Different Mechanisms of Intensification of Contractile Activity in the Proximal and Distal Portions of the Duodenum in Psychogenic Stress in Rabbits

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Psychogenic stress in rabbits induced by fixation of the animals to a frame was accompanied by an increase in contractile activity of the duodenum. Against the background of blockade of muscarinic and nicotinic cholinergic receptors and β_1/β_2-adrenoceptors this increase was observed in postpyloric portion, but not in the distal third of the duodenum. The increase in contractile activity was determined by the direct effect of the hormonal stress factor on smooth muscles in the first case and by the influence of circulating catecholamines on excitatory β-adrenoceptors of cholinergic neurons of the enteral nervous system in the second.

Key Words: *psychogenic stress; duodenum; myoelectrical activity; muscarinic and nicotinic cholinergic receptors; β-adrenoceptors*

We previously showed that psychogenic stress induced by rigid fixation of the rabbit to a frame suppresses motility of the anthral and pyloric portions of the stomach and increases contractile activity (CA) of the duodenum [1]. These data suggest that decelerated evacuation of the gastric content is determined by not only weakened propulsive force of the stomach, but also increased resistance of the duodenum due to its contraction. Contraction of the duodenum under conditions of open pyloric sphincter creates conditions for duodenogastric bile reflux. The latter is considered as a pathogenetic factor in the development of the gastric mucosa erosions. In light of this, it was interesting to study the mechanism of stress-induced potentiation of CA of the duodenum.

Here were studied the role of mechanisms mediated by muscarinic and nicotinic cholinoceptors and β_1/β_2-adrenoceptors in stress-induced potentiation of CA in the proximal and distal portions of the duodenum.

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MATERIALS AND METHODS

Chronic experiments were carried out on male rabbits weighing 2.5-3.0 kg. Bipolar electrodes were implanted to 12 animals with strict adherence to the rules for abdominal surgeries. Two sites were chosen for subserous implantation of the electrodes: the proximal (postpyloric) portion of the duodenum (5 cm from the pyloric sphincter) and in the distal third of the duodenum. The experiments were performed 10-12 days after surgery without food and water deprivation. Myoelectrical activity of the duodenum was recorded on an ERG-16s encephalograph (0.1 time constant, 7.5 mm/sec recording rate, and sensitivity of 250 μV per 1 cm pen deviation). After recording the baseline myoelectrical activity for 30 min, psychogenic stress was modeled in rabbits by catching and rigid fixation to a frame in the supine position. This induced a pronounced stress reaction manifesting in increased plasma levels of ACTH, corticosteroids, epinephrine, and norepinephrine [3,7]. All experiments were performed with strict adherence to ethical norms for animal experiments. The effect of stress on CA of the proximal and distal portions of the duodenum were

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studied under control conditions (5 experiments) and after blockade of muscarinic cholinergic receptors with methacin (0.5 mg/kg; 5 experiments), nicotinic receptors with benzohexonium (7 mg/kg, 5 experiments), and β_1/β_2-adrenoceptors with propranolol (1 mg/kg, 5 experiments). These substances were injected subcutaneously. For quantitative analysis of myoelectrical activity, two 30-min periods from the start of the stress exposure were chosen in each experiment (first and second phases of the reaction). CA was evaluated by the index of contractile activity (ICA, a product of the number of spikes over 40-sec period and mean spike amplitude (in mm) during the same period expressed in arbitrary units). The arithmetic means and standard error of the mean were calculated using Origin 6.1 software. Significance of the differences in independent samples was evaluated by ANOVA.

RESULTS

Stress increased CA in the proximal and distal portions of the duodenum: in phase 1 of the reaction ICA increased by 40 and 51%, respectively, while in phase 2 this parameter in both portions surpassed the initial level by 75% (Fig. 1, Table 1).

Under conditions of muscarinic receptor blockade, ICA in the proximal portion of the duodenum during phases 1 and 2 of the stress reaction surpassed the baseline values by 60 and 119%, respectively (Fig. 2, Table 1). In the distal portion, ICA during phases 1 and 2 of the stress reaction was below the initial values by 38 and 55%, respectively. Thus, blockade of muscarinic receptors had no effect on the stimulatory reaction in the proximal portion of the duodenum, while in the distal portion its inhibition instead of stimulation was observed.

