# POSTSTRESSOR CORRECTION OF MACROPHAGE FUNCTION BY TUFTSIN AND ITS DERIVATIVES

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The physiological control of the immune response by neuroendocrine mechanisms and the role of mediators of the immune system in the formation of resistance to stress are under intensive study [4, 7]. One type of biocontrol, responsible for communication between the nervous and immune systems is the system of endogenous peptides [3, 6]. The list of these highly active hormone-like peptides, with their marked pleiotropic activity, includes the tetrapeptide tuftsin (Thr—Lys—Pro—Arg) [8]. Behavioral and neurochemical analysis has revealed a central action of tuftsin on the nervous substrate of behavior [1, 3, 14]. Other targets for the action of tuftsin are cells of the mononuclear system, macrophages in particular [15]. Tuftsin binds specifically with macrophages, stimulates their secretion and phagocytic activity, stimulates migration of mononuclears, and potentiates the antigen-specific macrophage-dependent training of T lymphocytes [8, 15].

To search for approaches to the pharmacologic correction of poststressor disturbances of immunity and functions of the phagocytes, the effect of tuftsin and its derivatives were studied on the functional state of mononuclear phagocytes of rats with experimental neurosis.

## EXPERIMENTAL METHOD

A neurosis was induced in noninbred male rats weighing about 200 g by Hecht's method [11] for a period of 15 days. The severity of the neurosis was assessed by the change in weight of the immunocompetent organs, the weight of the adrenals, and the peripheral blood leukocyte count. The state of function of peritoneal macrophages was assessed by their ability to undergo adhesion and the nitro-blue tetrazolium (nitro-BT) test [2].

Tuftsin and its derivatives, obtained from the Institute of Molecular Genetics, Academy of Sciences of the USSR (synthesized by V. N. Nezavibat'ko and co-workers), were injected intraperitoneally once a day for 7 days after induction of the neurosis, in a dose of 300 μg/kg.

## EXPERIMENTAL RESULTS

The effect of long-term psychoemotional stress on the hematologic parameters and state of macrophage function after and in the absence of treatment are shown in Table 1. By evaluating the state of the adrenals, the immunocompetent organs, and macrophage function, the status of the animals at the end of induction of neurosis could be defined as a stage of adaptation to the stressor. By the 15th day of stress, against the background of an increase in weight of the adrenals, a tendency was observed for the development of compensatory hypertrophy of the lymphoid organs (thymus, spleen, lymph nodes), but the number of karyocytes in the peripheral blood remained low. The state of macrophage function, assessed by the nitro-BT test, also was depressed and amounted to about 60% of its level in the group of control animals. Reduction in the number of cells of the monocytic series in the peripheral blood and of phagocytic activity of the peritoneal macrophages to 50% of normal in the stressed rats is evidence of the high sensitivity of the monocytic—macrophageal branch of hematopoiesis to the action of stressor hormones.

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TABLE 1. Investigation of Stress-Protective Properties of Tuftsin and Its Derivatives (M ± m)

| Parameter studied | Native control (n = 12) | Chronic psychogenic stress | | | |
|---|---|---|---|---|---|
| | | without treatment (n = 10) | administration of tuftsin (n = 8) | administration of heptapeptide (n = 9) | administration of pentapeptide (n = 10) |
| Relative weight of organs, g | | | | | |
| thymus | 1,52±0,14 | 1,70±0,13 | 1,58±0,23 | 1,59±0,11 | 1,97±0,12* |
| spleen | 3,19±0,22 | 3,37±0,15 | 4,26±0,53* | 3,74±0,40 | 4,00±0,30* |
| lymph nodes | 0,122±0,013 | 0,144±0,019 | 0,313±0,053** | 0,148±0,007 | 0,132±0,01 |
| liver | 3,83±0,13 | 3,14±0,081* | 4,19±0,42** | 3,67±0,095** | 3,42±0,088* |
| adrenals | 0,177±0,014 | 0,211±0,015* | 0,338±0,036** | 0,250±0,018* | 0,213±0,022 |
| Blood karyocytes, No./mm$^3$ | 9916±1363 | 6784±610 | 10 188±793** | 8800±868** | 9250±708** |
| Leukocyte formula, % | | | | | |
| granulocytes | 7,88±2,32 | 7,70±3,27 | 13,0±3,16 | 5,0±2,12 | 11,56±2,43 |
| monocytes | 2,25±0,91 | 1,10±0,49 | 4,0±1,79 | 1,0±0,1 | 1,78±0,76 |
| lymphocytes | 89,88±2,71 | 91,2±3,23 | 83,0±7,37 | 94,0±2,12 | 86,67±2,78 |
| Peritoneal macrophages | | | | | |
| relative content, mm$^3$/body weight | 10,24±1,37 | 8,84±1,27 | 25,11±4,91** | 18,5±5,85** | 25,4±5,85** |
| percent adhesion | 54,5±6,56 | 55,2±8,13 | 82,2±3,96** | 57,8±6,67 | 77,53±8,2** |
| percent survival | 81,0±5,28 | 60,5±6,27 | 86,8±3,7** | 91,1±3,49** | 89,9±2,07** |
| CIA | 0,178±0,060 | 0,089±0,021 | 0,108±0,022 | 0,222±0,056** | 0,047±0,008* |

Legend. n) Number of animals in group; CIA) color index of macrophage activity in nitro-BT test (in $E_{490}$/45 min/$10^6$ macrophages). *) $p \leq 0.05$ compared with control; **compared with group of stressed rats.

