ROLE OF $ACTH_{5-8}$ and β-MSH_{5-8} FRAGMENTS IN ORGANIZATION OF THE SELF-STIMULATION REACTION

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KEY WORDS: $ACTH_{5-8}$; β-MSH_{5-8}; self-stimulation reaction.

The prehormonal adenohypophyseal protein pro-opiomelanocortin is a precursor of ACTH and β-lipotropin, and from the latter, in turn, are formed the opioid peptides (α, β, and γ-endorphins and Met-enkephalin) and β-melanocyte-stimulating hormone (β-MSH). Fragments of hormones ACTH and β-MSH, while not exhibiting hormonal activity, may act as modulators of CNS functions and may affect animals' behavior [14]. It has been shown that fragments $ACTH_{4-7}$, $ACTH_{4-9}$, $ACTH_{4-10}$, and $ACTH_{5-10}$ accelerate learning in rats, accompanied by both negative [11] and positive food [1, 4] and drink [2, 9] reinforcement. Peptides $ACTH_{5-9}$ and $ACTH_{7-9}$ have been shown to possess analgesic activity [6], β-MSH_{5-8} has a dipsogenic action [5], and so on. In experiments on rats [10] fragment $ACTH_{4-9}$, when injected subcutaneously, caused an increase in frequency of the self-stimulation (SS) reaction of the medial forebrain bundle and a decrease in the animals' motor activity; $ACTH_{4-10}$ also potentiated the SS reaction [7]. According to data in the literature [12], the intensity of the action of $ACTH_{4-10}$ depends on the region of stimulation and the original intensity of SS. In experiments with intraperitoneal injection of $ACTH_{5-10}$ [3] a decrease in the intensity of SS of the lateral hypothalamus was noted.

In the investigation described below the action of hitherto unstudied fragments $ACTH_{5-8}$ and β-MSH_{5-8} on the character of the SS reaction was studied in rats.

EXPERIMENTAL METHODS

Experiments were carried out on eight noninbred male albino rats weighing 230-260 g. Electrodes were implanted beforehand into the anterior hypothalamic nucleus (AP +1), the lateral hypothalamic area (AP +1), the fornix (AP +3), and the mammillothalamic tract (AP +3) in accordance with stereotaxic coordinates [8, 13]. The operation was performed under pentobarbital anesthesia (40 mg/kg, intraperitoneally). The rats were placed in a chamber with a lever 5-6 days after the operation and subjected to testing stimulation of points of their brain through the implanted electrodes in order to reveal the type of their response and to determine the threshold intensity of stimulation required to induce it. The animals were then taught the SS reaction. Volleys of square pulses with a frequency of 80-100 Hz, amplitude 0.8-4.0 V, and duration 0.5 sec, were used. The duration of a single stimulus was 0.5 msec. To stimulate the brain an ÉSL-2 stimulator with specially designed time relay, enabling volleys of stimuli of the necessary duration to be formed, was used. Sessions of SS were given for 10 min daily. The frequency of the SS reaction was recorded on an N 327-5 automatic writer and calculated by means of an electromagnetic counter (attachment to the B-3 radiometer) during 5-min intervals. After stabilization of the frequency of the SS reaction for 5-6 days, experiments were carried out with administration of the peptides. $ACTH_{5-8}$ (H-Glu-His-Phe-Arg-OH, synthesized by L. I. Leont'eva in the Department of Chemistry of Natural Compounds, Leningrad University) was injected intraperitoneally in a dose of 40 ng/100 g body weight, and β-MSH_{5-8} (H-Asp-Glu-Gly-Pro-OH, synthesized by E. I. Sorochinskaya at the same place) was injected in a dose of 20 ng/100 g body weight. The intensity of the SS reaction was recorded for 5 min every 30 min after injection of these peptides and the animals were

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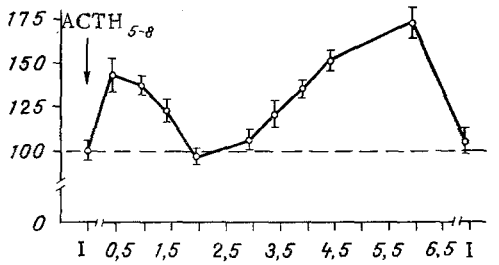

Fig. 1. SS reaction of anterior hypothalamic nucleus in a rat (in % of initial level, I), taken as 100, after administration of $ACTH_{5-8}$. Abscissa, duration of experiment (in h); ordinate, level of SS reaction during 5-min intervals (in %); five background experiments and three experiments with $ACTH_{5-8}$.

