CORRECTION OF DRUG-INDUCED BEHAVIORAL DISTURBANCES IN ALBINO RATS BY TUFTSIN

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The tetrapeptide tuftsin (Thr-Lys-Pro-Arg) has a broad spectrum of biological activity and, in particular, it has a brief excitatory effect [1, 2, 6, 7]. The mechanism of the neurotrophic action of tuftsin is not clear, although its influence has been demonstrated on activity of tyrosine hydroxylase [2-4], enzymes of energy metabolism of neurons [8, 9], and enzymes responsible for brain catecholamine metabolism [9].

In this investigation the action of tuftsin was studied on spontaneous motor activity (MA) and the orienting-investigative response (OIR) of albino rats, when disturbed by administration of drugs affecting brain biogenic amine metabolism. The possibility of using tuftsin to correct various behavioral disturbances and interaction between tuftsin and the monoaminergic system of the brain also were studied.

EXPERIMENTAL METHOD

Noninbred male albino rats weighing 150-200 g were used. MA was recorded on an "Animex" actometer (Serva, West Germany) for 30 min in darkness and quiet. OIR was studied in the open field (OF) test for 2 min and by the "holeboard test." The animals were kept in a transparent plastic box measuring 400 × 400 × 300 mm, with 13 holes in the floor, and the number of holes sniffed and the number of rearings on the hind limbs were recorded for 3 min in quiet and during illumination by a red lamp.

Tuftsin, synthesized in the Research Institute of Chemistry, Leningrad University, was injected intraperitoneally in a dose of 0.3 mg/kg body weight in aqueous solution 5 min before the beginning of the experiment. To change the functional state of the biogenic amine system the following drugs were given at the corresponding times before the experiment: chlorpromazine 1 and 5 mg/kg, 30 min; reserpine, 1 mg/kg, 20 h; diethyldithiocarbamate (DTC), 250 mg/kg, 30 min and 2 h; cyproheptadine, 1 mg/kg, 5 min; haloperidol, 0.1 and 0.5 mg/kg, 5 min; amphetamine, 1 mg/kg, 30 min; apomorphine, 1 mg/kg, 3 min. All substances were injected intraperitoneally in aqueous solution; solutions of DTC and reserpine were made up with Tween-80. Control animals received an equivalent volume of distilled water or Tween-80.

When each of the above-mentioned drugs was used the biogenic amine concentration was measured in the rats' forebrain by a fluorometric method, using column chromatography on Amberlite CG-50 [10].

The numerical data were subjected to statistical analysis by the Mann—Wilcoxon test.

EXPERIMENTAL RESULTS

Behavioral parameters recorded after administration of the drugs, alone and together with tuftsin, are summarized in Table 1.

Chlorpromazine (1 mg/kg) did not change the brain biogenic amine concentrations (Table 2) but inhibited MA and OIR. Tuftsin increased the depressed MA but OIR remained depressed. However, the effect of a larger dose of chlorpromazine (5 mg/kg), expressed as a marked decrease (by 64%) of MA, was not abolished by tuftsin.

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TABLE 1. Changes (in % of control) in Behavioral Parameters Recorded during OF Test, in the Holeboard Test, and on an Animex Actometer, after Administration of Drugs Alone or with Tuftsin (T)

Substance and dose	MA (Animex)	OF		Holeboard test	
		ambulation	number of rearings	Number of holes sniffed	number of rearings
Chlorpromazine	62*	69*	78*	75	75
Chlorpromazine + T	137**	80*	84	64	64*
Reserpine	73*	87	89	62	64*
Reserpine + T	67*	97	91	67*	75*
DTC	57*	77**	87*	86	63*
DTC + T	74**	47*	83	50***	80**
Haloperidol	91	81*	67*	82	65*
Haloperidol + T	59***	79*	71*	71	35***
Cyproheptadine	83	99	88	174*	101
Cyproheptadine + T	94	97	84	159	120*
Amphetamine	128*	131**	132*	93	109
Amphetamine +T	103**	115**	100	64*	120
Apomorphine	44*	67**	31*	173	44*
Apomorphine + T	84**	86	16*	66	28*

Legend. *P < 0.05 compared with control; **P < 0.05 compared with drug.

TABLE 2. Concentration (in % of control) of Biogenic Amines in Albino Rat Forebrain after Injection of Various Drugs

Biogenic amines	Chlorpromazine 30 min	Reserpine 4 h	DTC		Haloperidol 15 min	Cyproheptadine 5 min	Apomorphine 3 min	Amphetamine 30 min
			30 min	2 h				
Noradrenalin	97,7	52,0*	68,0*	61,0*	97,2	94,5	89,6	107,4
Dopamine	104,4	47,0*	95,1	144,4*	99,0	100,6	100,6	120,7*
Serotonin	104,2	43,5*	98,7	103,5	100,3	98,2	93,2	101,7

Legend. Times between administration of drugs and determination of biogenic amines are shown. *P < 0.05 compared with control.

