Morphological Features of the Lymphoepithelial Structures of the Jejunum after the Stress in Rats

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We studied the effect of acute emotional stress on functional status of lymphoid epithelial structures of the jejunum in rats with different behavioral activity. Morphological and functional characteristics of lymphoid tissue were assessed using morphometric, histological and electron microscopic methods. In behaviorally active and passive rats, reduction in villus height, area of the epithelium and lymphoid tissue of the jejunum was recorded on the third day after acute emotional impact. At that, the largest number of destructively modified lymphoid cells was identified by comparison with the other time points. Moreover, destruction of the apical part of the jejunal villi was observed on days 3 and 7 after stress exposure. Recovery of the lymphoepithelial structures of the jejunum after acute stress exposure was detected on day 14.

Key Words: *emotional stress; rats; lymphoid structures of the gastrointestinal tract*

In recent years, increasing attention has been paid to immune structures in the mechanisms of emotional stress [2,8,15]. The small intestine is the longest section of the gastrointestinal tract responsible for digestion and assimilation of food, where absorbed substances are subjected to immunological control [8]. We hypothesized that the involvement of the lymphoid structures of the small intestine in such a systemic response as the emotional stress may be due to its own intramural nervous system and endocrine cells located in the epithelial lining of the small intestine [4,6].

Hans Selye (1960) described the gastrointestinal tract and its immune structures as one of the main targets exposed to various chemical and physical influences [10].

Lymphoid structures associated with the small intestinal mucosa are considered as the first barrier of the immune defense facing the antigen [1,5,13,14]. In this case, the epithelium is believed to be a part of the microenvironment integrated into the lymphoid tissue, which is immersed directly into the mucose [12]. J. Jolli in 1953 regarded the presence of lymphocytes among the cells of the epithelium as lymphoepithelial symbiosis and pointed to the positive tropism of lymphocytes for epithelium [7].

Here we studied the effects of acute emotional stress on the functional status of jejunal lymphoepithelial structures in behaviorally active and passive rats.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats (n=72) weighing 220.0±5.2 g. The work was conducted in accordance with the Rules of the work using experimental animals (Annex to Decrees of the USSR Ministry of Health No. 755 of 12.08.1977 and No. 701 of 24.07.1978) and Good Laboratory Practice Rules (2003). The animals were kept the vivarium of the Institute of Normal Physiology under conditions regulated by the Decree of the USSR Ministry of Health No. 1179 from 10.10.1983.

Individual typological characteristics of rats were assessed in the open field test over 3 min [3]. Two groups were formed: active (n=36) with activity index of 4.5-6.0 and passive (n=36) with activity index of

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0.2-0.6. It has been previously shown that behaviorally active rats are more resistant to the stress effects than passive ones [3,9]. Further, active and passive rats were subdivided into 12 groups, 6 animals in each as follows: controls (group 1), immediately after 1-h stress exposure (group 2), and 1 day (group 3), 3 days (group 4), 7 days (group 5), and 14 days (group 6) after stress exposure.

Acute emotional stress was modeled by immobilization of the rats with simultaneous electrocutaneous stimulation [3,9]. The animals were placed in individual plastic boxes for 1 h; metal needle electrodes were fixed to the skin on the back. Electrocutaneous stimulation with alternating current was performed by stochastic pattern with pulse length of 1 msec, voltage of 4-6 V at 50 Hz. Stimulation strength was adjusted individually according to the vocalization threshold in response to electrical stimulation.

The animals were sacrificed by decapitation under ether anesthesia immediately after 1-h stress exposure and on days 1, 3, 7, and 14 after it. Control active and passive rats were decapitated simultaneously with the animals subjected to stress.

Samples of the jejunum (length of about 1.0-1.5 cm) were taken strictly at the same sites opposite the mesenteric lymph nodes between the branches of the mesenteric artery.

Histological slides were processed routinely and stained with hematoxylin and eosin. Five fields of view were examined in each histological slide randomly moving the ocular "node grid" modified by S. B. Stefanov (1976).

Morphometry of lymphoid structures in the small intestinal wall was performed using the stereological method proposed by G. G. Avtandilov (1990).

