Role of Serotoninergic System in the Development of Gastrointestinal Diseases

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Experimental gastroduodenal ulcer was simulated after pre-injection of serotonin; gastroesophageal reflux disease and duodenogastral reflux were induced. Activation of the serotoninergic system promoted the development of alteration processes in gastroduodenal ulcer.

Key Words: *serotonin; electromotor activity; experimental gastroduodenal ulcer; gastroesophageal reflux*

Along with the parasympathetic and sympathetic systems, the serotoninergic system is involved in regulation of the contractile activity of visceral smooth muscles, including those in the gastrointestinal tract, common bile duct, and vessels [10]. Smooth-muscle cells express 5-HT_1 and 5-HT_2 effector serotonin receptors [7,9]. Intramural ganglionar neurons and enterochromaffine cells have surface 5-HT_3 and 5-HT_4 receptors. Through these receptors serotonin (5-HT) regulates the contractile activity of smooth muscles [1,4,13,14]. Serotonin induces contractions of the smooth-muscle cells of the fundal compartment of the stomach during reaction with 5-HT_{2B} receptors [4,6,8,12]. The contractile effect of 5-HT towards the smooth muscles of the gastric fundal compartment is confirmed by other studies [6,8,12].

We tried to detect the role of 5-HT and serotoninergic system in the development of ulcerative lesions of the gastrointestinal tract and refluxes of the upper portions of the gastrointestinal tract.

MATERIALS AND METHODS

Gastric ulcer was simulated in 24 male and female Wistar rats (220-250 g) narcotized by Nembutal (40 mg/kg). Electromotor activity (EMA) of the fundal, antral compartments of the stomach and ascending portion of the duodenum was studied using bipolar silver electrodes [3]. Local chemical irritation was inflicted by 100% glacial acetic acid at the interface of the fundal and antral compartments of the stomach along the greater curvature. Electromotor activity was recorded during the moment of the irritant application, during the first 15 min, 1 and 3 h, 1, 4, and 5 days after reproduction of the model.

The protective effects of 5-$HT_{1,2}$ receptor blocker (sumatryptane) on the development of ulcerative process was studied in 6 animals. The drug was injected in a dose of 1 mg/kg 30 min before gastric ulcer reproduction. Gastric tissue was fixed in 10% formalin, dehydrated in ascending alcohols, and embedded in paraffin. The sections were stained by hematoxylin and eosin.

Duodenal ulcer was simulated in 12 female Wistar rats (250-270 g) by applying 100% glacial acetic acid onto the serous membrane of the duodenal bulb. Electromotor activity of the duodenal bulbar compartment, antral and fundal compartments of the stomach was recorded before and directly after application of the chemical irritant, and 10 days after the beginning of experiment.

Statistical analysis of the amplitude and frequency characteristics of slow-wave and spike activities of the gastroduodenal smooth muscles was

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carried out. Serotonin was injected in doses of 10^{-5}-10^{-4} mg/kg intraperitoneally 1-3 min before simulation of duodenal ulcer.

Gastroesophageal reflux (GER) was simulated in 12 male Wistar rats (220-280 g) narcotized by Nembutal. Methylene Blue (0.2-0.5 ml 1% solution) was injected with an insulin syringe once or twice into the upper portion of the fundal compartment of the stomach. After 1-5 min EMA was recorded in the lower third of the esophagus, prefundal compartment, fundal and antral compartments of the stomach, and the ascending portion of the duodenum using submerged and non-submerged electrodes (extracellularly, bipolarly) before and during injection of Methylene Blue and during 7-10 min after injection. Gastroesophageal reflux was also induced by Methylene Blue injection into the prefundal compartment of the stomach under conditions of experimental gastric ulcer.

Duodenogastral reflux was simulated in 10 female Wistar rats (220-250 g), narcotized by Nembutal, by applying 100% glacial acetic acid onto the serous membrane of the duodenal bulb. Methylene Blue (0.2-0.5 ml of 1% solution) was then injected once or twice into the prefundal compartment of the stomach. After 3-7 min the EMA in the lower third of the esophagus increased and the esophageal mucosa was stained. The height of the reflux was evaluated by the length of stained portion of the distal part of the esophagus. The EMA was recorded in the lower third of the esophagus, fundal and antral compartments of the stomach, and ascending portion of the duodenum by submerged and nonsubmerged electrodes (extracellularly, bipolarly).

