# Histochemical Characteristics of the Structures of Rat Lymphoid Organs in Stress

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Luminescence analysis and histochemical methods have shown that stress affects various structures of rat immunocompetent organs and decreases the contents of catecholamines, serotonin, and histamine in central and peripheral immune organs. The content of biogenic amines in thymic structures increased 10, 20, and 30 days after the administration of the immunostimulator polystim against the background of stress. The results obtained indicate that polystim displays stress-protective activity and can be used in clinical practice.

**Key Words:** *lymph node; stress; catecholamines; serotonin; histamine*

Stress is an emotional and behavioral disorder associated with inability to react properly under certain conditions. This term designates a wide range of human reactions to various extreme factors [1].

The immune system is one of the most important regulatory systems in the organism. It is involved in excretion, accumulation, and binding of bioactive substances. The immune system regulates homeostasis and plays the major role in stress reactions [2-5].

Here we studied amine-containing structures of the thymus, spleen, and lymph nodes in stress. We also tested a new preparation, polystim, as an antistress agent.

## MATERIALS AND METHODS

Experiments were performed on 50 outbred albino rats weighing 180-200 g. An overpopulated cage (20×30×40 cm) was used to model stress.

The rats were divided into three groups: group 1, stressed animals ($n$=10); group 2, stressed animals ($n$=15) injected intramuscularly with 0.5 ml polystim; and group 3, stressed animals ($n$=15) injected intramuscularly with 0.5 ml physiological saline. Intact animals ($n$=10) served as the control. The thymus, spleen, and mesenteric lymph nodes were excised under deep ether anesthesia 10, 20, and 30 days after stress (group 1) and administration of polystim (group 2) or physiological saline (group 3). Cryostat sections (15-20 μ thick) were analyzed by luminescent and histochemical methods of Falk-Hillarp to estimate the contents of serotonin (ST) and catecholamines (CA) and by the Cross' method (with *o*-phthalic aldehyde) to identify histamine. Cytospectrofluorometry was performed in a luminescent microscope equipped with a FMEL-1A photometric device at 600 V output voltage. The intensities of CA, ST, and histamine luminescence were measured using light filters 6, 8, and 7, respectively. The data were expressed in arbitrary (arb.) units of fluorescence by the scale of the recording device. The data were analyzed statistically using Student's $t$-test. The morphometry of lymph node sections stained with hematoxylin and eosin was performed in a MBI-3 light microscope equipped with a MOV-1 photometric device.

## RESULTS

The contents of ST, CA, and histamine in the amine-containing structures of immunocompetent organs decreased 10 days after stress. The contents of all neurotransmitters in polystim-treated rats (group 2) were 1.5 times higher than in rats of groups 1 and 3. The in-

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tensity of CA luminescence in thymic premedullary and mast cells in group 2 rats was 1.5- and 2.1-fold higher than in groups 1 and 3 respectively. The concentrations of ST and histamine in premedullary cells increased by 1.4 and 1.7 times, respectively (Table 1). The intensity of CA fluorescence in intrafollicular cells of the spleen in group 1 rats increased by 1.5 times compared with that in group 1 animals. Polystim induced a twofold increase in the content of histamine in intrafollicular cells of the spleen and a 2.6-fold increase in the content of ST in splenic red pulp macrophages.

Twenty days after the beginning of experiments the concentrations of biogenic amines in the red pulp macrophages, intrafollicular cells, and splenic white pulp decreased in rats of groups 1 and 2. The contents of neurotransmitters increased considerably in group 3 animals. The concentrations of biogenic amines in all structures of the thymus and spleen increased sharply on the 30th day after stress. The maximum increase in the CA content was observed in premedullary cells of the thymus and intrafollicular cells of the spleen in polystim-treated rats. The concentrations