For electron microscopy, tissue fragments (~1 cm) were fixed in 2.5% glutaraldehyde in cacodylate buffer, pH 7.2-7.4. Ultrathin sections were contrasted with uranyl acetate in 70° alcohol and Reynolds solution and examined under JEM 100 C electron microscope at accelerating voltage of 80 kV.

The experimental data were subjected to statistical and analytical processing. The significance of differences between the groups was determined by Student's *t* test.

RESULTS

Among registered parameters, only epithelium area in the jejunal wall in the control behaviorally active rats was higher by 25.8% ($p<0.05$) than in passive animals.

Immediately after stress exposure and on days 1, 3, and 7 we revealed extended and devastated blood vessels (vascular stasis), blood plasma and isolated erythrocytes in the intercellular space (plasmorrhagia) of the jejunal mucosa. According to some authors, the phenomenon observed by us can be associated with both systemic effects of stress hormones, and the impact on the innervation of blood vessels in the intestinal wall [2,4,10,14,15]. In addition, blood vessels and, in particular, postcapillary venules with cubic endothelial cells (or venules with high endothelium) provide infiltration of lymphocytes in the peripheral organs of the immune structure [6-8].

The increase in the area of the jejunal lymphoid tissue by 14% was revealed in behaviorally active rats immediately after 1-h stress; in contrast, this parameter in passive rats was decreased by 10% and the area of the epithelium by 18% in comparison with the controls.

The area of jejunal lymphoid tissue in behaviorally active rats increased by 20% on day 1 after stress exposure; on the contrary, this parameter in passive rats decreased by 28% and epithelium area by 22% in comparison with the controls.

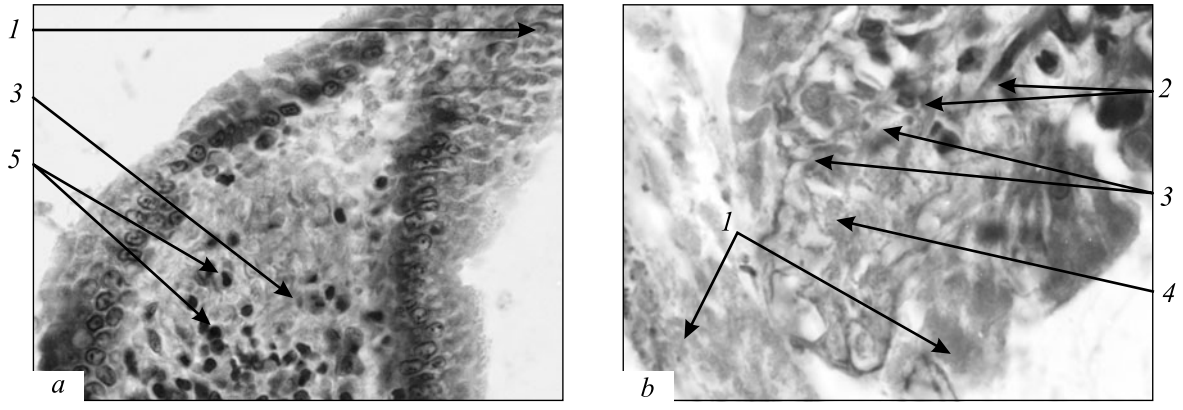

Fig. 1. The apical part of jejunal villi in passive (*a*) and active (*b*) rats 3 days after the stress. Destruction of epithelial cells (*1*), destructively changed cells (*2*). Granules of damaged eosinophils (*3*). Devastation of the upper part of the villous stroma (*4*). Destructively changed lymphoid cells (*5*). Staining with hematoxylin and eosin. ×600 (*a*), ×1000 (*b*).