The data were processed using Statistics 6.0 software and Student's *t* test.

RESULTS

Acetic acid, applied to the interface between the gastric antrum and fundus, sharply stimulated EMA of both gastric compartments (Table 1). When applied to the gastric serous membrane, it immediately activated EMA of the studied zones (Fig. 1, *a*).

Fifteen minutes after application of the irritant the EMA of the fundal and antral compartments was "painful" (Table 1) and remained high after 24 h. The duodenal EMA normalized after 24 h (Table 1).

Gastric EMA was high during 4 days, while the duodenal motor activity normalized. After 5 days gastric EMA increased and persisted high till day 10.

Due to pre-injection of sumatryptane the frequency of EMA slow waves in the gastric antral and fundal compartments decreased 2-fold in response to application of acetic acid (Table 1). Hence,

TABLE 1. Electromotor Activity of Various Gastroduodenal Compartments before, 15 min and 24 h after Application of Acetic Acid or Injection of Sumatryptane

Period of registration	Stomach								Duodenum	
	fundus				antrum				frequency per min	amplitude, mV
	frequency per min		amplitude, mV		frequency per min		amplitude, mV			
	M±*m*	%	*M*±*m*	%	*M*±*m*	%	*M*±*m*	%	*M*±*m*	*M*±*m*
Basal	4.7±0.3		0.12±0.02		4.0±0.8		0.35±0.05		28.5±0.7	0.22±0.02
Acid application	17.5±0.5	272*	0.35±0.06	191*	14.0±0.5	350*	1.1±0.1	210*	27.5±0.5 -4	0.25±0.03 13
After 15 min of simulation	9.0±1.0	-48.6*	0.1±0.05	-71.4*	13.6±0.3	-2.8	1.25±0.2	13.6	26.8±2.3 2.5	0.23±0.02 8.0
After 24 h of experiment	6.0±0.6	-30.9*	0.15±0.05	50*	8.5±0.4	-37.5*	0.2±0.03	-84*	32.0±2.0 16.0*	0.18±0.03 28.0*
Sumatryptane, 30 min before simulation	8.0±1.0	-54.2*	0.1±0.02	-71.4*	8.0±1.0	-42.9*	0.2±0.03	-81.8*	23.0±1.0 -16.4*	0.25±0.05 0
Sumatryptane injection, after 24 h	6.0±0.6	-25*	0.15±0.05	50*	4.0±0.5	-50*	0.2±0.4	0	32.0±2.0 39.1*	0.2±0.04 -20

Note. *$p<0.05$ *vs.* basal level.

