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Changes in rabbits' behaviour under the influence of corticotropin derivative analogue 4-10 in spontaneous conditions

Precursor molecule POMC localised in CNS, digestive tract and placenta decomposes into shorter peptide chains and becomes a precursor for corticotrophin, b-lipotropine, b-endorphine, g-melanotropine, which act as hormones, neurotransmitters, neuromodulators and mitogenic factors. Corticotrophin molecule gets decomposed into a-melanotropine, shorter peptides 1-22, 1-26 and CLIP and demonstrates multidirectional effects of their activity in the body (3). Many proteins of CNS act as neuropeptides and they seem to be involved in regulation of numerous homeostatic systems of the body (4). The aim of the study was to analyse the influence of the analogue 4-10 of corticotrophin on the rabbits' behaviour in spontaneous conditions.

MATERIAL AND METHODS

A. Animals. The experiments were conducted on 26 male rabbits (chinchilla breed) of 2,900-3,500g 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. B. Substances. The following chemical compounds were used in the experiments: the analogue 4-10 of the corticotrophin with the Met-Glu-His-Phe-Pro-Gly-Pro amino acid sequence, 1% Polocain. The analogue 4-10 was administered into the lateral cerebral ventricle (icv) in dose of 0.5 nmol/kg body weight of a rabbit. C. Methods. The local anaesthetic 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 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. Registration of behaviour of the rabbits in the spontaneous conditions. 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-minute intervals. The behavioural 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 of the 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. E. Characteristics of behaviour phases. The tension phase was manifested by the immobility of the rabbit, the important increase in 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 decrease in 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 – those were the aggressive reactions towards the environment like throwing food out from 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 in 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 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

Changes in the animals behaviour influenced by the analogue 4-10 of corticotrophin administered into icv during eighteen 10-minute intervals and six 30-minute intervals were evaluated.

1. The changes in the animals behaviour structure under the influence of analogue 4-10 corticotrophin in spontaneous conditions. The tension phase was decreased in the studied groups and was equal to 76.45% comparing to the group before administration of the preparation ($p<0.05$). The orientation-searching phase was considerably decreased to the value 34.26% comparing to the group before administration of the analogue ($p<0.05$). In comfort phase the analogue 4-10 caused a considerable increase to 125.54% like in the grooming phase, which was extended by 2.5 times to the value 288.39% comparing to the group before administration of the preparation. In aggression phase a considerable decrease was noted in length by 37.81% comparing to the animals before administration of the analogue. The eating and drinking phases were slightly

decreased comparing to the group before administration of the analogue. In the same experimental conditions the influence of analogue 4-10 in the control group was analysed. The control group was given the solvent into icv. The tension phase was changed and was longer by 9.40% comparing to the control. The orientation-searching reaction phase was decreased only by 3.33%, and the comfort phase was extended by 14.44% comparing to control. The grooming phase was extended to 119.27%, and the aggression phase decreased to 62.98% comparing to control. The eating phase was decreased to 77.34% as was the case with drinking phase by 35.12% comparing to control (Fig. 1).

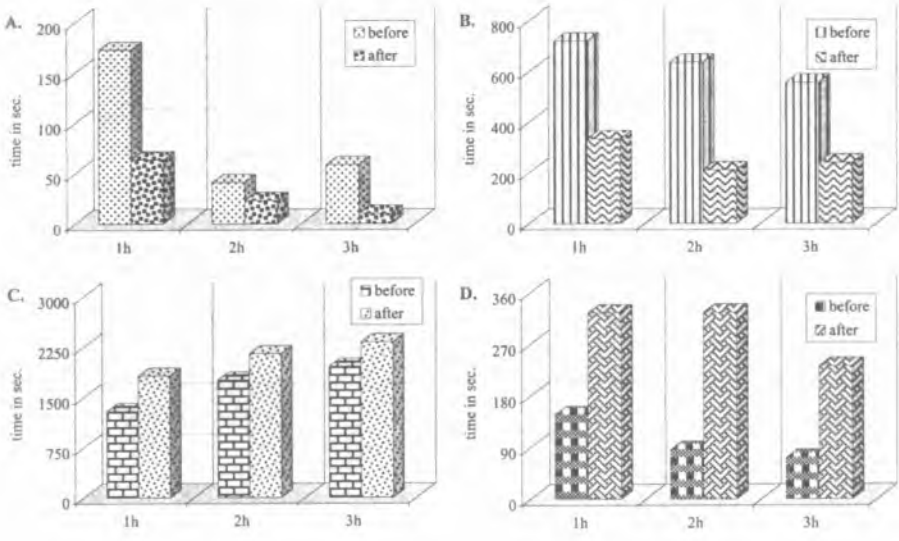

Fig. 1. The change of the behaviour structure in animals before and after the icv administration of analogue 4-10 of the corticotropine in the spontaneous conditions. The statistic importance for the differences between the mean values: * - $p<0.05$,** - $p<0.01$. A - tension phase; B - orienta--tion-searching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

