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CYCLIC ANALOGS OF ACTH FRAGMENTS IN THE ORGANIZATION OF SELF-STIMULATION AND GROOMING BEHAVIOR IN RABBITS

R. A. Burchuladze, I. K. Liepkaula,
P. Ya. Romanovskii, and G. I. Chipens

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ACTH fragments without hormonal activity play an important role in behavioral adaptation in the external environment [1, 6, 9]. ACTH fragments have been shown to be involved in learning and memory [1, 6, 9], analgesia [10], sexual behavior [10], sleep processes [11], and the development of tolerance and dependence [11]. In experiments on rats [3, 4, 8] fragments $ACTH_{4-9}$, $ACTH_{4-10}$, and $ACTH_{5-8}$ intensified self-stimulation (SS) behavior during stimulation of the medial forebrain bundle, whereas intraperitoneal injection of $ACTH_{5-10}$ [2] reduced the intensity of SS of the lateral hypothalamus. ACTH analogs, injected into the cerebral ventricles, evoked intensive grooming in rats, mice, and pigeons [7].

In the investigation described below hitherto unstudied cyclic analogs of ACTH fragments, incorporating in their structure specific and nonspecific active centers:

and their role in the organization of SS and grooming behavior in rabbits were investigated.

EXPERIMENTAL METHOD

Experiments were carried out on 20 male chinchilla rabbits weighing 3-3.5 kg. The animals were scalped, and 1 day later bipolar nichrome stimulating electrodes were implanted in the region of the lateral hypothalamus (P-2, L-2, H-15; 15.5). To inject ACTH fragments into the animals steel cannulas 14 mm long and 0.8 mm in diameter were implanted into the lateral cerebral ventricles. On the 2nd day after the operation the animals were placed in a chamber with a fixed metal ring and testing stimulation was applied to points of the brain in order to discover the type of responses and the threshold intensity of stimulation required to evoke it. The unrestrained animals, with free access to water and food, touched a metal ring with their nose and lips, and soon learned to close the electric circuit required to obtained electrical stimulation of the brain. The parameters of the stimulating current were: volleys of square pulses with a frequency of 100 Hz and duration 0.3 sec, strength of current 40-60 μA, pulse duration 1.4 msec. Sessions of SS

Laboratory of Physiology of Emotions, P. K. Anokhin Research Institute of Normal Physiology, Academy of Medical Sciences of the USSR, Moscow.

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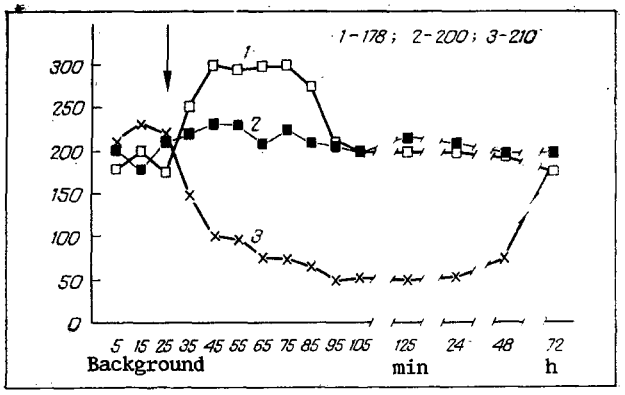

Fig. 1. Effect of cyclic ACTH fragment EHFRWGKPVG—NH_2 on time course of self-stimulation in rabbits. Arrow indicates time of injection of peptide and of physiological saline. 1) 0.1-2.5 μg (n = 14); 2) 10 μl of physiological saline (n = 8); 3) 4-5 μg (n = 12). $p < 0.05$ by Student's t test. Abscissa, time of experiment; ordinate, frequency of SS during 5-min time intervals.

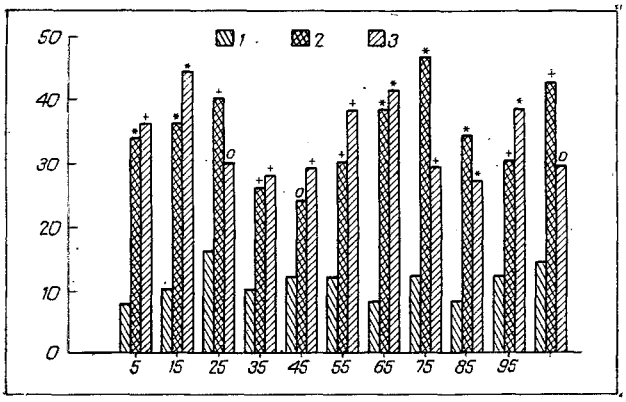

Fig. 2. Changes in duration of grooming in rabbits after intraventricular injections of cyclic ACTH fragments. 1) Control (physiological saline); 2) EHFRWGKPVG—NH_2 (0.1-5 μg); 3) KHFRWG—NH_2 (0.1-5 μg). Circles) $p < 0.05$, crosses) $p < 0.01$. Abscissa, time (in min); ordinate, duration of grooming (in sec).