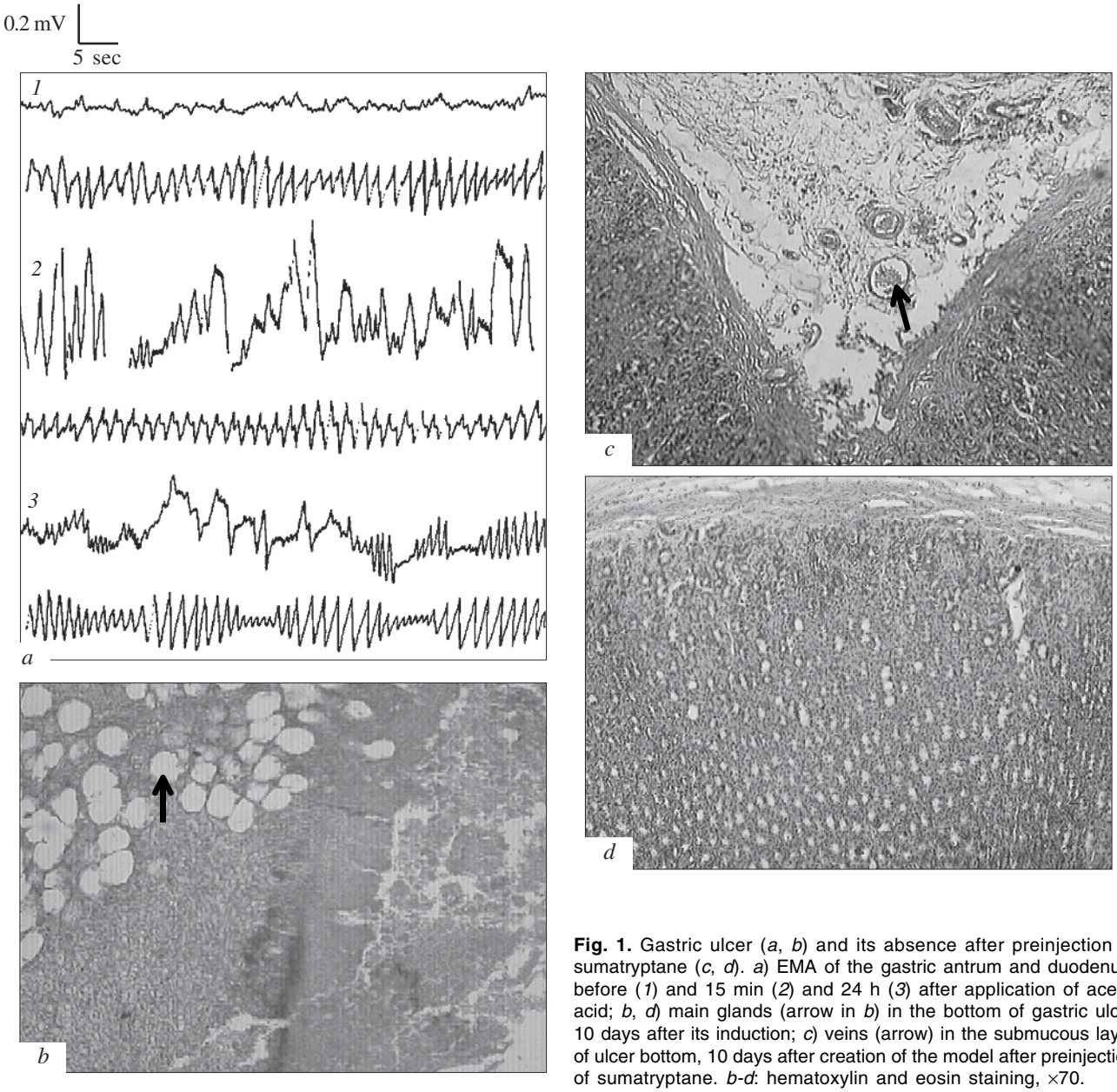


Fig. 1. Gastric ulcer (*a*, *b*) and its absence after preinjection of sumatryptane (*c*, *d*). *a*) EMA of the gastric antrum and duodenum before (*1*) and 15 min (*2*) and 24 h (*3*) after application of acetic acid; *b*, *d*) main glands (arrow in *b*) in the bottom of gastric ulcer 10 days after its induction; *c*) veins (arrow) in the submucous layer of ulcer bottom, 10 days after creation of the model after preinjection of sumatryptane. *b-d*: hematoxylin and eosin staining, ×70.

sumatryptane inhibited the development of high frequency high amplitude EMA waves in the gastric antrum and fundus.

Twenty-four hours after ulcer reproduction following preventive sumatryptane the amplitude and frequency of EMA slow waves in both gastric compartments exhibited a trend to normalization (Table 1), while the duodenal EMA somewhat decreased (Table 1). Hence, gastric ulcer leads to increase of its EMA and less so of the duodenal EMA, this activity being the most pronounced in the gastric antrum.

Preventive injection of sumatryptane promoted a marked reduction of the "painful" motoricity of the fundal and antral gastric compartments and the duodenum. Sumatryptane normalized the gastroduodenal motoricity in animals with experimental ulcer. This prompted analysis of the ulcer edges 10 days after the model creation. The veins of the submucous layer were dilated. Stasis signs were seen; the main glands were cystically dilated, the ducts were blocked (Fig. 1, *b*).

