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ANALYSIS OF NEUROCHEMICAL MECHANISMS OF THE PSYCHOTROPIC ACTION OF TUFTSIN AND ITS ANALOGS

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Previous investigations [2, 3] have demonstrated the activating effect of tuftsin and some of its analogs on behavior and emotional reactivity, on the basis of which the psychotropic effect of these peptides has been linked with their action on the monoaminergic systems of the brain. The aim of the present investigation was to make a more detailed study of the catecholaminergic mechanisms of the central action of tuftsin (Thr-Lys-Pro-Arg) and its analogs Thr-Lys-Pro-D-Arg (D-Arg^4-tuftsin) and Leu-Lys-Pro-Arg (Leu^1-tuftsin), synthesized by V. N. Kalikhevich at the Chemical Research Institute, A. A. Zhdanov Leningrad University, with special reference to their effect on dopamine-dependent behavior and to tyrosine hydroxylase activity.

EXPERIMENTAL METHOD

The action of the peptides on the dopamine systems was assessed on models of rotation behavior [14] in male Wistar rats weighing 200-250 g, with universal injury to dopamine terminals of the striatum (injection of 16 μg 6-hydroxydopamine — 6-OHDA — in 4 μl physiological saline, containing 0.2 mg/ml ascorbic acid, into the rostromedial part of the head of the right caudate nucleus 24 h previously). The number of rotations in 15 min was determined in a rotameter 35 cm in diameter. The character of stereotyped behavior [7] and the level of emotional reactivity [1] were estimated in points. Rats receiving an injection of 4 μl of the solvent served as the control. For statistical analysis of the data the nonparametric U criterion was used [4]. All drugs were injected intraperitoneally. Tyrosine hydroxylase (TH) activity was determined in structures of the corpus striatum and hypothalamus by a fluorometric method based on the rate of formation of the end product of the reaction — dopa. These results were subjected to statistical analysis by Student's t test.

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TABLE 1. Direction of Rotations and Their Number Following Action of the Tetrapeptide Tuftsin and Its Analogs (M ± m)

Substance	Ipsilaterally	Contralaterally
Physiological saline	14.6 ± 6.1	absent
Tuftsin (0.2)	23.7 ± 4.5*	absent
Leu^1-tuftsin (0.2)	19.0 ± 3.4	absent
D-Arg^4-tuftsin (0.2)	7.7 ± 3.0*	4.2 ± 1.8†
DTC (35)	8.3 ± 2.4	absent
DTC (35) + tuftsin (0.2)	27.1 ± 5.4†	absent
DTC (35) + Leu^1-tuftsin (0.2)	24.5 ± 4.1†	absent
DTC (35) + D-Arg^4-tuftsin (0.2)	6.1 ± 2.5	7.4 ± 3.0†
Amphetamine (1)	44.2 ± 8.6	absent
Amphetamine (1) + tuftsin (0.2)	27.4 ± 5.3†	absent
Amphetamine (1) + Leu^1-tuftsin (0.2)	24.5 ± 4.1†	absent
Amphetamine (1) + D-Arg^4-tuftsin (0.2)	9.0 ± 2.5†	13.8 ± 3.7†
Haloperidol (1)	absent	absent
Haloperidol (1) + tuftsin (0.2)	3.0 ± 0.9†	absent
Haloperidol (1) + Leu^1-tuftsin (0.2)	9.2 ± 2.6†	absent
Haloperidol (1) + D-Arg^4-tuftsin (0.2)	absent	4.0 ± 1.3†
Apomorphine (0.5)	absent	48.4 ± 10.1
Apomorphine (0.5) + tuftsin (0.2)	absent	55.3 ± 9.4
Apomorphine (0.5) + Leu^1-tuftsin (0.2)	12.0 ± 4.4	38.1 ± 7.5*
Apomorphine (0.5) + D-Arg^4-tuftsin (0.2)	absent	17.6 ± 5.0†

Legend. Here and in Table 2 differences are significant relative to administration of control test substances: *) $P < 0.05$, †) $P < 0.01$; dose of substance (in mg/kg) given in parentheses.

