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Kierownik: prof. dr hab. Andrzej Niechaj

MARIUSZ TETER, MARIOLA DEC-SZLICHTYNG

The influence of the ACTH-derived short regulatory peptide on the rabbit behaviour

Wpływ krótkiego peptydu regulującego pochodnego ACTH na zachowanie królików

The physiological defence mechanisms are of basic importance for the proper homeostasis and functioning of the organism. Stress releases a number of biochemical changes not only in the central nervous system but also in the endocrine system, the immunological and in the metabolism controlling system. It also activates the hypothalamus-hypophysis-adrenal gland system. As a result these changes determine the complex organism behaviour forms towards the environment (17,19). The important link of these reactions is releasing the corticoliberin (CRF) and ACTH which activates the adrenal gland directly and through the corticotrophin's catabolites shows the central and peripheral biological activity (4,10). There are many evidences of the fact that the ACTH secretion is also stimulated by many other substances: NA, A (9), β-adrenergic receptors, among the others, which are localised in the anterior lobe of the hypophysis (8). It was proved that the ACTH-derived fragments influence or modify different forms of the animal behaviour (12). The hypophysis peptides are supposed to play an important role in shaping and preserving the learned model of the animal behaviour (17). The ventromedial nucleus of the hypophysis (Vmh) has the particular meaning for the behaviour mechanisms. It was noted that the electric stimulation of Vmh caused the behavioural stress reaction in the rat; the releasing of the affective aggression was also observed (3). The present study has been carried out in order to investigate the influence of the ACTH 4-10 fragment on the behaviour of rabbits in the spontaneous conditions and acute stress,

which has been evoked by the electrical stimulation of Vmh and its influence on the changes of the latency of the escape reactions.

MATERIALS AND METHODS

A n i m a l s. The experiments were conducted on 26 male rabbits (chinchilla breed) of 2900-3500g body weight. The animals were kept under the standard laboratory conditions (temp. 20±2°C) with free access to water and food. There were 8 rabbits in each experimental group and 5 animals in the control one.

S u b s t a n c e s. The following chemical compounds were used in the experiments: the ACTH 4-10 fragment with the Met-Glu-His-Phe-Arg-Trp-Gly (Sigma) aminoacid sequence, 1% Polocain (Polfa). The ACTH 4 -10 was administered into the lateral cerebral ventricle (icv) in dose of 0.5nmol/kg b.w. of a rabbit.

M e t h o d s. The local anaesthesia was administered by subcutaneous injection of 10 ml of 1% Polocain into the frontoparietal area of the head. After uncovering the tectum of the cranium the position of the cannula, used for the electrode for Vmh insertion, was stated in accordance with the co-ordinates in the stereotactic atlas (Fifkova E. 1960. Cvietkova I.P. 1987): AP-1 (1 mm backwards from the bregma point), L-1 (1 mm laterally from the saggittal suture), V-15.5-16 (15.5-16 mm below the external cranium surface). The position of the second cannula used for the chemical substance administration (ivc) was demarcated in accordance with the coordinates: AP-1.5, L-2, V-7. The bipolar, chromo-niccolite electrode was inserted through the first cannula. The electric stimmulation of Vmh was performed by current of 100 Hz frequency, 0.3 ms the impulse width and 3-6 V voltage according to the excitability of the centre.

R e g i s t r a t i o n o f b e h a v i o u r o f t h e r a b b i t i n t h e s p o n t a n e o u s c o n d i t i o n s. The spontaneous conditions consisted in the standard laboratory environment for the rabbits, without the influence of the stress in this experimental model. The 3-hour observation period was divided into 10 minutes' time intervals. The behavioral structure of each animal was divided into the following phases: tension, orientation-searching reactions, comfort, grooming, aggression, eating and drinking. The duration of each phase was measured in the particular time intervals. The following stage of the experiment was performed after 24 hours. After 1-hour adaptation the examined substance was injected into the icv the of rabbit. The animal behaviour was observed through the 3-hour period according to the principles stated above, after the administration of the examined substance into icv. In the control group the solvent was administered into icv.