2. The analogue 4-10 of corticotrophin action profile during 3-h our observation of the animal behaviour in the spontaneous conditions. In this experimental model the tension phase under the influence of oligopeptide was decreased by 76.44% comparing to the group of animals before administration of the preparation ($p<0.05$). The orientation-searching reactions were decreased by 65.74% comparing to the group before administration of the preparation ($p<0.05$). The duration of comfort phase was extended by 25.54% ($p<0.05$), similarly as the grooming phase by 118.39% comparing to the group of animals before administration of the preparation ($p<0.05$). The aggression phase was decreased by 37.94%, and the eating and drinking phases were decreased by 2.94% and 12.28%, respectively, in relation to the group of animals before administration of the preparation. In spontaneous conditions the results were compared to control and here the tension phase under the analogue influence was extended by 8.63% and orientation-searching reaction phase was slightly increased by 3.33% comparing to control. The comfort phase was extended by the analogue by 14.44% and the grooming phase was extended by 19.28% comparing to control. The aggression phase was decreased by 37.94% similarly as the eating and drinking phases, respectively, by 22.65% and 35.12% and the changes were not significant (Fig. 2).

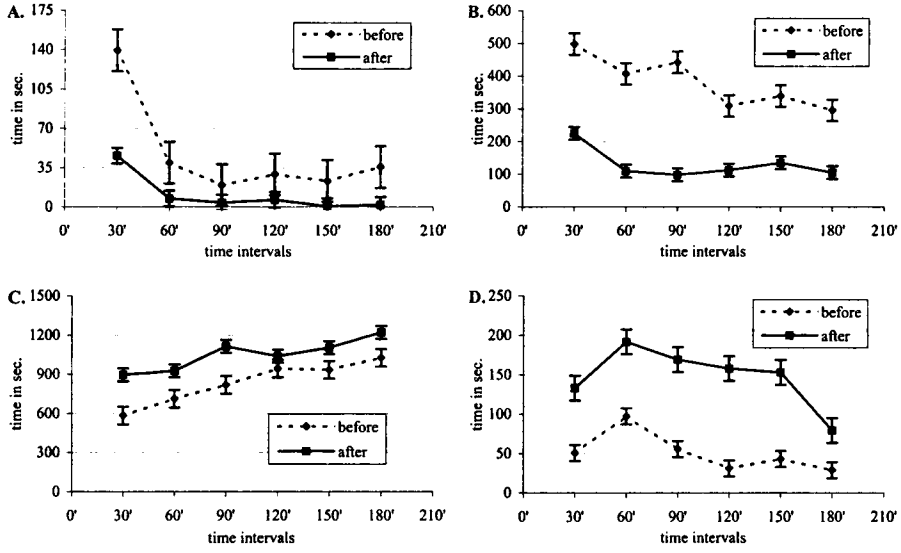

Fig. 2. The profile of the changes of the particular phases in animals behaviour in the spontaneous conditions before and after the analogue 4-10 derivatives of the corticotropine administration to the icv during the 3 hours of observation period. The statistic importance for the differences between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

DISCUSSION

Principal issues for regular functioning of the body are physiological defensive mechanisms which counteract/neutralise various pathogenic factors, and this is a necessary condition for normal metabolic processes in the tissues. The influence of stressors causes the disorder of balance and the release of non-specific reaction of the organism in the form of generalised adaptation syndrome, which in turn, activates the HPA system. Here one can see an important role of hypophysial peptides in formation and maintenance of learned model of animals' behaviour (11,13). The impaired behaviour of rats after hypophysectomy was considerably improved by using corticotrophin or its derivatives 4-10, a-MSH and b-MSH (11,13). Short peptides of corticotrophin additionally influenced the maintenance of earlier learned conditional reaction of avoidance in sick animals (13). The peptides released from the hypophysis act as neuropeptides that are involved in learning and maintaining new patterns of behaviour (5,13). Many effects of corticotrophin derivatives activity were noted: modulation of reverse memory, influence on the sexual behaviour, influence on the memorisation and learning processes, improvement of attention selectivity (11). It was confirmed that the fragments 1-24, 1-17, 4-10 of corticotrophin caused the growth and differentiation of astrocytes (15). The attempts at administration of analogue 4-9 (ORG2766) in diabetic neuropathy treatment were made due to its neurotrophic reaction. The derivative 4-10 of corticotrophin meets the minimal requirements that are important for strong behavioural influence with simultaneous marginal trophic activity (13). It is thought that the fragment 4-10 is the active core that is involved in receptors' stimulation of the whole corticotrophin molecule and phenyloalanine plays here a key role (13). The place of behavioural activity of corticotrophin oligopeptides is in mesencephalic of the limbic system (5,11). The influence of corticotrophin analogues on the conditional reaction of avoidance in the sick animals is of short

