# EFFECT OF TUFTSIN AND OXYMETHACIL ON MACROPHAGAL TYROSINE HYDROXYLASE DURING CHRONIC STRESS

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**Key words:** chronic stress; tuftsin; oxymethacil; tyrosine hydroxylase.

The two-way connection which exists between the immune and nervous systems coordinates the highly specialized functions of these systems [9, 10]. One of the mechanisms of coordination of their activity is the presence of receptors for catecholamines, involved in the regulation of the specific functions of these cells, on the membrane of lymphocytes and macrophages. An inseparable part of the catecholaminergic systems of the brain is tyrosine hydroxylase (TH), whose activity is controlled by specific presynaptic receptors, and which in turn is involved in the regulation of their activity. Recently, TH has been found in peripheral blood leukocytes [6], but the connection between tyrosine hydroxylase activity of leukocytes and their function is not clear. The aim of this investigation was to study the kinetic properties of TH from peritoneal macrophages of rats exposed to chronic stress, receiving injections of the tetrapeptide tuftsin, which possesses immunomodulating and psychotropic properties, and oxymethacil, an activator of phagocytosis with no marked psychotropic activity.

## EXPERIMENTAL METHOD

Male rats weighing 180-200 g were subjected to chronic (15-day) stress: for 15 min daily they were exposed in special containers to an electric current (10 sec) combined with flashes in accordance with a special program [12]. Tuftsin, in a dose of 300 $\mu$g/kg, and oxymethacin, in a dose of 50 mg/kg, were injected intraperitoneally for a period of 7 days after exposure to the psychostimulating procedure. Macrophages were obtained by irrigating the peritoneal cavity of the rats with medium 199 with the addition of 10% embryonic calf serum and heparin in a concentration of 5 U/ml [7]. The cell suspensions thus obtained were poured into plastic Petri dishes and incubated at 37°C for 4-6 h, after which nonadherent cells were removed by washing 3 times with warm medium. Macrophages were collected from the surface of the dishes by means of a rubber spatula, and resuspended in buffer, after which the cells were frozen in liquid nitrogen. Functional activity of the macrophages was determined by the nitro-BT test [8]. Before determination of TH activity the cells were thawed at room temperature and homogenized in a glass homogenizer; the homogenate was centrifuged at 8000$g$ for 10 min. The supernatant was used for determinations. The velocity of the tyrosine hydroxylase reaction was measured spectrophotometrically [4], by recording the increase in absorption at 335 nm continuously, at the point of absorption of oxidation products of the pterine coenzyme. The compound 6,7-dimethyl-5,6,7,8-tetrahydropterine ($DMPH_4$) was used as the coenzyme. The composition of the medium used to measure the reaction velocity was as follows: 0.05 M Tris-maleate, pH 6.2; L-tyrosine in concentrations of 1.6-550 $\mu$M; catalase, 33 units; dithiothreitol, 65 mM; $DMPH_4$, 183 $\mu$M. The measurements were made on an Aminco DB-2 differential spectrophotometer. Michaelis' constants ($K_m$) were calculated by the method in [2].

## EXPERIMENTAL RESULTS

On the assumption that tissue macrophages, which like peripheral blood leukocytes, belong to the myeloid series, possess tyrosine hydroxylase activity like leukocytes [6], and also since tyrosine hydroxylase activity has not been detected pre-

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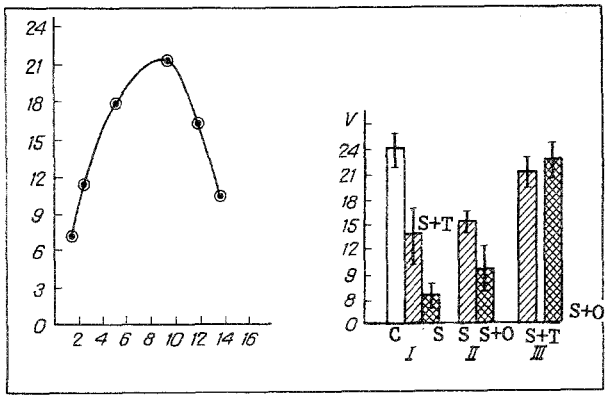


Fig. 1 Fig. 2

Fig. 1. Dependence of velocity of tyrosine hydroxylase reaction in homogenate of rat peritoneal macrophages on tyrosine. Abscissa, tyrosine concentration (in $\mu$M); ordinate, reaction velocity (in mmoles/min/mg protein).

