COMPARATIVE STUDY OF THE PSYCHOTROPIC ACTIVITY OF TUFTSIN AND ITS ANALOGS

A. V. Val'dman, N. A. Bondarenko,
M. M. Kozlovskaya, D. Yu. Rusakov,
V. N. Kalikhevich, and Z. A. Ardemasova

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Previous investigations [1] revealed a central stimulating component of the tetrapeptide tuftsin, a natural stimulator of phagocytosis liberated by the action of a proteolytic enzyme (leukokinase) from the C_H2 domain of the gamma-chain of immunoglobulin G [15]. Scores of analogs of tuftsin have now been synthesized and their effect on phagocytosis and immunologic reactivity studied [6].

The object of the present investigation was to compare the psychtropic activity of tuftsin (Thr-Lys-Pro-Arg) and some of its analogs (Leu-Lys-Pro-Arg, Thr-Lys-Pro-D-Arg, Thr-Lys-Pro-Pro-Arg), synthesized by V. N. Kalikhevich at the Chemical Research Institute of the A. A. Zhdanov Leningrad University.

EXPERIMENTAL METHOD

The activating action of the substances on behavioral depression in a stress situation was detected by the method in [16] on CBWA mice weighing 20 g. The number of squares enumerated, holes explored, vertical standing postures and acts of grooming [2] and also ability to solve an extrapolation problem depending on avoidance of an acute stress situation, as described in [9] in the modification in [3], were assessed in Wistar rats weighing 220-250 g in open field tests. Subgroups of "emotional" and "nonemotional" animals [4] were distinguished in the first place, both among intact Wistar rats and among rats after destruction of catecholamine terminals by injection of 6-hydroxydopamine (6-OHDA) in the neonatal period as in [12]. Behavioral manifestations were assessed quantitatively or by means of a point system. The significance of differences was determined by a nonparametric test [5].

EXPERIMENTAL RESULTS

On a model of behavioral depression, in an unavoidable stress situation [16] tuftsin, in doses of 20-250 μg/kg had an activating effect. The total duration of behavioral immobilization was reduced and the period of active behavior increased. With an increase in dose the stimulating action was weakened (Table 1). Leu^1-tuftsin exhibited its activating effect in doses of 25 to 750 μg/kg, but no dose-dependent effect was observed. $D\text{-}Arg^4$-tuftsin abolished behavioral immobilization in doses of 250-500 μg/kg. The pentapeptide Thr-Lys-Pro-Pro-Arg, known to inhibit the stimulating effect of tuftsin on phagocytosis, had no significant action on behavior in doses of 100-400 μg/kg.

Substances with an antidepressive and psychostimulant activity [14, 17] are known to exert an activating effect on animal behavior in Porsolt's test [16]. The basic neurochemical mechanism is activation of catecholaminergic (noradrenergic, dopaminergic) processes. Hence it can be postulated that the activating effect of tuftsin and certain of its analgos is connected with its action on the catecholaminergic mechanisms of the brain. A direct effect of tuftsin on tyrosine hydroxylase activity was demonstrated by the writers previously both *in vivo* and *in vitro*.

The reactivity of the animals in behavioral tests, especially under stress conditions or in conflicting situations, differed significantly depending on the type of their original

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TABLE 1. Effect of Tuftsin and Its Analogs on Duration of Immobilization in the Test of Porsolt et al. [17]

Substance	Dose, μg/kg	Duration of immobilization, sec (M ± m)	% of control
Physiological saline	—	268±8,8	—
Tuftsin	750	233±11,4	—13
	500	150±26,5†	—44
	250	137±55,0*	—49
	100	149±20,3*	—44
	50	95±18,5†	—64
Physiological saline	—	185,8±20,0	—
Leu^1-tuftsin	750	84,2±23,0*	—55
	500	78,0±19,6*	—58
	250	72,5±23,8*	—61
	100	65,8±23,9*	—65
	50	95,8±22,9*	—48
	10	72,5±9,7*	—61
Physiological saline	—	147,5±8,8	—
D-Arg^4-tuftsin	750	115,8±17,7	—22
	500	98,2±7,0*	—35
	250	91,7±19,4	—38
	100	102,5±3,5*	—31
	50	100,0±6,2*	—32
	10	113,3±11,5	—23

Legend. Differences compared with control values significant: *) $P < 0.05$, †) $P < 0.01$.