Under conditions of nicotinic receptor blockade, ICA in the proximal portion of the duodenum during phases 1 and 2 of the stress reaction surpassed the baseline values by 116 and 384%, respectively (Fig. 2, Table 1). In the distal portion, ICA did not change significantly during both phases of the stress reaction. Thus, nicotinic receptor blockade had no effect on stress-induced potentiation of CA in the proximal portion of the duodenum in both phases of the stress response. At the same time, significant CA potentiation observed in the control was absent against the background of nicotinic receptor blockade.

Under conditions of β_1/β_2-adrenoceptor blockade, ICA in the proximal portion of the duodenum during phases 1 and 2 of the stress reaction surpassed the baseline values by 53 and 60%, respectively (Fig. 2, Table 1). In the distal portion of the duodenum, ICA during both phases was below the initial values by 71 and 44%, respectively. Thus, β_1/β_2-adrenoceptor blockade had no effect on stimulatory reaction in the proximal portion of the duodenum. At the same time, β_1/β_2-adrenoceptor blockade prevented the CA increase and led to its inhibition in the distal portion of the duodenum.

In chronic experiments on dogs [6], immobilization-induced psychogenic stress considerably increased spike frequency in the duodenum, which was prevented by vagotomy. In our study, stress enhanced CA in both distal and proximal portions of the duodenum. Under conditions of muscarinic and nicotinic receptor blockade, CA remained enhanced in the proximal portion in both phases of the reaction. Since muscarinic receptor blockade excludes the stimulatory effect of neurotransmitter acetylcholine on smooth muscles, we can conclude that stress-induced activation of motility in the proximal portion of the duodenum has a non-cholinergic nature.

It was found that excitatory effector neurons of the enteral nervous system apart from cholinergic neurotransmitter acetylcholine contain substance P produc-

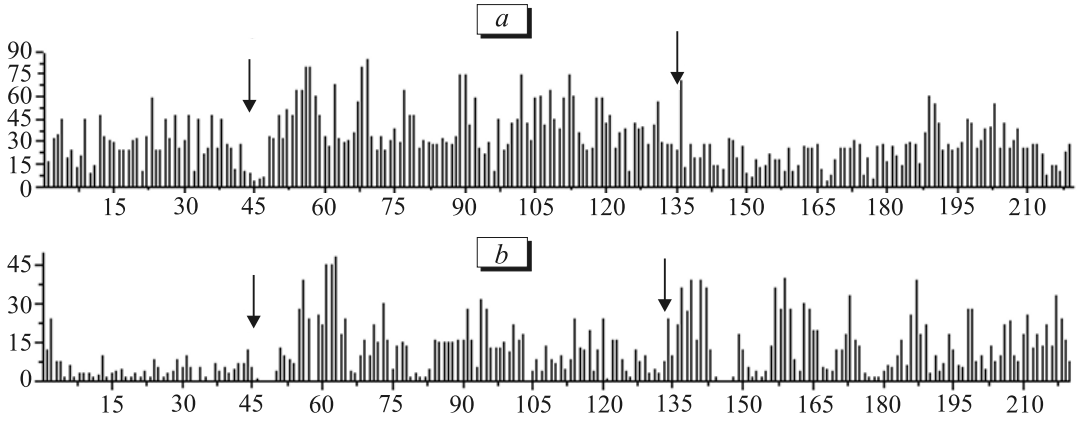

Fig. 1. Changes in ICA in the proximal (*a*) and distal (*b*) portions of the duodenum under the effect of psychogenic stress. First and second arrows indicate the start and the end of the stress exposure, respectively. Here and on Fig. 2: abscissa: number of 40-sec records of myoelectrical activity; ordinate: ICA.