EXPERIMENTAL RESULTS

Data on the effect of tuftsin and its analogs on rotation behavior and interaction with psychotropic agents acting on the dopamine systems of the brain are given in Table 1. Unilateral destruction of dopamine terminals of the nigro-striatal system by preliminary injection of the neurotoxin 6-OHDA created asymmetry of dopaminergic activity, manifested by rotatory movements towards the side of the injury. The action of direct (of apomorphine type) and indirect (of amphetamine type) dopamine agonists (DA) was demonstrated on this model. Ipsilateral rotations are due mainly to DA liberation from mesolimbic dopamine neurons (n. accumbens), whereas stereotyped behavior is due mainly to activity of nigro-striatal dopamine neurons. Destruction of the dopamine terminals of the corpus striatum by injection of 6-OHDA disturbs amphetamine stereotypy but does not activate motor activity [13]. Tuftsin significantly increased ipsilateral rotation (compared with the control), Leu^1-tuftsin has no significant effect, and D-Arg^4-tuftsin reduced ipsilateral and induced contralateral rotations. After preliminary (30 min beforehand) inhibition of dopamine-β-hydroxylase by diethyldithiocarbamate (DTC) the effect of tuftsin and its D-Arg^4 analog was unchanged, but Leu^1-tuftsin significantly increased the number of ipsilateral rotations. Against the background of amphetamine, a presynaptic liberator of catecholamine which potentiates ipsilateral rotations, tuftsin and, to a lesser degree, the Leu^1-analog reduced rotations, whereas D-Arg^4-tuftsin inhibited them to a very marked degree and, at the same time, induced contralateral rotations. After preliminary unilateral destruction of dopamine terminals apomorphine induced contralateral rotations as a result of its stronger action on hypersensitive (on the side of injury) dopamine receptors of the striatum. This effect was sharply weakened by D-Arg^4-tuftsin and, to a lesser degree, by the Leu^1-analog with simultaneous induction of ipsilateral rotations. Tuftsin itself potentiated the effect of apomorphine but not significantly. Against the background of blockade of the dopamine receptors by haloperidol, all peptides activated rotation behavior, indicating that they have a DA-mimetic effect, but the side of the movements differed: ipsilateral in the case of tuftsin and the Leu^1-analog, contralateral in the case of D-Arg^4-tuftsin. It can be concluded from analysis of the data that tuftsin and its Leu^1-analog do not act directly on postsynaptic dopamine structures, for they do not induce contralateral rotations even in the case of hypersensitivity of these structures due to destruction of dopamine terminals.

TABLE 2. Parameters of Stereotyped and Agressive Behavior of Rats under the Influence of Tetrapeptide Tuftsin and Its Analogs (M ± m)

Substance	Level of stereotypy, conventional units	Level of aggressiveness, conventional units
Physiological saline	absent	absent
Tuftsin (0.2)	absent	0.2 ± 0.04
Leu^1-tuftsin (0.2)	absent	1.3 ± 0.4
D-Arg^4-tuftsin (0.2)	absent	absent
DTC (35)	absent	absent
DTC (35) + tuftsin (0.2)	absent	2.3 ± 0.7†
DTC (35) + Leu^1-tuftsin (0.2)	absent	3.3 ± 0.9†
DTC (35) + D-Arg^4-tuftsin (0.2)	absent	absent
Amphetamine (1)	4.1 ± 1.2	1.5 ± 0.6
Amphetamine (1) + tuftsin (0.2)	7.7 ± 2.8*	0.4 ± 0.06*
Amphetamine (1) + Leu^1-tuftsin (0.2)	6.9 ± 2.0*	1.3 ± 0.5
Amphetamine (1) + D-Arg^4-tuftsin (0.2)	2.8 ± 0.9*	absent
Haloperidol (1)	absent	absent
Haloperidol (1) + tuftsin (0.2)	absent	1.3 ± 0.3†
Haloperidol (1) + Leu^1-tuftsin (0.2)	absent	0.6 ± 0.02
Haloperidol (1) + D-Arg^4-tuftsin (0.2)	absent	absent
Apomorphine (0.5)	7.1 ± 0.8	4.0 ± 1.6
Apomorphine (0.5) + tuftsin (0.2)	6.0 ± 3.1	2.2 ± 0.9*
Apomorphine (0.5) + Leu^1-tuftsin (0.2)	6.6 ± 3.5	1.4 ± 0.6†
Apomorphine (0.5) + D-Arg^4-tuftsin (0.2)	3.6 ± 0.7†	absent

Additional information on the character of the action of the peptides on dopaminergic brain mechanism is provided by an estimation of their effect on the behavioral manifestations of stereotypy and aggressiveness (Table 2). Stereotyped behavior is known to be more closely connected with activation of nigro-striatal dopamine neurons. Tuftsin and its Leu^1-analog potentiate amphetamine stereotypy (snuffing, vertical standing), and lengthens its duration. The effect is analogous to increasing the dose of amphetamine. Under these circumstances, just as after injection of a large dose of amphetamine, potentiation of stereotypy is not accompanied by activation of motor activity [10]; on the contrary, the number of ipsilateral rotations was reduced. It can thus be tentatively concluded that the action of these peptides is oriented toward the dopamine systems of the striatum, but not to n. accumbens, with which the induction of locomotor activity is connected. D-Arg^4-tuftsin weakened both amphetamine and apomorphine stereotypy. Apomorphine stereotypy is characterized by predominance of licking and biting [8]. D-Arg^4-tuftsin facilitated the conversion (inversion) of this behavior into an amphetamine-like stereotypy. Tuftsin had no significant action on the effects of apomorphine. Tuftsin and its Leu^1-analog (but not D-Arg^4-tuftsin), both in the control and after haloperidol and DTC, gave some increase in aggressiveness, the level of which is associated with the state predominantly of the noradrenalin systems of the hypothalamus, but did not facilitate manifestation of dopamine-dependent stereotypy. Meanwhile the aggressiveness induced by apomorphine was abolished by all peptides (but only D-Arg^4-tuftsin abolished the stereotypy).

