.0°

Note. $p<0.05$ compared to *baseline and +vagal stimulation. °Difference with vagal stimulation data is insignificant (no effect of sympathetic stimulation).

became 11.7±1.1 min^{-1} and 0.17±0.03 mV, respectively. Vagal stimulation increased the frequency of slow waves to 18.0±3.0 min^{-1} (by 57%, $p<0.05$). Surprisingly, addition of sympathetic stimulation slightly decreased this parameter to 15.5±3.5 min^{-1} ($p>0.1$).

Thus, sumatriptan completely eliminated the potentiating effect of sympathetic stimulation of vagal influences in Oddi sphincter.

Before injection of sumatriptan, the frequency and amplitude of slow waves in the duodenum was 12.9± 1.3 min^{-1} and 0.22±0.02 mV, respectively. Vagal stimulation increased the frequency to 18.0±1.3 min^{-1} (by 38%, $p<0.05$), did not affect the amplitude of slow waves and intensified spike activity to 0.17±0.02 per 100 slow waves. Addition of sympathetic stimulation potentiated the vagal effect and enhanced the frequency and amplitude of slow waves to 23.3±1.3 min^{-1} (by 28%, $p<0.05$) and 0.34±0.03 mV (by 96%, $p<0.05$), respectively. Moreover, it intensified spike activity to 0.33±0.04 per 100 slow waves. After injection of sumatriptan, the baseline frequency and amplitude of slow waves in the duodenum became 13.0±1.3 min^{-1} and 0.16± 0.02 mV, respectively. Vagal stimulation increased frequency and amplitude of slow waves to 17.0±1.3 min^{-1} (by 31%, $p<0.05$) and 0.22±0.02 mV, respectively. Addition of sympathetic stimulation produced no effect on the frequency (16.3±1.2 min^{-1}, $p>0.1$) and amplitude of the slow waves. Therefore, sumatriptan eliminated the potentiating effect of sympathetic stimulation on vagal influences in the gallbladder, Oddi sphincter, and duodenum.

The potentiating effect on vagal activation of EMA in the gallbladder, Oddi sphincter, and duodenum was observed, when sympathetic trunk was stimulated at the level of fifth and sixth cervical vertebra. The degree of this effect correlated with high content of serotonin in the tissues of Oddi sphincter and duodenum. Purine receptor blocker theophylline did not affect the examined sympathetic potentiation, which excludes the involvement of purine receptor in this effect. By contrast, injection of $5HT_{1,2}$ or $5HT_{3,4}$-blockers inhibited sympathetic potentiation of vagal-induced EMA in the gallbladder, Oddi sphincter, and duodenum, which attested to a mediatory role of intramural serotoninergic neurons in transmission of neural influences to serotonin receptors of biological actuators. Blockade of this effect with ganglionic or peripheral serotonin antagonists suggests that serotonin-bearing enterochromaffin cells are not involved in this phenomenon.

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