Fig. 2. Effect of tuftsin and oxymethacin on tyrosine hydroxylase activity in homogenates of rat macrophages exposed to different procedures. Horizontal axis — optimal concentrations of tyrosine (in $\mu$M) for high- (I), medium- (II), and low-affinity (III) forms of TH; vertical axis — reaction velocity (in mmoles/min/mg protein). C) Control, S) stress, T) tuftsin, O) oxymethacin.

viously in macrophages, the velocity of the tyrosine hydroxylase reaction was measured over a wide range of tyrosine concentrations: from 1.8 to 550 $\mu$M, i.e., almost to the limit of solubility of tyrosine. It was found that tyrosine hydroxylase activity can be detected in homogenates of peritoneal macrophages over a range of tyrosine concentrations from 1.8 to 16 $\mu$M (Fig. 1). It will be clear from Fig. 1 that dependence of velocity on tyrosine concentration for homogenates of peritoneal macrophages from intact (control) rats is characterized by a curve with a peak at 9 $\mu$M tyrosine. In higher tyrosine concentrations the reaction velocity fell sharply. Thus the tyrosine-hydroxylase activity of peritoneal macrophages, like that of peripheral blood leukocytes [6], is subject to substrate inhibition. Because of the presence of substrate inhibition of TH, the different forms of TH, differing in their affinity for tyrosine, can be clearly distinguished.

During exposure to stress changes took place in the kinetics of the tyrosine hydroxylase reaction relative to tyrosine: tyrosine hydroxylase activity in homogenates of peritoneal macrophages of stressed rats was discovered in the presence of higher tyrosine concentrations (36.7-135 $\mu$M) than in the control. Tyrosine hydroxylase activity also was found in macrophages of stressed animals, which had received tuftsin or hydroxymethyluracil (oxymethacil) for 7 days, after the end of exposure to stress, in the presence of tyrosine in concentrations of between 275 and 550 $\mu$M. Thus during exposure to stress, forms of TH differing in their kinetic properties from the TH found in macrophages of intact animals appear in the peritoneal macrophages after exposure to stress. Curves of dependence of reaction velocity on tyrosine concentrations for these two variants of the experiment were similar in shape to the curve obtained for the control animals, and they are therefore not given in the text. The results of a study of the TH activity of macrophages after exposure to stress, compared with the activity of these cells in intact animals are given in Fig. 2 in the form of reaction velocities for tyrosine concentrations optimal for each form of TH, and found experimentally during the study of dependence of the velocity of the TH reaction on the tyrosine concentration. It will be clear from Fig. 2 that the TH activity of macrophages of the control rats was discovered in the presence of tyrosine in an optimal concentration of 9 $\mu$M. The tyrosine concentration optimal for exhibition of TH activity, for homogenates of peritoneal macrophages of rats exposed to long-term stress, was 93 $\mu$M (activity of the enzyme was found in the presence of tyrosine in concentrations of between 36.7 and 135 $\mu$M). The reaction velocity, measured in the presence of this concentration of tyrosine, was only half of that observed in macrophages of the control animals, measured in the presence of 9 $\mu$M tyrosine. With tyrosine present in concentrations of 1.8-16 $\mu$M, hardly any TH activity could be found in the macrophages of the stressed animals. It will be clear from Fig. 2 that during long-term stress changes in the kinetic properties of TH are observed in the peritoneal macrophages, together with a reduction of its affinity for tyrosine. A similar phenomenon is known for TH of brain neurons: depending on the state of activation or rest of presynaptic catecholamine receptors, the TH of the corresponding neurons is in a high- or low-

TABLE 1. Effect of Tuftsin and Oxymethacil on Functional Activity of Peritoneal Macrophages (nitro-BT test) of Rats Exposed to Chronic Psychogenic Procedures

| Parameter | Group of rats | | | |
|---|---|---|---|---|
| | control | stress | stress + tuftsin | Stress + oxymethacil |
| CPA, rel. units | 118,0±40,05 | 59,5±13,8 | 72,3±19,3 | 71,9±12,9 |

**Legend.** CPA) Color index of macrophage activity.

TABLE 2. Kinetic Parameters of TH of Peritoneal Macrophages Relative to Tyrosine

| Parameter | Form of TH | | |
|---|---|---|---|
| | I | II | III |
| $K_m$ for tyrosine, μM | 4,2±0,12 | 47,0±0,33 | 270±2,4 |
| $V_{max}$, mmoles/min/mg protein | 30,2±1,2 | 18,4±1,8 | 27,8±1,0 |

affinity state [5]. Thus similarity with the regulation of brain TH activity is found in the regulation of TH activity of macrophages.