In animals pretreated by sumatryptane (Fig. 1, *c*) the ulcer during the same period was flat, healed, adhesions were negligible, and epithelialization was complete. The counts of fibroblasts in the marginal zone of the ulcer were low, as was the content of granulation tissue beyond the ulcer. Metachromasia was less pronounced. The main glands were present in the mucous membrane of the marginal zone of the ulcer. Epithelial proliferation was more pro-

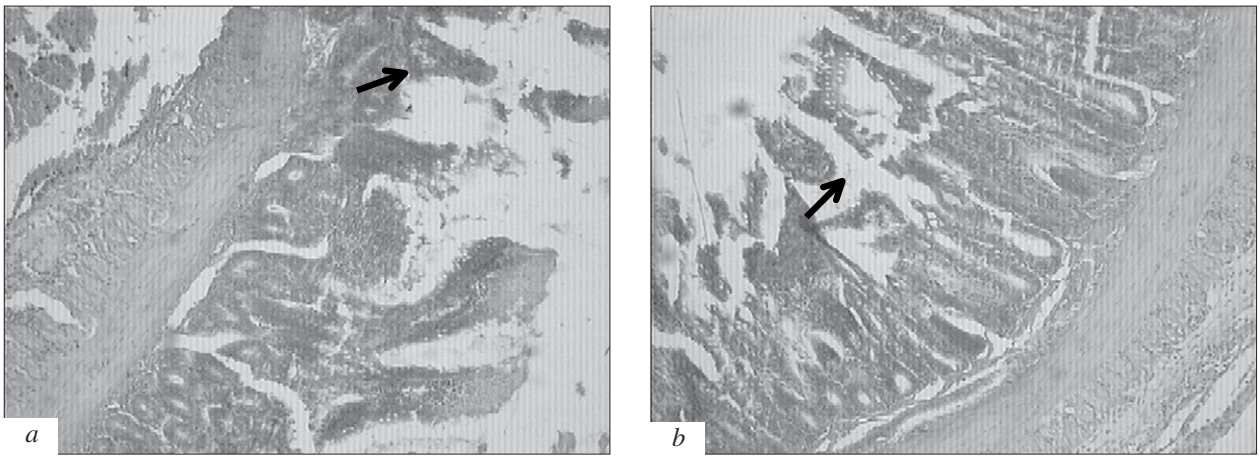


Fig. 2. Duodenal ulcer. Hematoxylin and eosin staining, ×120. *a*) ulcer edge, impairment of serous membrane, detritus (arrow); *b*) necrosis of villi (arrow).

nounced. The content of glucosaminoglycanes was higher in the ulcer edge mucosa.

Histological study of ulcer edges 10 days after its creation following sumatryptane injection (Fig. 1, *c*, *d*) showed functionally active vessels, which were not dilated; no stasis signs were seen; the main glands were of normal size, functionally active, the ducts were patent.

Hence, sumatryptane exhibited a gastroprotective effect, promoting reduction of the motor component of experimental gastric ulcer and stimulating its healing. The gastroprotective effect of sumatryptane prevention was confirmed.

Application of acetic acid to the duodenal bulbar zone resulted in activation of EMA slow waves. After 10 min the frequency increased from 30.0± 1.3 to 31.8±3.7/min (by 6%), the amplitude increased from 0.20±0.03 to 0.27±0.02 mV (by 35%, $p<0.05$). Spike activity also increased (from 0.04 to 0.16±0.04 spikes/100 slow waves, that is, by 300%; $p<0.01$); the amplitude of spikes increased from 0.22±0.03 to 0.43±0.12 mV (by 91%; $p<0.05$). The predominant increase of the spike activity indicates drastic activation of the motor component of the duodenal activity.

The gastric fundal EMA changed in the duodenum: the slow wave frequency increased from 10.2±3.2 to 13.0±3.5/min (by 27.4%), while the amplitude did not change (0.20±0.04 mV). Low-frequency low-amplitude spike activity was observed in 30.5% cases. Slow-wave activity of the gastric antrum was inhibited: the frequency decreased from 13.7±4.2 to 13.4±4.0/min (by 5.1%), the amplitude did not change (0.245±0.030 mV). Electromotor activity of the gastric antrum, the closest to the duodenal bulbar ulcer, somewhat decreased, which created conditions for reduction of the contractile activity of smooth-muscle cells and development of their atrophy. Presumably, the contractile function of the pyloric sphincter smooth myocytes suffered most of all, this being a prerequisite for subsequent development of pylorostenosis.