R e g i s t r a t i o n o f b e h a v i o u r d u r i n g t h e a c u t e s t r e s s, t h a t i s a f t e r t h e V m h s t i m u l a t i o n. The rabbit behaviour was observed according to the principles described above. Additionally, we evoked the escape reactions by the electrical stimulation of Vmh. The stimulation was performed every 10 min at the beginning of each 10 minutes' time interval within 3 hours. In the following stage, the next day, the substance examined was administered to icv and the rabbit behaviour was registered under the conditions of the acute stress as well as the latency period of the escape phase. The control group was given the solvent into icv.

C h a r a c t e r i s t i c s o f b e h a v i o u r p h a s e s. The tension phase was manifested by the immobility of the rabbit, the important increase of the tension of dorsum and limb skeletal musc-

les, the acceleration of the breathing, the frequent micturition and defecation. The orientation-searching phase was the increased motor activity with the cognitive aim, searching movements, environment examining mevements and smelling the cage. The comfort phase – the relaxation of the animal, very often somnolence, the decrease of the muscle tension and the decreased reactivity for the external stimuli. The grooming phase – the nursing activities: the paws and trunk licking, in that phase the animal was completely relaxed, calm, and assuming any body arrangement. The aggression phase – these are the aggressive reactions towards the environment like throwing food out of the container, spilling water, the typical hind paws striking the ground. The food phase was eating food and the coprophagy. The drinking phase was the free quenching their thirst. The escape phase was characterised as the motor reaction-taking place immediately after the Vmh stimulation. It was the sudden turn of the animal with the jumping from the cage trial, the hind paws stamping on the ground with the breathing acceleration and the increase of the muscle tension. The latency period of the appearance of the escape phase was the period from the moment of the Vmh stimulation beginning until the motor phase appearance. Immediately after the experiments were over the rabbits brains were subjected to micro- and macroscopic verification. The correctness of the electrodes location and the administration of the substances examined were evaluated.

Statistic analysis. The numerical data obtained were analysed statistically. It was checked if the differences between the control groups and the ones examined under the spontaneous conditions and the conditions of the acute stress exist and the following data were calculated: the arithmetic means (±SE), the standard deviations (SD) and the correlation coefficients. For the evaluation of the importance of the differences of the means the following statistic tests were used: Cochrane-Cox test, Wilcoxon test, the dignities sum test. The numerical data for the latency period of the escape phase were elaborated using t-Student test.

RESULTS

The evaluation of the changes of the rabbit behaviour influenced by the ACTH 4 -10 fragment administered into icv under the spontaneous conditions and the conditions of the Vmh electric stimulation in 18 ten minutes' intervals and 6 thirty minutes' ones was made.

The changes of the animal behaviour pattern influenced by the ACTH 4-10 fragment in the spontaneous conditions. Under the influence of the ACTH 4 -10 the tension phase was shortened during the whole observation period of the rabbit behaviour. The duration of this phase decreased to 3.2% as compared to groups of animals before the administration of the ACTH 4 -10 (i.e. to the control group) and this change was statistically significant. The orientation-searching phase was also shortened by 73.9% with reference to control group. The significance of these changes was noted through the whole experiment except the fourth 30 minutes' interval during the second hour of the experiment. The duration of the comfort phase was

also extended but there were no statistically significant changes found and the increase reached the magnitude of 142.7% as compared to the control group. The grooming phase was about 3-times lengthened (to 335.2%) under the influence of the oligopeptide in relation to control group and the statistic significant was noted in the following time intervals of the behaviour observation: 0-30'; 60-90'; 90-120'. The aggression and the eating and drinking phases were shortened but did not show any statistically significant differences during the period of the experiment (Fig 1). The solvent administration to icv did not change the animal behaviour pattern in these conditions.

