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# *The influence of TP-1 on the behaviour of rabbits in spontaneous conditions and after stimulation of ventromedial hypothalamic nucleus (Vmh)*

Wpływ TP-1 na zachowanie królików w warunkach spontanicznych i po stymulacji jądra brzuszno-przyśrodkowego podwzgórza (Vmh)

Tuftsin, a natural tetrapeptide with the sequence Thr- Lys - Pro-Arg is present in the Fc fragment of the IgG molecule (10, 11). It is known as the modulator of immunological processes (4, 11). Lately, its psychotropic properties have been found (1, 9, 14, 17). In living organisms there are also tuftsin natural analogs having properties depending on the quantity, kind, and sequence of aminoacids in a molecule (4, 5). Synthetic derivatives were produced on the basis of natural ones.

TP-1 is a synthetic analog of tuftsin having stronger psychotropic effect than tuftsin (13). It contains tuftsin sequence joined with tripeptide Pro-Gly-Pro. Like tuftsin, it influences emotional-motivating and adaptive reactions of the organism (19). It makes learning (9) and memorising (14) easier. It reduces negative and emotional tension. It increases motor activity (9). Tuftsin shows analgesic activity as well (8).

This study sought to determine spontaneous behaviour, typical of a rabbit and thus define side effects of tuftsin analog on this model of behaviour. We were also interested in the behaviour of a rabbit in extreme conditions, mainly in determining the effectiveness of sedative and anxiolytic effects of the studied substance in stress situation. Reaction of TP-1 on the escape reaction latency time was also studied.

## MATERIAL AND METHODS

The experiments were performed using 20 male Chinchilla rabbits of mean body weight 3250 grams, which were randomised for the study. Adaptation of the animal to the surrounding conditions lasted for 1 hour and then three-hour recording of the behaviour of the rabbit was initiated. To make the observation easier, the time of observation was divided into 10-minute intervals. Several phases of behaviour were estimated in the animals' behaviour: tension, orientation-searching behaviour, comfort, grooming, water and food uptake. Duration of each phase was measured in seconds with a stopwatch during individual 10-minute intervals.

The experiment in spontaneous conditions was carried out for the 3 subsequent days in a group of 10 rabbits. On the first day spontaneous behaviour was tested, on the second day 100 ml of distilled water into each nostril of the rabbit was administered with an automatic measuring pipette and on the third day TP-1 was administered with an automatic measuring pipette at a dose 200 $\mu$g per kg of rabbit body weight diluted in distilled water up to 200 $\mu$l into each nose opening, 15 minutes before initiation of the experiment.

The experiment in stress conditions was performed also in the group of 10 rabbits. The experimental model of stress situation was an active defence, described as the reaction of escape, induced by electrical stimulation of ventromedial hypothalamic nucleus (Vmh) (3, 15). After 24 hours since the electrode had been implanted into Vmh, the animal was placed in an experimental cage and the same scheme was used as in case of spontaneous conditions only with a difference concerning the beginning of each 10--minute interval when electric stimulation of Vmh was evoked for the whole time of the experiment until the escape reaction occurred. The escape reaction latency time was registered with digital time-meter connected with the stimulator.

Each time after completion of the experiment, macroscopic evaluation of electrode placement was controlled. The significance of dissolvent and substance influence on the duration of the phases and the latency time was checked with t-Student test.

## RESULTS

The reaction of dissolvent was excluded before testing the influence of substance. The duration of specific phase of behaviour was evaluated and it was expressed in ‰ as the share of a given phase in the 3-hour observation time.

Administration of TP-1 decreased the tension phase in both models of behaviour. In the spontaneous behaviour the reduction from 15‰ to 1.2‰ is not a statistically significant change probably due to small initial values. Yet, it is a reduction by more than 90%. However, reduction of tension phase in stressful state from 496‰ to 281‰ is significant.