The frequency of EMA slow-waves increased by 13% in duodenal ulcer simulated after injection of 5-HT, reaching 28.7±0.6/min in the bulbar compartment, the amplitude remaining unchanged (0.23±0.03 mV). Pattern activity, detected in 20% of control specimens, acquired a higher amplitude type: 1.1±0.1 mV at the beginning and 1.7±0.3 mV in the middle. Preinjection of 5-HT stimulated the duodenal EMA in experimental duodenal ulcer.

Hence, a duodenal ulcer is associated with activation of motor activity of its bulbar compartment in parallel with a retrograde reduction of EMA in the gastric compartments.

The results are comparable with clinical morphological findings [5] indicating that patients with ulcers in the duodenal bulb develop pylorostenosis. Presumably, its development is mediated through retrograde impairment of the pyloric sphincter smooth muscle EMA and subsequent atrophy of smooth muscle cells, development of connective tissue of increasing compactness (from elastic and collagen fibers with connective tissue cells to hyaline-like tissue).

The development of experimental duodenal ulcer is characterized by the presence of detritus in the ulcer, neutrophilic infiltration of the duodenal wall, dilatation of adventitial layer vessels, and hemorrhages (Fig. 2, *a*, *b*). These morphological changes are similar to those observed in gastric ulcer and are largely caused by the development of the hyperserotoninergic syndrome, associated with the development of edema, mucosal infiltration, and dilatation of veins [1].

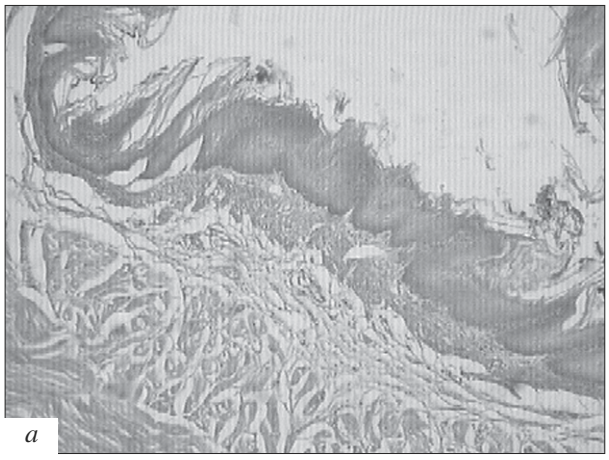
Before simulation of GER the basal frequency of slow-wave EMA in the gastric antrum was 7.5±1.5/min, with amplitude of 0.17±0.01 mV. In 25% cases slow waves were grouped in patterns of 3-4 to 9 slow waves with a 0.1 mV amplitude at the beginning of the pattern and 0.20 mV peaks in the middle of the pattern. Basal frequency of EMA slow waves in the ascending duodenum was 33.2±3.4/min with amplitude of 0.24±0.06 mV. A microphotograph of the normal esophagus is presented in Fig. 3, *a*.

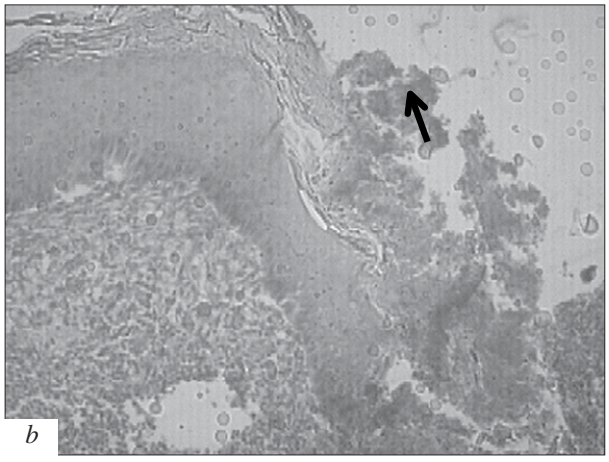
Methylene Blue stimulated EMA in the lower third of the esophagus, stained the esophageal mucosa, and led to emergence of foci of necrosis after 24 hours in some experiments (Fig. 3, *b*). The frequency of slow waves increased 2.4 times, amplitude by 40%, reaching 0.21±0.05 mV *vs.* basal frequency of EMA slow waves 19±4/min and amplitude of 0.15±0.02 mV (Fig. 3, *c*).

