# ACTION OF TUFTSIN ON BRAIN MONOAMINE OXIDASE AND ACETYLCHOLINESTERASE ACTIVITY

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Ability to modulate neurotransmitter processes in the brain is a feature of several short endogenous peptides which are fragments of peptide hormones and proteins [4, 5]. One of these regulatory peptides is the tetrapeptide tuftsin (thr-lys-pro-arg), an immunostimulator, the pharmacologic analysis of whose psychotropic activity has shown that immediately after injection it has a stimulating action, which is followed by an inhibitory effect, on motor activity, it exhibits stress-protective and antidepressant properties, and acts on the emotional-behavioral sphere in animals (increases aggressiveness and quarrelsomeness, and inhibition of fear) [9, 11].

The mechanisms of the central action of tuftsin have not been completely discovered. Meanwhile there is some evidence in the literature that the psychostimulant effect of tuftsin is due to its action on catecholaminergic systems. Tuftsin has been shown to influence the tyrosine hydroxylase activity of the hypothalamus and striatum [1, 6] and the reuptake of biogenic amines by rat brain synaptosomes. The direction and character of this effect depends on dose and time. There is also evidence of a phasic effect of tuftsin on the concentrations of components of the GABA system [13].

Accordingly, in the investigation described below, the effect of tuftsin was studied on activity of enzymes connected with various neurotransmitter systems in separate subcellular functions of animal brain at different times after injection.

## EXPERIMENTAL METHOD

Experiments were carried out on 60 rabbits weighing 1.5-2 kg. Tuftsin was injected intraperitoneally in a dose of 300 μg/kg body weight. Physiological saline was injected into the control animals. The animals were decapitated 30 and 75 min and 3 days after a single injection of tuftsin, and the sensomotor cortex and caudate nucleus were removed for investigation. These brain structures were used as a single morphophysiological system, connected with motor functions, and participating in the formation of cognitive mechanisms. Activity of acetylcholinesterase (AChE) [17] and of molecular forms of type A (substrate, serotonin) and type B (substrate, p-nitrophenylethylamine) monoaminooxidase (MAO) [7] was determined spectrophotometrically in fractions of synaptic membranes of light and heavy synaptosomes and of free mitochondria, isolated from these brain formations. The specific activity of the enzymes was expressed per milligram protein of the subfractions and in percentages of the change relative to the control, which was taken as 100%. The results were subjected to statistical analysis by Student's test.

## EXPERIMENTAL RESULTS

The writers showed previously [8] that the level of activity of the enzyme systems chosen for study in intact rabbits differs both in different brain structures and in different subcellular fractions of each formation. The time course of changes in enzyme activity connected with neurotransmitter utilization, under the influence of tuftsin at various times after its injection, in synaptosomes and mitochondria of the sensomotor cortex and caudate nucleus of the rabbits is illustrated in Fig. 1. Clearly the action of tuftsin is relatively specific for individual enzyme systems and neurotransmitters: Activity of type A MAO was statistically significantly depressed during short periods of action, whereas activity of type B MAO was

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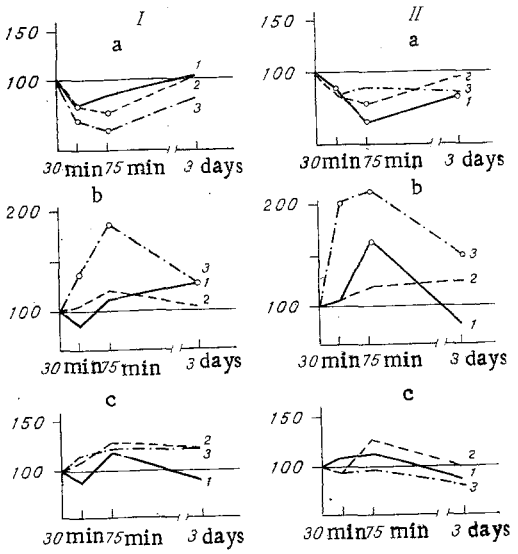


Fig. 1. Change (in % of control) of specific enzyme activity in subcellular fractions of different parts of the rabbit brain under the influence of tuftsin *in vivo*: a) type A MAO; b) type B MAO; c) AChE. I) Sensomotor cortex; II) caudate nucleus. 1) Light synaptosomes; 2) heavy synaptosomes; 3) mitochondria. Circles indicate statistically significant deviations from normal. Horizontal line — control.

increased, and this increase was particularly marked in subfractions of free mitochondria. The time course of activity of these enzymes reflects an increase in the changes observed 30 and 75 min after injection of tuftsin compared with the normal state, and this was particularly true of activation of type B MAO in subfractions of light synaptosomes and free mitochondria of the caudate nucleus (168 and 216%, respectively), whereas activity of type A MAO in these structures was depressed by 50 and 21%. Thus in the course of 75 min of action of the peptide, biochemical features of progressive activation of the catecholaminergic and depression of the serotoninergic systems were observed. AChE activity in all the structures tested showed only negligible changes under the influence of tuftsin.

According to the results of behavioral tests and observations on animals, after a 30-min period of activation the phase of a sedative action of tuftsin was observed [3, 10]. However, according to the experimental results, changes in levels of types A and B MAO were in the same direction for 1 h, and reached a maximum after 75 min. Some tendency was noted for AChE activity to increase during this same period, and this could evidently lead to a decrease in the acetylcholine concentration in synaptosomes of the brain formations studied and could bring about weakening of motor activity.

