MICROBIOLOGY AND IMMUNOLOGY

Adrenergic Regulation of Phagocytic Activity of Peripheral Blood Neutrophils, Monocytes, and Eosinophils in Stressed Rats

Yu. I. Shilov and E. G. Orlova

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 129, No. 5, pp. 563-566, May, 2000
Original article submitted October 25, 1999

Time course of phagocytic activity of peripheral blood neutrophils, monocytes, and eosinophils was studied in rats exposed to acute stress under conditions of propranolol blockade of β-adrenoceptors. The important role of β-adrenergic mechanisms in the regulation of phagocytic functions in stress was demonstrated.

Key Words: *stress; β-adrenoceptors; phagocytosis*

Neutrophilic leukocytosis, eosino- and lymphopenia are typical of stress [1], but their relationship with resistance mechanisms in stress is little known. Stress suppresses cell-mediated and humoral immunity and phagocytic activity of mononuclear phagocytes [2,4]. It is not clear how the opposite changes in neutrophil and eosinophil counts in stress modify their functional activities, including phagocytic activity (PA). Adrenergic mechanisms play an important role in the regulation of bone marrow hemopoiesis in stress [1,2,5]. Phagocytic cells express both β- and α-adrenoceptors [8,10], but adrenergic regulation of phagocytosis is little studied.

We investigated changes in integral parameters of peripheral blood neutrophil, monocyte, and eosinophil PA in rats exposed to acute stress under conditions of β-adrenergic receptor blockade.

MATERIALS AND METHODS

Male Wistar rats (169.8±6.05 g) were used. Group 1 rats (n=12) were exposed to acute 6-h immobilization stress combined with dosed blood loss (0.5 ml blood was collected from the caudal vein before immobilization and 0.5, 1, 3, 6, 24, and 72 h, 5 and 7 days after the beginning of immobilization). Group 2 animals (n=10) were similarly exposed to stress under conditions of β-adrenoceptor blockade (2 subcutaneous injections of 5 mg/kg propranolol hydrochloride at 3-h intervals, the first injection 30 min before immobilization). The dose and scheme of β-adrenoblocker injections were selected with consideration for previous findings [1,2]. Mean initial values were used as the control.

Leukocyte PA was evaluated as described previously [3] with modifications. Heparinized blood (25 μl) and suspension of formalin-treated sheep erythrocytes (25 μl, 100×10^6/ml in medium 199 with 10 mM HEPES and 2 mM L-glutamine) were incubated for 20 min at 37°C in microtubes with antiadhesive coating. The results were evaluated under a microscope. Parameters described previously were calculated [6]. Changes in hematological values were evaluated routinely. The significance of differences was evaluated using unpaired Student's t test.

RESULTS

Dosed blood loss decreased erythrocyte count and hemoglobin concentration (p<0.05 starting from the 60th min). These parameters remained reduced until day 7.

Laboratory of Ecological Immunology, Institute of Ecology and Genetics of Microorganisms, Ural Division of the Russian Academy of Sciences, Perm'; Department of Microbiology and Immunology, Perm' State University

0007-4888/00/0005-477$25.00

Erythrocyte count in group 1 was minimum on day 2 ($5.178\pm0.443\times10^6$ *vs.* $7.495\pm0.145\times10^6$ cells/μl in the control) and in group 2 on day 1 ($5.490\pm0.533\times10^6$ cells/μl), the differences between these groups were insignificant. Hence, moderate posthemorrhagic anemia can be regarded just as a factor potentiating the effect of immobilization stress, but not contributing to the differences in phagocyte functions.

In group 1, absolute neutrophilic leukocytosis was observed starting from the 3rd hour (Fig. 1). The absolute neutrophil count remained high until day 5. The blockade of β-adrenoceptors during stress increased neutrophilic leukocytosis 3 and 6 h after the beginning of immobilization in comparison with group 1, but decreased it after 48 and 72 h ($p<0.05$). Changes in the integral relative parameters of neutrophilic phagocytosis (percentage of phagocytosis and phagocytic number) in group 1 were biphasic. Phase I, coinciding with the immobilization period, was characterized by a decreased percentage of phagocytic cells (Fig. 1) and phagocytic number (0.345±0.027 after 6 h *vs.* 0.849± 0.137 in the control, $p<0.002$). However because of neutrophilic leukocytosis, the relative values do not adequately reflect the total changes in neutrophil phagocytic activity, and therefore the absolute count of phagocytosing neutrophils and the absolute number of phagocytosed particles were counted. The former parameter increased during immobilization: 2643.91± 371.29 after 3 h and 2262.00±321.46 cells/μl after 6 h *vs.* 1253.36±337.63 cells/μl in the control ($p<0.01$ and $p<0.05$, respectively). The latter parameter did not change (Fig. 1). Opposite changes in the relative and absolute values were apparently due to the release of functionally immature neutrophils from the bone marrow: the count of segmented neutrophils increased from 516.67±153.02 after 1 h to 562.67±93.82 after 3 h and to 1172.63±232.31 cells/μl after 6 h *vs.* 15.00±11.47 in the control ($p<0.005$, $p<0.001$, and $p<0.001$, respectively). Phase II (24 h of immobilization) was characterized by an increase in both relative and absolute parameters of neutrophilic phagocytosis (Fig. 1). It is noteworthy that the early changes in the function of phagocytic cells coincides with the anxiety stage of the general adaptation syndrome, while