The slow-wave activity of the gastric prefundal compartment also changed during this period: slow wave frequency increased 2.2 times (from 9.1±1.1 to 20.2±2.8/min), while the amplitude somewhat decreased (from 0.20±0.05 to 0.16±0.06 mV; by 20%). The disappearance of spike activity is worthy of note; it has been detected in 25% cases at a frequency of 0.1 spike/100 slow waves and amplitude of 0.25 mV. The frequency of EMA slow waves in the gastric fundus increased from 7.8±0.8 to 15.0±1.5/min (by 92.3%; $p<0.05$), while the amplitude decreased from 0.22±0.04 to 0.14±0.02 mV (by 36.3%; $p<0.01$). Low-amplitude spike activity emerged in 50% experiments; its frequency varied from 0.16 to 0.64/100 slow waves.

Gastric antral EMA increased 2.45 times in experimental GER (from 7.5±1.5 to 18.3±2.4/min; $p<0.05$), the amplitude remaining virtually unchanged (0.60±0.06; by 6%). The duodenal EMA tended to decrease during the first 30-40 min of GER simulation: the slow wave amplitude decreased by 8.3% (from 0.20±0.03 to 0.22±0.05 mV), the frequency decreasing by 6.6% (from 29.2±2.9 to 31.0±1.5/min). Spike activity, observed before creation of GER model, was retained after Methylene Blue injection.

The smooth muscle EMA in the lower third of the esophagus and upper portion of the gastric fundus were activated in experimental GER, which was paralleled by relaxation of the annular muscles (similar to the lower esophageal sphincter) due to the effect of NO, donated by Methylene Blue.





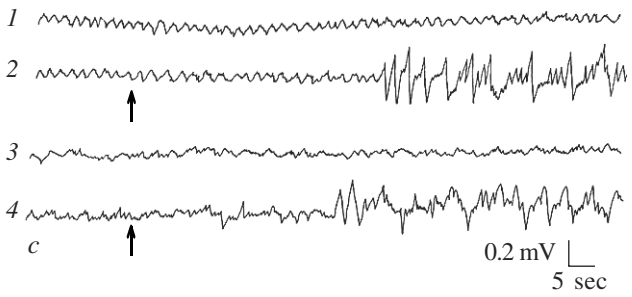


Fig. 3. Esophagus in health (*a*) and lower third of esophagus in experimental GER; necrotic focus (*b*); EMA (*c*). *a*) normal esophagus. Hematoxylin and eosin staining, ×120; *b*) lower third of esophagus in GER; necrotic focus (arrow). *a-b*: hematoxylin and eosin staining, ×120. *c*) esophageal EMA: in health (*1*), after injection of Methylene Blue into gastric cardia (*2*), in normal gastric cardia (*3*), and after injection of Methylene Blue (*4*). Arrow shows injection of Methylene Blue.

Activation of the smooth muscles in the fundal and antral compartments of the stomach was less pronounced than in the prefundal compartment, while the duodenal EMA virtually did not change in GER induced by Methylene Blue. It seems that the cholinergic and serotoninergic systems of the gastric intramural nervous system are involved in the development of GER. These systems promote the formation of motor hyperkinesia in the proximal part of the stomach, which is also characteristic of GER.

Methylene Blue, injected into the prefundal compartment of the stomach under conditions of gastric ulcer, led to development of GER. Basal frequency of EMA slow waves in the lower third of the esophagus was 11.1±1.0/min, amplitude 0.17±0.02 mV; basal frequency of EMA slow waves in the prefundal part of the stomach was 12.2± 1.8/min, amplitude 0.20±0.05 mV. Injection of the stain promoted EMA activation in the lower third of the esophagus and staining of its mucosa, in other words, led to electrophysiologically documented and visualized GER. The frequency of EMA slow waves in the esophagus increased by 129.8% and reached 25.5±6.1/min ($p<0.05$), the amplitude increased by 53%, reaching 0.26±0.04 mV ($p<0.05$). Spike activity with the rapid biopotentials frequency of 0.39±0.03/100 slow waves and amplitude of 0.19±0.02 mV emerged in 77.7% cases. The emergence of spike activity indicated a significant increase of the esophageal motor activity.