It was also shown histochemically that during 1 h after injection of tuftsin, activation of glucose-6-phosphate dehydroxylase and succinate dehydrogenase was observed in neurons of the caudate nucleus and of individual layers of the sensomotor cortex of rats [16], evidence that tuftsin stimulates the pentose phosphate shunt and the Krebs' cycle; this also indirectly confirms that the action of tuftsin is connected with regulation of neurotransmitter systems.

It can be tentatively suggested that the considerable inhibition of type A MAO activity within short times after injection of the peptide, causing accumulation of serotonin, particularly marked in subcellular fractions of the caudate nucleus, may lie at the basis of the antidepressive action of tuftsin.

The trend of changes in enzyme activity in individual subcellular fractions of the brain formations tested was similar, but the intensity of these changes differed: In the caudate nucleus changes in MAO activity were more marked than in the sensomotor cortex, but in subfractions of synaptosomes they were less marked within each formation than in free mitochondria. There is no doubt that cortico-subcortical interrelations in the integrative-triggering structures of the brain are more complex; however, in the modern view the neostriatum plays the role of a subcortical associative center, and controls processes of sensomotor integration. Meanwhile, dependence of motor automatisms on the state of the dopaminergic mechanisms in the caudate nucleus has been demonstrated. Behavioral effects observed in animals after injection of tuftsin are probably connected with these changes [2, 10].

All the processes studied had returned to normal 3 days after a single injection of tuftsin, with the exception of type B MAO activity in the subfraction of free mitochondria of the caudate nucleus, where it remained significantly higher than normal (150%). Evidently, some activation of the catecholaminergic system still remained after this long time in these brain formations.

The experimental results indicate that within short times after injection tuftsin acts on the turnover of neurotransmitters such as catecholamines and serotonin. As regards the serotoninergic system, significant lowering of the level of serotonin utilization was demonstrated, which could lead to the accumulation of this mediator. The activating effect of tuftsin observed on the catecholaminergic systems (type B MAO) is confirmed by data in the literature showing that injection of tuftsin compensates reserpine-induced exhaustion of neurotransmitters and, in particular, of dopamine, and it may be important in pathological states of the CNS and, in particular, in parkinsonism. There is a tendency for the dopamine concentration to rise and the 5-hydroxyindoleacetic acid level to fall [10]. On the basis of our data it can be postulated that this is the result, on the one hand, of depression of type A MAO activity and, on the other hand, of activation of dopa hydroxylase and type B MAO activity.

It can thus be suggested that tuftsin has rather selective modulating functions in relation to metabolism of biologically active substances in subcellular structures of the brain. Since changes in individual enzyme systems in response to administration of the neuropeptide are the result of summation of several processes, the precise mechanism of its modulating action requires further elucidation.

## LITERATURE CITED


1. N. A. Avdulov and V. V. Rozhanets, Pharmacology of Neuropeptides [in Russian], Moscow (1982), p. 40.
2. O. S. Adrianov, Principles of Organization of the Integrative Activity of the Brain [in Russian], Moscow (1976).
3. S. O. Andreev, K. V. Anokhin, L. V. Antonova, et al., Dokl. Akad. Nauk SSSR, 253, No. 2, 498 (1980).
4. I. P. Ashmarin, M. Yu. Eropkin, G. A. Kovaleva, and V. V. Rozhanets, Mol. Biol., No. 6, 965 (1978).
5. A. V. Val'dman, Vopr. Med. Khim., No. 3, 56 (1984).
6. A. V. Val'dman, I. P. Ashmarin, M. F. Mineeva, and K. V. Anokhin, Byull. Éksp. Biol. Med., No. 6, 31 (1981).
7. V. Z. Gorkin, I. V. Verevkina, L. I. Gridneva, et al., Modern Methods in Biochemistry [in Russian], Vol. 2, Moscow (1967), p. 155.
8. E. L. Dovedova, N. A. Popova, and L. M. Kachalova, Neirokhimiya, No. 2, 138 (1983).
9. A. A. Kamenskii, L. V. Antonova, N. A. Samoilova, et al., Byull. Éksp. Biol. Med., No. 7, 43 (1980).
10. M. M. Kozlovskaya, V. E. Klusha, and N. A. Bondarenko, The Neurochemical Bases of the Psychotropic Effect [in Russian], Moscow (1982), p. 95.
11. É. F. Lavretskaya, I. P. Ashmarin, V. N. Kalikhevich, et al., Khim.-farm. Zh., No. 1, 20 (1981).
12. M. D. Mashkovskii, Khim.-farm. Zh., No. 1, 33 (1981).
13. Z. S. Nikitina and I. A. Sytinskii, Neirokhimiya, No. 3, 276 (1984).
14. A. F. Nikiforov and G. G. Knyazev, Zh. Vyssh. Nerv. Deyat., No. 5, 962 (1983).
15. B. F. Tolkunov, The Striatum and Sensory Specialization of the Neuron Net [in Russian], Leningrad (1978).
16. M. B. Shishova, A. V. Sergutina, and L. M. Gershtein, Neirokhimiya, No. 4, 353 (1984).
17. S. Hestrin, J. Biol. Chem., 180, 294 (1949).
18. V. Popov, V. Rosler, C. Thiemann, and H. Molthen, Acta Biol. Med. Ger., 26, 239 (1971).