INFLUENCE OF OLIGOPEPTIDES, THE FRAGMENT AND ANALOGUE 4-10 OF CORTICOTROPIN, ON THE BEHAVIOUR OF RABBITS UNDER SPONTANEOUS CONDITIONS

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Received for publication June 21, 2004.

Abstract

Short peptides of corticotropin derivatives, fragment 4-10 and its analogue 4-10, were administered to rabbits into the lateral cerebral ventricle (icv) in a dose of 0.5 nmol/kg b.w. It was proved that the fragment and analogue significantly modified the animals' behaviour. The difference in the activity between the fragment and its analogue was noted. The slighter influence of the analogue was caused by the change of C-terminal fragment of oligopeptide sequence. The peptides significantly prolonged the comfort and grooming time in general structure of the behaviour by intensifying and conditioning the reactions that reduced the influence of stressing factors causing the changes in animal patterns of the behaviour. The tested synthetic tripeptide Pro-Gly-Pro showed a very weak activity and only slightly changed the behaviour profile.

Key words: rabbits, corticotropin, oligopeptides, behaviour, spontaneous conditions.

Corticotropin as the element of precursor particle of proopiomelanocortin (POMC) is widely distributed in the brain and 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 the central nervous system (CNS). Examinations of corticotropin structure in cerebral synaptosomes confirm the hypothesis that cerebral corticotropin is the secondary precursor of neuropeptides that modify animals adaptive behaviour (25). Many peptides in the 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 (13). In experiments, the applied fragments and analogues of corticotropin caused changes in behaviour of various animals. It was confirmed that the fragment 4-10 of corticotropin accelerates adaptation changes (23), the fragment 11-24 has a great similarity to human line of lymphocytes T by influencing the regulation of the immunological system (17), the fragment 4-7 influences the processes of learning and memorisation, similarly as tripeptide Pro-Gly-Pro (14). The present study is focusing on defining the way of influence of short peptides corticotropin derivatives on the behaviour of rabbits under spontaneous conditions, i.e. without the influence of stressing stimuli, as well as on the profile of behavioural activity of individual oligopeptides.

Material and Methods

Animals. The experiments were conducted on 29 male rabbits (chinchilla breed) of 2900-3500 g b.w. The animals were kept under the standard laboratory conditions (temp. 20±2°C) with free access to water and feed. There were 8 rabbits in each experimental group and 5 animals in the control one.

Substances. The following chemical compounds were used in the experiments: the fragment 4-10 of corticotropin with the Met-Glu-His-Phe-Arg-Trp-Gly amino acid sequence, its analogue 4-10 with the Met-Glu-His-Phe-Pro-Gly-Pro sequence, the synthetic tripeptide Pro-Gly-Pro, and 1% Polocain. The fragment 4-10, its analogue, and tripeptide were administered into the lateral cerebral ventricle (icv), and they were dissolved in saline, in a dose of 0.5 nmol/kg body weight.

Methods. The local anaesthesia was performed 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 was located in accordance with the co-ordinates in the
skeletal muscles, acceleration of breathing, and frequent micturition and defecation; orientation-searching phase - increased motor activity with the cognitive aim, searching movements, environment examining movements and sniffing the cage; comfort phase, the relaxation of the animal, - very often somnolence, decrease in the muscle tension and decreased reactivity to external stimuli; grooming phase - body care activity: licking the paws and trunk; complete relaxation of the animal, calm, and assuming any body arrangement; aggression phase - aggressive reactions towards the environment like throwing feed out from the container, spilling water, typical striking the ground with hind paws; eating phase - eating feed and coprophagy; drinking phase - free quenching the thirst.

