Role of the Serotoninergic System in the Development of Diseases of the Intestinal and Biliferous Tracts

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Primary sclerosing cholangitis and nonspecific ulcerative colitis were modeled before and after serotonin and spiperone injections. Activation of the serotoninergic system prevented the development of primary sclerosing cholangitis, but promoted more severe course of nonspecific ulcerative colitis.

Key Words: *cholangitis; ulcerative colitis; serotonin; spiperone; electromotor activity*

The serotoninergic system is presumably involved in the regulation of smooth muscle cells of the gastrointestinal tract and common bile duct. 5-HT_4 receptors involved in the regulation of motor activity of the gastrointestinal tract, including the large intestine, are present in the gastrointestinal tract [9]. Oral mosapride (5-HT_4 receptor agonist) stimulates motor activity of the small intestine and cecum in horses; this can be used for correction of small and large intestinal dyskinesias [10]. Dilatation of the cecum is associated with reduced levels of 5-HT_{1B}, 5-HT_{2B}, and 5-HT_4 receptor mRNA in cecum preparations. This confirms the involvement of the serotoninergic system in the regulation of cecal motor activity and tone [4]. Serotonin is present in enterochromaffine cells of the gastrointestinal tract [8]. It causes contractions of isolated common bile duct of guinea pig in a dose-dependent manner: its low doses activate 5-HT_2 receptors, while in high doses the contractions depend on acetylcholine release with participation of 5-HT_3 receptors [12].

Disorders in the nervous regulation of the intestine contribute to the pathogenesis of nonspecific ulcerative colitis (NUC) [1]. It seems that the progress of NUC and development of clinical symptoms depend also on the function of serotonin-containing cells constituting about 70% of the total population of intestinal neurons. The development of NUC is paralleled by an increase in the count of melatonin-secreting apudocytes (EC-2) and serotonin-containing mast cells, but the percentage of these cells decreases with the progress of NUC [2]. Serotonin excess is eliminated from the synaptic cleft by serotonin transporter (SERT) [6,8].

We studied the role of serotonin and serotoninergic system in the development of primary sclerosing cholangitis and NUC.

MATERIALS AND METHODS

Primary sclerosing cholangitis (PSC) was simulated in 28 Wistar rats (200-220 g) narcotized with Nembutal (40 mg/kg). The animals received a retrograde injection of picrylsulfonic acid (0.1 ml; 1:1 with 96% ethylene) into the common bile duct. The function of the common bile duct was evaluated by electromotor activity (EMA) of smooth muscles (amplitude and frequency of slow waves and spike activity) under conditions of laparotomy. In group 1 (n=14), EMA was recorded before the experiment, directly after injection of picrylsulfonic acid, and on day 15 of experimental PSC. Morphological studies of the liver and intrahepatic bile ducts were carried out on day 15 of PSC on routinely stained tissue specimens (toluidine blue).

Group 2 animals (n=7) received a retrograde injection of serotonin (0.1 ml; 10^{-4}-10^{-5} g/liter sa-

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line) into the common bile duct 2-3 min before injection of picrylsulfonic acid. The common bile duct EMA was recorded before drug injections, after serotonin injection, and during injection of picrylsulfonic acid. Morphological study of the liver and intrahepatic bile ducts in this experimental series was also carried out on day 15 of PSC after serotonin preinjection.

Possible involvement of 5-HT_2 receptors in the development of PSC was detected by a retrograde injection of 5-HT_2 receptor blocker spiperone in a dose of 1 mg/kg into the common bile duct (group 3; n=7). Primary sclerosing cholangitis was induced 20-30 min after injection of the blocker. The common bile duct EMA was recorded before injection of spiperone and during experimental PSC simulated after spiperone injection.

Nonspecific ulcerative colitis was simulated on 24 Wistar rats (250-270 g) narcotized with Nembutal (40 mg/kg) by injection of 45-50% solution of picrylsulfonic acid in ethylene into the cecal lumen with an insulin syringe. The hypo- and hyperkinetic status of the cecum and ileum was evaluated by EMA during NUC simulation and on day 10 of NUC.

