Serotoninergic System in the Development of Pyloric Stenosis and Pancreatitis

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 146, No. 8, pp. 226-232, August, 2008
Original article submitted August 13, 2007

Pyloric stenosis and pancreatitis were simulated before and after administration of serotonin and spiperone (5-HT_2 receptor blocker). Activation of the serotoninergic system prevented the development of pancreatitis, but led to more severe course of pyloric stenosis.

Key Words: *serotonin; 5-HT_2 receptors; electromotor activity; pyloric stenosis; pancreatitis*

Serotonin injection activates motility of the pancreatic duct via activation of 5-HT_1, 5-HT_2, 5-HT_3, and 5-HT_4 receptors [7,8]. Serotonin activation of 5-HT_2 receptors plays an important role in aggravation of acute pancreatitis in mice induced by choline-deficient and ethionine-rich diet [9]. However, activation of 5-HT_2 receptors by endogenous serotonin is not the only effect of this substance on the secretory motor activity of the pancreas and its duct. The role of serotonin-activated 5-HT_1 and 5-$HT_{3,4}$ receptors is not quite clear, and hence, the impact of serotonin in general for pancreatitis development cannot be completely characterized.

Propulsive activity of the gastrointestinal tract depends on the stimulatory effect of serotonin on smooth muscles [1] and on the stimulatory influences realized via acetylcholine [5]. Impairment of the sphincter function, for example, in duodenal ulcer, leads to dissociation of the gastroduodenal motility.

We studied the role of the serotoninergic system in the development of pyloric stenosis and pancreatitis.

MATERIALS AND METHODS

Pyloric stenosis was induced in 22 Wistar rats (220-250 g) during surgical stage of Nembutal narcosis (40 mg/kg). After fixation of the animal and opening of the abdominal cavity, the frequency and amplitude of electromotor activity (EMA) slow waves and spike activity of the antral part of the stomach, pyloric sphincter, and of the proximal portion of the duodenum were recorded via surface contact bipolar electrodes attached to the serosa (contact surface area 1.5-2 mm^2 and distance between electrodes 1.2-1.5 mm). Serotonin was injected intravenously in doses of 1×10^{-5}-1×10^{-4} mg/kg, spiperone (5-HT_2 receptor blocker) in doses of 0.5-1.0 mg/kg intraperitoneally 1-3 and 15-20 min, respectively, before simulation of pyloric stenosis. The effects of serotonin and spiperone on the course of pyloric stenosis were evaluated during the first minutes and on day 15 of the experiment.

Clearly discernible and well-pronounced pyloroduodenal sphincter is situated at the boundary between the pylorus and the duodenum [3]. A loose ligature was applied in this area, which later caused the development of pyloric stenosis. Subsequent recording of gastroduodenal EMA was carried out on days 5 (group 1; n=10), 10 (group 2; n=7), and 15 (group 3; n=5) of the experiment, after which the pyloric sphincter tissue was fixed in 4% paraform in Hanks saline for 2 h and postfixed in 1% OsO_4 for 2 h. After dehydration, the tissue was embedded in epon and araldite mixture. Tissue status was evaluated on 1-μ sections stained with methylene blue.

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0007-4888/08/1462-0258

Pancreatitis was simulated in 18 Wistar rats by retrograde injection of 0.1 ml picrylsulfonic acid with 96% ethanol (1:1) into the pancreatic duct. EMA of the pancreatic duct was recorded in the control, directly after injection of picrylsulfonic acid, and on day 15 of pancreatitis simulation. The morphology of the pancreas was studied on day 15 of pancreatitis. Tissue specimens were fixed routinely and stained with methylene blue.

The role of the serotoninergic system in the development of pancreatitis was studied in a series of the experiments (n=5) by a retrograde injection of serotonin into the pancreatic duct (0.1 ml of 1×10^{-4}-1×10^{-5} g/liter saline) 2-3 min before pancreatitis modeling (n=5) and by injection of spiperone (5-HT_2 receptor blocker) in a dose of 1 mg/kg 15-20 min before and on day 15 of pancreatitis simulation (n=5). The morphology of the pancreas was studied on day 15 of pancreatitis development after preinjection of serotonin.

The data were statistically processed using Statistica 6.0 software with evaluation of the confidence interval.