Orientation-searching reactions were not influenced by the tuftsin analog (slight reduction in spontaneous conditions from 116‰ to 107‰ and in stress conditions – from 90‰ to 88‰).

TP-1 minimally reduced the comfort phase in spontaneous behaviour from the initial value 708‰ to 679‰, it significantly increased the duration of the phase after stimulation of Vmh to 453‰, as compared to initial value 300‰.

Intra-nasal administration of TP-1 and of dissolvent caused caring reaction. The duration of grooming after administering dissolvent was increasing both in spontaneous conditions (from 31‰ to 62‰) and in stress conditions. The increase in stress conditions from 25‰ to 61‰ is statistically significant. After administering of TP-1 the observed changes were of the same value as after administering a dissolvent (the increase to 67‰ and to 61‰ respectively). The changes were not analysed.

Tuftsin analog did not influence significantly food uptake. In spontaneous behaviour it was increased from 108‰ to 118‰ and in stress conditions – from 72‰ to 94‰.

No significant influence of TP-1 on water uptake was reported, in spite of its slight increase in spontaneous conditions from 18‰ to 27‰ and in stress situation from 18‰ to 23‰.

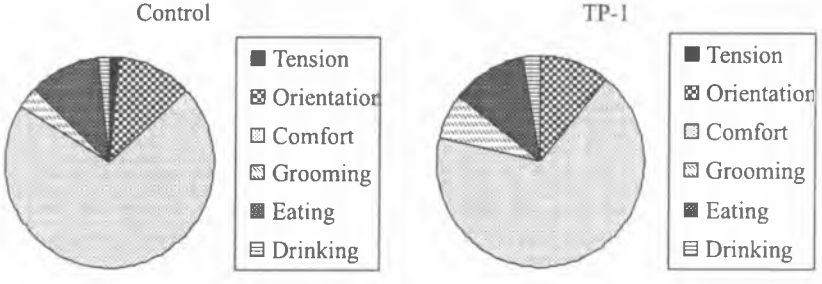


Fig. 1. Spontaneous conditions

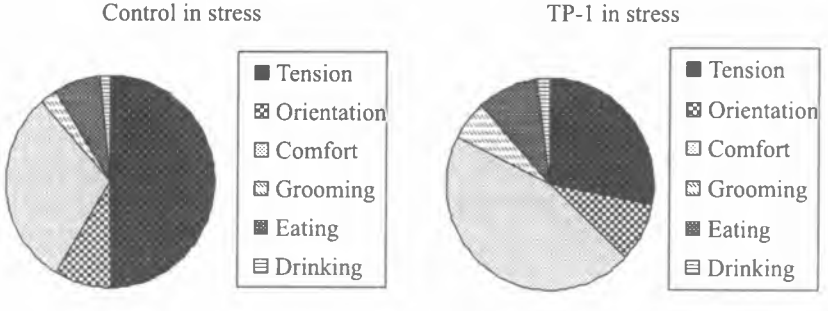


Fig. 2. Stress conditions

Administration of tuftsin analog significantly decreased the escape reaction latency time during the first and second hour of observation respectively from 1.9 s to 1.1 s and from 1.6 s to 1.1 s. Shortening of latency time during the third hour of observation from 1.5 s to 1.2 s is not a statistically significant change.

## DISCUSSION

The study proved that TP-1 increased the comfort phase in spontaneous conditions minimally. We may suppose, that it does not have a soporific influence. It does not cause heaviness and drowsiness either. In 200 microg/kg dose, it minimally decreased the orientation-searching phase and it means, that tuftsin analog does not influence life normal activity. It does not change the attention of the animal either.

