Catecholamines, α-, and β-Adrenoceptors Are Not Responsible for Activation of Duodenum Motility Induced by Sympathetic Nerve Stimulation

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Stimulation of sympathetic nerve in anesthetized dogs not treated with adrenergic blockers more frequently exerted stimulating rather than inhibitory effect on duodenal motility. Blockade of α- and β-adrenoceptors with phentolamine and propranolol, respectively, did not prevent the excitatory action of the sympathetic nerve stimulation, but even potentiated this effect. The data showed that catecholamines as well as α- and β-adrenoceptors are not involved in the excitatory effect of sympathetic origin.

Key Words: *duodenum; neural control; catecholamines; α- and β-adrenoceptors*

Activation of cholinergic or adrenergic structures during stimulation of the sympathetic nerve (SN) can possibly enhance motility in the gastrointestinal tract (GIT). Sympathetic activation of GIT motility can be mediated via α-adrenoceptors (α-AR) of smooth muscles. Apamin, a polypeptide found in bee venom, blocks purinergic and adrenergic inhibitory synaptic transmission in muscles and prevents the hyperpolarizing effect of norepinephrine and ATP on smooth muscles. Under the action of apamin, muscular cells in the circular and longitudinal intestinal layers generate not inhibitory, but excitatory postsynaptic potentials in response to intramural stimulation, which are resistant to blockers of acetylcholine, norepinephrine, and serotonin receptors. Under these conditions, ATP and norepinephrine (but not epinephrine) induce muscle depolarization instead of hyperpolarization. The norepinephrine-induced depolarization (similar to hyperpolarization in the apamin-free animals) can be blocked with phentolamine. These data are consonant with the discovery postsynaptic α_1-AR [7]. The presence of excitatory β-AR was revealed in interneurons of the small intestine, which realize the excitatory reactions of catecholamines of adrenal or neurotransmitter origin.

Therefore, under certain conditions the sympathetic postganglionic fibers in SN can exert not only the inhibitory, but also excitatory effect on GIT motility.

This study was designed to test the possible role of catecholamines and α- and β-AR in activation of duodenal motility caused by SN stimulation.

MATERIALS AND METHODS

Acute experiments were carried out on male and female dogs weighing 10-15 kg during the surgical stage of ethaminal narcosis (intramuscular nembutal, 60 mg/kg) with opened thorax and artificial ventilation. Cervical bilateral vagotomy was performed to eliminate the reflex influences. The sympathetic branch of ANS was blocked with intravenous ornid (20 mg/kg) to prevent the release of catecholamines from sympathetic nerve terminals, phentolamine (2 mg/kg) to block α-AR, and propranolol (4 mg/kg) to block β-AR.

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SN was stimulated in the thorax (in contrast to [5,6]) where this nerve contained no parasympathetic fibers. To this end, the thorax was opened on the right side in the intercostal space IX; the sympathetic chain was cut at T_{VI} level. The peripheral stump of SN was ligated and mounted on the bipolar stimulating electrodes spaced at 2 mm. The nerve was stimulated for 30 sec with rectangular electrical pulses (duration 1.5 msec, amplitude 10 or 15 V, repetition rate 10 or 20 Hz).

The abdominal cavity was opened along the midline and duodenum was exposed into the surgical wound. The anterior aspect of the duodenal wall was opened with a transversal section (<0.3 cm) made at a distance of 3 cm from the pyloric sphincter in the least vascularized region. A balloon with elastic tube was introduced into the duodenum towards the anal direction. The wall was closed with a purse-string suture, and the same ligature fixed the tube emerging from the duodenum. A 1-5-ml balloon was filled with warm (38°C) distilled water via the tube. Ingress of air was excluded. There was no deformation of the duodenal wall in the sutured area and the region of balloon projection.

RESULTS

Examination of transmitter nature of the nerve fibers and smooth muscle receptors involved in potentiation of duodenal motility during stimulation of SN was carried out in the experiments with blockade of various elements in the sympathetic chain.

The experiments with α- and β-AR blockers (respectively, phentolamine and propranolol) were performed on dogs (n=7); 35 reactions of the duodenum were recorded. These reactions were subdivided into three types: excitatory, inhibitory, and indifferent (no reaction).

The incidence of excitatory reactions increased with increasing SN stimulation. This phenomenon was also observed in weak range of SN stimulation under combined action of phentolamine and propranolol. Stimulation of SN with 10-V pulses in these experiments (block of α- and β-AR) induced the excitatory response in 100% animals (Fig. 1). When 10-V pulses were applied at a rate of 20 Hz under the action of both adrenergic blockers, they elevated the duodenal pressure from 10.2±3.8 to 23.4±3.0 mm Hg, *i.e.* by 13.20±2.79 mm Hg (223%; p<0.01).

