Neutrophilokins as Stress Inducers

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The response of the blood system to injection of neutrophilokin, a secretory product of latex-stimulated neutrophils, is studied. Neutrophilokin is shown to induce neutrophilic leukocytosis, eosinopenia, and lymphocytopenia and to reduce the cell count in the spleen. The ability of neutrophilokin to increase spontaneous oxidation of lipids is noted in the rat brain.

Key Words: *neutrophilokin; stress; lipid peroxidation*

Latex-stimulated neutrophils have been found to secrete a group of new transmitters of the immune response, which have been termed neutrophilokins (NK). These compounds raise the number of antibody-producing cells in the spleen, stimulate the host-graft reaction, and increase the functional activity of phagocytes [5-7]. On the other hand, immunization with sheep erythrocytes has been demonstrated to trigger in the blood system and immunocompetent organs a reaction which is similar to the effects of the general adaptive syndrome resulting from hypodynamia and hyperthermia [10]. In addition, immunization and immobilization stress cause unidirectional shifts in the content of biogenic monoamines in brain structures and the adrenals [11]. It should also be mentioned that immunogenesis is thought by some to be a particular case of adaptogenesis [2]. In addition to their immunostimulating effect, NK also elicit systemic biological effects (psychotropic and actoprotective effects and stimulation of wound repair) [8,9].

In the present study we investigated the role of NK in the induction of the general adaptive syndrome.

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MATERIALS AND METHODS

The study was caried out on 48 nonpedigree rats weighing 160-200 g. The animals were divided into three groups. The control group (1st) received physiological saline subcutaneously and the 2nd group was given NK in a dose of 7×10^{-7} mg/rat, subcutaneously. NK is a substance of peptide nature, which is isolated from the supernatant of stimulated neutrophils, as was described previously [6]. The rats of the 3rd group were injected subcutaneously with physiological saline, after which they were immobilized in the supine position by fixing the four extremities for 60 min. Two hours after the start of the procedure, blood was taken from the retroorbital sinus under ether anesthesia, and the animal was killed by transcervical dislocation. The leukocyte count in the blood and the karyocyte count in the spleen were determined routinely [4]. One day before the experiment the animals were deprived of food; water was available ad libitum. The effect of NK on lipid peroxidation (LPO) was assessed as the degree of spontaneous LPO of the rat brain homogenate in the presence of 0.9% NaCl (control) and NK in different concentrations (experiment). LPO was assessed as the accumulation of 2-thiobarbituric acid (TBA)-reactive substances [3]. The data obtained were statistically processed using Student's *t* test and nonparametric methods.

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TABLE 1. Effect of NK and Immobilization Stress on Cellular Composition of Peripheral Blood in Rats

Cells, units of measurement	Control	NK	1-h immobilization stress
Neutrophils, %	14.97±1.07 (n=15)	31.17±4.75** (n=15)	44.05±3.3*** (n=15)
Neutrophils, $\times10^9$/liter	1.85±0.23 (n=12)	3.47±0.70* (n=13)	3.77±0.52** (n=12)
Monocytes, %	1.83±0.33 (n=15)	3.02±1.03* (n=15)	0.96±0.37 (n=15)
Monocytes, $\times10^9$/liter	0.22±0.05 (n=12)	0.23±0.08 (n=13)	0.08±0.03* (n=12)
Eosinophils, %	0.73±0.25 (n=15)	0.44±0.27* (n=15)	0.17±0.1* (n=15)
Eosinophils, $\times10^9$/liter	0.10±0.05 (n=12)	0.03±0.02 (n=13)	0.013±0.01 (n=12)
Lymphocytes, %	82.46±1.03 (n=15)	65.36±5.13** (n=15)	54.82±3.39*** (n=15)
Lymphocytes, $\times10^9$/liter	10.67±0.95 (n=12)	6.34±0.66*** (n=13)	3.82±0.31*** (n=12)
Leukocytes, $\times10^9$/liter	12.84±1.13 (n=12)	10.07±0.70* (n=13)	7.68±0.66*** (n=12)

Note. n: number of animals in group. One, two, and three asterisks show the reliability of differences from the control for $p<0.05$, $p<0.01$, and $p<0.001$, respectively.

