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Chair and Department of Human Physiology, Medical University of Lublin

MARIOLA DEC-SZLICHTYNG, MARIUSZ TETER,
BARBARA BOROWICZ

The influence of tuftsine analog TP-1 on the behaviour of rabbits

Wpływ analogu tuftsyny TP-1 na zachowanie królików

One of the most important mechanisms determining functioning of animals and humans are the defensive reactions. These reactions (including escape) may be caused by stimulation of various structures of central nervous system (CNS) (13). The nucleus ventromedialis of the hypothalamus (Vmh) is especially important. Electrostimulation of this nucleus and the region adjacent to ventromedialis of the hypothalamus causes escapes, attacks and defensive reactions (11). Generation of defensive reactions is influenced by many neurotransmitter systems of CNS and by exogenic and endogenous chemical compounds. One of the short endogenous peptides that originated from enzymatic disintegration of IgG is tuftsine - a tetrapeptide with immunomodulatory properties and clear influence on CNS. Its shortcoming is a very short period of activity. The tuftsine analog – heptapeptide TP-1 – includes tuftsine sequence in its composition. It maintains many properties of tuftsine and its important feature as compared to tuftsine, is an extended time of activity. The aim of the study was to investigate the influence of tuftsine analog TP-1 on the behaviour of rabbits after electrostimulation of Vmh, without electrostimulation and to evaluate if rabbit's behaviour is subject to any significant changes at the time of experiment. The intention was also to analyse the influence of TP-1 on the escape reaction caused by electrostimulation of Vmh.

MATERIAL AND METHODS

Animals – 26 rabbits were used in the experiments, males of chinchilla breed with body weight 2900 – 3500 g. The animals stayed in standard laboratory conditions (temp.

20 $\pm 2^0$ C), with easy access to water and food. The experimental group included 8 rabbits, each control groups included 5 animals.

Substances – The following substances were used in the experiments:

Tuftsine analog – heptapeptide of TP-1 , synthesised in the Institute of Molecular Genetics of Russian Academy of Science, Moscow, Russia, contains tuftsin seguence joined with tripeptide Pro-Gly-Pro. Polocain hydrochloride (1% Solutio Polocaini hydrochlorici, Polfa). TP-1 was administered in 0.5 nmol/kg of body weight dose into the lateral ventricle of the rabbit brain (icv). In the control experiments the solvent was given.

Methods – Local anaesthesia was made to the animals by subcutaneous injection in the frontoparietal region of head of 10-20 ml of 1% Polocaini hydrochlorici. After removing the skull integument, there was established location of electrode introduction into nucleus ventromedialis of hypothalamus according to coordinates in stereotactic atlas. (Fifkova E. 1960, Cvietkova I.P. 1987). AP-1 (1mm backwards from the bregma point), L-1 (1 mm sideways from the sagittal suture), V-15.5-16 (15.5 – 16 mm below the outer surface of the skull) as well as the place of second cannula enabling administration of substance icv of the rabbit's brain AP-1.5; L-2; V-7. Chromium-nickel-plated bipolar electrodes were used in the experiment. Stimulation of Vmh was made with a current frequency 100Hz, impulse width 0.3 ms and threshold voltage 3-6 V, depending on individual excitability of the centre.

Observation of rabbit's behaviour in spontaneous conditions (standard experimental conditions, without stressing stimuli, with free access to water and food). The time of observation (3 h) was divided into 10-minute periods. The structure of behaviour was divided into the following phases: tension, observatory-cognitive, comfort, grooming, aggression, eating and drinking. The duration of these phases was measured during the particular periods. After 24 h and after administering the investigated substance icv, the behaviour of rabbits was studied according to the above mentioned principles. Every time observations of the rabbit's behaviour was initiated after 1h of adaptation. The control group was administered solvent icv.

Observation of rabbit's behaviour after stimulation of Vmh. The observation of rabbits behaviour was made according to the above mentioned principles and additionally Vmh was stimulated with electric current, causing the reaction of escape. Stimulation was carried out every 10 minutes at the beginning of each 10-minute time interval during 3 h. In the subsequent stage after 24 h, the investigated substance was introduced icv and the behaviour of rabbits was registered in stressful conditions, with simultaneous recording of latency time of escape reaction.

Evaluation of the phases of rabbits' behaviour. Reaction of escape – motor reaction occurring directly after stimulation of Vmh, characterised by a sudden turn of the animal's body, the attempt of jumping off the cage, stamping with back paws on the ground with accompanying acceleration of breath and increase of muscular tension. The latency time (LT) of escape reaction is the time from the stimulation of Vmh

until the reaction of escape occurs. Phase of tension – immobilisation of the animal, increase in muscular tension, pilo-erection, acceleration of breadth, frequent urination and defecation. Orientation-cognition phase – increased motor activity, movements of seeking, examination of the environment, interest in the surroundings. Comfort phase – relaxation of the animal, decrease in muscular tension, drowsiness, reducing of reactivity to exterior stimuli. Grooming phase – caring activities: licking of paws, trunk, cleaning of the mouth. Aggression phase – aggressive reactions with relation to the environment: spilling out the food and water from the vessels, back paws stamping against the ground. Eating phase – eating of food and coprophagy. Drinking phase – free quenching of their thirst. Directly after completion of the experiments the brains of the rabbits were subjected to macro and microscopic verification. Adequacy of electrodes localisation and of administration of the investigated substance was evaluated.

