PHYSIOLOGY

Morphofunctional Characteristics of Connective Tissue in Emotionally Stressed August and Wistar Rats

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August rats, which are predisposed to emotional stress, are shown to differ from Wistar rats, which are not so predisposed, in the morphofunctional organization of lymphoid and connective tissues. In August as compared to Wistar rats, the lymphoid tissue of the spleen, trachea, bronchi, intestine, and lymph nodes is less abundant, the pancreatic insular apparatus is developed much better, and loose connective tissue contains more mast cells without signs of degranulation. Emotional stress leads, in both strains, to strong connective tissue congestion, massive hemorrhages, edema of loose connective tissue in the renal medullary substance and in the liver, focal or focal/confluent connective tissue metachromasia, and progressive mast cell degranulation. These changes are all more pronounced in August rats.

Key Words: *emotional stress; connective tissue; glycosaminoglycans; mast cells*

Since the classical studies of Hans Selye, stress has been primarily associated with reactions of hypophyseoadrenal structures. Adrenal hormones exert marked effects on the connective tissue, altering metabolic rates in its major components [8,10]. Selye developed the concept of a "local adaptation syndrome" in which the leading role is ascribed to the mast cells of connective tissue [12].

However, the question as to whether and how connective tissue is implicated in stress reactions has been paid little attention in the literature, although there is published evidence of connective tissue involvement in the reactions of stress elicited by electrostimulation of various emotiogenic brain structures, prolonged immobilization [1-3], or exposure to low temperatures [11].

As our earlier studies showed, some members of an animal population are prone to develop disturbances of various physiological functions during emotional stress, whereas other members are not [4,5].

The most clear-cut differences in the resistance to experimental emotional stress have been demonstrated between two different rat populations, Wistar and August, the latter being more susceptible than the former to emotional stress [6,7,9].

We have developed special criteria for predicting the resistance of animals to emotional stress [7]. In the present study, we examined morphofunctional characteristics of connective tissue in various organs of August and Wistar rats before and after emotional stress in order to gain insight into the role this tissue plays in the mechanisms of such stress.

MATERIALS AND METHODS

The study was conducted on 20 male August rats (body weight 150-200 g) and 20 male Wistar rats (250-300 g). All rats were kept caged in groups of 5 and had free access to food and water.

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For a prognostic evaluation of their resistance to emotional stress, the rats were first tested in an "open field". This was a chamber 90 cm wide and 40 cm high with the floor divided into 37 sectors where objects for exploration (bars) were placed. During the test, the chamber was illuminated by a 100 W lamp located at a height of 100 cm from the center of the field.

The open-field test included measurement of the following parameters: latency of first movement, latency of advance to the center, horizontal movements (number of peripheral and central sectors crossed), vertical activity (number of upright postures at the periphery and in the center), exploratory activity (number of objects explored), grooming time, and indices of autonomic balance (defecation and urination). Each animal was tested once for 10 min.

Rats with short latencies of the first movement and advance to the center and with high motor activity at the periphery and particularly in the center were considered to be stress-resistant, whereas those exhibiting prolonged latencies, low activity both in the center and at the periphery, and high indices of autonomic balance were judged to be predisposed to emotional stress.

The experimental model of emotional stress we used was one of aggressive conflict behavior: the animals had their tail nonrigidly fixed at the base of the outer wall of the cage for 10 h daily during the nighttime on five successive days in cages containing 5 rats each.

Immediately after the last stressing session, the rats were decapitated, their internal organs were dissected out, the thymus and adrenals were weighed, and their relative weights (organ weight per 100 g body weight) were determined.

Hematoxylin-eosin- or toluidine blue-stained histological sections of the following organs or tissues were examined: lungs with trachea and lymph nodes, liver, kidneys, spleen, thymus, adrenals, pancreas, heart, skeletal muscle, intestine, thyroid, hypothalamus, loose connective tissue, and skin.

The criteria used to appraise the condition of connective tissue were metachromasia (which indicates the quantity of glycosaminoglycans and, primarily, hyalurone structures that have accumulated), edema, mast cell reaction, infiltration by histiocytes and lymphocytes, congestion, and hemorrhages. The results were evaluated by a semiquantitative method.