TABLE 1. Effects of Stress on ICA in the Proximal and Distal Portions of the Duodenum before and after Blockade of β_1/β_2-Adrenoceptors and Muscarinic and Nicotinic Cholinergic Receptors (*M*±*m*)

Blockade, portion of the duodenum		Baseline	Stress			
			phase 1		phase 2	
			abs.	Δ, %	abs.	Δ, %
Control	proximal (*n*=5)	14.7±1.2	20.6±2.2*	+40	25.8±2.3**	+75
	distal (*n*=5)	10.7±1.1	16.2±2.1*	+51	18.7±2.0**	+75
Muscarinic receptors	proximal (*n*=5)	9.4±1.3	15.0±2.0*	+60	20.6±2.3**	+119
	distal (*n*=5)	63.5±4.5	30.4±2.7***	-38	28.8±2.5***	-55
Nicotinic receptors	proximal (*n*=5)	3.8±0.9	8.2±1.5*	+116	18.4±1.6***	+384
	distal (*n*=5)	8.0±1.8	7.5±2.0	-6	11.6±1.6	+45
β_1/β_2-adrenoceptors	proximal (*n*=5)	16.7±1.4	25.6±2.0***	+53	26.8±1.4***	+60
	distal (*n*=5)	17.4±1.8	5.7±0.8***	-71	9.7±1.1**	-44

Note. *p<0.05, **p<0.01, ***p<0.001 compared to baseline.

ing a stimulatory effect on smooth muscle cells. The assumption that non-cholinergic potentiation of CA in the proximal portion of the duodenum during stress is a result of neurogenic excitation of effector neurons with the release of substance P was not confirmed in our experiments. We observed CA potentiation in the proximal portion of the duodenum under conditions of nicotinic receptor blockade disturbing cholinergic transmission in ganglia of the autonomic nervous system. The appearance of stress-induced stimulation of CA in the duodenum under these conditions suggests that this reaction is not the result of enhanced sympathetic and parasympathetic efferentation followed by influence on microganglia of the enteral nervous system. Enhanced release of not only acetylcholine, but also substance P from effector neurons of the enteral system is impossible under these conditions. Impossibility of neurogenic potentiation of CA in the proximal portion of the duodenum suggest that this reaction can be a result of direct activating effect of a hormonal stress factor on smooth muscles. It was demonstrated that corticotropin-releasing factor and urocortins play an essential role in the realization of stress effects on gastrointenstinal motility [4,5,8]. They probably participate in the formation of the stress-induced CA potentiation in the proximal portion of the duodenum.

Our experiments showed that muscarinic cholinergic mechanism played the key role in the formation of stress-induced CA potentiation in the distal portions of the duodenum, because blockade of muscarinic receptors led to inversion of the stimulatory effect into inhibitory one in both phases of the reaction. At the same time, significant potentiation of CA in the distal portion of the duodenum observed in the control was absent against the background of nicotinic receptor blockade. These findings suggest that not only effector cholinergic neurons, but also interneurons of the enteral nervous system contacting with them via nicotinic cholinergic synapses participated in the realization of the stress-induced potentiation of CA in the distal portion of the duodenum. As was mentioned before, β_1/β_2-adrenoceptor blockade prevented CA increase and led to its inhibition during both phases of the reaction. Thus, stress-induced potentiation of CA in the distal portion of the duodenum involved cholinergic neurons of the enteral nervous system and was mediated by β-adrenoceptors. We previously reported [2] that cholinergic neurons of the enteral nervous system (effector neurons and interneurons) have β-adrenoceptors mediating the stimulatory effects of catecholamines on intenstinal smooth muscles. Stress induces activation of the sympathoadrenal system and elevation of catecholamine content in the circulation. These findings suggest that CA potentiation in the distal portion of the duodenum during psychogenic stress can be determined by the influence of blood catecholamines on stimulatory β-adrenoceptors on cholinergic neurons of the enteral nervous system.

Thus, we showed that the stimulatory effect of stress on CA in the proximal and distal portions of the duodenum is mediated by different mechanisms. In the first case, the effect was realized without participation of central or local neurogenic mechanisms and involved a hormonal stress-related factor. In the