RESULTS

The initial frequency of EMA slow waves in the antrum was 7.00±0.73/min, amplitude 0.22±0.04 mV. EMA of the pylorus presented as slow-wave activity of 4.4±0.3/min frequency, the amplitude of each wave varying within 0.5±0.1 mV, with 11.3±1.5 low-amplitude waves on each wave (Fig. 1, *1*). Low-frequency spike activity was observed in some animals.

The frequency of EMA slow waves in the proximal portion of the duodenum in controls was 28.5±2.1/min, slow wave amplitude being 0.23±0.03 mV.

Ligature application at the interface between the pylorus and the duodenum led to modification of the slow-wave activity of the antral compartment of the stomach: the frequency of slow waves was 7.9±0.8/min, amplitude 0.20±0.03 mV (Fig. 1, *2*). In addition, low-amplitude spike activity was recorded in 13% cases, indicating high motor activity of the antrum. Hence, application of the ligature led to a minor (14%) increase in the frequency of EMA slow waves in the antral part of the stomach.

Ligation of the pyloric sphincter was associated with changes in the duodenal EMA: slow wave frequency decreased to 25.1±3.3/min with the slow wave amplitude 0.22±0.04 mV. Hence, application of the ligature led to a slight (12%) reduction of the duodenal slow-wave EMA frequency at a stable amplitude.

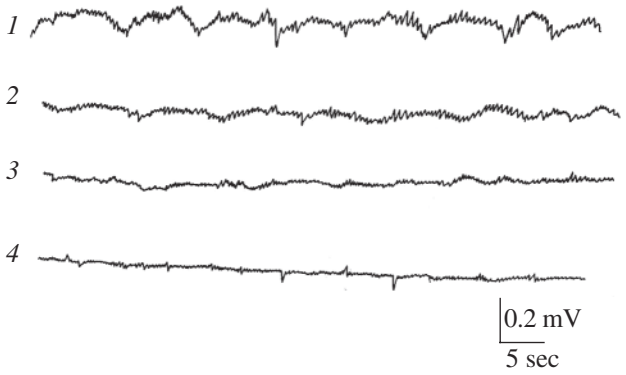

Fig. 1. Electromotor activity of the gastric pyloric sphincter in health (*1*), after ligature application (*2*), on day 15 of stenosis (*3*) and pyloric stenosis after preinjection of spiperone (*4*).

Creation of pyloric stenosis was associated with activation of the sphincter slow-wave EMA: slow-wave frequency increased to 4.8±0.4/min (by 9%), amplitude to 0.60±0.04 mV (by 9%); high-frequency and high-amplitude spike activity appeared. Initially high sphincter EMA was observed in 4 animals, and creation of stenosis led to suppression of slow-wave activity: slow-wave frequency decreased from 6.3±0.9 to 5.0±0.9/min (by 20.6%), while the amplitude increased from 0.55±0.70 to 0.7±0.1 mV (by 27.2%). Spike activity decreased.

Serotonin injection led to activation of the pyloric EMA: slow wave frequency increased to 4.9±0.4/min (by 11.4%), amplitude reached 0.75±0.09 mV (36.3%; p<0.05). Each slow wave carried 14.5±2.4 low-amplitude waves; the incidence of spike activity increased virtually 2-fold and was high-frequency, this indicating intensification of motor activity of the pyloric compartment of the stomach.

Creation of pyloric stenosis after serotonin injection increased the frequency of the sphincter EMA slow waves to 5.7±1.6/min (by 12.2%) and their amplitude to 0.85±0.09 mV (by 13.3%); high-amplitude spike activity was also frequently detected. Hence, creation of pyloric stenosis after serotonin injection was associated with activation of pyloric sphincter EMA.

Simulation of pyloric stenosis after preliminary (15-20 min before ligature application) injection of 5-HT_2 serotonin blocker was associated with a decrease in pyloric EMA to 4.5±0.5/min (8.2%) and its amplitude to 0.20±0.04 mV (73.3%; p<0.01; Fig. 1, *4*). This suppression of the pyloric sphincter smooth muscle EMA was associated with deterioration of animal status, which attested to an important role of the serotoninergic system in the development of pyloric stenosis.

The study of antral EMA in group 1 animals on day 5 of the experiment detected the following chan-

ges. The frequency of EMA slow waves was 8.2± 1.7/min (17% increase), amplitude 0.34±0.05 mV (54.5% increase; $p<0.05$).

