MORPHOLOGICAL AND FUNCTIONAL CHANGES IN THE REGULATORY SYSTEMS OF THE LARGE INTESTINE IN SALMONELLOSIS

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KEY WORDS: salmonellosis; large intestine; cholinergic structures; enterochromaffin and mast cells; integration.

Hormones and biogenic amines produced by the endocrine cells of the stomach and intestine play an important role in the regulation of functional activity of the digestive organs [1, 3, 4]. They are released under the direct influence of food or vagal stimulation or indirectly with the participation of local cholinergic reflexes [6, 8, 9].

The aim of this investigation was to study morphological and functional changes in the intestinal regulatory systems after salmonella infection.

EXPERIMENTAL METHOD

Experiments were carried out on 45 male albino rats weighing 130-170 g, of which 30 were infected with a virulent strain of *Salmonella typhimurium* ($2 \cdot 10^9$ microbial cells, in a single dose perorally), while the rest served as the control. All the animals were decapitated 1, 3, 7, 14, and 21 days after infection, all at the same time of day (10 a.m.), in the fasting state. Pieces of tissue taken from the same regions of the large intestine were fixed in 12% neutral formalin solution. The enterochromaffin (EC) cells were demonstrated by the argentaffin method. Cholinergic structures were determined in fresh frozen sections [13] counterstained with 0.1% thionine solution to reveal mast cells (MC). Pieces of the organ for electron-microscopic investigation were processed by the usual method and embedded in Araldite. Ultrathin sections were examined in the JEM-100B electron microscope. The granulation index was calculated from the number of EC cells counted [9]. The area of the cholinergic structures was determined by a dot counting method [8]. Ultrastructural identification of cholinergic endings [12] was carried out and their number counted in 50 nerve fibers at the preterminal and terminal level. The experimental results were subjected to statistical analysis by the usual methods.

EXPERIMENTAL RESULTS

After 1 day the area of the cholinergic structures and the number of nerve terminals with pale synaptic vesicles (SV) containing acetylcholine (ACh) were reduced (Table 1). Perhaps as a result of this, acetylcholinesterase (AChE) activity in the ganglia was reduced, and because of intensive unloading of ACh from the nerve terminals into the intestinal tissues in response to microbial toxins, the quantity of ACh in the nerve cells of the intramural ganglion also was reduced. In turn, the increase in the ACh concentration in the intestinal tissue led to stimulation of peristalsis, which could be the cause of the diarrhea observed in the experimental animals on the 1st-3rd day of salmonellosis. At the same time the number of EC cells detected and the granulation index (GI) both increased. Meanwhile the number of degranulated EC cells increased (to 30.0 ± 1.3 compared with 21.0 ± 0.4 in the control, $p < 0.05$). This was probably connected with the more rapid release of serotonin from the EC cells, which ultimately also led to stimulation of peristalsis and to the evacuation of profuse liquid stools. On the 3rd day of development of salmonella infection the morphometric parameters of the areas of the cholinergic structures and the number of terminals with pale SD were higher than on the 1st day, but they still remained lower than in the control, evidence of lowering of the

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TABLE 1. Time Course of Changes in Area of Cholinergic Structures and Number of Cholinergic Terminals and Enterochromaffin Cells of the Large Intestine in Salmonellosis

Time of investigation, days	Area of cholinergic structures, %	Number of cholinergic terminals, %	Number of EC cells in 50 longitudinally sectioned crypts	GI of EC cells
Control	38,7±0,1	67,9±0,9	61,0±0,4	50,0±0,4
1	30,2±0,2*	61,9±1,3*	69,0±1,3*	55,0±1,3*
3	35,9±0,3*	65,5±0,8*	54,0±1,7*	39,0±0,8*
7	42,8±0,2*	82,7±2,0*	52,0±1,3*	36,0±1,3*
14	37,6±0,1*	70,3±0,4*	53,0±1,7*	37,0±0,8*
21	38,5±0,1	68,7±0,5	60,0±1,7	49,0±0,8

Legend. *p < 0.05 compared with control.

