# Skin Glycosaminoglycans in Emotional Stress

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 127, No. 2, pp. 134-136, February, 1999
Original article submitted March 17, 1998

Here we studied the effects of water-immersion emotional stress on the components of several skin biopolymers in rats. The resistance of animals to stress was determined in preliminary experiments. This model of stress induced similar effects on the studied components in stress-resistant and stress-predisposed rats.

**Key Words:** *stress; skin; glycosaminoglycans*

Psychoemotional stress is a global reaction of the organism. In humans, emotions induce generalized effects on all tissues and cells of the body [2,9]. These effects are mediated by the somatic and autonomic nervous systems and hormonal factors.

The connective tissue is a structure penetrating all parts of the body. Its macromolecules are the components of the extracellular matrix. In the matrix, glycosaminoglycans of various types, proteoglycans, glycoproteins, and protein fibers interact with each other and with the membranes of adjacent cells according to complex physicochemical principles. These interactions contribute to tissue homeostasis [13].

Our findings [7] and the results of other researchers [1,3] show that experimental emotional stress (ES) causes considerable changes in the morphofunctional organization of connective tissue in rats. These changes are different in animals with different resistance to ES (for example, in Wistar and August rats) [10,11].

Here we studied changes in glycosaminoglycans, the main connective tissue components of the skin, in rats subjected to ES.

## MATERIALS AND METHODS

Experiments were performed on 20 male Wistar rats weighing 250-300 g. The animals were kept in cages (five rats per cage) under natural light-dark cycle (5.15-21.40, light phase) and *ad libitum* food and water supply.

All the rats were tested in the open field (OF) to estimate the resistance to the water-immersion ES. The OF was a circle (90 cm in diameter) surrounded by walls (40 cm in height) and illuminated with a 100-W lamp positioned at a distance of 100 cm from the OF center. The floor of the OF was divided into 37 squares. The posts set upright into these squares served as the objects for exploration. We recorded the following behavioral parameters: the latency of the first movement, the latency of visiting the center of the OF, horizontal activity (the number of peripheral and central squares crossed), vertical activity (the number of peripheral and central rearing postures), exploratory behavior (the number of posts explored), the time of grooming, and the indices of vegetative balance (defecation and urination). The behavior of each rat was studied for 10 min.

Our previous studies showed that the rats displaying short latencies of the first movement and entrance into the center of the OF and high ambulatory activity in the periphery and especially in the center of the OF are resistant to ES [4]. By contrast, the rats with long latencies of the first movement and entrance into the center of the OF, low activity scores in the center and at the periphery of the OF, and high indices of vegetative balance are predisposed to ES. Stress-induced gastric ulceration was more pronounced in passive rats than in active rats [5].

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0007-4888/99/0002-0120$22.00 

**TABLE 1.** Contents of Glycosaminoglycan Components, Hydroxyproline Levels, and Activities of Skin Enzymes in Control Rats and in Rats Subjected to Water-Immersion ES (*M±m*)

| Group | Uronic acids | Hexosamines | Hydroxyproline | β-Galactosidase | β-Glucoronidase |
|---|---|---|---|---|---|
| | mg/g dry skin | | | activity units | |
| Control (*n*=10) | 2.33±0.15 | 3.28±0.15 | 54.90±6.40 | 17.27±1.61 | 13.50±1.57 |
| Stress (*n*=10) | 1.74±0.27* | 3.21±0.18 | 55.30±6.43 | 21.13±4.81 | 14.98±0.15 |

**Note.** Here and in Table 2: *$p<0.01$ compared with the control.

**TABLE 2.** Contents of Glycosaminoglycan Components, Hydroxyproline Levels, and Activities of Skin Enzymes in ES-Resistant and ES-Predisposed Rats (*M±m*)

| Group | Uronic acids | Hexosamines | Hydroxyproline | β-Galactosidase | β-Glucoronidase |
|---|---|---|---|---|---|
| | mg/g dry skin | | | activity units | |
| Control (*n*=10) | 2.33±0.15 | 3.28±0.15 | 54.90±6.40 | 17.27±1.61 | 13.50±1.57 |
| Resistant (*n*=3) | 1.79±0.37* | 3.32±0.14 | 52.93±4.06 | 19.10±4.72 | 14.40±0.00 |
| Predisposed (*n*=4) | 1.65±0.24* | 3.09±0.22 | 59.53±8.24 | 19.26±4.56 | 14.65±0.63 |

Active and passive animals were divided into two groups. Experimental rats ($n$=10) were subjected to water-immersion ES [14]. Control rats ($n$=10) were kept in vivarium under normal conditions.

Rats of both groups were deprived of food for 24 h before the experiment but had free access to water. Experimental rats were immobilized in plastic tubes (16.5 cm length and 5.5 cm inner diameter) and immersed in water (23°C) up to the level of the xiphoid process of the sternum for 2 h. Then, the animals were returned to their cages for 2 h. Stressed and control rats were then decapitated.

Skin samples (4×4 $cm^2$) for biochemical studies were taken from the back and frozen in liquid nitrogen. Subcutaneous fat and hair were removed, the samples were then minced (to 1-2 $mm^2$ in size), lyophilized, and stored at -60°C. For analysis of structural components the skin was delipidated with chloroform-methanol mixture, and organic solvents were removed. The contents of uronic acids, hexosamines, and hydroxyproline (mg/g dry skin) and the activities of β-galactosidase and β-glucoronidase were measured [6,12,15]. Data were analyzed statistically by single-factor ANOVA using a Mikrostat software.

## RESULTS

Control and stressed rats differed only in the contents of uronic acids (Table 1). The content of uronic acids in stressed rats was lower than in control animals ($F_{1:17}$=37.46). The content of skin hydroxyproline did not differ in stressed and control rats. The activities of β-galactosidase and β-glucoronidase, were insignificantly higher in stressed rats.

Stressed rats ($n$=10) were divided into behaviorally active (resistant to ES) and behaviorally passive (predisposed to ES) subgroups. The first subgroup included 3 rats displaying short latencies of the first movement (less than 5 sec) and high central activity scores. The second subgroup included 4 rats displaying long latencies of the first movement (more than 5 s) and the absence of central activity.

Rats of these subgroups did not differ in the contents of the studied glycosaminoglycans.

After stress, the contents of uronic acids in ES-resistant and ES-predisposed rats were significantly lower than in control animals (Table 2). We have concluded that water-immersion ES induces similar effects in the animals of both subgroups.

These data indicate that water-immersion ES leads to changes in skin glycosaminoglycans. The content of uronic acids decreased significantly in ES. It is known that uronic acids are the components of the connective tissue carbohydrates which change during activation of the pituitary-adrenal axis [8]. These changes probably reflect depolymerization of biopolymers, particularly, of skin glycosaminoglycans. Specific types of glycosaminoglycans and/or glycoproteins which change in stress, their quantitative ratio, the nature of qualitative changes, and the mechanisms of these processes are the subjects for our further studies.

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