According to Siemienova's et al. report tuftsin and its analog TP-1, that are administered to rats intraperitoneally, intensify searching activity in an open field. The duration of this influence is 6 hours and it is longer after administering of TP-1(14). Intensification of motor activity due to tuftsin influence is demonstrated by Waldman et al. (18, 19). According to Waldman et al. with the increase of tuftsin dose its stimulating reaction of motor activity gets reduced (18) and at dose 500 $\mu$g/kg administered intraperitoneally it does not influence the rats' behaviour in an open field (19). However, according to Herman et al. tuftsin reacts in two phases to the activity of animals, initially by inhibiting the activity and then by intensifying it (7). Probably it stimulates searching motivation (18). Ashmarin et al. say that tuftsin partly normalises the motor activity and orientation behaviour of rats that was changed due to administration of drugs influencing their system of biogenic amines. The observed effect during the first minutes after tuftsin has been administered, is combined with its (tuftsin) influence on the dopaminergic system (4).

Siemienova et al. report a positive influence of tuftsin and TP-1 on the learning and memorising processes. Both peptides make the creation of conditional reaction easier (14) and they reduce the number of wrong answers (13, 14).

TP-1 reduced insignificantly the time of tension phase and it minimally increased the duration of such natural activities as eating and drinking. It proves lack of influence of this substance on the normal activity of animals.

The influence of tuftsin analog on the grooming is difficult to interpret due to the method of its application. The fact that TP-1 did not eliminate reflexive cleaning reaction resulting from nostrils irritating activity of the administered substance may show that it does not weaken the interest in grooming and it does not influence the reflexive reactivity.

Active and defensive defence (active fear) was an experimental model for stress situation, that was described as an avoidance reaction or an escape reaction (3, 15). This reaction was induced by electrical stimulation of ventromedial hypothalamic nucleus (Vmh) (3, 15).

There was a minimal influence of TP-1 on comfort phase in spontaneous conditions and a strong one after stimulation of Vmh. Therefore, specificity of tuftsin analog reaction on the comfort phase in stress conditions should be emphasised. Probably TP-1 reduces susceptibility to stress. TP-1 also reduces the duration of tension phase resulting from Vmh stimulation. The obtained results suggest anxiolytic and sedative character of the tested substance and it complies with the studies in this field that are described by other authors.

Siemienova et al. report that tuftsin and its analog significantly weaken negative reaction of animals in stress conditions, particularly by decreasing the feeling of anxiety (14). Waldman et al. support the increase of resistance to stressing factors after administering tuftsin. Those authors think that tuftsin induces animals initiative, which can create the opportunity of correcting pathologic changes in CNS (central nervous system) activity which take place due to stress (18). As mentioned before, tuftsin makes conditional reaction occurrence easier, however this reaction in extreme conditions gets disturbed (9, 14). Inoziemcev et al.. state, that administering of tuftsin or of TP-1 significantly increases the duration of created situation in stress conditions (9). Seredenin et al. think that TP-1 has a stronger anti-anxiety and psycho-stimulating activity as compared with tuftsin. Both peptides cause a weaker reaction to stress (12).

The leading mechanism of central activity of tuftsin and of TP-1 is their influence on biogenic amines level: noradrenaline, dopamine and serotonine (1, 13, 14, 17). According to Waldman et al. the influence of tuftsin on noradrenaline and dopamine levels may be through the reaction on hydroxylase activity – the enzyme of biosynthesis of those amines (17, 19). Hydroxylase catalyses the reaction of tyrosine transformation into 3,4-dihydroxyphenylalanine (DOPA). The substrate tyrosine protects the enzyme against peptide activity (19). Tuftsin had a double influence on tyrozine hydroxylase activity according to the dose and time that lapsed since its administration (17). As Waldman et al. report in the 500 μg/kg dose tuftsin increases the activity *in vivo* of the enzyme in hypothalamus and in striatum during the first 10 minutes. During the subsequent 20 minutes the enzyme activity gets increased in hypothalamus and gets slightly decreased in striatum. However also in this structure this activity is higher as compared to control conditions. The authors support direct inhibiting influence of tuftsin on hydroxylase *in vitro*. The changes of animals behaviour concern emotional-motivating and adaptive reactions, that are controlled by hypothalamus and the motor activity related to striatum activity (19). At the 200 μg/kg dose tuftsin initially increases and then inhibits the enzyme activity in hypothalamus, however it only inhibits its activity in striatum (17).The influence of tuftsin and of TP-1 on the noradrenaline and dopamine in brain by tyrosine hydroxylase is described by Siemienova et al. The authors say, that TP-1 decreases noradrenaline concentration in hypothalamus and in brain stem and it increases the amount of dopamine in the hypothalamus. Due to the fact that dopamine is a precursor of noradrenaline, Siemienova et al. suggest that tuftsin and TP-1 influence dopamine beta-hydroxylase activity (13). Beta-hydroxylase catalyses reaction of dopamine metamor-

