CENTRAL EFFECTS OF THE TETRAPEPTIDE TUFTSIN

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Evidence is continuing to accumulate that not only neuropeptides, but also some oligopeptides which participate in the regulation of peripheral functions also affect activity of the CNS. The tetrapeptide tuftsin (L-threonyl-L-lysyl-L-prolyl-L-arginine) has been isolated and previous studies [4, 5] showed it to be a stimulator of the phagocytic activity of leukocytes. It is a noteworthy fact that the Lys-Pro-Arg fragment, i.e., a tripeptide found in the structure of tuftsin, is also present in such highly active neuropeptides as substance P and neurotensin, which act in various ways on regulation of central functions [6]. It was accordingly decided to study the action of tuftsin on emotional-behavioral reactivity in animals.

EXPERIMENTAL METHOD

Noninbred male rats weighing 200-230 g were used. The animals' behavior was assessed in an "open field" with several holes, by counting vertical and horizontal activity (the number of squares crossed), the number of holes investigated, and the number of acts of grooming in the course of 6 min (the results were recorded every 2 min). The animals' vertical investigative activity was studied in a small space (a box) in the course of 20 min, thresholds of the pain response (the appearance of vocalization) to electric shocks applied to the paws, and thresholds (in mA) of development of aggressiveness (fights) between two rats placed on an electrode floor were studied, taking into account the duration of emotional excitation after the end of stimulation, and the animals' ability to hold on to a revolving rod (for 2 min) and to be pulled on a horizontal crossbar was determined. Formation and preservation of a conditioned passive avoidance reaction to single aversive stimulation with testing after 24 h were evaluated. Tyrosine hydroxylase (TH) activity was determined spectrophotometrically [1] in experiments *in vitro* and colorimetrically, by measuring the dopa concentration by the method in [7]. In the experiments *in vivo*, the coarse mitochondrial fraction of the hypothalamus and striatum of the rats, solubilized with Triton X-100, was used. In experiments *in vitro*, the purified enzyme, obtained by the method in [2], was used.

EXPERIMENTAL RESULTS

Data showing the effect of tuftsin on the rats' open field behavior are summarized in Table 1. In a dose of 500 μg/kg (intraperitoneally) tuftsin had no definite effect on the indices of behavior in an open field: investigative activity (horizontal, vertical, number of holes investigated) was slightly reduced, the animals stayed longer in a static, "frozen" posture, and emotional defecation increased. The response of the rats to handling was intensified. Open field behavior is known to be the resultant of two opposite motivations: investigation and fear (an open, well lit space). The behavioral changes observed could be connected with increased emotional tension in a stress situation. The animals' behavior in a less stress-producing situation (in the confined space of the box) was activated by tuftsin (Table 1).

Tuftsin had no distinct muscle-relaxing action and did not produce ataxia, but in the revolving rod and crossbar tests 20% of animals obtained lower scores.

Determination of the effect of tuftsin on the animals' emotional reactivity in response to electric shocks revealed a definite stimulating action. The threshold of perception of

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TABLE 1. Mean Data Reflecting Rats' Behavior in Open Field and in a Small Box

Index	Time, min	Control (physiological saline)	Experiment (tuftsin, 500 μg/kg)
Horizontal activity	2	22,8	16,2
	2	6,5	6,3
	2	4,5	4,6
	Σ	33,7	29,2
Vertical activity	2	6,8	5,1
	2	3,2	4,0
	2	1,8	1,1
	Σ	11,9	8,8
Looking into holes	2	4,5	6,3
	2	2,0	2,2
	2	1,5	0,8
	Σ	8	9,5
Grooming	2	1,6	1,1
	2	1,6	3,0
	2	2,5	1,0
	Σ	5,7	4,6
Defecation	6	2,7	6,7
Vertical activity (in box)	20	13,2	37,5

TABLE 2. Velocity of Tyrosine Hydroxylase Reaction in Rat Brain after Injection of Tuftsin (M ± m)