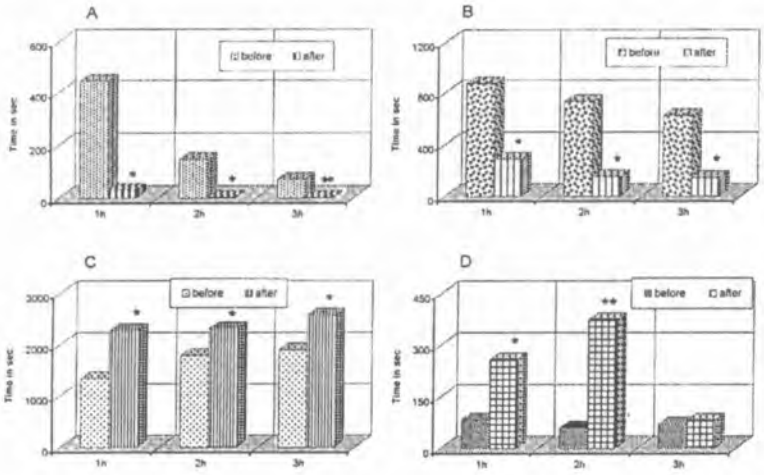

Fig. 1. The change of the behaviour structure in rabbits before and after the icv administration of the ACTH 4-10 fragment in the spontaneous conditions. Statistically significiant differences between the mean values: * $p<0.05$; ** $p<0.01$; A – tension phase; B – orientation-searching phase; C – comfort phase; D – grooming phase

The changes of the animal behaviour pattern influenced by the ACTH 4-10 fragment in the Vmh electric stimulation conditions. The administration of the ACTH 4 -10 in the Vmh electric stimulation conditions shortened the tension phase to 10.6%. The largest shortening of that phase was noted within the first hour of the experiment; in the second and in the third one the decrease was considerably lower but also statistically significant. The orientation-searching reactions of the animals in the examined groups of animals were weakened to 36.5% comparing to the control group and except the 0-30 minutes' time interval of the first hour these changes were not statistically significant. Under the influence of the acute stress and the oligopeptide the comfort phase was lengthened, which makes 152.6% comparing to the control group and this change was statistically significant through the whole

observation period except the 0-30' time interval of the first hour of the experiment. The grooming phase was over 5-times lengthened (to 595.2%) as compared to the control group. The aggression phase influenced by the Vmh stimulation was shortened and the differences between the groups were not statistically significant similar to the changes in the eating and drinking phase (Fig 2). Administration of the solvent to *icv* did not change the animal behaviour in the acute stress conditions.

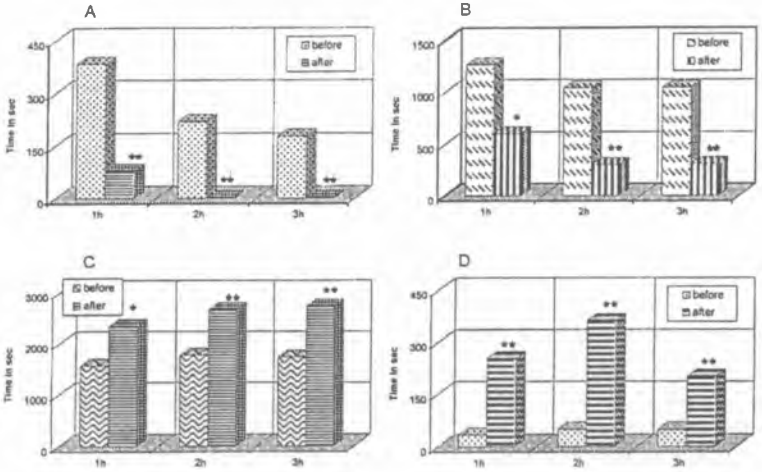

Fig. 2. The change of the behaviour structure in rabbits before and after the ACTH 4-10 fragment administration of the lateral ventricle of the brain in the acute stress conditions. Statistically significiant differences between the mean values: * $p<0.05$; ** $p<0.01$; A – tension phase; B – orientation-searching phase; C – comfort phase; D – grooming phase