**Influence of corticotropin derivatives on general structure of rabbits' behaviour.** The tension phase under the influence of fragment 4-10 was decreased by 96.93%, of analogue 4-10 by 76.44% and of tripeptide Pro-Gly-Pro by 40.54% as compared to the group before administering the preparations (P<0.05). The orientation-searching reactions under the influence of fragment 4-10, analogue 4-10, and tripeptide Pro-Gly-Pro were shortened by 73.92%, 65.74% and 42.98%, respectively, with relation to the animals before the administration of the preparations (P<0.05). The comfort phase was prolonged by fragment 4-10 by 42.72%, by analogue 4-10 by 25.54%, and by tripeptide Pro-Gly-Pro by 13.81%, as compared to the animals before administering the preparations (P<0.05). The grooming phase was increased by fragment 4-10 by 235.22% (P<0.05), analogue 4-10 by 188.39% (P<0.05), and tripeptide Pro-Gly-Pro – by 54.58%, with relation to the animals before the administration of the preparations. The aggression phase was completely eliminated by fragment 4-10. The analogue 4-10 decreased the aggression phase by 37.94% (P<0.05), similarly the tripeptide Pro-Gly-Pro by 63.64%, as compared to the animals before the administration of the preparations. The eating phase was slightly inhibited by fragment 4-10 by 7.60%, its analogue – by 2.94%, and tripeptide Pro-Gly-Pro by 14.70%, as compared to the animals before the administration of the preparations. The drinking phase was increased by the fragment and analogue 4-10 by 29.52% and 12.38%, respectively and by tripeptide Pro-Gly-Pro by 17.96%, with relation to animals before administering the preparations.

In comparison to the control group the tension phase was shortened by fragment 4-10 by 64.89% and increased by analogue 4-10 and tripeptide Pro-Gly-Pro by 8.63% and 43.75%, respectively. The orientation-searching reactions were reduced by Fragment 4-10 by 28.49%, analogue 4-10 – by 3.33% and tripeptide Pro-Gly-Pro by 17.15%. Fragment 4-10 increased the comfort phase by 30.40%, analogue 4-10 – by 14.44%, and tripeptide Pro-Gly-Pro by 29.36%. Grooming phase was reduced by 3.72% by fragment 4-10 and by 37.75% by tripeptide Pro-Gly-Pro and increased by analogue 4-10 by 19.28%. The aggression phase was shortened under the influence of fragment 4-10, its analogue, and tripeptide Pro-Gly-Pro, by 97.17%, 37.94%, and 95.98%, respectively (P<0.05). The applied oligopeptides reduced the eating phase in the following way: fragment 4-10 by 36.03%, analogue 4-10 by 22.65%, and tripeptide Pro-Gly-Pro by 45.76% (P<0.05). Similarly, the drinking phase was reduced by 40.88%, 35.12%, and 24.17%, respectively.

The presented data allow us to state that the corticotropin derivatives: fragment 4-10 and its analogue influenced the rabbits’ behaviour under spontaneous conditions. Fragment 4-10 suppressed the aggression and tension as well as orientation-searching reactions, appetite and thirst and intensified the comfort and grooming phases. Analogue 4-10 suppressed aggressive behaviour, tension phase, and orientation-searching reactions and extended comfort and grooming phases. The influence of oligopeptides was most vivid during the first 30 min of the experiment, starting from the moment of application of the preparation. The effect of corticotropin fragment 4-10 was dominating with comparison to its analogue and tripeptide Pro-Gly-Pro. Analogue 4-10 showed less activity due to the modified sequence in position 8,9,10 of the chain. The synthetic tripeptide Pro-Gly-Pro influenced marginally the modification of the accepted model of behaviour.
Discussion

Pituitary peptides have a significant role in creating and maintaining learned behaviour of animals. Hypophysectomy in rats was leading to generalised disorders – not only endocrineal, metabolic, but also serious disturbances in the getting and conditioning of evasive reactions (24, 26). Deteriorated reactions of rat behaviour could be considerably improved by application of corticotropin or its derivatives. The derivatives not only improved the disordered behaviour of rats after hypophysectomy but also influenced maintaining earlier acquired reactions of evasion in healthy animals (26). The hypophysis synthesises short peptides playing the role of neuropeptides which are involved in acquiring and maintaining new behavioural patterns (15, 26). Many behavioural effects of corticotropin derivatives have been noted. They concern modulation of early memory while solving complex problems, they make recovery of memory easier after amnesia induced by application of CO\(_2\), they intensify sexually motivated behaviour and influence learning and memorisation processes (8, 24, 26). Corticotropin fragment 4-10 facilitates learning and acquiring the earlier experience during performance of tasks; it intensifies memorisation processes by directly reacting with neurocytes that cause their stimulation, protein synthesis, and modification of synaptic transmission (16). In a human being this oligopeptide facilitates selective visual attention (24) and in animals it influences the change of theta waves rhythm that are registered in the septum, hippocamp, and thalamus (15, 24). The shortest aminoacid sequence, that has a strong behavioural activity, is closed in formula 4-7 (H-Met-Glu-His-Phe-OH) which determines this activity (26). Fragment 4-10 is considered to be the core involved in receptors activation of the whole corticotropin particle, while the other elements of the chain are only for affinity (26). In oligopeptide sequence 4-10 behavioural activity is included in the sequence 4-7, and phenylalanine is a key word - tranducon (26). However, the sequences Phe-D-Lys-Phe and DPhe-Arg-Trp-Gly demonstrate significant behavioural activity and the same information seems to be transmitted also by the sequence 7-9 of the chain (26). Both fragments 4-7 and 7-9 have a common Phe in position 7 as the common word that initiates activity (26). The location of corticotropin analogues activity is in centres of the limbic system (7, 8, 15, 24). These peptides enter the CNS by circulation or reverse transport along the peduncle of the pituitary (24, 26). Corticotropin analogues facilitate transmission in limbic structures. It suggests that neuropeptides intensify the excitation of these centres and define the motivation influence of environmental stimuli, which in turn, facilitate reaction that is specific for the stimulus (26). Biochemical tests point to the mechanism of corticotropin analogues influence through the cell membranes of intracerebral structures, facilitating nervous impulsation, and this causes the increase in likelihood of generation of reactions specific for a given stimulus (24, 26).