TABLE 1. Area of Lymphoepithelial Structures in the Wall of Rat Jejunum after the Stress per 880 μ² Histological Section Area (*S*±*s*, min-max)

Condition of experiment	Area of the epithelium, μ²		Villi heigh, μ		Area of the lymphoid tissue, μ²	
	active rats	passive rats	active rats	passive rats	active rats	passive rats
Controls	56.76±4.67⁺	45.12±4.75	26.55±2.61	26.14±2.76	13.34±1.02	15.81±1.42
Stress	54.12±4.12⁺	40.40±3.88	25.76±1.54	24.32±2.02	15.44±1.32	14.22±0.86
After stress						
day 1	47.68±3.95⁺	37.00±2.74*	23.90±2.12	22.00±1.86	16.44±1.42⁺*	12.33±1.02*
day 3	44.27±4.16⁺*	35.64±2.83*	20.97±1.76*	17.78±1.55*	11.00±0.95	10.84±0.54*
day 7	48.15±4.32⁺	37.22±2.44*	20.44±2.03*	19.61±1.36*	11.26±0.82	12.35±0.76*
day 14	55.12±5.16⁺	44.63±4.06	26.02±1.75	26.42±2.16	12.56±1.23	14.00±0.75

Note. $p<0.05$ compared with *controls, ⁺passive rats.

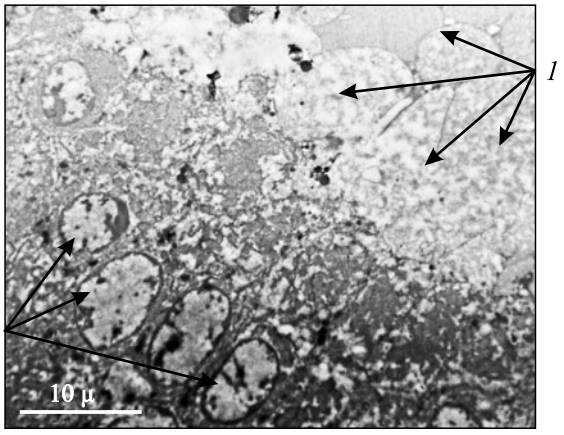

Fig. 2. Jejunal mucosa in a passive rat on day 3 after stress exposure (electron micrograph), ×7600. Homogeneous anuclear structures without organelles (*1*), in the lower part part of the picture: preserved ("live") enterocytes with swollen nuclei (*2*).

In active and passive rats, different density and height of the villi were recorded on day 1 after acute stress in some sites of the jejunal mucosa. At the same time, dilated lymph capillaries were observed in some villi. This was probably a result of hormonal imbalance and impaired innervation of some components of the villous stroma (lamina muscularis of mucosa, neurovascular elements) [1,4,8,10,11].

On days 3 and 7 after the stress, the height of jejunal villi in active rats was lower than in control animals by 22%, the epithelium area decreased by 23 and 15%, respectively, and the area occupied by the lymphoid tissue decreased by 18 and 16%, respectively. At the same time, the corresponding parameters in passive rats decreased to a greater extent than in active animals. Thus, reduced by 32% and 25% height of jejunal villi was found in passive animals compared with the control rats. The epithelium area decreased by 21 and 18%, respectively, and the area of the lymphoid tissue by 31 and 22% (Table 1).

Destruction of the apical part of jejunal villi and devastation of their stroma in active and passive animals was observed on days 3 and 7 after acute stress (Fig. 1). These morphological changes after stress exposure were not reported previously. Ultrastructural study showed local vacuolization or complete absence of microvilli in the jejunal epithelium, swelling of mitochondria, clarification (lysis) of the cytoplasm, destruction of the nuclei and intracellular structures of epithelial cells at these terms (Fig. 2).

We can assume that destruction of the apical part of villi and devastation of their stroma could be related to innervation and microcirculation disorders caused by combined effects of hormones released in response to stress exposure [2,4,11,14], impaired protective properties of the intestinal mucus [5,10,15] and qualitative and quantitative changes in the parietal microflora [12,13].

On day 14 after stress, the studied morphometric characteristics of jejunal mucosa and the area of lymphoid tissue in the villous stroma returned to baseline values both in active and passive rats.

Our data will improve understanding of specific and nonspecific defense mechanisms in the walls of the small intestine in subjects with different behavioral activity and contribute to the further development of modern concepts about the structural basis of the organization of lymphoid tissue in the walls of the digestive system.

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