duration and by the increase of cyclic-AMP level, synthesis of peptides it makes the creation of new patterns of animals' behaviour easier (13). Peptides derived from maternal corticotrophin give various behavioural effects and the majority of these effects seem to be localised in the fragment 4-10. Modification of molecule sequence gave oligopeptides, which are less susceptible to the enzymatic degradation and have a different influence on the changes in rabbits' behaviour (14). W o l t e r i n k and co-workers (14) indicated double activity of the corticotrophin analogue 4-9; the N-terminal part of the analogue chain produced the effect that made the rats' behaviour faster, and the part C-terminal of heptapeptide included information for the inhibitory effect. Similar observations can be assigned to the analogue 4-10 in which the modified C-terminal molecule suppressed behavioural activity in the rabbits. In our studies the analogue 4-10 of corticotrophin when administered icv, led to the decrease of tension, significant attenuation of aggression in the rabbits with simultaneous significant increase of grooming. The analysis of the intensity and duration of 4-10 analogue showed that the effect turned out to be weaker as compared to non-modified heptapeptide molecule. The mechanism of the occurrence of grooming being the activating effect of corticotrophin activity is thought to originate in the central dopaminergic system and GABA-ergic system (1, 8). D e W i e d and co-workers (11) noted that the intensification of grooming in the rabbits was always preceded by SYS syndrome (stretching and yawning syndrome), and it was also observed in our study. V a n E r p et al. (9) indicated the zone around the subthalamic nucleus that participates on this level in the organisation of occurrence of this type of behaviours by integrating it into one pattern of behaviour. Many researchers suggest common participation of muscarine receptors and cholinergic neurons (2). In the present paper the analogue 4-10 of corticotrophin caused shortening of the time of orientation-cognition reaction and the decrease of animals' interest in the stimuli coming from the environment, which is also noted by P o s h i v a l o v (6). In the experiments considerable prolongation of the comfort phase under the influence of analogue 4-10 was observed, however, the effect was weaker from hepapeptide of non-modified fragment of corticotrophin 4-10. The studies on biotransformation of the hypophyseal hormones and hypothalamus indicated that peptides as the precursor molecules of neuropeptides, constitute the generation of compounds with psychostimulatory and neuroleptogenic properties (12). Corticotrophin analogue of sequence H-Met-Glu-His-Phe-D-Lys-Phe-NHCH2-8NH2 has a very strong neuromodulatory activity and passes the blood/brain barrier (7). It is very difficult to evaluate the role of the analogue 4-10 with reference to thirst and hunger. The achieved results indicate the decrease of drinking and eating phases in spontaneous conditions, but this influence was very weak. The compulsion of licking the cages during drinking or eating was observed; it was also noted by W e i j n e n (10). The analysis of results confirms the behavioural activity of hypophyseal oligopeptides, which also have a wider influence on creation and modification of the patterns of behaviour in various species of animals.

CONCLUSIONS

1. Analogue 4-10 of corticotrophin derivative with modified aminoacid sequence influenced significantly the behaviour in spontaneous conditions when was administered icv.

2. Analogue 4-10 caused the suppression of aggression, decreased the tension and orientation-cognition reactions, however, it prolonged the comfort and grooming in the general structure of behaviour. In a way the analogue significantly emotionally increased the positive reactions of animals with simultaneous weakening of stressing reactions.

3. The time of biological reaction of analogue 4-10 of corticotrophin was 1 hour during 3-hour observation of the animals' behaviour.

4. Later changes in the behaviour could result from the reaction of the products of analogue 4-10 of corticotrophin molecule decomposition.

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SUMMARY

The influence and the role of corticotrophin analogue 4-10 on the rabbits' behaviour in spontaneous conditions was analysed. The data analysis indicated that analogue 4-10 with modified aminoacid sequence in 8, 9,10 positions significantly influenced the behavioural changes in the general model of behaviour. The 4-10 corticotrophin derivative caused the suppression of aggression, considerably reduced the tension and orientation-cognition reactions, however, it prolonged comfort and grooming significantly. This may prove the noticeable reaction of analogue 4-10 in modification of behavioural pattern, which was expressed by restraining the reaction of stressogenic character with simultaneous attenuation and prolongation of appetite reactions.

Zmiana zachowania królików pod wpływem analogu 4-10 pochodnego kortykotropiny w warunkach spontanicznych

Przeanalizowano wpływ i rolę analogu 4-10 kortykotropiny na zachowanie królików w warunkach spontanicznych. Podczas analizy statystycznej danych wykazano, że analog 4-10 ze zmodyfikowaną sekwencją aminokwasową w pozycji 8, 9,10 posiadał istotny wpływ na zmiany behawioralne w ogólnym modelu zachowania zwierząt. Analog 4-10 podawano icv tuż po rozpuszczeniu oligopeptydu. Pochodna 4-10 kortykotropiny spowodowała tłumienie agresji, istotnie skracała naprężenie i reakcje orientacyjno-poszukiwawcze, natomiast znacząco wydłużała komfort i reakcje pielęgnacyjne. Świadczyć to może o zauważalnym oddziaływaniu analogu 4-10 w modyfikacji ogólnego wzorca zachowania, który wyrażał się poprzez ograniczenie reakcji o charakterze stresogennym, z równoczesnym wzmacnianiem i wydłużaniem reakcji apetytywnych.