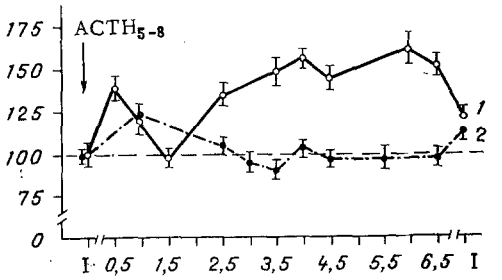

Fig. 2. SS reaction of fornix (1) and mammillothalamic tract (2) of a rat (in % of initial level, taken as 100) after injection of $ACTH_{5-8}$ (six background experiments and three experiments with $ACTH_{5-8}$). Legend as to Fig. 1.

kept under observation for 4-6 h. Injection of the peptides into each rat was repeated 2-3 times at intervals of 6-8 days. The experiments with $ACTH_{5-8}$ and β-MSH_{5-8} were conducted on the same animals. After the end of the experiments the location of the implanted electrodes was verified. For this purpose, point coagulation of zones around the electrode tips was produced by passage of a current of 0.5-1 mA through them for 30 sec. The brain was fixed in formalin for 8-12 days, after which frontal sections were cut to a thickness of 60-90 μ on a freezing microtome. The sections were placed between coverslips and slides and photographed by the contact method, using a photographic enlarger for illumination. The localization of the lesions was determined by reference to maps in sterotaxis atlases. The numerical data were subjected to statistical analysis by Student's test.

RESULTS

An SS reaction was obtained in the rats during electrical stimulation of the anterior hypothalamic nucleus, the lateral hypothalamic area, the fornix, and the mammillothalamic tract. The intensity of the SS reaction of the anterior hypothalamic nucleus, on average for 5-min intervals, was 90 ± 5.5 presses, that of the lateral hypothalamic area varied in different rats from 109 ± 13.4 to 232 ± 11.5, of the fornix 268 ± 17.0, and of the mammillothalamic tract 137 ± 19.8 presses.

$ACTH_{5-8}$ considerably altered the character of the SS reaction and the rats' behavior. As early as 15 min after its injection, the animals' investigative activity was increased. The rats began to press the lever in the absence of stimulation. When the stimulator was switched on the rats actively and regularly pressed the lever and the intensity of SS increased. The effect was similar after each injection of $ACTH_{5-8}$. Besides intensification, the SS reaction also became ambivalent in character: Having pressed the lever the rats ran away, then returned and pressed it again. The animals exhibited marked unease and sometimes even ran out of the chamber. Visually, the animals' respiration was quickened. By the end

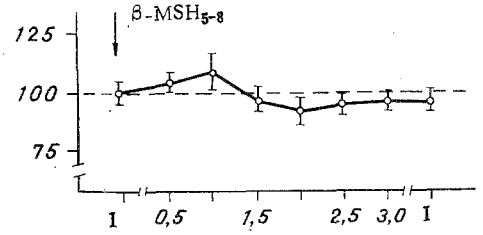

Fig. 3. SS reachtion after injection of β-MSH_{5-8}. Data pooled for three rats with electrodes located in lateral hypothalamic area; six background experiments and four experiments with injection of β-MSH_{5-8}. Legend as to Fig. 1.

of the first hour of the experiment the rats' excitation had diminished. Next day the "aversive" components of the SS reaction had disappeared completely. In three of the five rats it was possible to distinguish two phases of activity: the first, 30 min to 1 h, and the second, 4.5-6 h after injection of $ACTH_{5-8}$. In the interval between the 2nd and 4th hours of the experiment the frequency of SS declined (Fig. 1). In one rat the SS reaction was observed during stimulation of two points in the brain — the fornix and mammillothalamic tract. $ACTH_{5-8}$ intensified SS of the fornix, whereas SS of the mammillothalamic tract between the 3rd and 4th hours of the experiment was below its initial level (Fig. 2). In three rats stimulation of the lateral hypothalamic area induced an investigative response but no stable SS could be formed. Single presses on the lever were observed (15 ± 4 during 5 min). Injection of $ACTH_{5-8}$ into these rats caused a sharp increase in the animals' motor activity: They began to press the lever (40 ± 8 presses in 5 min). The reaction also was ambivalent.