Intensification of EMA was noted in the prefundal part of the stomach: the frequency of slow waves increased by 154% and reached 31.0±0.5/ min ($p<0.05$), while the amplitude changed negligibly (0.21±0.03 mV). Hence, injection of Methylene Blue into the gastric prefundal portion led to development of reflux into the lower third of the esophagus with staining of its mucosa.

The gastric fundal portion reacted to intracavitary injection of Methylene Blue by an increase in the slow wave frequency from 12.5±4.5 to 24.8± 3.4/min (by 98.4%; $p<0.05$) and of amplitude from 0.15±0.03 to 0.26±0.09 mV (by 73.3%), and also by emergence of spike activity in 25% cases with spike frequency of 0.24/100 slow waves and amplitude of 0.21±0.3 mV, which fact indicated an increase of EMA in parallel with development of GER. During the same period the gastric antrum reacted to Methylene Blue by increase of the amplitude and frequency characteristics of EMA slow waves. Frequency increased from 7.0±1.2 to 12.5± 1.3/min (by 78.5%; $p<0.05$), amplitude increased from 0.20±0.01 to 0.38±0.04 mV (by 90%; $p<0.05$). Previously detected probable early development of the "macro re-entry" phenomenon in the stomach in ulcer simulation after Okabe [2] suggested the participation of this mechanism in GER intensification in response to Methylene Blue under conditions of experimental gastric ulcer. The stain injected during the same period into the gastric prefundal portion slightly modified the duodenal slow-wave activity: the frequency increased by just 3.9% (from 29.3±1.0 to 30.5±2.5/min), amplitude by 9.1% (from 0.33±0.07 to 0.36±0.06) mV. Hence, intensification of GER after injection of Methylene Blue under conditions of gastric ulcer could be explained by the development of the macro re-entry phenomenon.

Gastroesophageal reflux developing under conditions of gastric ulcer in 27.7% cases was higher, with regurgitation of stained gastric contents into the upper airways, which caused the death of 2 animals. These observations were confirmed by the results of esophageal electromyogram analysis in experimental GER and GER under conditions of ulcer. In the former case the frequency of esophageal smooth muscle slow waves increased by 70.6% and the amplitude by 46.1%, in the latter by 129.8 and 53%, respectively. Hence, Methylene Blue injection to animals with gastric ulcer was associated with an additional elevation of EMA in the lower portions of the esophagus, which fact confirms the involvement of the macro re-entry phenomenon and the relevant intensification of EMA in the antegrade and retrograde directions. Peptic ulcer stimulates the electrophysiological manifestations of GER, which is observed in clinical practice. Morphological findings confirmed the presence of lesions in the esophageal mucosa in experimental GER.

The frequency of slow-wave activity in the lower third of the esophagus in intragastric administration of Methylene Blue 5 days after duodenal ulcer induction increased from 11.4±0.9 to 16.5±2.8/min (by 44.7%; $p<0.05$), the amplitude increasing from 0.25±0.09 to 0.32±0.03 mV (by 28%; $p<0.05$). Moreover, the bile was detected in the pyloric and antral compartments of the stomach (development of the duodenogastral reflux). Increase of the slow-wave activity of the esophagus during GER development in the presence of duodenal ulcer was less pronounced than in gastric ulcer.

The fundal portion EMA decreased slightly in response to Methylene Blue in duodenal ulcer: the frequency decreased from 18.3±2.2 to 15.5±1.8/ min, the amplitude decreased from 0.23±0.02 to 0.18±0.02 mV.

Infusion of Methylene Blue into the gastric cavity was associated with intensification of the antral EMA: slow wave frequency reached 17.0±2.5/min (143% increase; $p<0.01$), amplitude reached 0.28± 0.02 mV (40% increase; $p<0.05$).

Changes in EMA in the antral portion of the stomach under conditions of duodenal ulcer consisted in an increase of the EMA frequency component and reduction of the amplitude in comparison with the data obtained in GER simulation under conditions of gastric ulcer. This indicates modification of the chronoinotropic proportions in the smooth muscles of the gastric antrum. Hence, a descending gradient of EMA activation (from the antral to fundal compartment and lower third of the esophagus) is forming in duodenal ulcer. This, in turn, leads to development of the duodenogastral and rather low gastroesophageal reflux. Activation of the serotoninergic system leads to an increase in the reflux heights, promotes the development of alteration processes in gastroduodenal ulcer.

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