The influence of corticotropin analogues 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 (26). In the light of the above mentioned data it is purposeful to compare the influence and reaction profile of corticotropin fragment 4-10, its analogue, and tripeptide Pro-Gly-Pro under spontaneous conditions, i.e. without the influence of stressing stimuli. Fragment 4-10 reduced the tension phase and inhibited the aggression phase, resulting in peace and indifference, and it was especially clear under stressing conditions in another experimental model. Analogue 4-10 demonstrated a similar influence, however, its action was weaker and shorter with relation to corticotropin fragment 4-10. Wolterink et al. (27) proved that analogue 4-10 had a variable activity depending on its amino-acid sequence: N-terminal fragment of the chain resulted in the effect of making behaviour of rats easier, and C-terminal fragment had in its particle information for the inhibiting effect. Similar effects of the activity were noted in case of analogue 4-10 with a modified C-terminal fragment of the chain.

Application of corticotropin derivative icv reduces active zoo-social reaction of rats, and inhibits aggression and intercommunication between species. Analogue 4-10 when administered for a long time maintained the social attention, influenced sleep and the effect was long-lasting (11), and had neurotropic properties (27). The analogue influenced functional convalescence after fronto-cortical injury, improved and shortened the time of tests performance (3,10), facilitated learning of labyrinth task and improved the memory (9). It is thought that reduction of aggression results from direct influence on the CNS (4). Fragment 4-10 when applied icv caused the intensity of grooming, similarly as analogue 4-10, however, its effect was much weaker. After icv application of corticotropin fragments: 4-7, 4-10, 1-24, a SYS syndrome occurred in many animal species. This syndrome is manifested by yawning and pandiculation (24). In rodents there is excessive grooming that anticipated this syndrome (6, 19, 22). Activities of that type were noticed also in our studies. Many authors point out that the mechanism of grooming occurs due to the influence of corticotropin on the central dopaminergic and GABA-systems (5, 12, 18). Van Erp et al. (21) indicate that the Vmh nucleus participates in the organisation of such behaviour development by integrating it in one new or modified model of behaviour. In the present study, the fragment 4-10 caused suppression of seeking-orientation reactions. This has been confirmed by Antonova et al. (2), who point to the reduction of observation time in “open field” test and a decreased interest in other individuals. The comfort time was considerably extended under the influence of fragment 4-10, and the analogue 4-10 was less actively involved. The influence of corticotropin derivatives on eating and drinking phase is difficult to evaluate explicitly, however, there is a tendency to weaken appetite and thirst and this has been confirmed by other studies (1, 20). Tripeptide
differentiation of the brain because the increase in Corticotropin may be a neuromodulator during gender psychostimulating and neuroleptic properties (25). Corticotropin may be a neuromodulator during gender differentiation of the brain because the increase in ACTH inhibited sexual behaviour in males to whom corticotropin had been administered before birth and it was associated with the increase in serotonin in the area of tectal lamina. The observed psychotropic and psycho-stimulating activity make us to apply corticotropin or its fragments (4-9, 4-10) in the attempts of treating schizophrenia with depression symptoms, in SM, and in attacks of fear. Analogue 4-9 (ORG2766) was applied in the treatment of diabetic neuropathy due to its neurotropic activity.

References