The time course of TH activity under the influence of peptides administrated *in vivo* (in a dose of 200 µg/kg, intraperitoneally) is shown in Fig. 1. Changes were assessed 7, 15, and 30 min after the injection. Under these conditions both tuftsin and its Leu^1-analog caused a progressive decrease in TH activity with time in the striatum and, to a lesser degree, in the hypothalamus. D-Arg^4-tuftsin significantly activated TH. It can be concluded from a comparison with the behavioral data that D-Arg^4-tuftsin affects dopamine receptors mainly in the manner of an antagonist, for it abolishes or weakens the effects of apomorphine (contralateral rotations, stereotypy, aggressiveness), but does not abolish the effects of haloperidol, it weakens ipsilateral rotations induced by amphetamine, a DA liberator, and considerably depresses TH activity in the striatum. This peptide also exhibits DA-mimetic effects, but the problem of whether the ability of D-Arg^4-tuftsin to induce contralateral rotations is due to its direct action on dopamine receptors or not requires further analysis. Tuftsin also has a dual action on dopaminergic mechanisms, the character and direction of which depend both on

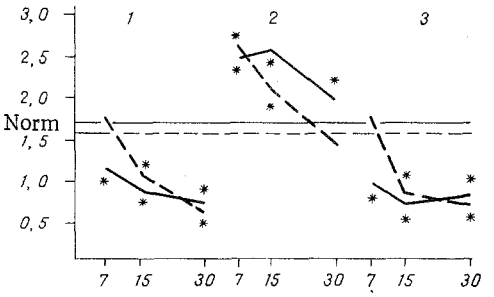

Fig. 1. Changes in TH activity (in nmoles/mg protein/15 min) in structures of rat hypothalamus and striatum at different times after injection of tuftsin and its analogs in a dose of 200 μg/kg. Continuous line represents TH activity in striatum, broken line — in hypothalamus. 1) Changes in TH activity after injection of tuftsin, 2) of D-Arg^4-tuftsin, 3) of Leu^1-tuftsin. *) Differences significant compared with normal at $P < 0.01$ level. Ordinate, TH activity; abscissa, time (in min).

dose and on time after administration. TH activity in the hypothalamus was initially activated by tuftsin in a dose of 200 μg/kg and by a greater degree in a dose of 500 μg/kg [2], but later it was depressed, and this can be compared with the parallel general activation of emotional-behavioral reactivity. Depression of TH activity in the striatum may be the result either of presynaptic activation of dopamine receptors (directly or through increased liberation or reduced reuptake of DA), or of strengthening of inhibitory nigro-striatal influences directed toward the corpus striatum (directly or through serotoninergic mechanisms).

It has been shown [9] that activity of dopamine receptors is under the modulating influence of substance P (SP), which is found in a high concentration in the substantia nigra [6, 12] and in axon terminals of the strionigral tract, the feedback channel for regulation of nigro-striatal interactions. SP behaves as a modulator of dopamine neuronal chains, intensifies DA metabolism in the striatum [15], and increases the number of vertical standings and snuffings in the stereotyped behavior of the rats (i.e., the amphetamine-like manifestations of stereotypy). Both effects disappear after destruction of dopamine terminals of the caudate nucleus by 6-OHDA [11]. In this connection it is important to note that the amino-acid sequence Lys^2-Pro^3-Arg^4 in the tuftsin molecule is the retrosequence of the N-terminal fragment 1-3 (Arg-Pro-Lys...) of the SP molecule, and also of the bradykinin-potentiating peptide (segments 6-8) and of fibrinopeptide II (segment 13-15). The structure of tuftsin thus is similar to that of the "common fragments" [5] of several low-molecular-weight peptides. It may be assumed that in an apolar biophase the tuftsin molecule adopts a quasicyclic structure, and in this form it interacts with the receptor or its surroundings [5]. It may be that replacement of the C-terminal amino acid Arg by its D-stereoisomer modifies the conditions of interaction, as a result of which the pharmacologic effect of D-Arg^4-tuftsin differs significantly from the effects of the natural tetrapeptide.

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