The role of the serotoninergic system was studied in a series of experiments on 8 rats by intravenous injections of serotonin (0.1-0.2 ml; 10^{-5}-10^{-4} g/liter) 1-4 min before induction of NUC and in a series of experiments on 8 rats by intravenous injection of spiperone in a dose of 1.5-2 mg/kg 15-20 min before induction of NUC. The morphology of the cecum was studied on day 10 of the experiment. Tissue specimens were fixed in 4% paraformaldehyde, postfixed in 1% osmium tetroxide, dehydrated in ascending alcohols, and embedded in epon and araldite. The sections were stained with toluidine blue. Tissue biopsy specimens for histological study were fixed in 10% neutral formalin, dehydrated in ascending alcohols, and embedded in paraffin by the standard method. Histological sections were stained with hematoxylin and eosin. The data were statistically processed using Statistica 6.0 software with evaluation of the confidence interval.

RESULTS

EMA frequency of the common bile duct in intact controls was 21.0±4.6/min, with amplitude of 0.78±0.30 mV. Injection of picrylsulfonic acid increased EMA frequency to 30.3±4.9/min (44.3%; p<0.05) and decreased the amplitude to 0.14±0.01 mV (82%; p<0.05). This seemed to indicate changes in the ino-chronotropic relationship in the smooth muscle. EMA of the common bile duct after 15 days of PSC was characterized by a slight increase in the frequency (32.3±2.1/min; 6.6%; p>0.1) and a 2-fold increase in the amplitude (0.28±0.02 mV; p<0.05) of slow-wave activity.

Morphological study of the liver carried out 15 days after injection of picrylsulfonic acid detected sharp plethora of the hepatic tissue. Dilated sinusoids contained numerous blood cells. Small hemorrhages were seen in different places of the hepatic parenchyma. Vacuolar degeneration of hepatocytes was detected.

Hence, the function of the common bile duct in experimental PSC was characterized by modification of the ino-chronotropic relationships in the duct smooth muscles against the background of moderate degenerative process in hepatocytes. No intrahepatic bile ducts were detected, which indicates the presence of the main morphological sign of NUC.

Injection of serotonin to experimental animals increased the frequency and amplitude characteristics of the common bile duct: EMA frequency reached 21.8±3.3/min (increased by 4%) and the amplitude reached 1.08±0.30 mV (increased by 38.6%, p<0.05). Simulation of PSC after serotonin injection further increased the frequency of EMA slow waves (27.4±5.4/min; by 25.6%) and reduced the amplitude (0.9±0.2 mV; by 20%), in other words, modified the ino-chronotropic relationships. By day 15 of PSC, EMA frequency in these animals reached 31.0±3.0/min, while the amplitude decreased to 0.68±0.05 mV.

Morphological study on day 15 of PSC simulated after serotonin injection showed moderate lymphoid infiltration along the common bile duct. The liver was characterized by total, though uneven dilatation of sinusoids. The widest of them were filled with blood cells. Solitary small hemorrhages were seen. The sinusoidal endotheliocytes had large bubble-like nuclei. Kupffer's cells were often seen. Cells accumulating lipids were seen at the interface between sinusoids and hepatocytes. Moderately pronounced degenerative changes (cytoplasm vacuolation and formation of lipid droplets) were detected in hepatocytes. The intrahepatic bile ducts were dilated and deformed.

Preinjection with serotonin prevented significant decrease in EMA slow wave amplitude in experimental PSC and hence, preserved excitability of the ductal smooth muscle cells and the capacity of cell membrane to depolarization and repolarization. Morphological study revealed intrahepatic bile ducts, which attested to a certain cholangioprotective effect of serotonin.

Injection of spiperone (5-HT_2 receptor blocker) before PSC simulation in group 3 (Fig. 1) led to a negligible reduction of EMA slow wave frequency to 27.8±3.1/min (by 8.9%) and to an increase of its amplitude to 0.9±0.2 mV (543%, $p<0.01$) in comparison with EMA in PSC. Morphological study of the liver on day 15 showed sharply dilated plethoric sinusoids and dilated veins in this group. Cells with clear vacuolated cytoplasm and pyknotic nuclei were frequently seen among hepatocytes.