emotional response, and in particular, this correlates with individual variations in brain catecholamine concentration and turnover [2]. Accordingly the effect of tuftsin and its analogs on behavioral manifestations was compared in open field tests on intact rats, previously divided into "emotional" and "nonemotional" subgroups on the basis of our own criteria [3], and on animals after preliminary destruction of brain catecholamine terminals by treatment with 6-OHDA in the early neonatal period. On the whole the animals receiving 6-OHDA showed increased emotional-behavioral reactivity compared with the control. However, in this group also it was possible to distinguish "emotional" and "nonemotional" subgroups of animals which differed significantly with respect to all the parameters studied [2]. The data in Table 2 indicate that the tetrapeptides tested differed in their effects on behavior depending on the type of emotional-behavioral reactivity of the animals. Behavior in an open field is known to be the resultant of two opposite motivations: exploration and fear [13]. If the two competing motivations are equally strong, "mixed activity" (self-grooming) arises, but if one of them is weaker, predominance of the specific motivation is found with weakening of grooming [6]. Tuftsin increased exploratory activity (the number of holes inspected) in animals of all subgroups, especially the "nonemotional," but increased the number of vertical standings in the "emotional" subgroup. For this reason the total number of squares crossed decreased. These shifts reflect the activating effect of tuftsin on the exploration motivation while, at the same time, they reflect weakening of the opposite motivation of fear. By contrast to this Leu^1-tuftsin reduced investigative, vertical, and horizontal activity but increased the number of acts of grooming, and this can be interpreted as a manifestation of frustration [8], replacing activity, and on the whole reflecting enhancement of emotionally negative reactivity. D-Arg^4-tuftsin caused no significant shifts of behavior in "nonemotional" animals, but had a regulating effect (both activating and sedative) in the "emotional" animals, leading to an increase in investigative activity and a decrease in the number of acts of grooming.

The qualitative assessment of the character of the action of tuftsin and its analogs on goal-directed behavior under conditions of emotional stress was carried out in a situation in which the animal, placed for the first time in a glass cylinder, was lowered into a vessel containing water, and in order to escape from the stress situation the animal had to extrapolate the parameters of the obstacle, to overcome fear, to dive beneath the wall of the cylinder, and to leave the experimental chamber, using netting lowered into the water [9]. Success in the performance of the task was inversely proportional to the level of the affective reactions exhibited by the animal when placed in the cylinder. Elevation of the initial emotional reactivity in animals receiving 6-OHDA, due to disturbance of the balance of activity

TABLE 2. Behavioral Parameters of Effect of Tuftsin and Its Analogs in "Emotional" (1) and "Nonemotional" (2) Rats of Control and Experimental (receiving 6-OHDA) Groups in Open Field Test (M ± m)

Substance	Dose, μg / kg	Group of animals	Subgroup	Horizontal activity (No. of squares crossed)	Vertical activity (No of standings)	Number of acts of grooming	Exploratory activity (number of holes sniffed)
Physiological saline		Control	1	84,1±6,2	1,7±0,6	4,1±1,1	2,7±0,4
			2	63,4±5,9	12,4±4,2	2,1±0,3	6,5±1,5
		Exptl.	1	103,6±11,2*	3,6±1,0*	6,4±1,9	2,1±0,5
			2	75,2±5,5	7,4±2,1*	2,8±0,8	4,2±1,8
Tuftsin	200	Control	1	75,9±5,1	7,8±3,1 †	6,2±2,3	3,8±1,0
			2	48,4±6,0†	14,3±5,1	1,9±0,4	9,0±2,6 †
		Exptl.	1	97,3±7,6	6,4±2,3 †	7,3±1,6	5,1±1,7*
			2	50,0±6,4	8,1±2,6	3,9±1,1	7,3±2,0
Leu^1-tuftsin	200	Control	1	64,5±7,0	2,6±0,8	6,9±2,2*	1,3±0,6*
			2	55,2±8,3	6,2±2,1†	4,4±1,7*	2,7±0,8†
		Exptl.	1	74,6±8,1*	1,3±0,3*	8,3±2,0	0,9±0,2*
			2	65,8±6,2	5,3±1,6*	3,6±1,4	1,4±0,7*
D-Arg^4-tuftsin	200	Control	1	63,6±6,8*	4,2±0,9*	2,0±0,8	5,6±1,1†
			2	54,0±4,6	7,0±1,6 †	1,1±0,7	6,3±2,0
		Exptl.	1	107,2±12,3	2,7±1,6	3,2±1,5†	6,4±2,3 †
			2	75,6±7,1	6,1±3,0	1,9±1,0	4,9±1,6