The ACTH 4-10 fragment action profile during the 3-hour observation of the animal behaviour in both experimental models. In the spontaneous conditions the tension phase influenced by the oligopeptide was significantly shortened from 37.4±9.8 s to 1.2±0.1s comparing to the control group ($p<0.05$). The orientation-searching reactions were weakened significantly from 123.6±6.6s to 32.3±4.7s in relation to the control group ($p<0.05$). The duration of the comfort phase was lengthened from 279.6±17.8s to 399.0±18.5s ($p<0.05$) similar to the grooming phase: from 11.8±2.6s to 39.7±6.0s comparing to the control group ($p<0.05$). The aggression phase totally disappeared ($p<0.05$) and the eating and drinking phases were shortened from 116.1±10.0s to 107.3±15.4s and from 29.3±3.3s to 20.6±2.4s respectively, comparing to the control group and changes were not significant (Fig. 3). In the acute stress conditions the tension phase under the influence of ACTH 4-10 was significantly shortened from 43.3±4.5s to 4.6±2.9s comparing to the

control group ($p<0.05$) similar to the orientation searching reactions phase from 185.8±8.5s to 67.9±10.1s ($p<0.05$). On the other hand, the comfort phase was significantly lengthened from 281.5±11.2s to 429.8±17.8s and the grooming phase from 7.4±0.9s to 44.0±5.1s as compared to the control group ($p<0.05$). The

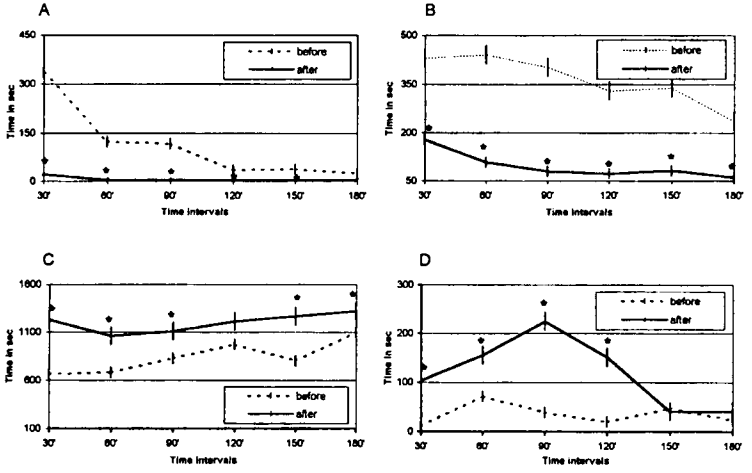

Fig. 3. The changes of the particular phases in the rabbit behaviour general model in the spontaneous conditions before and after the ACTH 4-10 fragment administration to the *icv* during the 3-hour observation period. The statistic importance for the differences between the mean values: $p<0.05$; A – tension phase; B – orientation-searching phase; C – comfort phase; D – grooming phase

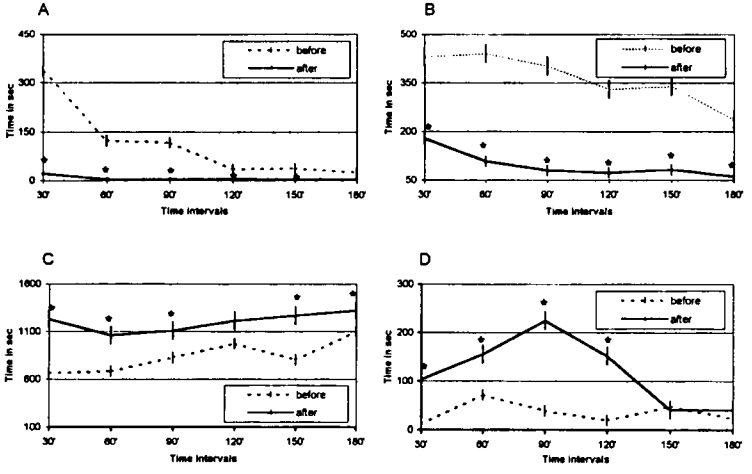

Fig. 4. The changes of the particular phases in the rabbit behaviour general model in the acute stress conditions before and after the ACTH 4-10 fragment administration to the icv during the 3-hour observation period. The statistic importance for the differences between the mean values: $p<0.05$; A – tension phase; B – orientation-searching phase; C – comfort phase; D – grooming phase

aggression phase was completely restrained from 0.6±0.2s to 0.0s and the change was statistically significant ($p<0.05$). The eating and drinking phase was shortened and it was of no statistic importance (Fig 4).