were carried out daily, and the frequency of SS during 5-min intervals was investigated every 10 min during 2 h of the experiment. SS was recorded on an N327-5 automatic writer. After three recordings of SS during 5-min intervals before injections, aninals of one group (n = 8) received $\overline{\text{EHFRWGKPVG}}$—$NH_2$, and animals of the other group (n = 7) received $\overline{\text{KHFRWG}}$—NH_2. The peptides were dissolved in 10 μl of sterile 0.9% physiological saline in doses of 0.1-5 μg. Intraventricular injections of 10 μl of physiological saline were given in the control experiments (n = 5). Injection of the peptides into each rabbit was repeated once or twice after 6-7 days. After the end of the experiments the location of the electrode tips in the hypothalamus was determined by passing a direct current of 0.5-1 mA through them for 30 sec. The brain was fixed in 10% formalin solution. The location of the lesions was determined on maps in stereotaxic atlases. The results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

Intraventricular injection of $\overline{\text{EHFRWGKPV6}}$—$NH_2$ in doses of 0.1-2.5 μg increased the frequency of SS on average by 42-45% 5 min after the injections and led to an even greater increase (by 50-75%; $P < 0.05$) 15 min after the

injection (Fig. 1). The frequency of SS decreased 65-75 min after injection of EHFRWGKPVG—NH_2, although it exceeded the background level, but not significantly (up to 10%), and reverted to the initial value after 48-72 h. Injection of EHFRWGKPV6—NH_2 in doses of 4-5 μg caused the frequency of SS to fall by 25-30% 5 min after the injections, and this was followed by a further decrease in the frequency of SS by 100-125% ($p < 0.05$) 15-25 min after injection (Fig. 1). During the next 24-72 h the frequency of SS was low until the end of the experiment. The initial level of SS was restored after 72 h. During the first 15 min after injections of KHFRWG—NH_2 in doses of 0.1-5 μg, the frequency of SS fell by 25-30%. It then returned to the background level, after which it fell again. SS became strong after 24 and 48 h, exceeding the background level by 5-8%, and during the next 2-hour period it was virtually unchanged. Besides the changes described above, both ACTH fragments increased the duration of grooming in the animals by 200-400% compared with that in animals receiving physiological saline (Fig. 2).

The cyclic analogs of ACTH fragments EHFRWGKPV6—NH_2 and KHFRW6—NH_2 which we investigated thus differed in their action on SS behavior in rabbits. The strongest action was exerted by EHFRWGKPVG—NH_2. After injection of KHFRWG—NH_2 the intensity of the SS response did not change statistically significantly.

Comparison of the results of this investigation with those of a study of the action of $ACTH_{4-10}$, $ACTH_{4-9}$, and $ACTH_{5-8}$ on SS showed that cyclic analogs of ACTH fragments had a more prolonged action on SS — up to 72 h. These properties of cyclic ACTH analogs also are manifested in relation to grooming. The increase in the intensity and duration of grooming described in the literature in different animals after injection of linear molecules of ACTH fragments [7] did not last longer than 1-2 h, whereas in the present experiments the potentiating action of cyclic fragments of ACTH analogs on grooming was observed even on the 2nd or 3rd day.

It has been shown [5, 12] that the delay of extinction of the conditioned active avoidance reaction is mainly conencted with the structure of the cyclic part of the vasopressin molecule. This conclusion was drawn by the authors cited from data obtained by the study of individual fragments of the neuropeptide. Like vasopressin, the cyclic part of the oxytocin molecule also contains basic structural elements essential for modulation of learning and memory consolidation processes [5, 12]. The prolonged modulating action of cyclic analogs of ACTH fragments which we observed can be explained by delayed enzymic degradation or, perhaps, by specific physiochemical properties promoting accumulation of the peptide in the brain.

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