COMPARISON OF THE EFFECTS OF TUFTSIN AND SOME OF ITS ANALOGS ON IMMUNOGENESIS

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Tuftsin (Thr-Lys-Pro-Arg), a biologically active peptide [9], has attracted the attention of research workers because of its marked stimulating action on macrophages and on polymorphonuclear granulocytes [3, 4]. The ability of this tetrapeptide to stimulate certain functions of cells of the monocytic macrophagal systems — mobility [7], phagocytosis [9], bactericidal [10] and antitumor [8] activity — has been demonstrated. The effect of tuftsin on parameters of the immune response has received less study. There have been only isolated investigations in which its stimulating effect on antibody production has been established in response to injection of various thymus-dependent and thymus-independent antigens [6, 12]. The results of these investigations have necessitated further research in the same direction. In particular, more precise clarification of schemes of administration of the peptide in order to obtain the maximal immunotrophic effect is required. In addition, there is good reason to study the character of the effect of a number of tuftisin analogs, whose action on specific antibody formation has not yet been investigated.

The object of this investigation was to compared the action of tuftsin and of some of its analogs on the humoral immune response.

EXPERIMENTAL METHOD

Experiments were carried out on CBA mice weighing 18-21 g which were immunized by a single intraperitoneal injection of a suspension of 3×10^8 sheep's red blood cells. The number of antibody-forming cells (AFC) in the spleen was determined by the local hemolysis method in special microchambers [5] on the 5th and 7th days after antigenic loading. The viability of lymphoid cells was assessed by the trypan blue test. The substances for investigation (tuftsin, Thr-Lys-Pro-D-Arg, Thr-Ala-Val-Arg, Leu-Lys-Pro-Arg) were injected intraperitoneally in a dose of 20 mg/kg in physiological saline. The peptides were synthesized at the A. A. Zhdanov Leningrad University by V. N. Kalikhevich and Z. N. Ardemasova. In the experiments of series I tuftsin or one of its analogs was used for 3 days before immunization of the animals. In series II it was used for 3 days starting from the day of injection of the test antigen (i.e., in the inductive phase of immunogenesis), and in series III it was given on the 3rd and 4th days after antigenic loading (i.e., in the productive phase of immunogenesis). Physiological saline was injected into the control animals.

EXPERIMENTAL RESULTS AND DISCUSSION

Tuftsin and Thr-Lys-Pro-D-Arg had a weak stimulating action on the humoral immune response in mice into which these substances were injected before antigenic loading. As Table 1 shows, a statistically significant increase in the number of AFC was observed in the spleen of these animals compared with their number in the control mice. The other two tetrapeptides, differing from tuftsin in one of two amino acids, had no effect on antibody formation under similar experimental conditions.

Injection of the tetrapeptides in the inductive phase of immunogenesis was accompanied by a stronger immunostimulant effect. Differences only in the degree of activation of antibody formation by the various substances were found. The maximal effect was observed in ex-

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TABLE 1. Effect of Tuftsin and Some of Its Analogs on Number of AFC in Mouse Spleen

Substance	Series of experiments	Number of animals	Number of AFC per 10^6 splenocytes	
			5th day	7th day
Thr-Lys-Pro-Arg	Control	16	245±28,4	90±8,6
	I	8	382±33,6*	98±11,0
(tuftsin)	II	10	746±59,3‡	186±21,8†
	III	12	293±30,2	82±9,3
Thr-Lys-Pro-D-Arg	I	9	368±27,4*	114±12,2
	II	12	620±48,5‡	162±14,8†
	III	10	255±23,0	97±10,4
Leu-Lys-Pro-Arg	I	10	296±25,8	78±6,3
	II	10	485±38,3†	135±12,8*
	III	11	264±23,2	80±9,4
Thr-Ala-Val-Arg	I	9	259±31,4	96±7,5
	II	10	380±28,5*	118±13,6
	III	10	288±25,3	85±10,5

*$P < 0.05$.
†$P < 0.01$.
‡ $P < 0.001$.

periments with tuftsin, in which the number of AFC in the spleen at the peak of the immune response was about 3 times higher than in the control. Thr-Lys-Pro-D-Arg had rather weaker, although still quite considerable, stimulating activity. The remaining tetrapeptides had a weaker effect on the development of the humoral immune response.