Hence, serotonin exhibited a cholangioprotective effect; pharmacological blockade of 5-HT_2 receptors modified excitability of the common bile duct smooth muscle cells, led to development of degenerative changes in hepatocytes, and prevented the cholangioprotective effect of serotonin.

Basal EMA of the cecum was characterized by slow wave activity at a frequency of 10.7±1.9/min and amplitude of 0.24±0.04 mV (Fig. 2). Three or four low-amplitude waves were recorded among low-frequency slow waves in 25% cases, while in 75% cases high-amplitude (0.81±0.22 mV) and high-frequency (0.90±0.16/min/100 slow waves) spike activity was recorded.

Basal EMA of the ileum was characterized by high-frequency (20.8±3.2/min) and medium-amplitude (0.52±0.07 mV) slow waves. Waves with a lower frequency (6.1±0.9/min) with low-amplitude waves of 6.8±1.2/min on them were observed in 62.5% cases. The amplitude of these low-frequency waves was lower (0.39±0.09 mV). Spike activity was observed in 37.5% cases; the frequency of spikes was 0.34±0.05/100 slow waves, amplitude 0.32±0.04 mV. Hence, the ileum was characterized by intensive basal motor activity, because spike activity was recorded in a sufficiently high percentage of cases (Fig. 2).

Simulation of NUC by injection of picrylsulfonic acid was associated with the following changes in cecal EMA: the frequency of slow waves decreased to 7.6±1.2/min, the amplitude virtually did not change (0.27±0.05 mV). Three or four low-amplitude waves were recorded among slow waves. Highly variable spike activity with the mean frequency of 1.00±0.15/100 slow waves and amplitude of 0.83±0.09 mV was recorded (Fig. 2). Hence, simulation of NUC was associated with suppression of slow-wave frequency of EMA of the cecum.

The ileac EMA changed less markedly in NUC. The frequency decreased to 18.1±1.2/min (12.5%), amplitude to 0.34±0.03 mV (34.6%, $p<0.05$). Spike activity was detected more often and was characterized by an amplitude of 0.34±0.02 mV and frequency of 0.44±0.02/100 slow waves (29.4%, $p<0.05$; Fig. 2). Hence, injection of picrylsulfonic acid was associated with a slight initial stimulatory effect on the ileac muscle, paralleled by an increase in spike activity, and was caused by reflex motility activation in this compartment of the small intestine.

Injection of serotonin (10^{-4}-10^{-5} g/liter) stimulated cecal EMA: the amplitude increased to 0.33± 0.04 mV (37.8%, $p<0.05$); the frequency did not change. High-frequency (1.27±0.20/100 slow waves; 41.1%, $p<0.05$) high amplitude (1.17±0.15;

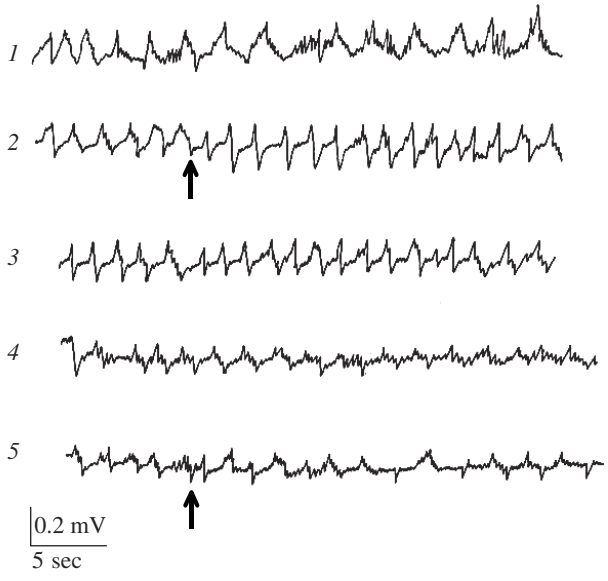

Fig. 1. Bile duct EMA normally (*1*), after injection of serotonin (*2*), picrylsulfonic acid (*3*), on day 15 of PSC (*4*), and on day 15 of PSC induced after preinjection of spiperone (*5*).