Reserpine and DTC depressed the behavioral parameters. Reserpine exhausts depots of biogenic amines, whereas DTC, which inhibits dopamine-β-oxidase, prevents noradrenalin synthesis. The noradrenalin level fell 30 min after injection of DTC but the dopamine concentration remained unchanged (Table 2). If injected 30 min after DTC or 20 h after reserpine, tuftsin gave opposite effects. Actometer studies showed that it abolished the depression of MA induced by DTC, but had a weaker effect on it in OF. Tuftsin also restored the OIR level, as shown by the number of rearings in OF and by the holeboard test. However, the behavioral disturbances induced by reserpine were actually worsened by tuftsin.

Two hours after injection of DTC, when the dopamine level was raised and the noradrenalin level remained low (Table 2), the action of tuftsin on MA was distorted: the number of movements made by the animals after combined administration of the substances was reduced to 28% of the control level ($P < 0.05$) and it differed significantly ($P < 0.05$) from the parameter obtained after injection of DTC alone.

Haloperidol (0.1 mg/kg), which blocks dopamine receptors, did not change the biogenic amine levels (Table 2) but reduced horizontal MA and OIR in OF and in the holeboard test, but spontaneous MA, recorded on the Animex, remained close to the control level. After administration of tuftsin plus haloperidol the effect was reversed: instead of motor excitation, observed after injection of tuftsin into the intact animals, depression of MA was observed on the Animex and the number of rearings in the holeboard test was reduced. If the dose of haloperidol was increased to 0.5 mg/kg, this led to a reduction of MA by 72% ($P < 0.01$). In this case the peptide did not abolish the abnormalities which appeared.

Injection of apomorphine, an agonist of dopamine, caused stereotyped behavior, accompanied by depression of MA and OIR. In this case, tuftsin restored the ambulation of the animals but did not change the number of rearings.

Amphetamine did not change the biogenic amine concentrations (Table 2) but caused an increase in MA and OIR. Against the background of amphetamine, tuftsin did not exhibit any excitatory effect on the behavioral parameters studied, but abolished the effect of amphetamine. The effect of the peptide on OIR in the holeboard test also was reversed in sign.

Cyproheptadine did not affect biogenic amine levels in the brain (Table 2). It did not change motor activity but increased OIR recorded in the holeboard test. After administration of cyproheptadine the stimulating effect of tuftsin on MA was not exhibited, but the action of the peptide on OIR in the holeboard test did not change its sign.

In the dose used, and at the time indicated, according to the results described above tuftsin thus did not change the brain biogenic amine levels but was able to restore the normal DA level if it was depressed (by DTC, chlorpromazine, and apomorphine), and also when it was raised (amphetamine). The effect of tuftsin on MA in this case was opposite in direction to the action of the drugs.

However, in the presence of more severe behavioral and neurochemical disturbances, such as were observed after injection of a large dose of chlorpromazine or reserpine, tuftsin did not restore MA.

The results confirm the hypothesis that the neurotrophic action of tuftsin is realized through the biogenic amine system.

Haloperidol blocks postsynaptic dopamine receptors, which play a leading role in the behavioral effects of this neurotransmitter; however, because of the increased rate of both synthesis and breakdown, the dopamine level changes only very little as a result of administration of haloperidol [12], and this was confirmed also by the results of the present experiments. The behavioral effect of tuftsin against a background of dopamine receptor blockade was reversed and a marked decrease of MA was observed. For tuftsin to exhibit its psychostimulating action, normal functioning of the postsynaptic dopaminergic receptors is therefore necessary.

Apomorphine in small doses mainly activates presynaptic dopamine receptors, which leads to inhibition of tyrosine hydroxylase and, consequently, to inhibition of dopa formation.

Under these circumstances, according to some data, dopamine synthesis is depressed, but according to other data, as a result of inhibition of dopamine turnover its level in the brain structures is raised somewhat [11]. In this case, MA is considerably depressed. Injection of tuftsin against this background restored MA, i.e., the excitatory action of tuftsin was exhibited also when presynaptic dopamine receptors were blocked. The behavioral effect of tuftsin was reversed even when DTC was injected 2 h before MA was measured. Under these circumstances not only was the noradrenalin level lowered, but the dopamine level also was raised.

These results confirm the conclusions that [2, 5] the neurotrophic effect of tuftsin, observed during the first few minutes after its injection, is mediated through the dopaminergic system of the brain.

