Changes in the Rat Thymus Cytoarchitectonics during Repeated Exposure to Hypergravitation

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Comparative study of the structural and functional changes in rat thymus after 5-day and repeated 5-day exposure to hypergravitation (2*g*) showed less pronounced changes after repeated exposure. Proliferative potential of thymic lymphocytes was higher and their loss (death) was lower after repeated exposure to hypergravitation, which attested to better adaptation of the thymus during repeated prolonged exposure to hypergravitation than after single exposure of the same intensity and duration. Our findings suggest the possibility of the formation of gravitation memory in thymic lymphocytes during repeated long-term exposure to hypergravitation.

Key Words: *thymus; lymphocyte; hypergravitation; adaptation*

Exposure to artificial gravitation is now regarded as a prophylactic means for prevention of undesirable effects of some factors of space flights, and special attention in modern airspace biology and medicine is paid to investigation of changes in the structure and functions of organs resulting from repeated exposure to moderate hypergravitation. Repeated overload trainings is a positive factor improving body resistance to hyperexercise, which manifests in readiness of the structural adaptation systems to rapid mobilization [5]. Training prevents possible disorders in the bloodflow system and other untoward symptoms [6].

The possibility of the formation of gravitation memory in animals is discussed in modern science. Functional activity of structural elements of the cerebral somatosensory cortex, neuroepithelium of the otolithic system, and cerebellar nodular cortex in rats repeatedly exposed to prolonged 2*g* hypergravitation is higher than after a single exposure. Some authors [3,4] showed that repeated 2*g* exposure stimulates the synthesis and release of somatotropin, thyroid hormones, and vasopressin. These changes facilitate adaptation to repeated hypergravitation exposure. However, the role of the immune organs in these processes was not studied until present.

We compared the morphology and function of rat thymus after prolonged single and repeated exposure to hypergravitation.

MATERIALS AND METHODS

The study was carried out on adult male Wistar rats (*n*=30, 10 per group). Hypergravitation exposure was realized by rotating in peripheral cells of a CFKB-365 centrifuge (radius 1.41 m) at 33.3 rpm. The gradient of gravitation increase and decrease was 0.02*g*/sec. During rotation the animals remained free in the cells. The rotation was permanent with daily 20-min stops for cleaning the cells and giving the fodder. The duration of the first exposure was 19 days, after which the animals were kept in a vivarium for 30 days for re-adaptation to normal gravitation and then were repeatedly exposed to 2*g* for 5 days simultaneously with rats exposed to 2*g* for 5 days (for the first time). Controls (*n*=10) were kept in cages in the centrifuge room.

After the end of hypergravitation exposure the animals were guillotined. The thymus was fixed in

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10% neutral formalin, treated with ethanol, and embedded in paraffin. Histological sections (4-6 μ) were stained with hematoxylin and eosin, Azur II-eosin, and by Foot's method. The area of structural components of the thymus on histological sections was evaluated by the dot-count method [1] and the corticomedullary index was estimated. For evaluation of cytoarchitectonics of the structural and functional components of the thymus, the number of cells per area unit on histological section (880 μ^2) was counted and their percent ratio was evaluated. The significance of differences was evaluated using Student's *t* test. The results of examination of rat thymus after 5-day exposure to 2*g* were reported previously [2].

RESULTS

Changes in thymus structure after repeated exposure to hypergravitation were in general similar to changes observed after single exposure [2], but were less pronounced. The total area of histological section of the thymus after repeated exposure to 2*g* hypergravitation decreased 1.5 times compared to the control, but its histological structure did not change. The corticomedullary interface was clearly seen. The medulla contained mainly small thymic bodies, their number was 2.6 times below the control value ($p \leq 0.001$). Repeated exposure to hypergravitation, similarly to single exposure of the same intensity, reduced the total specific area of blood vessels on histological sections of the thymus in comparison with the control. However, the vascular lumen was dilated after repeated exposure. Presumably, this can be regarded as a compensatory adaptive reaction to repeated hypergravitation exposure. Along with this, we detected a sharp decrease in the density of cell disposition in the intralobular perivascular spaces of the thymus in rats repeatedly exposed to hypergravitation. We consider that this indicates activation of lymphocyte migration from the thymus.

Repeated 5-day exposure to hypergravitation produced less significant changes in the cellular composition of all structural and functional components of rat thymus (compared to single exposure). After repeated exposure the total cell count at a unit of histological section area in the subcapsular zone of the cortical matter and in the medulla decreased 1.1 and 1.2 times in comparison with the control, respectively ($p \leq 0.01$). Cell distribution density in deep layers of the cortical matter virtually did not change. The number of blasts, large and medium-size lymphocytes decreased significantly in the subcapsular and deep zones of the thymic cortex (Fig. 1). The percentage of blasts in the subcapsular zone of the cortical matter decreased 4.5 times ($p \leq 0.01$) and was 0.85±0.31 *vs.* 5.82± 1.27% in the control, the count of medium-size lymphocytes

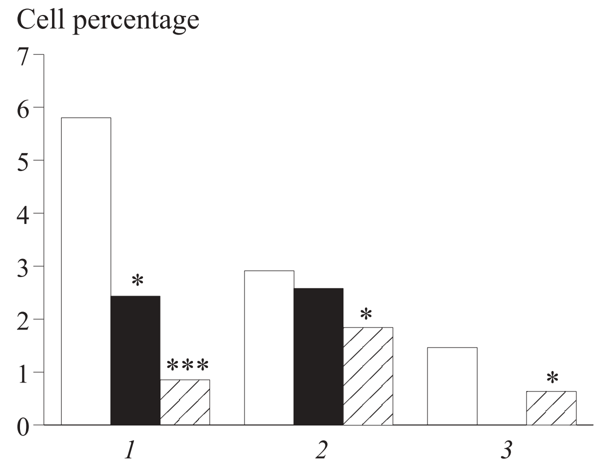

Fig. 1. Content of lymphocyte blast forms in the structural functional components of rat thymus after exposure to 2*g* hypergravitation in different modes. Here and in Figs. 2, 3: *1*) subcapsular zone of the cortex; *2*) deep layers of the cortex; *3*) medulla. Light bars: control; dark bars: 5-day exposure; cross-hatched bars: repeated 5-day exposure. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$ *vs.* the control.

