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The influence of the analogue 4-10 corticotropin derivative on the general structure of the rabbits behaviour in stress

Wpływ analogu 4-10 pochodnego kortykotropiny na ogólną strukturę zachowania królików w stresie

Neuroendocrynic system plays a significant role in modification of the psychological model of reaction to distinct functional changes in rabbits behaviour and stress is the cause of many significant changes in structures and functions of CNS (1,4). Hypophysis synthesises peptides which play the role of neuropeptides that are physiologically engaged in producing and maintaining new patterns of animals behaviour (13). Corticotropin is not only a peculiar agonist of adrenal receptors, it has also induced other biological effects in the organism in a wider aspect. When administered into the lateral cerebral ventricle (icv) it significantly lengthens grooming and it may act as the mediator in CNS; in hepatocytes it intensifies the speed of corticosteroids disintegration, it influences the memory and learning processes and significantly improves selectivity of attention and causes anorexia. So far there have been reported many other effects of behavioural reaction of fragments and analogues of corticotropin which concern: modulation of reverse memory when performing a complex task; making regaining memory easy after amnesia caused by electroshock therapy (11) and making sexual motivation easy and delaying cessation of approach behaviour as well as the processes of learning and memory (11,13). The present study has been carried out in order to investigate the influence of the analogue 4-10 of corticotropin, with modified amino acid sequence, on the behav-

iour of animals in the acute stress, which has been evoked by the electrical stimulation of the ventromedial nucleus of the hypothalamus.

MATERIAL AND METHODS

A. Animals. 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 eight rabbits in each experimental group and five animals in the control one.

B. Substances. The following chemical compounds were used in the experiments: the analogue 4-10 of corticotropin with the Met-Glu-His-Phe-Pro-Gly-Pro amino acid sequence, 1% Polocain (Polfa). The analogue 4-10 was administered into the lateral cerebral ventricle (icv) in the dose of 0.5 nmol/kg body weight of a rabbit.

C. Methods. 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 located in accordance with the co-ordinates in the stereotactic atlas (Fifkova D. 1960, Cvietkova I.P. 1987): AP-1 (1 mm backwards from the bregma point), L-1 (1 mm laterally from the sagittal 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 (icv) 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 stimulation of Vmh was performed by current of 100 Hz frequency, 0.3 ms the impulse width and 3-6V voltage according to the excitability of the centre. The control group was given the solvent into icv.

D. The recording of the behaviour during the acute stress that is after the Vmh stimulation. 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-minute 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. There was also investigated the latency period of the escape phase. The control group was given the solvent into icv.

E. Characteristics of behaviour phases. The tension phase was manifested by the immobility of the rabbit, the important increase of the tension of dorsum and limb skeletal muscles, 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 movements and smelling the cage. The comfort phase - the relaxation of the animal, very often somnolence, the de-

crease of the muscle tension and the decreased reactivity to the external stimuli. The grooming phase - the nursing activities: the paws and trunk licking; in that phase the animal was completely relaxed, calm, and not assuming any body arrangement. The aggression phase - these are the aggressive reactions to the environment like throwing food out of the container, spilling water, the typical hind paws striking the ground. The eating 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 which occurred immediately after the Vmh stimulation. It was a 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 animals were sacrificed by the lethal dose of the anaesthetic). The correctness of the electrodes location and the administration of the substances examined were evaluated.

F. 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 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 significant 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 animals behaviour influenced by the analogue 4-10 of corticotropin administered into icv under acute stress (i.e. electrostimulation of the ventromedial nucleus of the hypothalamus - Vmh) during eighteen 10-minute intervals and six 30-minute intervals, has been made.

THE CHANGES OF THE ANIMAL BEHAVIOUR STRUCTURE DURING ACUTE STRESS UNDER THE INFLUENCE OF CORTICOTROPIN ANALOGUE 4-10.

The tension phase in these experimental conditions was decreased to 35.47% at the period of registering the behaviour comparing to the group of animals before administration of the preparation ($p<0.05$). The duration of orientation-searching reaction was decreased to 70.75% in relation to the group before administration of the preparation. The comfort phase for the studied groups was slightly extended and it made up insignificant