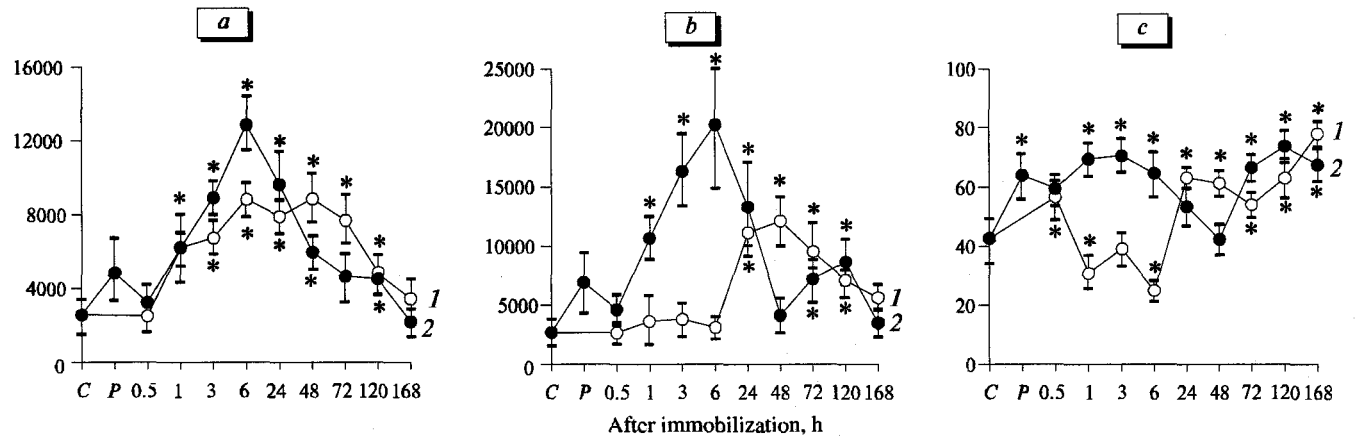

Fig. 1. Time course of phagocytic activity in rats exposed to acute stress (*1*) and stress under conditions of β-adrenoceptor blockade (*2*). Here and in Figs. 2 and 3: *C*: control (baseline values); *P*: 30 min after injection of propranolol. *$p<0.05$ *vs.* the control. Ordinates: absolute number of phagocytizing cells (*a*) and phagocytosed particles (*b*) per μl blood, and percent of phagocytosis (*c*).

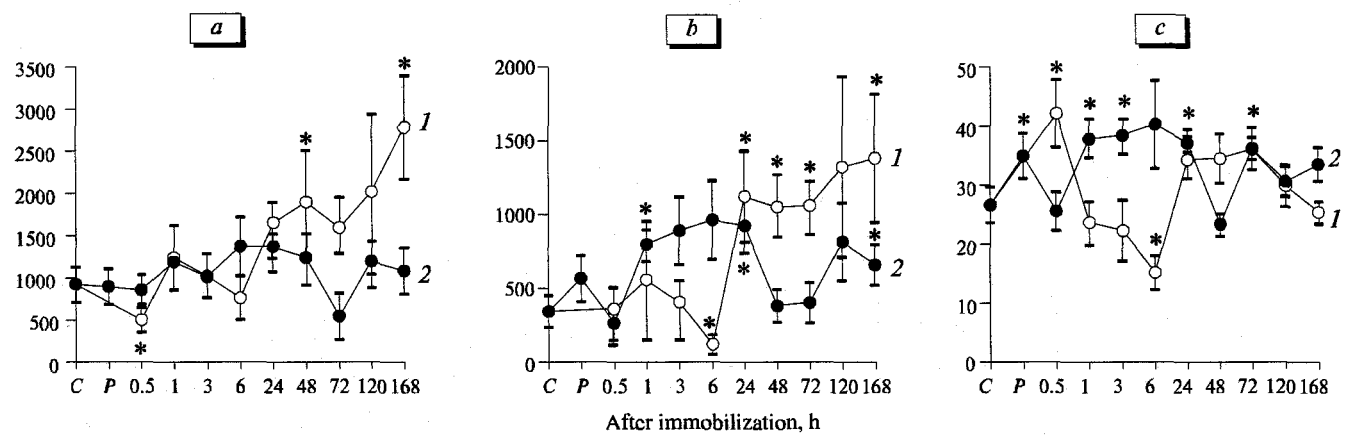

Fig. 2. Time course of phagocytic activity of monocytes in rats exposed to acute stress (*1*) and stress under conditions of β-adrenoceptor blockade (*2*).