Legend. Here and in Table 3 differences between groups for respective subgroups are significant in control test and between control test and action of substances; *) $P < 0.05$, †) $P < 0.01$.

of the brain catecholamine systems (a significant decrease in the catecholamine concentration in the forebrain and diencephalon and a twofold increase in its concentration in the midbrain [12], a decrease in tyrosine hydroxylase activity [7]), considerably interfered with avoidance behavior (an increase in the number of unsuccessful attempts, lengthening of the latent period of avoidance). Tuftsin improved avoidance behavior and facilitated solution of the extrapolation problem both in the control "emotional" and "nonemotional" animals and also in rats with destruction of their catecholamine terminals. This distinguishes tuftsin sharply from other compounds which exert their effect through catecholaminergic mechanisms (amphetamine, haloperiodol, clonidine), and which have different, sometimes opposite, effects on animals that differ in their level of emotional reactivity, especially after preliminary destruction of catecholamine terminals. Leu^1-tuftsin raised the level of effective manifestations, increased the number of unsuccessful attempts at avoidance, and impaired the avoidance reaction considerably. D-Arg^4-tuftsin weakened affective reactions in animals of all groups and led to the more rapid realization of avoidance behavior while reducing sharply the number of unsuccessful attempts (Table 3). These changes correlated with those of behavioral manifestations observed under the influence of this peptide in open field tests.

Comparison of the psychotropic properties of the various tetrapeptides with their effect on phagocytosis and immunogenesis shows that D-Arg^4-tuftsin lacked the ability, which tuftsin itself possesses, of stimulating phagocytic activity of leukocytes, but does stimulate immunogenesis to the same degree, whereas Leu^1-tuftsin had stronger phagocytic activity than tuftsin itself [6]. On intraventricular injection (200 μg) the D-Arg^4-analog induced stronger and more prolonged analgesia than tuftsin itself, whereas the D-Leu^1-analog was close to tuftsin in its activity [10, 11]. Different derivatives of tuftsin differ in their stimulating effect on phagocytosis and immunogenesis, and sometimes they have the opposite effect, indicating that they are brought about by different mechanisms.

There are as yet no experimental data which can directly explain the connection between phagocytosis stimulation processes and the central effects of tuftsin. However, the data showing that tuftsin inhibits tyrosine hydroxylase (TH), that tyrosine protects TH against the inhibitory effect of tuftsin, and that the allosteric regulator of TH activity, flufenazine, almost completely abolishes the inhibitory effect on tuftsin [1], lead to the conclusion that tuftsin interacts with TH not through the active site of the enzyme, but possibly on account of conformational changes in the surrounding lipid components of the membrane. Activation of phagocytosis is known to take place primarily through interaction of the positively charged groups of the tuftsin molecule with the negatively charged lipid components of the leukocyte

TABLE 3. Effect of the Tetrapeptide Tuftsin and Its Analogs on Behavior during Avoidance of an Acute Stress Situation in "Emotional" (1) and "Nonemotional" (2) Rats of Control and Experimental Groups (M ± m)

Substance	Dose, μg/kg	Group of animals	Subgroup	No. of unsuccessful attempts at avoidance	Level of affective manifestations, conventional units	Latent period of avoidance
Physiological saline		Control	1	16,4±3,8	7,2±3,0	39,8±5,0
			2	None	None	11,7±3,7
		Exptl.	1	26,4±5,3*	14,1±2,7*	68,2±6,1†
			2	7,1±2,2†	4,5±1,6†	34,3±4,6†
Tuftsin	200	Control	1	4,8±1,6†	5,0±1,7	23,2±4,8†
			2	None	None	5,1±1,7*
		Exptl.	1	12,3±4,0†	7,5±3,9*	41,3±4,4*
			2	None	2,9±0,8	19,2±3.5*
Leu^1-tuftsin	200	Control	1	28,8±7,6*	16,4±5,1†	∞†
			2	13,1±3,8†	3,2±0,9†	24,3±4,5†
		Exptl.	1	36,4±5,2*	18,0±3,9	74,1±10,3
			2	8,4±3,3	7,9±2,6	39,7±6,2
D-Arg^4-tuftsin	200	Control	1	5,9±2,0*	None	18,5±4,3†
			2	None	None	6,7±1,6*
		Exptl.	1	6,3±1,3†	2,1±0,4*	28,1±4,4†
			2	None	None	22,7±5,6*

membrane [7]. Changes in the amino-acid sequence of the tuftsin molecule lead both to changes in phagocytosis-stimulating activity and to the appearance of different psychotropic properties.

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