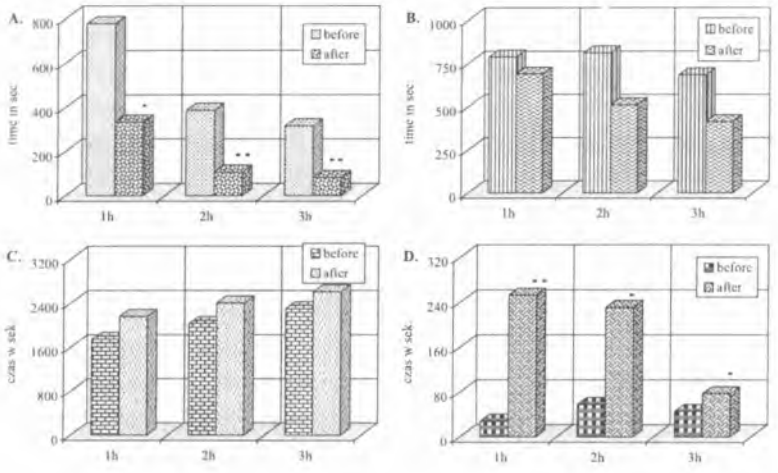

Fig. 1. The change in behaviour structure in animals before and after the icv administration of analogue 4-10 of corticotropin in acute stress conditions. The statistic importance for the differences between mean values: * - $p<0.05$,** - $p<0.01$. A - tension phase; B - orientation-searching phase; C - comfort phase; D - grooming phase

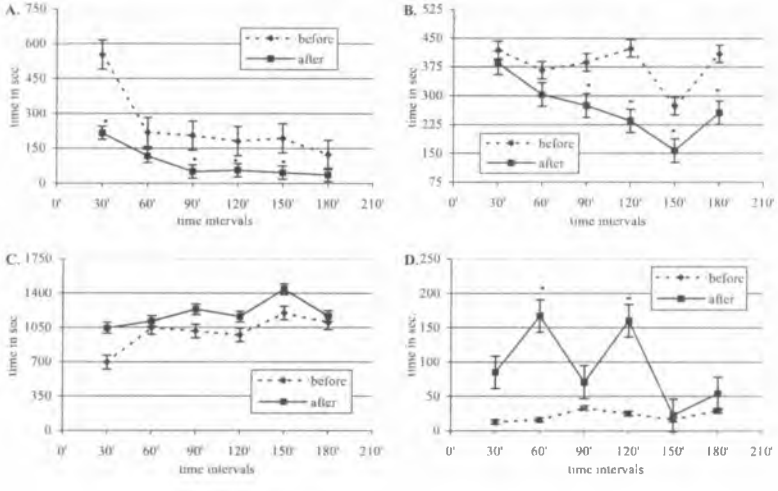

Fig. 2. The profile in changes of the particular phases in animals behaviour in acute stress conditions before and after the analogue 4-10 corticotropin derivatives administration to the icv during 3 hours of observation period. The statistic importance for the differences between the mean values: * - $p<0.05$. A - tension phase; B - orientation--searching phase; C - comfort phase; D - grooming phase

increase to 118.77%, and the grooming phase was increased by four times up to 424.13% in relation to groups before administration of the preparation ($p<0.05$). The time of aggression phase was reduced to zero and the eating phase was slightly extended and was equal to 118.36%, similarly as the drinking phase to 124.65% in relation to the group of animals before administration of the preparation. A comparative analysis concerning the influence of the analogue 4-10 of corticotropin in the control group of animals under acute stress was made. The tension phase was slightly decreased by 15.22%, similarly as the orientation-searching reactions, and was equal to 34.67% comparing to the control group. The comfort phase in the structure of behaviour was considerably increased to 158.52% comparing to control group. The change of duration of this phase is statistically significant within the limits 30-60' and 120-150' of the total period of observing the behaviour ($p<0.05$). The grooming phase was decreased by 25.12% comparing to control group and the significance was noted within the limit 120-150' ($p<0.05$). The period of aggression phase was almost doubled to the value 193.75% comparing to the control group ($p<0.05$). The eating phase was significantly decreased to 38.42% and was significant within the time limits: 0-30', 30-60', 90-120' of the whole period of observation ($p< 0.05$), and the drinking phase was decreased by 62.30% comparing to control group (Fig. 1).

THE CHANGES OF THE LATENCY TIME OF ESCAPE REACTION UNDER THE INFLUENCE OF THE CORTICOTROPIN ANALOGUE 4-10 IN THE ACUTE STRESS

Latency time of the escape reaction under the influence of the analogue 4-10 was extended 3.5 times from 14.77 ± 0.17s to 51.41 ± 0.67s, and it was significant comparing to the group before administration of the preparation ($p<0.01$). The analysis of latency time as compared to control under the influence of the analogue was increased from 14.26 ± 0.94s to 51.41 ± 10.67s, and the increase was by 260.51% comparing to control ($p<0.01$).