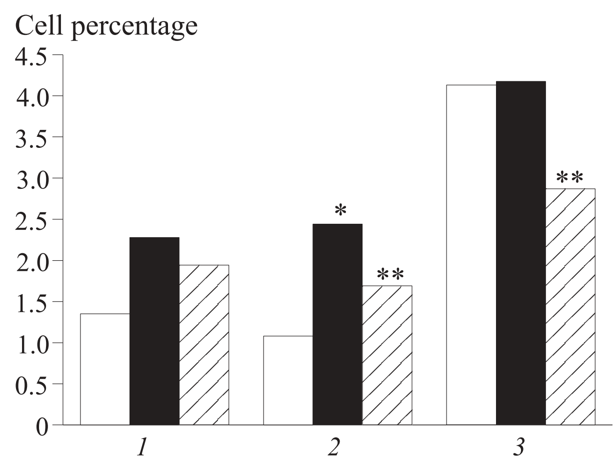

Fig. 2. Content of destructively changed cells in structural functional components of the rat thymus after exposure to 2*g* hypergravitation in different modes.

decreased 2-fold (12.73±1.23 *vs.* 19.43± 1.32%, $p \leq 0.001$). The decrease in the percentage of blast cells and large lymphocytes was less pronounced in the deep layers of the cortex and medulla (1.3 and 1.2 times respectively). The percentage of minor lymphocytes in the subcapsular zone of the cortical matter increased significantly and was 62.45±2.54 *vs.* 54.29± 2.79% in the control ($p \leq 0.05$), while in the deep layers of the cortical matter it almost did not differ from the control. The content of minor lymphocytes in the medulla decreased 1.2 times (38.16±3.68 *vs.* 45.89±3.13%, $p \leq 0.05$). The degree of cell destruction in the thymus was less pronounced after repeated exposure to hypergravitation (Fig. 2). The number of destroyed cells in the subcapsular and deep zones of the cortical matter of the thymus increased 1.4 and 1.6 times in comparison with the control ($p \leq 0.05$), respectively, while

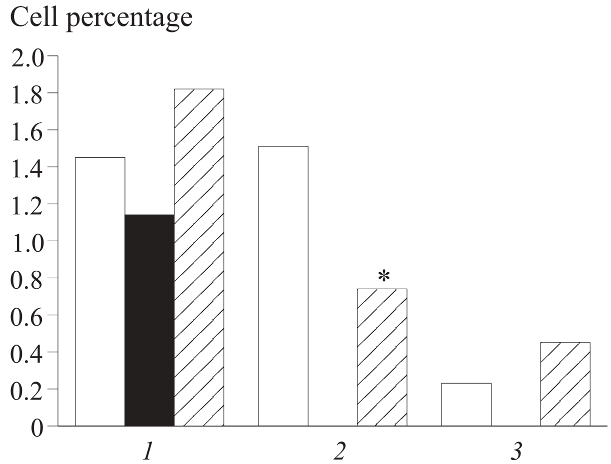

Fig. 3. Content of cells in mitotic stages in the structural functional components of the rat thymus after exposure to 2*g* hypergravitation in different modes.

after single exposure it increased 1.7 and 2.25 times ($p \leq 0.01$). The level of cell destruction processes in the medulla after repeated exposure was lower than in the control (1.4 times, $p \leq 0.01$).

According to our data, repeated hypergravitation modified proliferative activity of lymphocytes in the thymus (Fig. 3). After single hypergravitation exposure the number of dividing cells in the thymus decreased significantly, while after repeated exposure their count was higher in the subcapsular zone of the cortex (by 1.3 times) and in the medulla (2-fold) compared to the control ($p \leq 0.05$). Proliferative activity of cells in the deep layers of the cortical matter was 2-fold below the control. However, the percentage of dividing cells in the thymus after repeated exposure was 2-fold higher ($p \leq 0.05$) than 30 days after the initial 19-day exposure, that is, surpassed the level before repeated hypergravitation exposure [2]. Hence, repeated exposure did not decrease proliferative activity of lymphocytes in the thymus, as was observed after the first hypergravitation exposure, but on the contrary, increased the percentage of dividing cells.

Hence, repeated 5-day exposure to hypergravitation induced structural and functional changes in the thymus of Wistar rats, similar to those after single 5-day hypergravitation exposure of the same intensity. However, these changes were less pronounced and were in general compensatory adaptive. Repeated exposure to hypergravitation caused a less significant loss of lymphocytes as a result of destruction, while the level of mitotic activity was higher than in controls and animals during 30-day adaptation after the first 19-day exposure to hypergravitation.

Our experiments demonstrate higher adaptation of the thymus during repeated long-term exposure to hypergravitation compared to single exposure of the same duration and intensity, which suggests the formation of gravitation memory in thymic lymphocytes during repeated exposure to hypergravitation and attests to the involvement of the immune system in delayed potentiation reaction developing as a result of repeated exposure of animals to hypergravitation [6].

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