The $ACTH_{5-8}$ fragment thus has a dissimilar action on the behavior of rats and their SS reaction. On the one hand, investigative activity and the intensity of the SS reaction are increased, whereas on the other hand, negative components of the SS reaction are clearly manifested. The stimulating effect of $ACTH_{5-8}$ on SS is more marked when the electrodes are located in the anterior hypothalamic nucleus, lateral hypothalamic area, and fornix, and is much weaker when the electrodes are located in the mammillothalamic tract.

β-MSH_{5-8}, in the dose used, had no appreciable effect on the SS reaction or the animals' behavior. After injection of β-MSH_{5-8} the intensity of the SS reaction increased very slightly in the first hour, but decreased toward the third hour. The differences were not statistically significant (Fig. 3). Injection of β-MSH_{5-8} into three rats (without an SS reaction) caused no visible changes in their behavior.

$ACTH_{5-8}$ thus activates the SS reaction, whereas β-MSH_{5-8} has no appreciable action on it. Comparison of these results with those of a study of the action of $ACTH_{4-10}$ and $ACTH_{4-9}$ on SS [7, 10] reveals that the ACTH region adequate to potentiate SS was shorter still: only four amino acid residues — Glu-His-Phe-Arg. The results also agree with those of another investigation [12] which showed that the action of fragments of the ACTH hormone depends on the region of stimulation. Besides activating SS, $ACTH_{5-8}$ also causes a change in its character — the appearance of new ambivalent properties. Properties of negative reinforcement are exhibited in the SS reaction.

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ROLE OF THE CARDIOPULMONARY BLOOD VOLUME IN CHANGES IN LEFT VENTRICULAR OUTPUT DURING STIMULATION OF SOMATIC AFFERENT NERVE FIBERS

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KEY WORDS: left ventricular output; arterial pressure; venous return to the heart; blood flow in the pulmonary artery; somatic afferent nerve fibers.

Vessels of the pulmonary circulation contain about 20%, and the right and left chambers of the heart up to 10% of the circulating blood volume [5]. Investigations on dogs have shown [7] that during the first 5-6 min of muscular work the deficiency of the venous return to the heart is concealed mainly by the pulmonary reserve and the end-systolic volume. The main source responsible for the change in left ventricular output is the functional residual capacity, which amounts to between 46 and 65% of its diastolic volume, and consists of the reserve and residual volumes [4]. The increase in stroke volume during muscular work in the period preceding the increase in the venous return to the heart takes place on account of the reserve volume [7]. During muscular work activation of afferent fibers of groups III-IV (mainly of type C) of somatic nerves, which react to high-voltage and high-frequency stimulation [9], takes place. Muscular contraction is essential for activation of muscle afferents [8]. The writers showed previously [3] that during the pressor response to stimulation of afferent C-fibers of the tibial nerve, the left ventricular output (LVO) exceeded the venous return to the right atrium in one-third of the experiments. However, the degree of participation of the blood volume in the pulmonary vessels and in the right ventricle and right atrium in the formation of the inflow of blood to the left ventricle during coupled reflexes has virtually not been studied. An increase in the blood volume in a lobe of the lung has been demonstrated [1] during stimulation of the central end of the divided tibial nerve.

The aim of this investigation was to study the importance of the capacitive properties of the pulmonary vessels and chambers of the right heart, and also of neurogenic influences on the right ventricle for changes in LVO in response to electrical stimulation of C-fibers of a somatic nerve. For this purpose the character and magnitude of synchronized changes in blood flow in the ascending aorta, pulmonary artery (PA), and posterior vena cava (PVC) were compared.

EXPERIMENTAL METHODS

Acute experiments were carried out on cats with a closed chest and artifically ventilated and anesthetized with chloralose (40 mg/kg) and pentobarbital (10 mg/kg), intraperitoneally. Artificial respiration was applied by the DP-8 apparatus. The LVO was estimated as volume velocity of the blood flow in the ascending aorta, measured with a vascular transducer (diameter 7 mm) of an RKÉ-1 electromagnetic blood flowmeter, and the blood flow in PA and PVC also was recorded by vascular transducers of an RKÉ-2 electromagnetic blood flowmeter

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