THE ANALOGUE 4-10 OF CORTICOTROPIN ACTION PROFILE DURING 3-HOUR OBSERVATION OF THE ANIMAL BEHAVIOUR IN THE ACUTE STRESS CONDITIONS

The tension phase under the influence of the analogue 4-10 was decreased by 64.54% ($p<0.05$), similarly as orientation-searching reactions phase by 29.25% comparing to the group of animals before administration of the preparation ($p<0.05$). However, the comfort phase was considerably extended by 18.76% ($p<0.05$), and the grooming phase was three times extended (by 324.01%) comparing to the group before administration of the preparation. ($p<0.05$). The aggression phase was almost entirely inhibited because it was decreased by 97.42% ($p<0.05$). The eating phase was slightly increased by the analogue

by 18.34%, similarly as the drinking phase by 24.70% comparing to the animals before administration of the preparation. The activity of the analogue 4-10 was compared with reference to the control group. The analogue 4-10 caused the decrease of tension phase by 15.23%, similarly as the orientation-searching reaction phase by 34.66% comparing to the control. The comfort phase was increased by 58.51% ($p<0.05$), and the grooming phase was increased by 25.11% comparing to the control. The aggression phase was decreased by 83.33% ($p<0.05$), similarly as eating and drinking phases respectively by 61.58% and 62.29% comparing to the control ($p<0.05$; Fig. 2).

DISCUSSION

Corticotropin as a fragment of precursor particle POMC, widely common in brain, is the source of behaviourally active neuropeptides. Biotransformation of POMC chain which takes place in the pituitary gland is different from the parallel process in the brain, and the location of corticotropin release may ultimately define its function as a hormone or a neurotransmitter in CNS. The studies of the corticotropin structure in cerebral synaptosomes confirm the hypothesis that cerebral corticotropin is the secondary precursor of neuropeptides that modify animals adaptive behaviour (12). Many peptides in CNS have been described in vertebrates, which are considered to play the role of neurotransmitters or neuromodulators, and they seem to be engaged in the regulation of many homeostatic systems of the organism. In contemporary research works there are used fragments and analogues of corticotropin in order to trace the changes they induce in animals. It has been proved that corticotropin has its total bioactivity in sequence 1-24, whereas the corticotropic properties are in 11-24 chain and the neurotrophic properties are in 1-10, 4-10, 1-13 fragments (15). Corticotropin fragments when administered into cerebrospinal fluid of various rodents caused the behaviour that was characterised by excessive grooming and intensified sexual behaviour. Attempts at application of corticotropin derivatives in the form of fragments or analogues in pharmacotherapy have been made. The corticotropin analogue 4-9 (ORG2766) applied in autistic children caused a visible positive clinical reaction (3). Attella et al. (2) indicated that the analogue 4-10 when administered to rats with frontocortical defects, shortened the time of finding the platform in the Morris labyrinth and decreased the number of attempts at reaching the goal. The effects of such activity were long-lasting as the rats after a month from the time of the last administration of the preparation still proved to be better and more effective in performing their tests in the labyrinth. This improvement of memory is connected with direct reaction of the analogue to the neurones, causing acceleration of proteins synthesis and modification of synaptic transmission of neurones; interaction with serotonin synthesis and metabolism may play some role here as well. In rats under the influence of 4-10 chain the increase of latency time of avoidance reaction was noted (10) and the experiments proved that the oligopeptide also accelerated the initiation of adap-