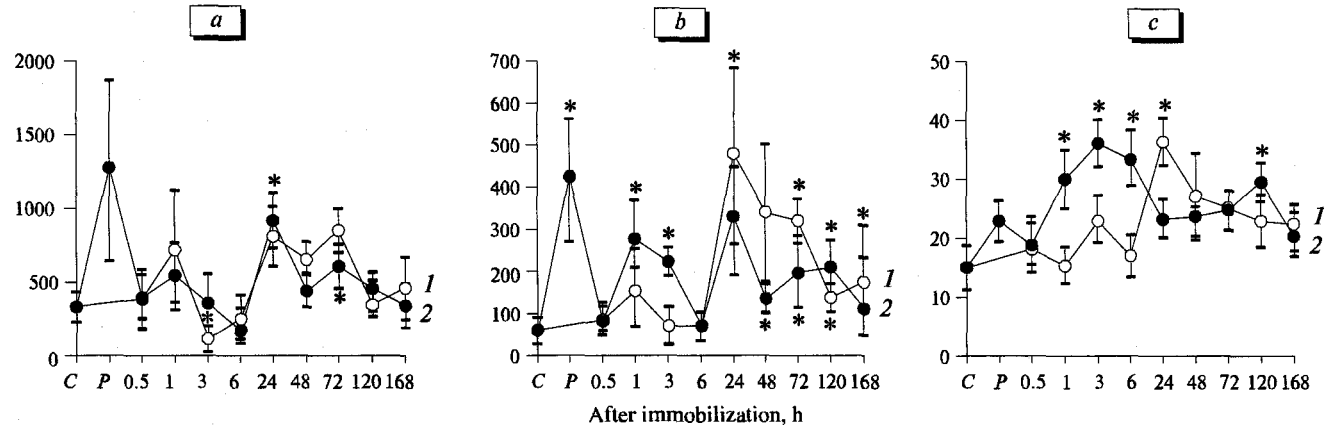

Fig. 3. Time course of phagocytic activity of eosinophils in rats exposed to acute stress (*1*) and stress under conditions of β-adrenoceptor blockade (*2*).

later changes corresponded to the increased resistance stage [9].

β-Adrenoceptor blockade with propranolol did not prevent the early decrease in relative parameters of neutrophilic phagocytosis; moreover, it led to activation of phagocytosis before immobilization and during stress (Fig. 1). Activation of neutrophilic phagocytosis during the first 6 h of stress in group 2 may be due to stress-induced release of endogenous catecholamines [1] and stimulation of α-adrenoceptors by these compounds in the absence of inhibitory effect mediated through β-adrenoceptors. β-Adrenergic agonist terbutaline sulfate suppresses *in vitro* phagocytic activity of peripheral blood phagocytes in rats, while epinephrine under the same conditions and in the same concentrations activates it [7]. Excessive activation of neutrophilic phagocytosis in the presence of β-adrenoceptor blockade during phase I modifies the time course of parameters during phase II (Fig. 1).

The same time course was observed for monocytic and eosinophilic phagocytosis (Figs. 2 and 3). The differences consisted in the decrease in not only relative, but absolute values of monocyte phagocytosis during the first 6 h and the absence of depression of relative parameters of eosinophils phagocytosis. The blockade of β-adrenoceptors notably increased the monocyte and eosinophil phagocytosis parameters. In contrast to previous data on changes in blood parameters during the first 24 h of stress [1], we revealed the development of eosinophilia on day 3 (Fig. 3). Transient monocytopenia 30 min after the beginning of immobilization was followed by absolute monocytosis, which was most pronounced on days 2 and 7 (Fig. 2).

Hence, adrenergic mechanisms play an important role in changes of phagocytic activity of peripheral blood neutrophils, monocytes, and eosinophils in acute stress. β-Adrenergic receptors can limit excessive activation of phagocytic cells induced by stress.

REFERENCES

1. P. D. Gorizontov, O. I. Belousova, and M. I. Fedotova, *Stress and Blood System* [in Russian], Moscow (1983).
2. Yu. I. Zimin, *Itogi Nauki i Tekhniki VINITI, Immunology*, **12**, Moscow (1983), pp. 41-52.
3. V. N. Kaplin, *Nontraditional Immunology of Infections* [in Russian], Perm' (1996).
4. D. N. Mayanskii, N. P. Voronina, and A. Yu. Voronin, *Byull. Eksp. Biol. Med.*, **100**, No. 9, 324-326 (1985).
5. I. A. Khlusov, A. M. Dygaii, and E. D. Gol'dberg, *Ibid.*, **116**, No. 12, 570-572 (1993).
6. Yu. I. Shilov, V. P. Vladykina, and A. T. Atnaguzina, *Permskii Med. Zhurn.*, **15**, No. 2, 3-9 (1998).
7. Yu. I. Shilov, E. G. Orlova, A. T. Atnaguzina, and D. Yu. Shilov, *Receptors and Intracellular Signalization* [in Russian], Pushchino (1998), pp. 63-65.
8. K. S. Madden and D. L. Felten, *Physiol. Rev.*, **75**, No. 1, 77-106 (1995).
9. H. Selye, *Endocrinology*, **21**, No. 2, 169-188 (1937).
10. R. N. Spengler, S. W. Chensue, D. A. Giacherio, *et al.*, *J. Immunol.*, **152**, No. 6, 3024-3031 (1994).