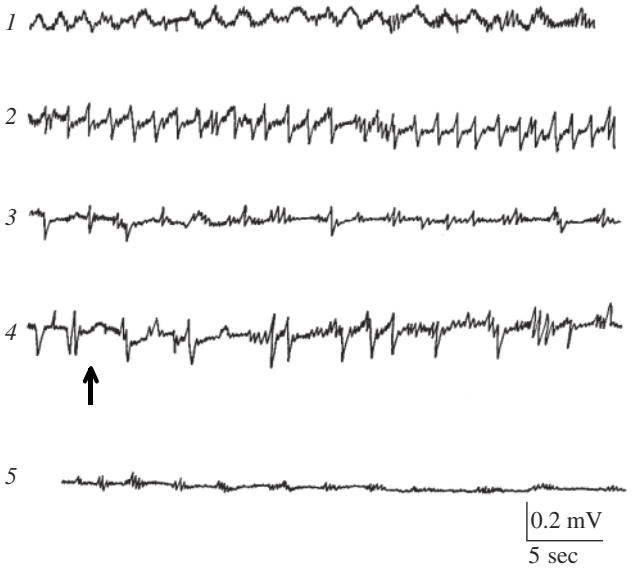

Fig. 2. Ileac and cecal EMA normally (*1*, *2*, respectively) and after injection of picrylsulfonic acid (*3*, *4*, respectively). Cecal EMA on day 15 of experimental NUC (*5*).

TABLE 1. Slow-Wave Electromotor Activity of the Cecum in Experimental NUC under Conditions of Serotoninergic System Stimulation and Blocking (Frequency: per min; Amplitude: mV)

Basal EMA		NUC		Serotonin+NUC		Spiperone+NUC	
frequency	amplitude	frequency	amplitude	frequency	amplitude	frequency	amplitude
10.7±1.9	0.24±0.04	7.6±1.2	0.27±0.05	7.0±1.2 12.7±0.5	0.12±0.02 0.25±0.03	11.5±2.3	0.12±0.03

increase by 44.4%, $p<0.05$) spike activity was observed in all animals. Hence, serotonin injection stimulated slow-wave and spike activity of the cecum.

Injection of serotonin also modified ileac EMA: the frequency of slow waves changed negligibly (19.2±0.5/min at an amplitude of 0.37±0.03 mV). The incidence of spike activity increased to 50%. The frequency of spikes reached 0.92±0.07/100 slow waves (171%, $p<0.05$) at a stable amplitude of 0.37±0.03 mV. Hence, serotonin also stimulated motor activity of the ileum.

Simulation of NUC after preinjection of serotonin was prolonged in a chronic experiment. After 10 days of experimental disease, the animals looked dormant, appetite decreased by 60-70%.

Simulation of NUC after preinjection of serotonin led (after 1-2 min) to the development of two opposite reactions of cecal EMA: inhibition and activation (Table 1), which were observed in animals with different basal EMA. Inhibition of cecal EMA was similar to toxic dilatation of the large intestine in clinical NUC.

Injection of spiperone (5-HT_2 receptor blocker) modified cecal EMA: the slow wave frequency increased to 14.8±2.4/min (39.2%, $p<0.05$) at amplitude of 0.23±0.04 mV. Spike activity with an amplitude of 0.98±0.02 mV (18%, $p<0.05$) and frequency of 1.16±0.04/100 slow waves (32%, $p<0.05$) was recorded in all observations. Hence, injection of spiperone stimulated cecal motility mainly at the expense of spike activity.

Injection of spiperone inhibited ileac EMA: the frequency was 14.5±5.5/min, with up to 12 low-amplitude waves on low-frequency waves, the amplitude of slow waves varying in a wide range (from 0.3 to 1.2 mV). Low-frequency and low-amplitude spike activity was observed.

Simulation of NUC after spiperone injection modified cecal EMA within 2-3 min: the frequency of EMA slow waves was 11.5±2.3/min (54%, $p<0.05$), amplitude 0.12±0.03 mV (55.5%, $p<0.01$). Spike activity of 0.87±0.11 mV amplitude and 0.86±0.09/100 slow waves frequency was recorded in all cases. Hence, spiperone leveled the destructive effect of picrylsulfonic acid during the first 10 days of NUC development.