tation changes (9). The analogue 4-9 (HOE27), corticotropin derivative with the strongest behavioral influence, without endocrine activity, when administered intravenously, caused extension of latency period, decreased slow waves of dream during the first three hours, caused symptoms of global increase of activity (8), it made recovery quicker in animals with slight defects of the central or peripheral nervous system (5,6,15). Chronic administration of analogue 4-9 (ORG2766) analysed in time of experiments, confirmed that after 2-month therapy it improved memory in old rats and the observed effects of this analogue activity have a long-lasting character (7). They may be engaged in creation and maintenance of a new model of animals' behaviour or they may be products of enzymatic disintegration of polypeptides of hypophysis in peripheral blood that after entering the brain, influence its central target structures. The location of behavioural activity of corticotropin analogues is in the mesencephalic centres of the limbic system (11). Biochemical tests indicate the mechanism of corticotropin analogues on the level of cell membranes of mesencephalic structures, causing passage of nervous impulses, and this increases the likelihood of specific reactions generation that would be adequate to the definite stimulus (11,13). The influence of corticotropin analogues on the conditions of reaction of the escape has a short-term character and through stimulation of cyclic AMP and synthesis of some proteins, it makes creation of new patterns of behaviour easier (13). Wolterink et al. (14) pointed to the dual activity of analogue 4-9, which presumably fixes to the structure of its particle, i.e. the terminal N-final part of the chain caused effects making behaviour reaction easier and C-final part of hexapeptide included information for the suppression effect; similar remarks may concern the analogue 4-10 of which the C-final fragment suppresses behavioural activity. De Wied et al. (13) presented modified analogues: heptapeptide 7-D-Phe-ACTH 4-10 and tetrapeptide 7-D-Phe--ACTH 4-7 by showing their inhibitory influence on both passive and active reaction of escape, and their pharmacological action turned out to be the same. Modification of particle of corticotropin analogue 4-10 produced an oligopeptide that is less susceptible to enzymatic degradation and therefore may have a different influence on the behavioural changes (14). The analogue 4-10 decreased tension and calmed the aggression phase slightly less than the fragment 4-10 without sequence modification and was clearer when the animals were under acute stress. Similar observations may be ascribed to the corticotropin analogue 4-10 in which modified C-final particle probably suppresses the behavioural activity. The analogue 4-10 when administered icv, caused a considerable intensification of grooming in rats. This analogue caused the decrease of duration of observation-searching reactions of rabbits' interest in external stimuli under acute stress. This complies with the reports from other centres which indicate the decrease not only of observations with reference to another animal under the influence of oligopeptides (fragments 4-10, 1-24, D-Phe-ACTH 4-10); they also reduce observation time in open field test and in new experimental conditions. In our experiments we noted a considerable extension of comfort phase under the influence of the analogue 4-10, which has a considerably stronger effect in acute stress than in the conditions of elimination of any stressors.

It seems that in the mechanism of active influence of stress, there is a great role of increased production and release of ACTH into blood which itself stimulates neurocytes synthesising noradrenaline and simultaneously accelerates adaptation processes in central adrenergic and/or dopaminergic receptors (9). It is very difficult to evaluate the influence of analogue 4-10 with reference to hunger and thirst, and our results prove that the analogue showed ambivalent influence on these phases in the structure of rabbit behaviour. Some compulsion of licking the utensils during eating and drinking water was noted, which was observed also in the present experiments. Data concerning latency time of the escape reactions in which the analogue 4-10 showed a strong influence on the increase of the period of latent stimulation of Vmh, are interesting and this complies with the results of other centres (10,11).

CONCLUSIONS

1. The analogue 4-10 of corticotropin with modified amino acid sequence when administered icv, influences significantly the behaviour of animals under acute stress.

2. The analogue 4-10 suppressed aggression and significantly reduced tensions and orientation-searching reactions as well as extended considerably grooming and comfort phases in the structure of the animals behaviour. It is the influence of the analogue 4-10 of corticotropin on significant reduction of the reactions which are stressogenic for animals.

3. The strongest and most important influence of the analogue 4-10 on the behaviour was noted during the first hour of observation of the behaviour, which falls on the period of alarm reaction to stress, whereas later there develops a period of animals adaptation. Later changes probably result from the activity of products of hydrolytic disintegration of the analogue 4-10.

4. The analogue 4-10 of corticotropin derivative causes the significant extension of latency time of the escape reaction in the acute stress conditions.

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STRESZCZENIE

Zbadano wpływ analogu 4-10 pochodnego kortykotropiny na zachowanie zwierząt w warunkach ostrego stresu oraz w warunkach spontanicznych, a także oddziaływanie analogu 4-10 na czas trwania latencji reakcji ucieczki. Stres wywoływano poprzez elektryczną stymulację jądra brzuszno-przyśrodkowego podwzgórza. W toku analizy okazało się, że analog 4-10 podany icv miał wpływ na zmianę ogólnej struktury zachowania w obu przyjętych modelach doświadczalnych. Analog 4-10 tłumił agresję, znamiennie skracał fazę naprężenia i reakcje orientacyjno-poszukiwawcze oraz znacząco wydłużał fazę komfortu i reakcje pielęgnacyjne. Analog 4-10 również istotnie wydłużał czas latencji reakcji ucieczki w warunkach ostrego stresu. Świadczy to o znaczącym skracaniu emocjonalnie negatywnych reakcji zwierząt w ogólnej strukturze zachowania, co było szczególnie istotne w pierwszej godzinie dokonywanych obserwacji zachowania.