RESULTS

It was shown that NK markedly altered the cellular composition of the peripheral blood in the rats (Table 1). NK caused a pronounced neutrophilia, the absolute number of neutrophils attaining 188.16% of the control. The fraction of eosinophils statistically reliably dropped and constituted 60.27% of the control, the absolute eosinophil count being just 26.27% as compared to the control group (the differences were statistically unreliable). The lymphocyte count per liter also dropped and constituted 59.42% of the control. A relatively mild stressor - a one-hour immobilization - was used as the reference model. This intervention resulted in a blood response which was similar to the response caused by the injection of NK: neutrophilic leukocytosis, eosinopenia, and lymphocytopenia. It should be mentioned that the karyocyte count in the spleen after administration of NK in a dose of 7×10^{-7} mg/rat reliably dropped as compared to the control group and constituted $522.35+39.91\times10^6$ (81.74% of the control, p=0.05). As is well known, the splenocyte count reflects the early response of the organism to diverse stressors [4].

Thus, 2 h postinjection the test substance causes neutrophilic leukocytosis, eosinopenia, lymphocytopenia, and a reduction of the karyocyte count in the spleen. Since the above-mentioned shifts are characteristic of the organism's response to stress factors [4], this testifies to the ability of NK to trigger the development of the general adaptive syndrome.

The aim of the next stage of the study was to assess the possible mechanisms underlying the ability of NK to cause stress. Stimulated neutrophils are known to generate active forms of oxygen which contribute to the bactericidal and cytotoxic effect of neutrophilic granulocytes [12]. On the other hand, when the accumulation of LPO products exceeds a certain basal level, it becomes a "primary transmitter" of the stress response [1]. In this connection, we studied the ability of NK to act upon LPO *in vitro*. As follows from the data presented in Table 2, in all test concentrations NK raised the content of TBA-reactive products. The maximum activation of LPO (by 29.51%) was noted for NK in a dose of 10^{-5} mg/ml. However, the lowest dose (10^{-7} mg/ml) of NK also reliably stimulated LPO and increased it by 17.67%.

Thus, NK-induced stress is evidently an "oxidative" stress, which possibly stems from the prooxidant effect of NK.

TABLE 2. Effect of NK on LPO *in vitro*

Concentration of NK, mg/ml	n	Increment of TBA-reactive products, % of initial level	p vs. control
Control	5	220.44±3.88	
10^{-5}	4	285.5±6.44	<0.001
10^{-6}	5	280.94±11.57	<0.002
10^{-7}	5	259.39±4.42	<0.001

Note. n: number of repeated experiments.

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Changes of the Level of Cytochrome P-450 and NADPH-Cytochrome P-450 Reductase in Rats with Hereditary Degeneration of the Retina

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In animals with hereditary degeneration of the retina the level of cytochrome P-450 in the brain microsomal fraction is found to be higher than that in healthy animals. In rats with hereditary degeneration of the retina the activity of NADPH-cytochrome P-450 reductase is unchanged in all tissues examined except for the retina, where it is markedly higher than in healthy animals on postnatal day 90.

Key Words: *hereditary degeneration of the retina; cytochrome P-450; NADPH-cytochrome P-450 reductase; hemoglobin; microsomes*

In studies performed on rats with hereditary degeneration of the retina (HDR) we established that in the early postnatal stages (days 10-20) some metabolic changes found in the retina and pigment epithelium (PE) of the eye of diseased animals also manifest themselves in the cerebral cortex [3], evidently due to the fact that these tissues develop from the same presumptive region [5]. Specifically, it has been shown that the rate of induced lipid peroxidation is higher in the retina, PE, and cerebral cortex of affected animals. Research into the possible causes of this phenomenon (experiments have been performed on different subcellular fractions of the animal brain) has shown that in the early stages of postnatal ontogenesis (day 20) the total content of heme-free iron in the microsomal fraction of the cerebral cortex drops, and the ratio between the oxidized and reduced forms of iron markedly changes (in favor of the latter). In addition, in the retina and cerebral cortex the activity of glucose-6-phosphate dehydrogenase, a major enzyme of the hexose-monophosphate shunt producing NADPH, an effective iron-reducing agent in the cell, was found to be higher in diseased than in healthy animals.

In view of the data on changes in the content of heme-free iron in microsomes of the cerebral cortex of rats with HDR (probably the same changes also occur in the retina), it seemed of interest to elucidate whether the heme-containing components, along with the related NADPH-dependent systems, are altered in the microsomal membrane of affected animals. Accordingly, the aim of the present study was to determine the content and

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