Ileac EMA under conditions of NUC simulation after preinjection of spiperone was characterized by slow waves with high frequency of up to 27.0±3.5/min (49%, $p<0.05$) and amplitude of 0.53±0.06 mV (56%, $p<0.05$). Spike activity was detected in 66% cases and was characterized by 0.30±0.04 mV amplitude and 0.55±0.05/100 slow waves frequency (32%, $p<0.05$).

Hence, spiperone blockade of 5-HT_2 receptors leveled the destructive effect of the serotoninergic system on the cecum by day 10 of NUC simulation.

On day 10 of NUC development, an extensive zone of chronic inflammation was detected in the rat cecal mucosa. The cecal mucosa was unevenly edematous. Foci of epithelial cell desquamation and destruction were seen in the surface epithelium. The subepithelial area was presented by granulation tissue infiltrated with various cells: many polymorphonuclear leukocytes and lymphocytes, few fibroblasts, plasma cells, and macrophages. Accumulations of polymorphonuclear leukocytes were located near separate crypts, penetrating into the epithelium and into the cryptic lumen. Crypt abscesses were detected. Capillaries contained erythrocytes.

Granulation tissue of the deep compartments has a multicellular structure with well-developed network of blood vessels (Fig. 3, *a*). The cellular composition of granulation tissues was rich. The majority of cells were polymorphic, cells with processes and elongated cells predominated, due to which they were referred to stromal cells (fibroblasts and smooth muscle cells). A characteristic feature of these cells was bubble-like nucleus with one or two large nucleoli. Some smooth muscle cells were atrophic. Lymphocytes, macrophages, polymorphonuclear leukocytes, and mast cells were scattered among stromal cells (Fig. 3, *b*). Mast cells (mainly in a state of degranulation) were detected in the deep compartments of the granulation tissue. The veins were sharply dilated, many of them plethoric; lymphocytes and polymorphonu-