**TABLE 1.** Effect of Stress and Biostimulator Polystim on the Content of Biogenic Amines (arb. units)

| Structures | | Intact rats | Period, days | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 10 | | | 20 | | | 30 | | |
| | | | Group | | | | | | | | |
| | | | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| **Thymus** | | | | | | | | | | | |
| Premedullary cells | ST | 10 | 10* | 14* | 8* | 11* | 11 | 9** | 44 | 40* | 60* |
| | H | 8 | 10 | 12* | 11** | 13* | 15* | 14* | 46* | 61* | 71 |
| | CA | 12 | 8* | 15* | 13* | 9** | 11* | 8** | 42* | 38 | 46** |
| Mast cells | ST | 14 | 27** | 32* | 18* | 23* | 28* | 22* | 12 | 18** | 14 |
| | H | 12 | 25 | 27** | 24* | 20* | 30 | 27 | 20* | 24 | 17* |
| | CA | 10 | 12* | 26 | 17* | 20* | 18** | 16* | 10* | 12* | 14 |
| Adrenergic nerves | ST | 40 | 17* | 23* | 33** | 19** | 21* | 21 | 21 | 37* | 17* |
| | CA | 34 | 16* | 23** | 23* | 17* | 21 | 19 | 41 | 39* | 37* |
| **Spleen** | | | | | | | | | | | |
| Intrafollicular cells | ST | 12 | 13** | 11 | 16* | 10* | 11* | 12 | 66* | 57* | 75** |
| | H | 7 | 11* | 10* | 14* | 6 | 7 | 14* | 70* | 61* | 76* |
| | CA | 8 | 9 | 10* | 12* | 7* | 6* | 14** | 72 | 73 | 56* |
| Red pulp macrophages | ST | 12 | 21* | 18 | 32** | 12 | 10 | 30* | 36* | 35* | 23 |
| | H | 15 | 17 | 13* | 14* | 14* | 12* | 17** | 40 | 39* | 22 |
| | CA | 19 | 14* | 14* | 20 | 18** | 16 | 18* | 27* | 28* | 25 |
| Germinative zone | ST | 20 | 22* | 17** | 23 | 21* | 33 | 23 | 80 | 74* | 44* |
| | CA | 15 | 15* | 10* | 21** | 20* | 34* | 25* | 76 | 38* | 56* |
| **Lymph nodes** | | | | | | | | | | | |
| Intrafollicular cells | ST | 7.9 | 39 | 29.8* | 58* | 44* | 26.5* | 26* | 14.8* | 31.2* | 46.6** |
| | H | 37.5 | 60 | 51** | 43* | 81.5* | 54.5* | 57** | 30.2* | 24.2* | 56.7* |
| | CA | 20.7 | 15.9 | 21.4** | 15.7** | 6.6* | 4.9* | 3.7 | 2.1* | 3.4* | 6.7* |
| Paracortical cells | ST | 14.2 | 59.8** | 13 | 52* | 58.8* | 38* | 54.6* | 26.9** | 52.3* | 16.2* |
| | H | 69.9 | 75.9** | 40* | 61.3* | 88* | 60.6* | 90** | 111.5* | 121.2* | 92.2* |
| | CA | 34.4 | 10.2 | 20* | 18* | 9* | 4.8** | 5.9* | 5.8 | 4.4* | 7.8* |
| Marginal cells | ST | 6.7 | 14.5* | 16* | 29* | 26* | 12.5* | 16.5* | 10.4 | 7.2* | 5** |
| | H | 46.2 | 19.7* | 36* | 45.4* | 16.4* | 21.3* | 15.3* | 25.2* | 35.1* | 23.6* |
| | CA | 15.4 | 21** | 10* | 21.2* | 3* | 2.9* | 3.6* | 1.6 | 1.2* | 1.6* |

**Note.** H: histamine; *$p<0.001$ and **$p<0.01$ compared with intact animals.

Fluorometric analysis of the mesenteric lymph nodes containing biogenic amines showed that the administration of polystim affected insignificantly the dynamics of histamine in intrafollicular cells (Table 1). The content of this diamine in paracortical macrophages of the T-dependent zone decreased by 1.5 times compared with the control 10 days after the neurotransmitter injection. However, on the 30th day the content of histamine was 2-fold surpassed the control. Intact animals demonstrated low contents of ST in all luminescent structures of the lymph node. A 10-day stress induced a 5-fold increase in the monoamine content in the T-dependent zone. After administration of polystim, the content of ST increased gradually till the 20th day, reaching the maximum on the 30th day. Injections of polystim and physiological saline under conditions of stress did not affect the redistribution of ST in the B-dependent zone.

Intact animals had high CA contents in the studied structures. Under effects of all these factors, the intensity of CA fluorescence in the T-dependent zone decreased to the 10th day and did not exceed 9 arb. units to the 20th and 30th days of experiments.

In stressed animals, polystim and physiological saline decreased the contents of CA (compared with control) only on the 20th day of experiments. The CA concentration remained at this level to the end of experiments.

Stress considerably increased the size of follicles in the lymph node. Physiological saline did not change this parameter in stressed rats. Polystim increased the size of follicles by the 20th day and decreased this parameter by the 30th day of experiments (compared with control level).

Thus, our results indicate that stress affects immunocompetent organs in rats. A long-term stress decreased the contents of CA, ST, and histamine in central and peripheral immune organs. This effect was accompanied by an increase in the number of luminescent structures. The concentrations of biogenic amines returned to the control levels after termination of stress. The immunostimulator polystim increased the contents of biogenic amines in all thymic structures. These findings show that polystim displays stress-protective effects and can be used in clinical practice.

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