The tetrapeptide tuftsin, a fragment of the immunoglobulin G molecule, besides its immunostimulating properties, also has a psychostimulating effect [1]. It was shown previously that tuftsin can act directly on brain TH [1], evidence of its affinity for the catecholaminergic systems of the brain, for TH is an essential part of these systems [5], and according to data in [12] TH and specific proteins of the presynaptic membrane are coded by common genes. It will be clear from Fig. 2 that macrophages of animals receiving tuftsin after days of exposure to stress do not possess activity of the form of TH (optimal tyrosine concentration 93 μM) found in stressed animals, but activity of TH for which the optimal tyrosine concentration was 9 μM could be detected in the stressed animals, and in addition, TH for which the optimal tyrosine concentration was 530 μM also could be found. If the enzyme preserved its substrate inhibition, activity of TH could not be found under these conditions [5]. Thus after injection of tuftsin into stressed rats, on the one hand a picture similar to that found in the brain was observed: TH changed from the low-affinity form observed during stress into the high-affinity state. At the same time, a low-affinity form of TH not known in the brain was found. It can be tentatively suggested that the appearance of this form of TH is connected with activation of specific receptors of phagocytes. To test this hypothesis, oxymethacil, an activator of phagocytes, without any marked psychotropic activity [3], was injected into the stressed rats. It will be clear from Fig. 2 that after injection of oxymethacil a tyrosine hydroxylase reaction with high velocity was observed in the presence of a high concentration of tyrosine (530 μM). Comparatively low TH activity (6.3 nmoles/min/mg protein) was observed in the presence of tyrosine in a concentration of 93 μM. Thus oxymethacil, when administered systemically, did not cause the appearance of a high-affinity form of TH, by contrast with tuftsin; like tuftsin, however, it caused the appearance of a low-affinity form of TH. Both preparations increased the functional activity of the macrophages of the stressed rats under these circumstances, as is shown in Table 1.

Table 2 gives the kinetic parameters of TH from peritoneal macrophages relative to tyrosine. It will be clear from Table 2 that TH of the macrophages may be present in three kinetically different forms with $K_m$ values of 4.2, 47, and 270 μM, respectively. No TH with $K_m$ for tyrosine of 270 μM was found in the brain.

The results show that TH from peritoneal macrophages can modify its kinetic properties relative to tyrosine depending on intravital exposure to psychogenic factors, and also on the state of the phagocytic apparatus. The discovery of a low-affinity form of TH in the macrophages of animals after injection of the phagocytosis activators suggests that this form of TH is bound with the system of specific phagocytosis receptors, and that it is an enzyme modified by a method which is not exhibited in the brain. The change to the low-affinity state of TH probably leads to inhibition of its activity, for the tyrosine concentration optimal for this form of TH is several times higher than the tyrosine concentrations determined in mammalian cells and tissues.

The results can be summed up as follows. The discovery of tyrosine hydroxylase activity in peritoneal macrophages, together with the previous discovery of TH in peripheral blood leukocytes, proves that cells of the myeloid series possess tyrosine hydroxylase activity, which hitherto was considered to be a specific attribute of nerve cells.

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# IMMOBILIZATION OF MYOCARDIAL TROPOMYOSIN


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Tropomyosin (TM) is a regulatory myofibrillary protein with mol. wt. of 68,000-70,000 daltons [5]. Many workers have demonstrated its role in autoimmunity in patients with rheumatic diseases [3-7]. It was accordingly decided to study the isolation of TM and its use as an antigen in immunologic analysis. Immobilization of biologically active substances in the structure of a carrier polymer is a method of obtaining preparations that are resistant to various biophysical and biochemical action, with a long storage life [1].

The aim of this investigation was to create a granulated immobilized preparation of TM with a long keeping life and to study its properties and possible fields of application.

## EXPERIMENTAL METHOD

TM was isolated from the cadaveric heart muscle of the clinically healthy person who had died as a result of an accident, and obtained not later than 8-10 h after death, by Bailey's method, followed by isoelectric reprecipitation at pH 4.3 [2]. The protein concentration was determined by Lowry's method [10]. The purified myocardial TM was incorporated into a polyacrylamide gel (PAAG) space lattice by emulsion polymerization [9]. The number of protein molecules incorporated into the space of the lattice of the gel and the rate of diffusion of the immobilized TM were monitored by the use of radioactive indicators. Radioactive labeling was carried out as in [8]. Since immobilized granulated myocardial TM with magnetic properties can be


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