Stronger stimulation with 15-V pulses at 20 Hz elevated the duodenal pressure from 10.19±3.90 to 24.50±8.36 mm Hg, *i.e.* by 14.28±8.50 mm Hg (240%; p<0.05).

The experiments shown in Fig. 1 were carried out under identical conditions. However, directivity of the reactions and their strength were different. In case of inhibitory reaction type, we observed a weak inhibitory reaction to SN stimulation before AR blockade (Fig. 1, *a*), which agreed with the known facts. Blockade of α- and β-AR transformed this reaction into the excitatory one (Fig. 1, *b*).

In case of excitatory reaction type, we observed a pronounced excitatory reaction of the duodenum with a small inhibitory phase at the beginning of SN stimulation (Fig. 1, *c*). The excitatory response became even more pronounced under blockade of α- and β-AR (Fig. 1, *d*). Under these conditions, the initial inhibitory phase disappeared, which probably resulted from elimination of the inhibitory sympathetic influences by adrenoblockers. It is noteworthy that under normal conditions (without adrenergic blockers), the incidence of inhibitory reaction to SN stimulation was lower (30%) than that of the excitatory response (55%). Moreover, the inhibitory reactions were weaker than the excitatory ones (Fig. 1).

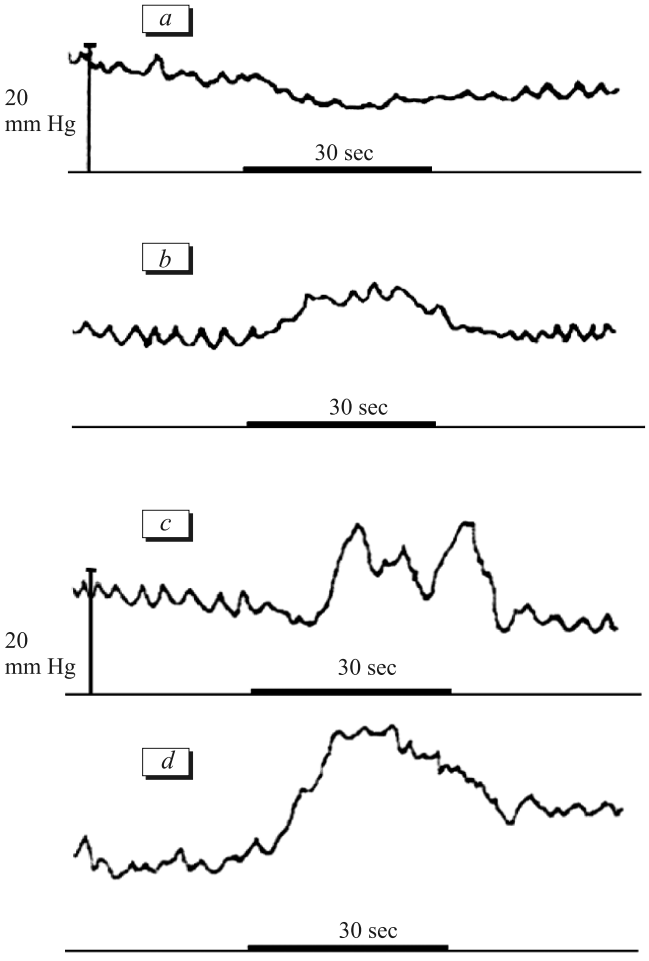

Fig. 1. Duodenal reactions to stimulation of SN (10 V, 20 Hz) before (*a*, *c*) and after (*b*, *d*) intravenous injection of adrenergic blockers phentolamine and propranolol. Here and in Fig. 2: thick segment on the abscissa shows stimulation period.

TABLE 1. Incidence of Excitatory and Inhibitory Reactions of the Duodenum in Dogs Evoked by SN Stimulation at 10 Hz with Electrical Pulses of Various Amplitudes under the Action of Adrenergic Blockers

Experimental conditions	*n*	Response to SN stimulation			
		10 V		15 V	
		excitatory	inhibitory	excitatory	inhibitory
Intact	31	15 (49%)	12 (39%)	17 (55%)	12 (39%)
Ornid	13	11 (84%)	0	13 (100%)	0
Phentolamine+ Propranolol	7	7 (100%)	0	7 (100%)	0

Note. Percent of dogs is given in brackets.

Thus, blockade of α- and β-AR did not prevent, but even promoted the appearance of SN-induced excitatory reactions of the duodenum. Under combined action of phentolamine and propranolol, the incidence of the excitatory effects attained 100% during stimulation of SN with weak (10 V) pulses. In contrast, under the action of ornid, the excitatory reactions were induced in all dogs only by strong electric pulses (15 V, Table 1).

