Changes on the Surface of Erythrocyte Membrane during Chronic Stress in Rats

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Binding of galactose-specific lectin PNA to the surface of erythrocyte membrane decreased by more than 90% in stressed rats. In the presence of sodium dodecyl sulfate, the lectin-binding fraction was electrophoretically separated into 3 major glycoprotein subfractions with molecular weights of 25, 37, and 50 kDa.

Key Words: *stress; hemagglutination; lectin*

Excess information causes stress in humans. Psychoemotional strain disturbs homeostasis and serves as a serious risk factor for various diseases. Stress can induce or increase the severity of tumors, heart diseases, and cerebrovascular disorders in humans. Stress conditions are associated with the development of gastric ulcer, diabetes, asthma, irritable bowel syndrome, depression, *etc*. [2,3,5].

Stress is accompanied by significant changes in the structure of some blood cells. Here we studied changes in surface properties of the erythrocyte membrane during chronic stress.

MATERIALS AND METHODS

Experiments were performed on outbred male albino rats weighing 100-150 g. Stress was modeled for 4 weeks by the method of Miczer with modifications [4]. The rats were maintained in individual cages at the 1:23 light/dark regimen and 15-18°C. Control animals were kept together in one cage at the 14:10 light/dark regimen (normal circadian rhythm).

The animals were decapitated after 1 month. The erythrocyte mass was obtained by centrifugation at 700*g* for 10 min and 3-fold washout with physiological saline. The erythrocyte membrane surface was examined using lectins with different specificity for carbohydrates: VAL, mistletoe lectin from *Viscum album* L. (galactose and sialic acid); PNA, peanut lectin from *Arachis hypogaea* L. (β-D-galactose and lactose); PSA, pea lectin from *Pisum sativum* L. (mannose and glucose); WGA, wheat lectin from *Triticum aestivum* L. (N-acetyl-D-glucosamine, N-acetyl-D-glucosamine oligomers, and sialic acid oligomers); and SNA, elder lectin from *Sambucus nigra* L. (sialic acid). We used the preparation of VAL synthesized at the Department of Plant Physiology and Anatomy (I. Dzhavakhishvili Tbilisi State University) and standard lectins.

Binding of lectins to blood erythrocytes was measured [1]. Specific activity (SA) of lectins was calculated as follows:

$$SA=T/C,$$

where SA is limit dilution of 1 mg protein causing hemagglutination; T is hemagglutination titer or protein dilution in the last well with agglutination; and C is protein concentration (mg/ml).

Lectin-binding activity of glycoconjugates of erythrocyte ghosts was studied by the method of hapten inhibition [1]. Lectin-binding activity was estimated as the ratio between lectin and lectin-binding protein. Lectin-binding glycoconjugates of erythrocyte ghosts were extracted with Triton X-100 in phosphate buffered saline [5]. Erythrocyte ghosts

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were homogenized with 0.1% Triton X-100 on ice for 1 h. The suspension was centrifuged at 16,000*g* for 20 min. The supernatant was dialyzed against agglutination buffer (40 mM K^+ phosphate and 0.9% NaCl, pH 7.4) for 24 h. The pellet was treated with 0.5% Triton X-100. After centrifugation and dialysis, the pellet was treated with 1% Triton X-100. Centrifugation and dialysis were repeated.

Analytical electrophoresis was performed in the presence of 0.1% sodium dodecyl sulfate under dissociation conditions. The study was conducted in a PAAG gradient (10-25%) on a Hoefer Scientific Instruments SE-200 device. The gels were stained with 0.2% Coomassie G-250. Carbohydrate concentration in proteins was estimated in the Schiff reaction. Protein concentration was measured by the method of Lowry.

The results were analyzed by Student's *t* test.

RESULTS

In stressed rats D-galactose-binding lectin PNA caused less pronounced agglutination of erythrocytes compared to that in control animals; specific activity of PNA significantly decreased. In case of Man/Glc-binding lectin PSA no changes were found. Activity of other lectins decreased less significantly compared to PNA. Activity of NANA-binding lectin SNA remained practically unchanged. These results suggest that the decrease in lectin VAL activity is associated with its specificity for glucose, but not for sialic acid (Table 1). Experiments with binding of galactose-specific lectins showed that stress is accompanied by changes in the carbohydrate composition of the outer surface of the erythrocyte membrane.

Then we studied binding of PNA to erythrocyte glycoconjugates.

The fraction obtained by treatment of ghosts from stressed rats with 0.1% Triton X-100 (fraction 1) was less potent in binding PNA compared to the control sample (0.30±0.03 and 0.61±0.08, respectively, $p<0.01$). Similar results were obtained for intact erythrocytes.

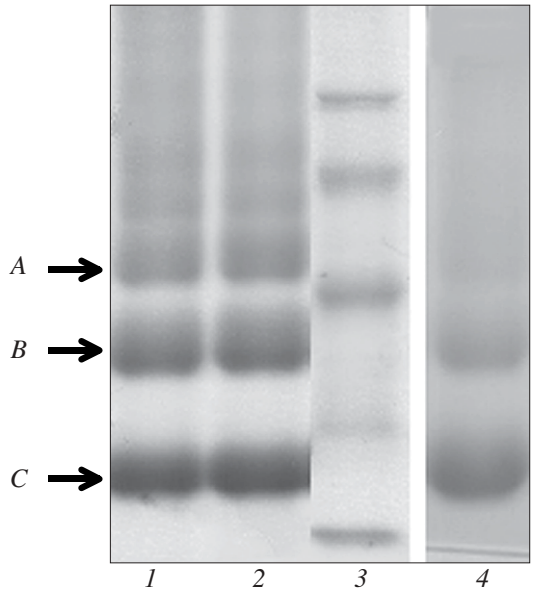

Fig. 1. Electrophoresis of the fraction from rat erythrocyte ghosts extracted with 0.1% Triton X-100 in a PAAG gradient (10-25%) under dissociation conditions. *1*) control; *2*) stress; *3*) standard proteins phosphorylase B (94 kDa), albumin (67 kDa), ovalbumin (45 kDa), carboanhydrase (30 kDa), and trypsin inhibitor (20.1 kDa); *4*) Schiff test. *A*, *B*, and *C*: subfractions (25, 37, and 50 kDa).

Fraction 1 was electrophoretically separated into 3 major subfractions with molecular weights of 25, 37, and 50 kDa (Fig. 1). They consisted of glycoproteins (Schiff reaction). By the molecular weight, protein fractions were similar to erythrocyte membrane glycophorins. Stress was not accompanied by qualitative changes in these compounds. We conclude that chronic stress leads to qualitative changes in galactose-binding glycoproteins of rat erythrocytes, which plays an important role in the maintenance of homeostasis.

TABLE 1. Binding of Lectins to Rat Erythrocytes under Normal Conditions and after Stress

Lectin source	Lectin	Carbohydrate specificity	Specific activity	
			control	stress
Viscum album L.	VAL	Gal, NANA	533	33
Arachis hypogaea L.	PNA	βDGal, Galβ(1,4)Glc	1024	8
Pisum sativum L.	PSA	Man, Glc	64	64
Triticum aestivum L.	WGA	GlcNAc, GlcNAc-oligomers, NANA-oligomers	128	64
Sambucus nigra L.	SNA	NANA	256	128

Note. Gal, galactose; NANA, sialic acid; Man, mannose; Glc, glucose; GlcNAc, N-acetyl-D-glucosamine.

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