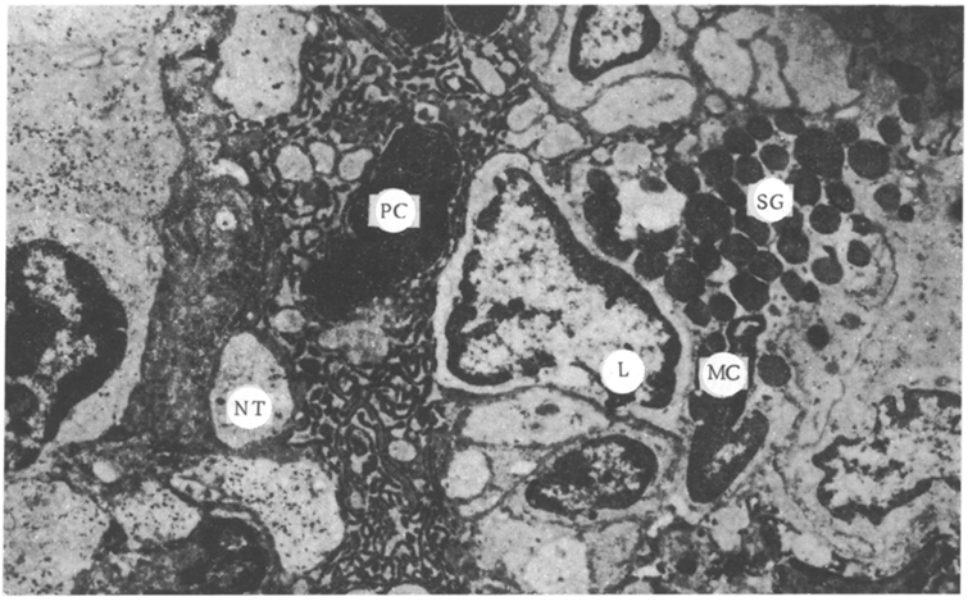

Fig. 1. Stroma of mucous membrane of rat large intestine 7 days after salmonella infection. Cooperation between stromal cells. L) Lymphocyte, PC) plasma cell, NT) nerve terminal, SG) secretory granules. 6500 ×.

mediator activity of the ganglia of the large intestine. The number of EC cells and GI were lower than on the 1st day and than in the control.

By the 7th day the area of the cholinergic structures and the number of cholinergic terminals reached a maximum in the course of salmonellosis, evidence of the functional strain on the nerve cells and the development of compensatory and adaptive processes in them, for release of the ACh reserves which had accumulated in the ganglia and an increase in AChE activity were necessary in order to maintain the mediator balance. At the same time, when the number of EC cells discovered was sharply reduced, GI fell even more. On the 21st day the morphometric parameters of the intramural ganglia and the number of EC cells returned close to the control values.

Salmonella infection is accompanied also by structural and functional changes in the cells of the intestinal stroma. A sharp decrease in the number of MC was discovered in the intestinal stroma 1 day after salmonella infection. On the 7th day after salmonella infection the most marked feature was close contact of MC with plasma cells, eosinophils, lymphocytes, blood vessels, and nerve terminals of the rat large intestine (Fig. 1). In the later stages

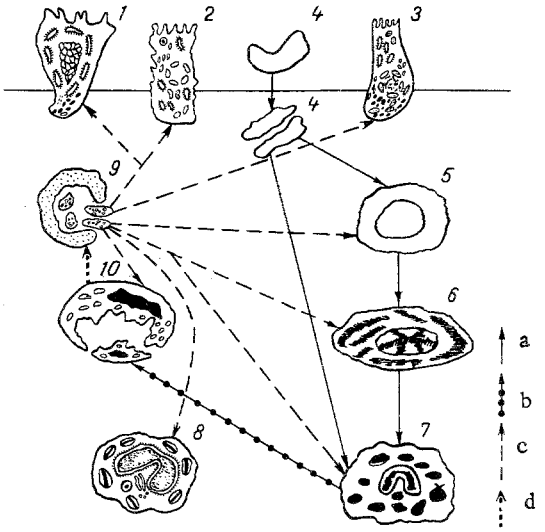

Fig. 2. Integration of intercellular co-operation in mucosa of large intestine. a) Mechanism of activation of immunologic reactions, b) biologically active substances with local action, c) neurotransmitters with local action, d) hormones and neurotransmitters with distant action. 1) Goblet cell, 2) enterocyte, 3) EC cell, 4) microorganisms, 5) lymphocyte, 6) plasma cell, 7) MC, 8) eosinophil, 9) nerve terminal, 10) capillary.