The frequency of the duodenal proximal portion EMA slow waves at this stage of the experiment somewhat decreased (23.5±3.4/min), while the amplitude increased to 0.31±0.04 mV. High-amplitude spikes were recorded in 12.5% cases, indicating certain activation of the motor constituent of duodenal activity.

Macroscopic examination showed that pyloric mucosa on day 5 of pyloric stenosis was pale pink, the wall was thickened. The antral and fundal mucosa had normal color, the size of the stomach was unchanged. The duodenum looked unchanged.

Morphological study of the pyloroduodenal sphincter detected extension of the connective tissue layer between muscle layers and penetration of the connective tissue into the muscle layers (Fig. 2, *b*). Solitary smooth muscle cells or their groups at some places were surrounded with the connective tissue. Some smooth muscle cells were characterized by pronounced degeneration.

On day 10 of pyloric stenosis development, dilatation of the stomach was observed. The pyloric channel mucosa was pale, the channel was sharply stenosed. The antral and fundal compartments of the stomach were pale. The duodenum was of normal color, without contents. An increase of the amplitude and frequency characteristics of the antral compartment was detected at this stage of pyloric stenosis development: the frequency of EMA slow waves was 9.3±0.8/min (32.8% increase), amplitude 1.20±0.06 mV (445% increase; $p<0.05$). Hence, the status of the pylorostenosed region was subcompensated: an increase of the EMA amplitude and frequency characteristics was paralleled by dilatation of the stomach.

Morphological study of the pyloric sphincter on day 10 revealed changed structure of the connective tissue between muscle layers (Fig. 2, *c*). Groups of fine collagen fibers appeared against the background of honeycomb structure of the connective tissue. Groups of severely atrophic smooth muscle cells and dilated vessels (mainly capillaries and veins) were seen in wide layers between the bundles. This dilatation of the capillary and venous microvessels is characteristic of local increase of serotonin content in the studied tissue. The connective tissue penetrated more extensively into the