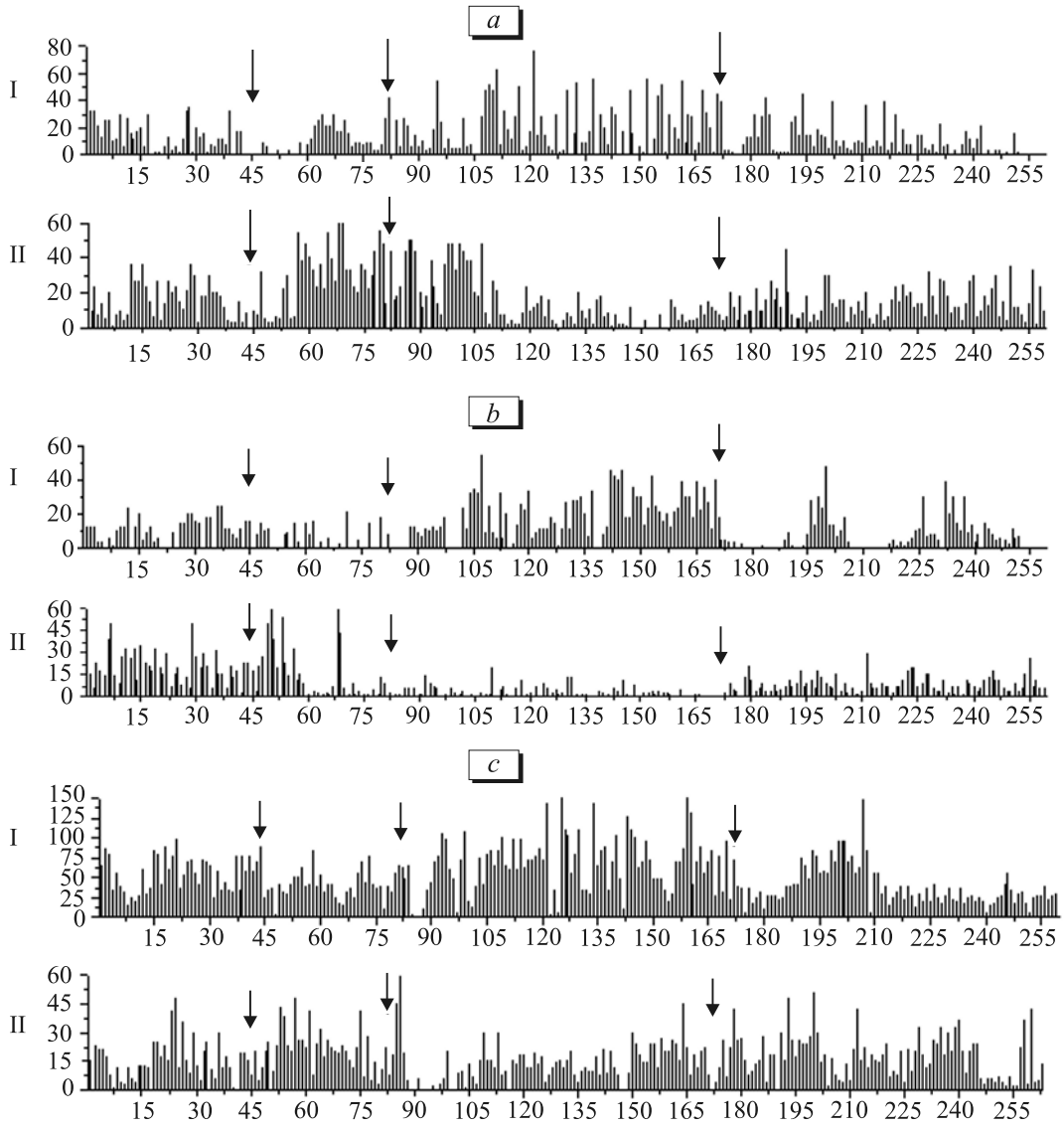

Fig. 2. Changes in ICA in proximal (I) and distal (II) portions of the duodenum under the effect of psychogenic stress against the background of muscarinic receptor blockade with methacin (*a*), nicotinic receptors with benzohexonium (*b*), and β_1/β_2-adrenoceptors with propranolol (*c*). First arrow shows the moment of injection of the corresponding receptor blocker, second and third arrows show the start and the end of the stress exposure, respectively.

second case, the humoral component was also present (endocrine effect of catecholamines released by the adrenals), but the stimulatory effect of catecholamines on CA in the distal portion of the duodenum was mediated by cholinergic neurons of the enteral nervous system.

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