RESULTS

On the basis of the open-field test, 10 of the 20 Wistar rats were classified as markedly resistant to emotional stress. These 10 rats were characterized by short latencies of the first movement ($\leq$3 sec) and advance to the center ($\leq$10 sec), high horizontal activity (>80 peripheral and 50 central sectors crossed), vigorous vertical activity (>20 peripheral and >10 central upright postures), high exploratory activity (>5 explored objects), and low indices of autonomic balance (0-1 pellet and no urination).

Of the 20 August rats, 9 were identified as markedly predisposed to emotional stress. These rats were characterized by long latencies (>5 sec and >50 sec for the first movement and advance to the center, respectively), low horizontal activity ($\leq$40 and $\leq$20 peripheral and central sectors crossed, respectively), low vertical activity (no upright postures in the center and $\leq$5 at the periphery), and high indices of autonomic balance (>8 pellets per 10 min plus urination).

The remaining 10 Wistar rats and 11 August rats showed intermediate results in the open-field test and were excluded from the main part of the study.

The rats judged to be most resistant to emotional stress (5 Wistar rats) and most susceptible to it (4 August rats) made up the control group. These 9 animals were not subjected to emotional stress. In the control group, the mean relative weights of the adrenals and thymus were 6.45±1.21 and 92.2±16.1 mg/100 g body weight, respectively, in the Wistar rats and 19.5±2.29 and 188.4±24.4 mg/100 g in the August rats.

The control Wistar and August rats also differed in the structure of the lymphoid (immunocompetent) tissue and Langerhans islets and in the degree of loose connective tissue infiltration by mast cells. In the August rats as compared to their Wistar counterparts, lymphoid tissue in the spleen, lymph nodes, and tracheal and bronchial lymphoid structures was less abundant, the insular apparatus was much better developed (Langerhans islets made up one-third of a pancreatic lobule), and the loose connective tissue was found to contain many more mast cells without signs of degranulation.

The histological appearance of connective tissue in the two strains was similar: a loose tissue composed of delicate fibers predominated over fibrous tissue, there was slight congestion, and focal infiltration by histiocytes and lymphocytes as well as occasional hemorrhagic areas were present.

The test group consisted of 5 stress-resistant Wistar rats and 5 stress-predisposed August rats. After 5 successive days of stressing for 10 h/day as described above, the relative weight of the adrenals was increased by 13.63% in the August rats and by 5% in the Wistar rats, while the thymus had undergone almost complete involution in the August rats, its weight having decreased by 98.7% as compared to 18.4% in the Wistar rats. Connective tissue from both strains showed the following morphological changes: strongly increased congestion, extensive and massive hemorrhages, and edema of loose connective tissue in the renal medullary substance (pyramid papilla) and in the liver (widened perisinusoidal spaces). In addition, focal or focal/confluent metachromasia of connective tissue (notably in vessel walls), its moderate infiltration by histiocytes and lymphocytes, and signs of progressive mast cell degranulation were noted. The above changes were all more pronounced in the August rats, which also differed from the Wistar rats in that the degranulation of mast cells was so severe as to deplete them of their contents; in contrast, mast cell degranulation in the Wistar rats was only moderate and these cells were present in even greater numbers than in the control group.

The experiments indicate that stress-resistant Wistar rats and stress-predisposed August rats differ in the original (prestress) morphofunctional organization of lymphoid and connective tissues. In both strains, emotional stress leads to congestion, massive hemorrhages, and edema of connective tissue. The liver of stressed animals has widened perisinusoidal spaces while their loose connective tissue accumulates glycosaminoglycans (primarily hyaluronic structures, judging by the nature of metachromasia). Emotional stress also induces mast cell degranulation with the result that in August rats mast cells appear almost totally depleted of their contents. Apparently, the degranulation process leads to the release of biologically active substances that make tissues and blood vessels more permeable.

In conclusion, the results of this study show that emotional stress in rats causes substantial changes in the morphofunctional organization of connective tissue in various organs and possibly impairs the metabolism of glycosaminoglycans, primarily of hyaluronic acid (hyalurone). Further in-depth studies are required to pinpoint the changes which connective tissue undergoes in emotional stress.

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