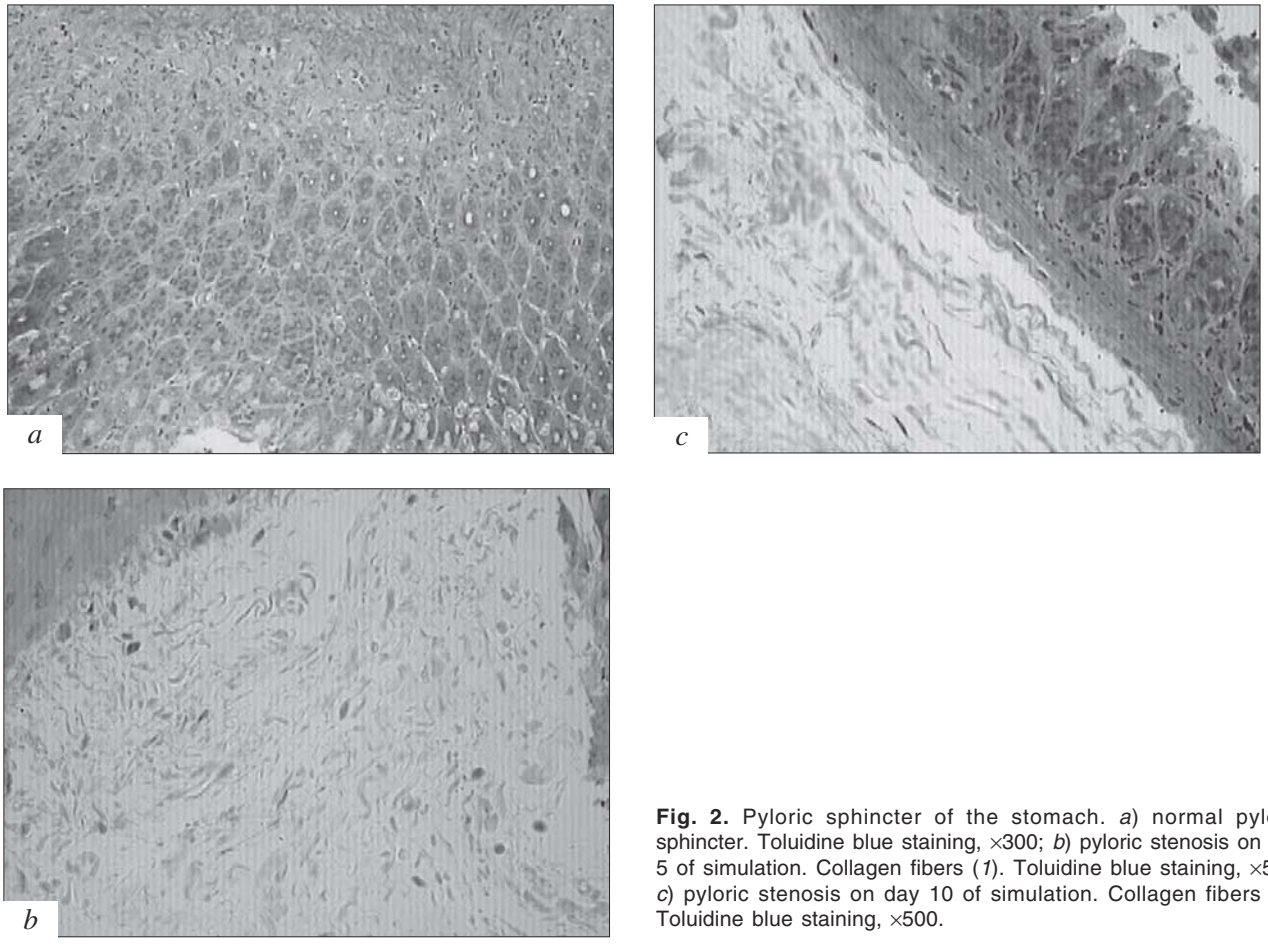

Fig. 2. Pyloric sphincter of the stomach. *a*) normal pyloric sphincter. Toluidine blue staining, ×300; *b*) pyloric stenosis on day 5 of simulation. Collagen fibers (*1*). Toluidine blue staining, ×500; *c*) pyloric stenosis on day 10 of simulation. Collagen fibers (*1*). Toluidine blue staining, ×500.

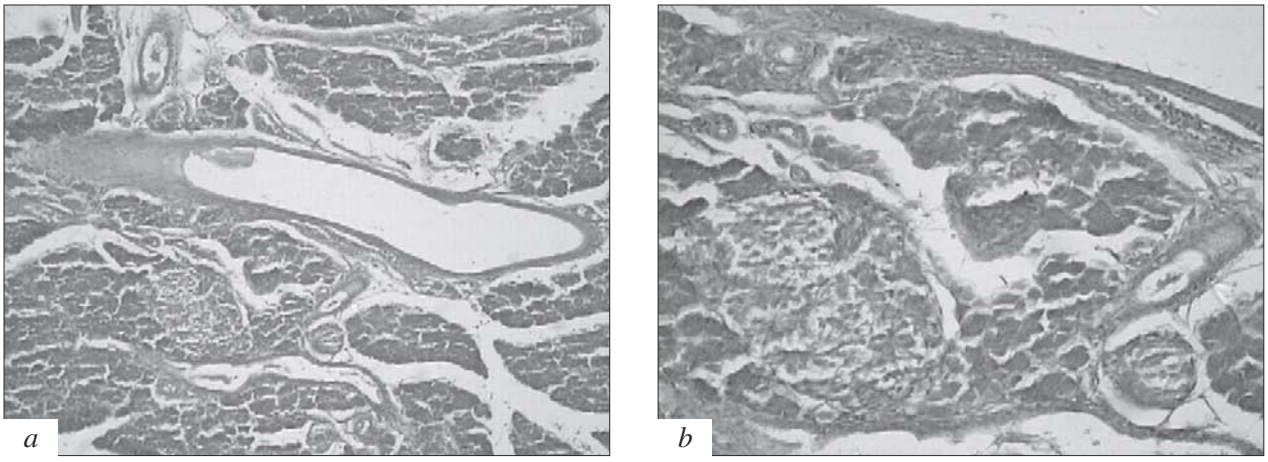

Fig. 3. Pancreatitis after preinjection of serotonin. *a*) sharp vascular dilatation. Hematoxylin and eosin staining, ×120; *b*) dilated vessel. Lobular edema (*1*). Sharp vascular dilatation. Hematoxylin and eosin staining, ×120.

depth of the muscle tissue, separating the bundles of smooth muscle cells. Atrophic muscle cells were seen at the periphery of muscle layers. On the other hand, many smooth muscle cells in the depth of muscles were hypertrophic.