When tuftsin or its analogs were given in the productive phase of immunogenesis, the substances injected had no effect whatever on antibody formation. No statistically significant increase in the number of antibody-producing cells was found in the spleen in the experiments of this series under the influence of any of the low-molecular-weight peptides studied.

The results of these experiments thus demonstrate that tuftsin and some of its analogs can exert a modulating action on the humoral immune response, the character of which depends on the time of injection of the substances relative to the time of immunization, i.e., the phase of immunogenesis. As regards the possible mechanisms of the immunotrophic activity of these tetrapeptides, it can be tentatively suggested that the observed stimulation of antibody formation is largely due to the influence of the injected substances on the immunogenic function of macrophages. This suggestion is based on the results of an investigation by Tzehoval et al. [13], who showed that this function of the peritoneal macrophages is activated by the *in vitro* action of tuftsin on these cells. This action evidently also takes place *in vivo*, more especially because macrophages carry specific receptors on their surface for tuftsin [11]. Our own data showing that the substances of peptide nature which we studied had their strongest immunotrophic action in the initial stage of the humoral immune response, also agree with this hypothesis. Macrophages are known to play an important role in presenting the antigen to immunocompetent cells in the inductive phase of immunogenesis [12]. Definite correlation is observed between the structure of the tuftsin analogs and their immunotrophic effects. Replacement of D-Arg^4 by Arg^4 did not lead to any significant change in immunostimulant activity, whereas replacement of one or two of the amino acids forming tuftsin is accompanied by a distinct decrease in its effect on immunogenesis. Conversely, the central effects of tuftsin and D-Arg^4-tuftsin and the particular features of their effects on the neuroclinical systems of the brain differ substantially, whereas mono- and disubstituted analogs of tuftsin do not exhibit such sharp differences [1, 2].

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BINDING OF [^{3}H]-L-GLUTAMATE WITH SYNAPTIC MEMBRANES ISOLATED FROM THE CEREBRAL CORTEX AND HIPPOCAMPUS OF KRUSHINSKII—MOLODKINA RATS

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The appearance of epileptiform seizures in Krushinskii—Molodkina (KM) rats with a hereditary predisposition to audiogenic seizures is usually used as a model with which to study the inheritance and manifestations of human epilepsy. The biochemical mechanisms of the audiogenic seizure are considered to be based on disturbances of the mediator systems of the brain and, in particular, the glutamatergic systems [2]. Synaptic membranes isolated from the cortex, hippocampus, and certain other structures of the brain have been shown to be rich in high-affinity, stereochemically specific glutamate binding sites which, according to certain physiological and chemical-pharmacological characteristics, correspond to glutamate receptors [8]. Studies of the properties of membrane-bound receptors *in vitro* provide a biochemical approach to the evaluation of the state of the postsynaptic membrane, which determines the level of excitation of the nerve cell.

The aim of the present investigation was to compare binding of [^{3}H]glutamate with synaptic membranes isolated from various brain structures of KM rats at rest and after an audiogenic seizure.

EXPERIMENTAL METHOD

Experiments were carried out on adult male KM and Wistar rats weighing 200-250 g. The KM rats were obtained from the Laboratory of Genetics of Higher Nervous Activity, I. P. Pavlov Institute of Physiology, Academy of Sciences of the USSR.* Seizures were induced by application of an acoustic stimulus (60 dB, duration 5-10 sec). Synaptic membranes were isolated from the rats' cortex and hippocampus by differential centrifugation [10]. The protein content in the samples was determined by Lowry's method [7]. Binding of [^{3}H]-L-glutamate with the synaptic membranes was carried out as described in [3]. The membranes (50 µg protein) were incubated with 10-200 nM [^{3}H]-L-glutamate ("Izotop," USSR, 45 Ci/mmole) in 10 mM Tris-citrate buffer, pH 7.4, for 15 min at 37°C. The reaction was stopped by filtration through membrane filters (Synpor, Czechoslovakia, pore diameter 0.6 µ). The filters were washed with 2.5 ml of cold buffer. Nonspecific binding was determined in an incubation mixture containing 0.1 mM

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