It must also be pointed out that the effect of tuftsin in the body is manifested against the background of all the drugs that were used, although the direction of its action depended on the functional state of the recipient, a characteristic feature in general of many peptide regulators of physiological functions. Finally, abolition of certain drug-induced behavioral disturbances by tuftsin is evidence of the new prospects for its therapeutic use.

LITERATURE CITED

1. S. M. Andreev, K. V. Anokhin, L. V. Antonova, et al., Dokl. Akad. Nauk SSSR, 253, 498 (1980).
2. A. V. Val'dman, M. M. Kozlovskaya, I. P. Ashmarin, et al., Byull. Éksp. Biol. Med., No. 7, 31 (1981).
3. A. V. Val'dman, D. Yu. Rusakov, V. N. Kalikevich, and Z. A. Ardemasova, Byull. Éksp. Biol. Med., No. 4, 49 (1982).
4. A. V. Val'dman, N. A. Bondarenko, V. A. Kamysheva, et al., Byull. Éksp. Biol. Med., No. 4, 57 (1982).
5. A. V. Val'dman and M. M. Kozlovskaya, Patol. Fiziol., No. 3, 60 (1984).

6. A. A. Kamenskii, L. V. Antonova, N. A. Samoilova, et al., Byull. Éksp. Biol. Med., No. 7, 43 (1980).
7. É. F. Lavretskaya, I. P. Ashmarin, A. A. Kamenskii, et al., Farmakol. Toksikol., No. 3, 275 (1981).
8. Rok'ya Begam and Ya. B. Maksimovich, Farmakol. Toksikol., No. 2, 41 (1985).
9. M. B. Shishova, A. V. Sergutina, and L. M. Gershtein, Neirokhimiya, No. 4, 353 (1984).
10. J. Barchas, E. Ardelyi, and P. Angwin, Anal. Biochem., 50, 1 (1972).
11. A. Carlsson, The Basal Ganglia, New York (1976), pp. 181-189.
12. A. Carlsson and M. Lindqvist, Acta Pharmacol. Toxicol. (Copenhagen), 20, 140 (1963).

CHANGES IN BENZODIAZEPINE RECEPTOR LIGAND AFFINITY IN THE PRESENCE OF 4,5,6,7-TETRAHYDROISOXAZOLO-(5,4-c-)-PYRIDIN-3-OL (THIP)

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At least three groups of ligands of benzodiazepine (BD) receptors have now been described [3]. These groups of ligands, depending on their pharmacological activity, can be differentiated into agonists, opposite agonists, and antagonists [3, 8]. Many experiments to study radioligand binding have shown that GABA agonists or barbiturates increase the affinity of receptors for agonists of BD-receptors [1, 3-7, 11] and reduce affinity for inverse agonists; affinity for antagonists of BD-receptors (Ro 15-1788, CGS 8216), moreover, is unchanged [3]. It has been suggested that the ability of GABA, muscimol, or barbiturates to increase or decrease the affinity of BD receptor ligands may reflect the pharmacological activity of these substances [3, 5, 7].

A group of highly specific agonists of $GABA_A$ receptors has recently been described [9, 10], including 4,5,6,7-tetrahydroisoxazolo-(5,4-c-)-pyridin-3-ol (THIP), isoguvacine, and piperidine-4-sulfonic acid which, unlike GABA and muscimol, do not increase BD binding [2, 9, 10]. In the absence of chloride ions at 0°C, moreover, these substances inhibited BD binding and antagonized the stimulating action of muscimol or GABA [2, 9, 10]. The inhibitory action of THIP or isoguvacine was manifested best if binding took place at 0°C with unwashed (intact) membranes in Tris-HCl buffer of low molarity [2, 10]. These effects were explained on the grounds that THIP possesses a mixed agonistic-antagonistic action or through its effect on a special population of GABA receptors linked with BD receptors [2, 9, 10].

In this paper we give data showing that BD receptor ligands change their affinity in the presence of THIP, and that the shift of affinity induced by THIP can be used to predict the activity of these substances in vitro.

EXPERIMENTAL METHOD

The forebrain of adult male Wistar rats was homogenized (1100 rpm, 10 passages) in 50 volumes of 25 mM Tris-HCl buffer in a glass homogenizer with Teflon pestle (Braun Melsungen). The homogenate was diluted to 600 volumes (w/v) and used directly in the binding experiments. Aliquots of the homogenate in a final volume of 1 ml were incubated with 1.25-1.35 nM ^{3}H-flunitrazepam (^{3}H-FNZ, 74-83 Ci/mmole, Amersham International, England) at 0°C for 1 h with corresponding concentrations of the substances and in the presence or absence of THIP (100-200 μM). Nonspecific binding was determined as the difference between binding in the absence and presence of 5 μM diazepam. At the end of incubation the bound and free radioligand was separated by filtration through GF/B (Whatman, England) filters. The filters were washed twice with 5 ml

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