On day 15 of the pyloric sphincter stenosis, the stomach was hyperstretched, its wall was sharply thinned, the antral and fundal portions were pale. The pyloric channel was closed, its wall was compact, the mucosa was pale. The duodenum was of its common color, spasmodic (Fig. 1, *3*). The amplitude of the antral EMA slow waves dropped to 0.15±0.01 mV, their frequency remaining sufficiently high (8.7±0.8/min); the frequency of slow wave activity in the pylorus was 6.3±0.5/min, amplitude 0.10±0.01 mV. The frequency characteristics of the duodenal EMA slow waves increased to 33.0±4.5/min at a negligible amplitude of 0.25± 0.04 mV.

Morphological study of the pyloric sphincter on day 15 of pyloric stenosis detected massive degeneration of smooth muscle cells, hypertrophic muscle elements, dark smooth muscle cells, and more pronounced stratification of smooth muscle cell bundles as a result of connective tissue growth.

Electrophysiological study of the pyloroduodenal area and pyloric sphincter during the formation of stenosis revealed significant changes in EMA activity of the gastroduodenal smooth muscles. Electrophysiological study of the pyloroduodenal region at the stage of compensated pyloric stenosis (day 5) showed a significant increase in slow wave activity of antral smooth muscles, the duodenal smooth muscle EMA being changed just negligibly. Widening of the connective tissue layer between the muscle layers corresponded to these changes; pronounced degeneration of some smooth muscle cells developed. Hence, day 5 of pyloric stenosis was characterized by complete functional compensation.

The progress of pyloric stenosis (day 15 of the experiment) led to further reduction of contractile activity of the gastroduodenal smooth muscles. Electrophysiological characteristics of the gastric antral smooth muscles sharply decreased. The stomach was dilated, the patency of the pyloric channel was impaired. All these processes created conditions for the development of decompensation. Importantly that creation of pyloric stenosis after preinjection of serotonin was paralleled by activation of the pyloric sphincter EMA. Simulation of pyloric stenosis after preinjection of spiperone was associated with suppression of the gastric pyloric EMA, indicating an important role of the serotoninergic system in the development of pyloric stenosis.

Experimental data on the possible role of serotoninergic systems in the development of pyloric stenosis was confirmed by other authors [5]. The development of pyloric stenosis in stubborn gastroduodenal ulcers is associated with intensive release of serotonin stimulating the serotonin receptors of the effector tissues in the pyloric channel and antral portion of the stomach. The effects of serotonin on smooth muscles (similarly as its effects on the vessels) can lead to the development of a long spasm of the pyloric sphincter with subsequent formation of stenosis. Dilatation of the gastric antrum during the development of pyloric stenosis seems to be caused not only by mechanical blocking of the gastric contents passage into the duodenum, but also by a known dilating effect of serotonin on the smooth muscles, for example, myocytes of the veins. Diverse effects of serotonin on the smooth muscles of the gastric antrum and pylo-

ric sphincter are mediated by its interactions with 5-HT_2 and presumably 5-HT_1 receptors in the effector tissues [4,9].

Control frequency of the pancreatic duct EMA in intact rats is 30.5±3.7/min at amplitude 0.4±0.1 mV. In 75% cases the EMA slow waves are grouped in 9-12 waves, carrying 3-4 low-amplitude waves each, in the rest cases pattern activity is observed. Each pattern includes 7-10 waves; 2-3 patterns/min are recorded. The amplitude of the waves at the beginning of a pattern is 0.15±0.03 mV, in the middle 0.27±0.05 mV.

Injection of picrylsulfonic acid led to an increase of the pancreatic duct EMA: the frequency of slow waves increased to 39-42/min. In 6 animals the frequency of slow-wave activity was 13.0±1.0/min at an amplitude of 0.5±0.1 mV, the waves being grouped in patterns. Each pattern consisted of 7-8 waves, with an amplitude of 0.10±0.02 mV at the beginning and 0.20±0.03 mV in the middle of the pattern. Hence, increase of the frequency and amplitude of slow wave activity were observed during pancreatitis simulation. The pattern activity became more smooth and uniform, this indicating stabilization of de- and repolarization processes on the smooth muscle cell membranes in the duct.

The frequency of pancreatic duct EMA on day 15 of pancreatitis simulation was 10.1±0.2/min (22.3% less) at 0.78±0.20 mV amplitude (56% increase). Mean amplitude spike activity was detected in 33% cases, each slow wave being associated with a spike.