Statistical analysis. Numerical material was analysed statistically. The significance of differences and correlation was checked with appropriate tests (t-Student, Wilkoxon, sum-range, variance analysis of two factors was also performed). The differences between groups was considered statistically significant when $p < 0.05$.

RESULTS

REACTION OF ESCAPE

After administration of TP-1 analog the latency time of escape reaction after each of 18 stimulations was increasing significantly. The mean latency time was 1.77 ± 0.11s before administration the preparation and 3.59 ± 0.36s after administration. Thus the administration of tuftsine analog TP-1 influenced extension of latency time by 1.82 s on the average. Administration of solution did not influence the latency time of escape reaction.

THE INFLUENCE OF TP-1 ON THE RABBIT'S BEHAVIOUR

Data analysis was performed in 1-h time intervals, during the 1st, 2nd and 3rd h of observation.

Depending on TP-1 administration (before and after administration) and on duration of observation (1, 2, 3 h), the time (in %) for particular phases amounted: for comfort phase 53.3-72.6%, for observation phase 8.1-18.7%, for eating phase 10.6-16.3%, for grooming phase 1.7-12.4%. For each of the remaining phases it was 0.0-4.4%. After TP-1 administration the changes in duration of grooming, eating and drinking phases did not show any significant changes during the experiment. The remaining phases changed sig-

nificantly with the time of experiment. The phase of tension during 1st h has decreased by 72 s, during 2nd h – only by 3 s and during the 3rd h after TP- 1 administration it did not change, so with the time of the experiment the influence of the preparation on the decrease of tension phase was not clear. The observation-cognition phase after administration of tuftsine analog during the 1st h has extended by 288 s but during the 2nd h it has decreased by 114 s. The comfort phase during 1st h has decreased by 562 s but during the 2nd h it has extended by 282 s. The aggression phase during the 2nd h has decreased by 2.5 s and during the 3rd h it did not change (and even this difference is statistically significant). Therefore the following statistically significant changes after administration of TP-1 were confirmed: the decrease in tension phase (excluding 3rd h), the decrease in comfort phase (only during 1st h) and of eating phase (during 2nd h) as well as the extension of observation phase (only during 1st h) and of grooming phase (irrespective of the duration of the experiment). Administration of solution had no influence on the change in the structure of animals' behaviour.

THE INFLUENCE OF TP-1 ON THE RABBITS' BEHAVIOUR AFTER STIMULATION OF VMH

Depending on TP-1 administration (before or after administration) and on the duration of observation (1, 2, 3 h), the time expressed in % for particular phases amounted to: for comfort phase 61.8-72.5%, for observation phase 10.9-20.0%; for tension phase 0.0-18.3%; for eating phase 2.6-8.9%; for grooming phase 0.3-7.6%. For each of the remaining phases it was 0.0-4.4%. The duration of the experiment did not have any significant influence on the range of changes caused by administration of tuftsine analogue TP-1 for comfort, grooming, eating and drinking phases. The tension phase during the 1st h of observation decreased after administration of TP-1 by 590 s, during the 2nd h by 233 s and during 3rd h by 97.5 s. The differences between the three h are significant, and the range of decrease is significant even in the 3rd h. The observation phase in the 1st h has increased by 75 s but in the 2nd h it has decreased by 135 s. In the 3rd h the decrease was by 329 s. The comfort phase after TP-1 administration is longer on average but in neither of the 3 h the observed differences are statistically significant ($p<0.05$). Regardless of the time of observation there is an extension of the grooming phase. As a matter of fact in the 1st h a decrease in aggression phase by 6.25 s was confirmed and it is significantly bigger than the decrease in duration of this phase in 2nd (0.25 s) and in 3rd h (3.1 s). However, in any hour no significant influence of administration of TP-1 on the decrease in duration of this phase was confirmed. Thus administration of TP-1 with simultaneous stimulation of Vmh, influences significantly the decrease in tension phase (though the smaller it is the longer the experiment lasts) and the decrease of observation phase (but significant only in 3rd h) and the extension of grooming phase irrespective of the duration of observation. Administration of solution did not influence the structure of behaviour of animals after stimulation of Vmh.