Experimental conditions	Reaction velocity, μmoles/min/mg protein	
	hypothalamus	striatum
Control	16,0±1,0	33,3±2,8
Tuftsin		
10 min	25,0±6,5	87,1±6,2
20 min	44,3±14,4	55,7±11,1

TABLE 3. Effect of Tuftsin *in vitro* on TH from Rat Hypothalamus (M ± m)

Experimental conditions	Reaction velocity, μmoles/min/mg protein
Control	355±10,0
Tuftsin	
10^{-4} M	90±4,5
10^{-5} M	255±15,3
10^{-4} M	
Fluphenazine, 10^{-4} M	278±13,9

Legend. Here and in Table 3: 0.05 M Tris maleate buffer, pH 6.1, 0.11 mM tyrosine, 0.17 mM dimethyltetrahydropterine; temperature 30°C.

aversive stimulation (as shown by vocalization) fell significantly (0.43 ± 0.03 mA in the control, 0.13 ± 0.02 mA after tuftsin, n = 13, $P \leqslant 0.01$). The thresholds of development of aggressiveness and the number of fights between two rats on an electrode floor fell significantly (control 2.3 ± 0.25 mA, after tuftsin 0.25 ± 0.03 mA, n = 13). The duration of after-excitation, reflected in maintenance of the "boxer's posture," shivering, and standing up on the hind limbs in response to gentle tapping, was doubled after administration of tuftsin.

The increase in emotional defecation in the open field and lowering of thresholds of aggressiveness, fighting, and perception of negative (nociceptive) stimulation, suggest that tuftsin potentiates (activates) manifestations connected with the negative reinforcing system of the brain.

Tuftsin did not improve the formation of a conditioned passive avoidance reaction (tested after 24 h) when given 10 min before testing (1st day) in a dose of 500 μg/kg. Conversely, the number of animals with a fixed passive avoidance reaction was reduced, the latent

period of the first entry into the dark compartment (where the animal had previously received aversive stimulation) was shortened (from 194 to 39 sec, $P \leqslant 0.05$), and the duration of stay in the lit (safe) compartment was increased (from 100 to 240 sec, $P \leqslant 0.05$).

In accordance with current views that oligopeptides play the role of modulators of neurochemical processes in the brain [6] and since many of them possess dopaminomimetic properties, the effect of tuftsin on TH, the limiting enzyme of catecholamine biosynthesis, was investigated. The effect of tuftsin on the velocity of the tyrosine hydroxylase reaction was estimated in the hypothalamus and striatum *in vivo* and *in vitro*, using purified TH for these experiments.

An increase in TH activity in the hypothalamus and, in particular, in the striatum (Table 2) was observed 10 min after injection of tuftsin (500 μg/kg) into the animals. TH activity in the hypothalamus continued to rise after 20 min, but in the striatum it had fallen a little, although it was still higher than in the control animals. Tuftsin also had a direct inhibitory effect on TH, as shown by the results of experiments *in vitro* (Table 3). The substrate (tyrosine) protected TH against the action of tuftsin. The inhibitory effect of tuftsin was reduced by 80% by fluphenazine, which can be regarded as an allosteric regulator of TH activity [3].

Tuftsin thus has a direct action on TH and can influence catecholaminergic processes in the brain. An increase in TH activity usually corresponds to a decrease in sensitivity of catecholaminergic receptors and to depression of the functional activity of the corresponding brain structure, as has been shown in the case of neuroleptics.

This investigation showed that tuftsin has a definite central action on the emotional behavioral reactivity of animals. Comparison of the animals' behavior after receiving tuftsin with the dynamics of their brain pH activity shows that the central action of tuftsin largely determines changes in catecholaminergic processes in the hypothalamus (the functional role of which is linked with the formation of emotionally motivated and appropriate adaptive reactions), and also in the striatum (which participates in the control of the animal's motor activity). Potentiation of the negative motivational reactions is evidently connected with the action of tuftsin on catecholaminergic processes.

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