The above data on duodenal reaction under blockade of α- and β-AR showed that adrenergic nerves are not involved in the realization of the excitatory duodenal response. However, to strengthen this inference, other experiments were carried out with block of norepinephrine release from the sympathetic terminals.

To this end, we used sympatholytic ornid known to abolish the cardiotropic sympathetic effects evoked by stimulation of the stellate ganglion in a dose of 20-30 mg/kg [1]. Based on this fact, we performed a series of animal experiments (n=13) and examined 65 duodenal reactions to SN stimulation under the action of ornid in a dose of 20 mg/kg.

The parameters of electric stimulation were the same as in the previous series (amplitude 10 V, repetition rate 7-20 Hz), because such stimulation evoked the responses with maximum increment of duodenal pressure. The reactions were distributed as follows: excitatory (n=34), indifferent (n=31), and inhibitory (n=0). The absence of inhibitory reactions resulted from the block of inhibitory adrenergic sympathetic fibers.

Under the action of ornid, stimulation of SN with 10 V pulses at 20 Hz elevated duodenal pressure in 11 of 13 dogs from 8.4±2.8 to 14.3±3 mm Hg, *i.e.* by 5.9±1.4 mm Hg (70%, p<0.05). The use of 15 V pulses under the same conditions elevated duodenal pressure in all the animals (n=13) from 7.9±2.7 to 14.4±3.1 mm Hg, *i.e.* by 6.7±2.9 mm Hg (82%, p<0.01; Table 2, Fig. 2).

In the experiments with ornid known to inhibit the release of catecholamines from the sympathetic terminals, the incidence of excitatory reaction induced by SN stimulation at 15 V was 100%. However, the incidence of excitatory reaction induced by 5-10 V stimulation of SN was lower than that observed during blockade of α- and β-AR, which can be explained by incomplete action of this agent on norepinephrine release from sympathetic terminals preserving the inhibitory effect of norepinephrine on the duodenum at some diminished level.

The data obtained exclude the hypothesis on the involvement of catecholamines (and α- and β-AR) in the realization of duodenum-directed excitatory influences of SN stimulation. They also rule out the hypothesis advanced in previous papers [2,4] on possible transmission of preganglionic sympathetic excitation to the neurons of other nature (specifically, to the cholinergic neurons) involved in stimulation of duodenal motility, because the ganglionic blockers (antagonists of N-cholinoreceptors) of autonomic ganglia do not abolish the excitatory effect and even promote it [2-

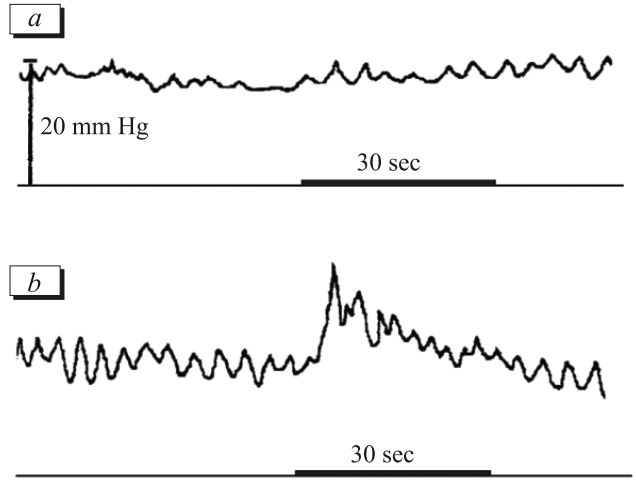

Fig. 2. Duodenal reactions to stimulation of SN (10 V, 20 Hz) before (*a*) and after (*b*) intravenous injection of sympatholytic ornid.

TABLE 2. Duodenal Pressure Increase (mm Hg) Evoked by SN Stimulation (*M*±*m*)

Experimental conditions	*n*	Parameters of stimulation	
		10 V, 20 Hz	15 V, 20 Hz
Intact	20	5.9±1.0 (+68%)	6.0±3.1 (+66%)
Ornid	13	5.9±1.4 (+70%)	6.7±2.9 (+82%)
Phentolamine+ Propranolol	7	13.2±2.8 (+223%)	14.3±2.5 (+240%)

4,6]. Our studies and the reported data also exclude the parasympathetic pathways in the realization of the examined phenomenon, since SN has no vagal fibers. Moreover, the reflex enhancement of duodenal motility via vagal branches was also excluded because these nerves were cut.

In summary, our findings attest to the presence of non-cholinergic and non-adrenergic fibers in the sympathetic trunk that up-regulate duodenal motility. Moreover, their stimulatory effects on the duodenum are stronger than the inhibitory influences of adrenergic nerve fibers.

The further studies of nerve fibers producing the stimulatory effect on the duodenum should employ the selective blockers of various receptors.

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