Morphological study of the pancreas 15 days after injection of picrylsulfonic acid showed a slight increase in the interlobular and intralobular connective tissue and lobular edema (Fig. 3, *b*). Thin intralobular connective tissue layers separated small groups of cells or individual cells. Unchanged arteries and slightly dilated veins with marginal stasis of erythrocytes were seen in the interlobular space. The density of endocrinocytes in the islets was slightly lowered: spaces between cells were dilated and filled by connective tissue with solitary capillaries. The majority of exocrinocytes contained zymogen granules. Small accumulations of destroyed cells were seen in some areas of the gland. Cells with degenerative changes (vacuolated cytoplasm, no secretory granules, nuclei of normal structure or with different degree of condensation) were seen in some sites of the gland. Unchanged chromatin pattern in intact nuclei was seen against the background of clear karyoplasm. Cells modified by destruction contained few zymogen granules. Capillaries were dilated (Fig. 3, *a*).

Serotonin injection 2-3 min before pancreatitis creation led to modification of the frequency and amplitude characteristics of the pancreatic duct EMA: the frequency of slow waves was 12.6±2.7/min at unchanged amplitude (0.40±0.05 mV), with 4-5 waves of medium amplitude grouped on each slow wave. High frequency (37.0±3.2/min) and high amplitude (1.0±0.2 mV) waves were detected in 16.6% cases.

The pancreatic duct EMA activity on day 15 of simulated pancreatitis after preinjection of serotonin was characterized by decrease of the amplitude and frequency characteristics of EMA slow wave activity: frequency of 10.5±0.9/min (16.5%) at 0.35±0.05 mV amplitude (12.5% reduction), with 3-4 low-amplitude waves located on each slow wave.

Morphological study of the pancreas under conditions of serotonin preinjection showed that small groups of exocrinocytes were separated by thin intralobular connective tissue layers. Dilated vessels with marginal stasis of erythrocytes were seen in the interlobular space. No homogenous protein substance was detected in the ducts.

Injection of spiperone during pancreatitis simulation blocked serotonin effect on pancreatitis development and motility of the pancreatic duct: slow wave frequency was 30.0±5.2/min at an amplitude of 0.45±0.10 mV. Spike activity with a frequency of 4 per 10 waves and amplitude of 1.2±0.2 mV manifested in half of experiments.

Study of the pancreatic duct EMA on day 15 under conditions of spiperone treatment with subsequent pancreatitis simulation showed reduction of EMA slow wave frequency to 9.1±0.8 at an amplitude of 0.70±0.08 mV; spike activity was detected in 66% cases with a frequency of 0.35±0.05/min and amplitude of 0.30±0.04 mV — hence, the contractile activity of smooth muscle cells of the duct reduced.

Hence, pancreatitis simulation was associated with a certain increase of the amplitude and frequency characteristics of the pancreatic duct, which however, did not reach the threshold level and were not associated with pronounced changes in the motor activity of the duct. Changes in the ductal smooth muscle cell function were paralleled by destruction of part of exocrinocytes, accumulation of viscous secretion in the pancreatic small ducts, which, together with reduced motor function of the duct, can lead to activation of proteolytic enzymes in the tissue of the gland proper with probable development of its further destruction.

Exogenous serotonin activates serotoninergic ganglionar neurons and pre- and postsynaptic serotonin receptors. Activation of postsynaptic receptors leads to stimulation of smooth muscle cells. Activation of presynaptic receptors leads to inhibition of serotonin release by the nerve terminals. It seems

that exogenous serotonin, reacting with stimulatory postsynaptic receptors, leads to depolarization of the smooth muscle cell membranes, and this mechanism of action predominates. Reacting with inhibitory presynaptic receptors, serotonin suppresses release of the mediator by the nerve terminals, which leads in some experiments to suppression of spike activity. The latter fact is proven by the results of our experiments with injection of 5-HT_2 receptor blocker. Spiperone promoted a suppression of spike activity of the ductal smooth muscle cells and stimulated the duct motility.

Hence, activation of the pancreatic duct motility in pancreatitis created after preinjection of serotonin does not lead to development of severe morphological changes in the structure of the pancreas and its exocrinocytes; no accumulation of viscous secretion, impeding the discharge of pancreatic secretion into the duodenum, was detected in small pancreatic ducts. Serotonin exhibited a protective effect during the development of pancreatitis. Activation of serotoninergic system prevented the development of pancreatitis, but promoted a more severe course of pyloric stenosis.

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