phosis into noradrenaline. Moreover, the authors prove that administration of both peptides modifies serotonine concentration in hypothalamus and brain stem. However, the changes are ambiguous and at present they are difficult for interpretation. Probably serotoninergic system reacts quickly during the first 10 minutes from the substance administration, however the changes in catecholaminergic take place later, within the time limit from 30 minutes to two hours (13). Yet, Ashmarin et al. state that tuftsine influences dopaminergic system during the first minutes after administering (1). The influence of tuftsin and TP-1 on the serotoninergic system was confirmed by Seredenin et al. (12).

The duration of orientation-searching activity phase after Vmh stimulation did not differ from spontaneous behaviour. In both conditions the attention and activity of the animal were similar. According to Waldman et al. tuftsin increases the duration of searching activity (18). Waldman et al. think, that tuftsin activates motivation of observation and searching of the environment (18). Siemienova et al. report, that also TP-1 has a stimulating influence on rats' searching activity (14).

Our results proved that grooming phase after TP-1 administration in spontaneous conditions was extended due to irritating reaction of the substance on the nostrils, however, significantly stronger extension of this phase in the stress conditions should be emphasised.

No significant influence of TP-1 on the duration of water and food uptake phases was confirmed.

The latency time of escape reaction after tuftsin analog administration got decreased during the first and second hour of the experiment. According to Barret et al. and Gunne et al. the defensive reactions induced by stimulation of VMH are regulated by adrenergic system (2, 6). Sweidan et al. point out the significant role of dopaminergic system in regulation of this type of behaviour (16).

## CONCLUSIONS

1. TP-1 shows sedative and anxiolytic activity. Its influence concerns a considerable extension of comfort phase and decrease of tension phase in extreme conditions.

2. Administering of TP-1 does not influence the activity and attention of the animal (orientation-searching phase).

3. Tuftsin analog makes defensive reactions easier (decreasing of latency time of escape reaction).

4. No reaction of TP-1 on water and food uptake phases was confirmed.

5. It seems that a less effectiveness of TP-1 is compensated by a greater specificity of the influence of this analog in stress conditions. There are smaller side effects confirmed by the level of changes observed in spontaneous model of behaviour.

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## STRESZCZENIE

Doświadczenia przeprowadzono w dwóch grupach zwierząt, po 10 królików każda. W pierwszej grupie rejestrowano zachowanie spontaniczne zwierząt, w drugiej zachowanie w warunkach stresu doświadczalnego oraz czas latencji reakcji ucieczki. Wyodrębniono sześć rodzajów zachowań. W każdej grupie pierwszego dnia nie podawano substancji, drugiego kontrolowano ewentualny wpływ rozpuszczalnika, trzeciego podawano donosowo TP-1, rozpuszczony w wodzie destylowanej w dawce 200 μg/kg. Stwierdzono, że TP-1 cechuje specyficzność działania uspokajającego i przeciwlękowego, dotycząca warunków stresu oraz niewielkiego stopnia skutki uboczne (poziom zmian obserwowany w modelu zachowania spontanicznego).