The changes of the latency of the escape reaction under the influence of the oligopeptide in the acute stress conditions. After ACTH 4 -10 administration the latency time of the escape phase after each of 18 stimulations was lengthened in a significant way through the period of 3-hour observation of the animal behaviour. On the average, the latency time of 18 stimulations before the administration of the substance was 20.8±0.9s and 38.5±1.8s after the administration of the oligopeptide so the ACTH 4 -10 administration caused 1.5-times statistically important lengthening of the latency time ($p<0.01$).

DISCUSSION

Many experimental studies show the neurotransmitters role including the ACTH-derived fragments, in modifying the behavioural effects which are especially under the stress conditions (6,21). The ACTH 4 -10 fragment is the shortest oligopeptide which shows strong behavioural activity that can be compared with the mother ACTH molecule strength while the ACTH 4 -7 tetrapeptide sequence of this molecule contains the essential elements which determines this activity (19). The ACTH 4-10 behavioural action seems to be a part of the aminoacid sequence 4 -7, which is regarded as a piece entering the action (17). The ACTH derivatives, including the 4 -10 fragment, can be directly involved in creating and keeping the new model of the animal behaviour or they may undertake the enzymatic degradation in the peripheral blood and the new risen elements, after entering the brain through the blood/brain barrier, can influence the structures of the central nervous system (17,19). They may also be the hypophysis hormones precursors or the units created as ones in the gland and liberated as a response to stress and this is related to shaping the new model of the animal behaviour (17,19). ACTH derivatives not only completed the handicapped rat behaviour after hypophisectomy but they also seemed to have influence on creation and preserving the conditioned avoidance reaction of healthy animals which was acquired earlier in time (19). Many other consequences of the corticotropine analogues and fragments of behavioral action were noted and demonstrated (7, 13, 16). The ACTH fragment makes it easier to gain the acquired experience during the appetitive task, it intensifies memory, most probably by

acting on the nervous cells causing their stimulation, the proteins synthesis and modifying synaptic transmission The ACTH 4-9 and ACTH 4-10 have both the influence on the motivate involvement in the animal self-stimulation (17). The ACTH 4-9 analogue (HOE427) which is the strongest corticotropine behavioural derivative is not endocrinologically active. When administered it caused the paraendocrinic behavioral effects: it lengthened the period of the latent narcosis, produced the effects (signs) of the increase in the general activity, strengthened the behavioural reactions of the passive avoidance in small doses and weakened them in larger ones (20). In human it facilitates the selective visual attention, in animals it influences the change of the theta waves, which are recorded in the septum, the hippocamp and the thalamus of the central nervous system rhythm (17). The place of the behavioral action of the ACTH analogues is in the intracerebral structures of the limbic system (17). The diencephalic area and especially the parafascicular nucleus is also of the essential importance for the ACTH 4 -10 analogues behavioral effects (17,19). In our own experiments we showed that ACTH 4 -10 caused the intensification of grooming in rabbit both in the spontaneous conditions and in conditions of the stress. The ACTH 4 -10 administered to icv caused the stretching and yawning syndrome (SYS syndrome) which was followed by the full picture of grooming in animals (17). The similar phenomena could also be observed under the influence of ACTH 4 -7 and 1-24 (17). Many authors suppose that the grooming genesis mechanism should be explained as the activating ACTH effect on the central dopaminergic and GABA-ergic systems (11,13). Van Erp et al. (17) pointed to the area around the paraventricular nucleus of the thalamus which participates, at that level of the central nervous system, in the organisation of the particular type of behaviour formation, integrating it in one model of the animal behaviour. Some suspect the participation of the muscarin receptors and the cholinergic neurones (5). In rabbits the Vmh plays very important role in the mechanisms activating the escape reaction caused by the electric stimulation (20). In rats the stimulation of Vmh causes the stereotypic prolonged motor reaction (running). In our experiments ACTH 4-10 shortened the duration of the orientation-searching reactions and weakened the animals interest in the external stimuli under the acute stress and the spontaneous conditions. This observation is in accord with data showing not only the decrease in the orientation-searching reactions but also the decrease in the interpersonal interactions influenced by ACTH 4-10, D-Phe-ACTH 4-10, ACTH 1-24 and they also reduce the observation time in the "open field" test and in some new experimental conditions (16). De Wied et al. (19) presented the modified analogues: the heptapeptide 7-D-Phe-ACTH 4-10 and the tetrapep-