During evaluation of the stress-protective properties of the peptides attention was paid to their ability to stimulate leukopoiesis, to correct functional hypertrophy of the adrenals and the central and peripheral lymphoid organs, and to restore the depressed functional activity of the peritoneal macrophages. It will be clear from Table 1 that all three peptides have about equal ability to stimulate leukopoiesis and the migrating ability of the leukocytes, initiating their departure from the blood stream into the tissues (an increase in hypertrophy of the spleen and lymph nodes) and cavities (an increase in the relative number of cells in the peritoneal fluid). Tuftsin and pentapeptide exhibit their greatest affinity for the myeloid blood and of macrophages in the peritoneal cavity, and significantly stimulating the adhesive prope-ties of the peritoneal macrophages. The heptapeptide stimulates leukopoiesis mainly on account of the lymphoid branch, and does not affect adhesion of macrophages. All three peptides increase the poststressor survival rate of the peritoneal macrophages (staining with trypan blue), evidence of their ability to stabilize the structural integrity of macrophages during stress.

Tuftsin did not stimulate the nitro-BT activity of the peritoneal phagocytes when depressed under stress conditions. The absence of recovery of functional activity of the macrophages is evidently connected with tuftsin-induced activation of the adrenal system, as assessed by the increase in the relative weight of the adrenals in the treated rats (Table 1). The effect of activation of adrenal function may be connected with stimulation of secretion of interleuken 1 by activated macrophages by tuftsin [15]. Subpyrogenic doses of interleukin 1 (0.5-1 μg, corresponding to the daily production of $10^6$ macrophages) are known to cause a four- to fivefold increase in the serum levels of ACTH and corticosterone in rats and mice [5]. The absence of restoration by tuftsin of the nitro-BT activity of the macrophages, which were observed experimentally, may be the end result of a consecutive chain of events including activation of macrophages and an increase in secretion of interleukin 1; interleukin 1 stimulates the pituitary–adrenal system and the release of ACTH and corticosterone which, by the negative feedback principle, depresses the metabolic functions of the macrophages. Evidence of the increased release of interleukin 1 under the influence of tuftsin, together with an increase in weight of the adrenals, is given indirectly by an increase in the number of granulocytes and monocytes in the peripheral blood and of macrophages in the peritoneal cavity, as a result of activation of proliferation of precursors of the granulocytic–macrophagal branch, for interleukin 1 is known to stimulate the production of colony-stimulating factors [9]. The similarity of the behavioral effects of tuftsin [3, 14] and ACTH [10] also indicates that the psychotropic effects of tuftsin are realized through the release of interleukin 1 and ACTH.

Unlike tuftsin, the heptapeptide caused only a small increase in weight of the adrenals and restored the functional activity of the macrophages, depressed under stress conditions,

only weakly. The pentapeptide did not affect the state of the adrenals and aggravated the stress-induced depression of nitro-BT activity of the macrophages. The increase in lymphotropism and increased ability to stimulate phagocytosis, observed in the tuftsin heptapeptide, with its protected C-end, and inhibition by the pentapeptide of the phagocytic activity of the macrophages are connected with modifications to the structure of tuftsin and are in good agreement with data in the literature [13].

The data showing the ability of tuftsin to induce functional hypertrophy of the adrenals and to stimulate the granulocytic—monocytic branch of hematopoiesis under conditions of adaptation to stress confirm the results of experiments in [5] and suggest the existence of regulatory feedback between the system of phagocytes and the pituitary—adrenal axis through interleukin 1. The latter acts as an afferent, and corticosterone as an efferent, hormonal signal. Most known stimulators of phagocytosis increase interleukin 1 secretion by macrophages [15]; corticosterone, on the other hand, is an inhibitor of secretion because of its ability to reduce the stability of mRNA and to inhibit transcription of the interleukin 1 gene [12].

The "provocative" effect of tuftsin, inducing additional hypertrophy of the adrenals and disturbance of hematopoietic function in stressed rats, discovered in this investigation, is an indication of the need to evaluate pharmacologic and, in particular, stress-protective properties of psycho- and immunostimulants in both intact and stressed animals.

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