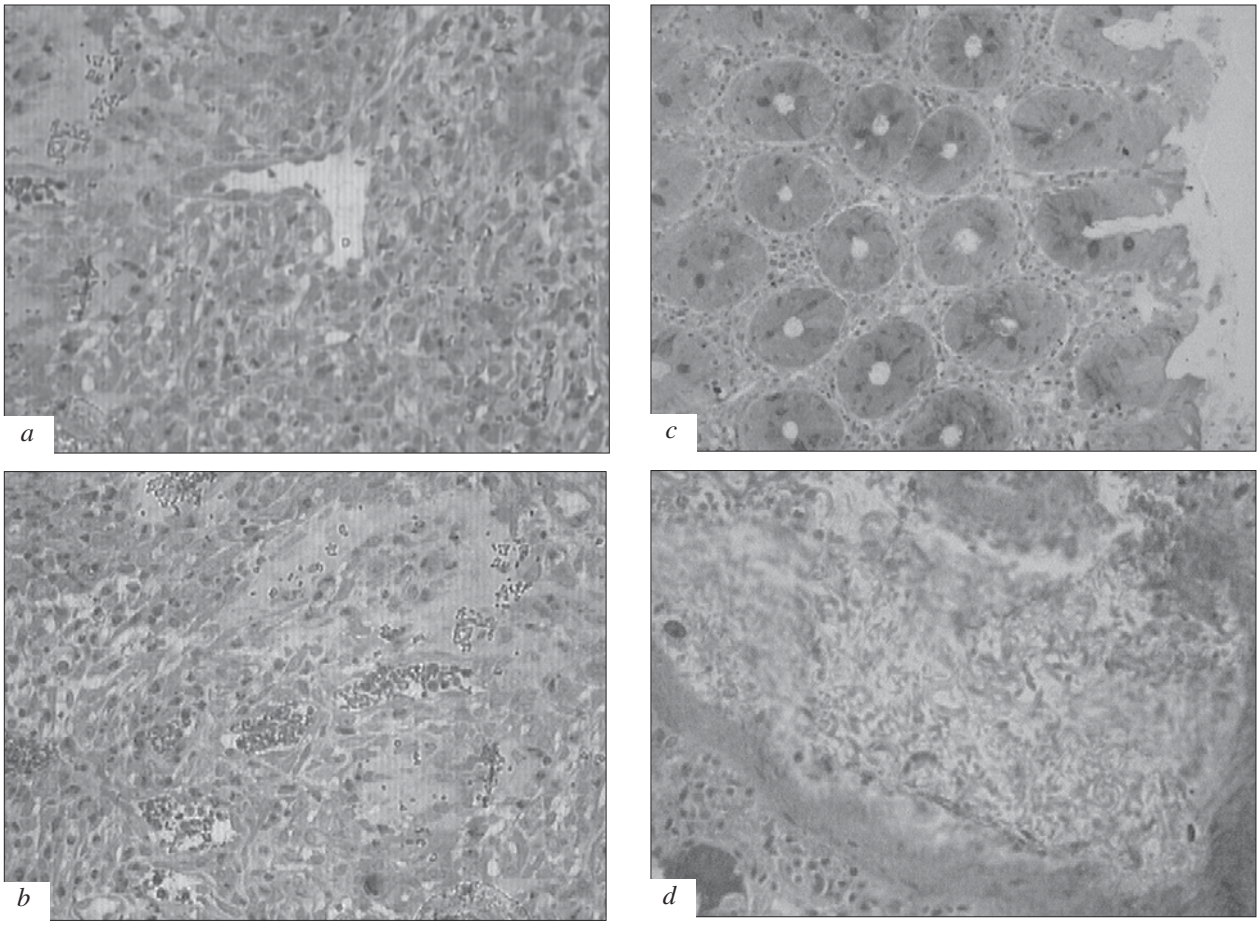

Fig. 3. NUC under different conditions. *a*) vessel without endothelial lining, ×500; *b*) mast cells, ×500; *c*) preinjection of serotonin, ×300; *d*) preinjection of spiperone. Cicatrix on day 10, ×500.

clear leukocytes were seen in the vascular lumen along with erythrocytes. Parietal microclots formed in some of dilated veins. Some veins contained no blood cells, their walls being sharply modified, with fragments of desquamated endothelium seen in the lumen. Local or extensive fragmentation of the basal membrane led to complete destruction of the wall.

The cecal mucosa (Fig. 3, *c*) was characterized by well-developed granulation tissue in NUC developing after preinjection of serotonin. It contained many cells, primarily lymphocytes and fibroblasts. Macrophages and plasma cells were rare. The intercellular matrix consisted of homogenous poorly stained substance and contained no collagen fibers. Hemorrhagic foci of different sizes were scattered in the granulation tissue.

The surface epithelium and crypts containing mucus-forming cells were retained in the adjacent mucosal areas. The cryptic epithelium contained interepithelial lymphocytes (3-5 cells per transverse section of the crypt). The intercryptic connective tissue lamina contained appreciable numbers of stromal and immunocompetent cells. Few collagen fibers enveloped the crypts, touching their basal membranes.

The cecal mucosa in NUC developing after preinjection of spiperone was in fact cicatricial tissue (Fig. 3, *d*): a conglomeration of collagen fibers oriented in different directions with scanty cells (mainly stromal fibroblasts and rarely lymphocytes). The cicatricial tissue penetrated into the submucosa and reached the muscle membrane. Blood vessels were rare.

The morphology of cecal changes in NUC suggests that spiperone stimulated cicatrization of the ulcerative defect of the cecal mucosa.

Simulation of NUC in the group of experimental animals was associated with high (37.5%) mortality. Preinjection of serotonin with subsequent simulation of NUC reduced mortality in this group to 25%. The signal pathways of serotonin are impaired in NUC, the bioavailability of the bioamine being increased, while its reuptake is reduced. Changed availability of serotonin, presumably resulting from loss of sensitivity by serotonin receptors, can

cause disorders in the motor activity of the intestine in its inflammatory diseases [6].

Preinjection of serotonin promotes activation of dendritic cells, penetration of lymphocytes, fibroblasts, plasma cells involved in the realization of the immune response into focus of inflammation. Activated DC, in turn, stimulate primary T-lymphocytes, initiating the primary immune reaction [3,5,7,11]. Activation of the serotoninergic system prevents the development of primary sclerosing cholangitis. Activation of 5-HT_2 receptors promotes more severe course of NUC.

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