DISCUSSION

For defining effective pharmaco–therapy of aggressive behaviour numerous studies were undertaken in order to explain the role of various neurotransmitter systems of CNS and of endogenic peptides in regulating the reactions of emotional defence. The research of Barret and co-workers (2) indicates that noradrenergic system stimulation makes the emotional defence easier. However, Sweiden and co-werkers (10) suggest that a similar result is reached by stimulation of dopaminergic system. Yokawa and co-werkers (13) report that γ-aminobutyric acid causes inhibition of some components of the escape reaction. According to Sudakov (9) $ß_2$ – endorphin and opiate peptides also have inhibitory effect on the escape reaction. Emelienova (4) proved inhibitory influence of angiotensine II on the reaction caused by electrostimulation of Vmh.

In our study the defensive reaction of escape caused by electrostimulation of Vmh in rabbits was the experimental model. The results indicate that tuftsine analog, TP-1 heptapeptide, clearly delays the reaction of escape. After TP-1 administration, after each of 18 stimulations, there is a statistically significant extension of latency time of escape reaction. It is likely that icv administration of TP-1 in rabbit causes the decrease in excitability of Vmh. This results in the extension of latency time and delay of defensive reaction. In the accessible literature there are a few data on the reaction of TP-1.

Herman and co-workers (5,6) investigated the influence of tuftsine itself on emotional and behavioural activity of rats. Tuftsine influences the behaviour of rats in two phases. During 15 min since administration it inhibits motor, cognition and spontaneous reaction and then after 60-90 min it increases both types of motor activity. Waldman and co-workers (12) proved that after tuftsine administration locomotion activity of rats, the aggressiveness and ability of passive avoidance during single augmentation increased. Arefieva (1) proved that the above mentioned effects of tuftsine on the animals' behaviour are due to tuftsine bonding with plasmatic membranes of cerebral cells in rats. According to Arefieva (1) this bond meets the majority of criteria for receptor bond. She also suggests that tuftsine analog TP-1 has a very great (similarly as tuftsine itself) affinity to the described locations of tuftsine bonds in plasmatic membranes of cerebral cells. Nieber and co-workers (7) report that tuftsine itself has anti–stress properties. Siemienova and co-workers (8) prove that synthetic analogue of tuftsine – TP-1 heptapeptide has similar properties. Our research indicates that TP-1 when administered icv, causes a significant decrease in tension phase (with the exception of 3rd h), decrease in comfort phase (in 1st h) and in eating phase (in 2nd h) as well as extension of observation phases (in 2nd h). There are reports (7, 12) stating that tuftsine through its activity on catecholaminergic processes in brain, influences emotional reactivity of animals by enabling performance of problems during emotionally stressing conditions. Siemienova and co-workers (8) propose similarly with reference to both tuftsine and its analogue TP-1, and she adds that TP-1 reaction is stronger than the reaction of tuftsine itself. The study indicates that TP-1 in stressful conditions caused by the stimula-

tion of Vmh, results in significant decrease in tension phase (during 3h of the experiment), in aggression phase (though insignificant) and in observation phase (with the exception of first hour). However TP-1 extends the grooming phase and the phase of eating and comfort (insignificantly). The performed investigations confirm reports concerning beneficial reaction of TP-1 in emotionally stressing conditions (3,7,8). It is possible that the binding sites for analogues of tuftsine are present not only in cerebral cells of rats but also in rabbits. It is likely that TP-1 by reacting with these sites may influence the behaviour of animals, and the influence is the strongest in stressful conditions. The obtained results indicate the need of further research concerning the mechanism of behavioural reaction of TP-1.

CONCLUSIONS

1. Tuftsine analog TP-1 causes the anxiety-relieving effect and prolongs the latency period of escape reaction caused by electrostimulation of the nucleus ventromedialis of hypothalamus.
2. Heptapeptide TP-1 influences significantly behaviour of rabbits both in spontaneous and in stressful emotional conditions induced by electrostimulation of Vmh.
3. Reaction of TP-1 is stronger with electrostimulation of Vmh than in spontaneous conditions. This means that it has anti-stress effects and extends emotionally positive reactions of animals.
4. The time factor has a significant role for observed changes in animals behaviour after TP-1 administration.

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STRESZCZENIE

Badano wpływ analogu tuftsyny TP-1 na zachowanie królików w warunkach spontanicznych oraz w warunkach elektrycznej stymulacji jądra brzuszno-przyśrodkowego podwzgórza (Vmh), a także jego wpływ na czas latencji reakcji ucieczki. Ogólną strukturę zachowania podzielono na fazy: naprężenie, reakcje obserwacyjno-poszukiwawcze, komfort, agresja, gruming, jedzenie, picie. Za czas latencji reakcji ucieczki przyjęto czas od momentu stymulacji Vmh do wystąpienia reakcji motorycznej. Z przeprowadzonych badań wynika, iż TP-1 wydłuża czas latencji reakcji ucieczki oraz wywiera istotny wpływ na zachowanie królików, skracając fazę naprężenia i agresji a wydłużając fazę grumingu, komfortu i jedzenia. Działanie TP-1, polegające na wyeliminowaniu emocjonalnie pozytywnych reakcji zwierząt, jest silniej zaznaczone w warunkach stymulacji Vmh.