tide 7-D-Pe-ACTH 4-7 showing their inhibitory influence on the passive and active escape reaction and the biological action strength showed to be the same. The experiments on ACTH 4-10 proved that the oligopeptide also accelerated the beginning of the adaptative changes. We noted the significant extension of the comfort phase under the influence of ACTH 4-10, which showed much stronger effect under the acute stress conditions than in the spontaneous ones. It seems that in the mechanism of the stress influence the increased ACTH production and liberation into the blood plays the important role; ACTH itself stimulates the neurocytes which synthesise noradrenaline and it also accelerates the adaptative processes in the central adrenergic and/or dopaminergic receptors (6,13). The experiments on the structure and biotransformation of the thalamic and the hypophyseal hormones proved that these peptides as the neuropeptide precursory molecules make up the group of the compounds which have properties similar to those of the psychostimulating and the neuroleptogenic drugs (18). The ACTH 4-10 fragment in the stress conditions shows the strong effect on the lowering of the tension and the almost complete aggression extinction in animals. The ACTH 4-10 administration to icv lowers the active zoosocial effect in rat, inhibits the aggression and interpersonal communication. It is suspected that the lowering of the animals aggression can be conditioned by the direct central effect of the ACTH 4-10 action. Adamec et al. (11) showed the great importance of the Vmh, the amygdala and the hippocampus in the aggressive and defensive behaviour of the cat. The experiments on the ACTH biotransformation in the brain synaptosomes prove that ACTH is the precursor of the second-order neuropeptides which are involved in the adaptative processes, the ones connected with concentration, motivation, insomnia, learning and preserving the animal behaviour model (18). Many peptides in the central nervous system which are considered as ones which play the role of neurotransmitters or neuromodulators can be involved in the regulation of many homeostatic system. The ACTH fragment through the mediation of the dopaminergic system causes the biochemical changes in the brain, which accelerate the development of the adaptative changes in the area of the limbic system of the mesencephalon (15). On the grounds of these facts Volosin et al. (15) suggest that the specific effects of the ACTH depend on its central activity, which agrees with the results of our experiments and with the adrenal cortex glikocorticoides secondarily liberated.

CONCLUSIONS

1. The ACTH hormone derivative fragment 4-10 administered to *icv* exerts significant influence on the animal behaviour in the spontaneous conditions and under the acute stress conditions.

2. The ACTH 4-10 fragment causes the aggression extinction, significant inhibition of the tension and the orientation-searching reactions and the significant prolongation of the duration of the grooming and comfort phases in the animal behaviour general structure. That is the influence of the short peptide on the extension of the emotionally positive animal reactions.

3. The strongest and most significant ACTH 4-10 influence on the animal behaviour was noted during the first hour of the experiment, which occurred during the period of the peptide activity.

4. The ACTH 4-10 fragment significantly prolongs the latency of the escape reaction under the acute stress conditions.

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STRESZCZENIE

Badano wpływ fragmentu 4-10 pochodnego ACTH na zachowanie królików w warunkach spontanicznych i ostrego stresu, czyli w warunkach elektrycznej stymulacji jądra brzuszno-przyśrodkowego podwzgórza, oraz jego wpływ na czas latencji reakcji ucieczki zwierząt. W toku badań okazało się, że fragment ACTH 4-10 podany *icv* wywierał istotny wpływ na zachowanie zwierząt w obu przyjętych modelach doświadczalnych: tłumił fazę naprężenia i agresji, a wydłużał czas fazy grumingu i komfortu oraz znamiennie wydłużał latencję reakcji ucieczki w warunkach ostrego stresu.