(14th and 21st days) after salmonella infection, intercellular cooperation in the intestinall mucosa was weaker. The processes taking place in the stroma of the large intestine are illustrated in Fig. 2. In the course of the primary immune response some B lymphocytes were transformed into antibody producers, and those most active in this immune response were transformed into typical plasma cells, synthesizing IgE [2]. The IgE attaches itself to the surface of MC because MC have surface receptors for what is called the constant region of the antigen [5]. When the chemotaxic mediator (ECF-A) is released from MC, it selectively attracts eosinophils [14]. In turn, the eosinophils contain several enzymes which degrade the mediators released by MC [11]. Consequently, one of the main factors responsible for collaboration between cells of the stroma is MC. Close contact of cholinergic endings with MC, eosinophils, lymphocytes, and plasma cells suggest that ACh, as mediator, integrates and coordinates activity of the cooperating cells (Fig. 2).

In salmonella infection a link is thus observed between the mediator activity of the cholinergic structures and the functional activity of the EC cells. In the early period (after 1 day), the period of maximal reduction of mediator activity of the neural structures, the number of EC cells increases and GI rises. The increase in GI of the EC cells is evidence of their enhanced functional activity. Serotonin can inhibit AChE activity and thereby potentiate the effects of ACh, and changes in serotonin metabolism lead to changes in AChE activity [7]. During the period of maximal strain on the nerve cells (after 7 days), however, the number of EC cells falls but GI rises. In the same period, intercellular cooperation is most marked in the intestinal stroma. MC, which are a system-forming factor in the process of intercellular cooperation in the intestinal stroma, evidently modulate and adapt the local cellular assemblies to the action of neurotransmitter stimuli, and thus increase the reliability of the protective mechanisms of the intestine. In the later stage (21 days) relative morphological and functional stabilization of the components of the mucosa and intramural ganglia is observed.

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ULTRASTRUCTURAL LOCALIZATION OF THIAMINE PYROPHOSPHATASE ACTIVITY IN EPITHELIOCYTES IN DUODENAL BIOPSY MATERIAL IN PEPTIC ULCER

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The presence of a developed thiamine pyrophosphatase (TPPase) system in the epithelial cells of the gastrointestinal tract regulates the supply of thiamine (vitamin B_1) to the tissues [4]. An important role in the metabolism and conversion of thiamine is played by the small intestine, in the mucosa of which this vitamin is absorbed [11, 12, 14]. However, the ultrastructural localization of TPPase activity has been studied in the epithelial cells of the small intestine mainly in experimental animals [13, 15, 16]. In peptic ulcer, as in other diseases of the gastrointestinal tract, thiamine absorption is disturbed [1, 2]. The chief results of investigations of thiamine metabolism in peptic ulcer have been obtained by biochemical methods [3, 8]. The use of electron-microscopic cytochemistry to investigate lysosomal enzymes, and TPPase in particular, in human cells in pathology [7] can shed light on the pathogenesis of several diseases at the subcellular level. There have been few such investigations in peptic ulcer [9].

The aim of this investigation was the ultrastructural cytochemical determination of the localization of TPPase activity in the epitheliocytes of the human small intestine in patients with peptic ulcer in phases of exacerbation and remission.

EXPERIMENTAL METHOD

An electron-cytochemical study was made of the TPPase content in biopsy material from the duodenal mucosa obtained from patients with peptic ulcer (men aged 25-45 years). Gastrointestinal fiberendoscopes (Olympus Optical Co., model JF type B_2 and B_3, side-viewing endoscopes, and type K forward viewing endoscope) were used for the endoscopic investigation of the mucosa of the gastrointestinal tract. According to the results of the endoscopic and histopathological investigations 17 patients had ulcers of the duodenal bulb in the remission phase, 13 had ulcers of the bulb in the exacerbation phase, and nine had superficial gastritis without any changes in the mucosa of the small intestine. Biopsy material was obtained from the mucosa of the visually least altered areas of the descending part of the duodenum for ultrastructural cytochemical and electron-microscopic investigation. Biopsy material was taken at endoscopy by the use of forceps contained in the duodenoscope outfit. Tissue sam-

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