EFFECT OF "MEMORY PEPTIDE" $ACTH_{5-10}$ ON SELF-STIMULATION REACTION IN RABBITS

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KEY WORDS: self-stimulation reaction; $ACTH_{5-10}$; mechanism of reinforcement.

Pituitary adrenocorticotrophic hormone (ACTH) and its individual peptide fragments have been shown to accelerate learning in animals in a model of active avoidance of nociceptive reinforcement [5, 9, 10]. It has also been shown that in positive types of reinforcement based on natural needs, peptides such as $ACTH_{4-10}$ and $ACTH_{5-10}$ stimulate learning and memory processes [2, 4, 11]. Adaptive behavior, in Anokhin's view, is based on a number of central components, the most important of which are motivation, memory, and effectiveness of reinforcement as the result of agreement or disagreement between the expected useful results and those actually obtained [1]. However, most of the attention of research workers studying the mechanisms of action of ACTH fragments has been directed toward the study of memory and attention processes only [2, 9, 11]. More recently the action of these peptides on the general motor activity of animals, and on individual autonomic parameters and electrical activity of the brain has been investigated [6, 7]. Effectiveness of learning can also largely depend on the functional state of the central reinforcement apparatus. It has thus become necessary to investigate the effect of $ACTH_{5-10}$, one of the "memory peptides," on the nonspecific positive reinforcement apparatus, for which the best experimental model is self-stimulation behavior with electrical stimulation of deep brain structures by Old's method.

EXPERIMENTAL METHOD

Experiments were carried out on 13 male rabbits weighing 2.5-3.0 kg. Bipolar nichrome electrodes were inserted into the lateral hypothalamus. For stimulation, bipolar square pulses (duration 1 msec, frequency 50 Hz) were used. The duration of stimulation was 500 msec. The voltage was chosen individually between 2.5 and 6.0 V. The self-stimulation reaction appeared in response to the animals' grasping a lever with their teeth or muzzle. Fuller details of the technique of self-stimulation in rabbits were described by the writer previously [3]. Two types of injections of $ACTH_{5-10}$ (from Serva, West Germany) were used in the experiments: intraperitoneally (50 μg/kg body weight in 1.0 ml physiological saline) and intraventricularly (100 ng per rabbit, equivalent to 50 pmoles/kg, in 5 μl of physiological saline) through a permanently implanted metal cannula with coordinates AP = −2.0 mm laterally, according to an atlas of the rabbit's brain [8]. Usually the cannula was located ipsilaterally relative to the stimulating electrodes. Experiments with injection of physiological saline, intraperitoneally (1.0 ml) and intraventricularly (5 μl), served as the control. All injections were given not more often than once a week. The frequency of self-stimulation was counted during consecutive 5-min intervals for 10 min before and 30 min after the injection. For statistical analysis of the data Student's test was used and the standard deviation was calculated ($P < 0.05$).

EXPERIMENTAL RESULTS

The self-stimulation reaction in rabbits differed in intensity depending on the individual characteristics of the animals and the location of the electrodes. The mean frequency of the instrumental acts in 5-min periods at the beginning of the experiments was between 80

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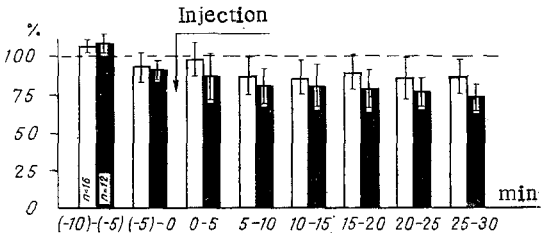

Fig. 1. Time course of self-stimulation reaction in control experiments (unshaded columns) and after intraperitoneal injection of peptide $ACTH_{5-10}$ (shaded columns). Abscissa, time (in min), ordinate, intensity of self-stimulation reaction (in % of intensity before injection). Numbers with minus sign indicate time before beginning of injection. Confidence intervals at $P < 0.05$ level. n) Number of experiments in each series.

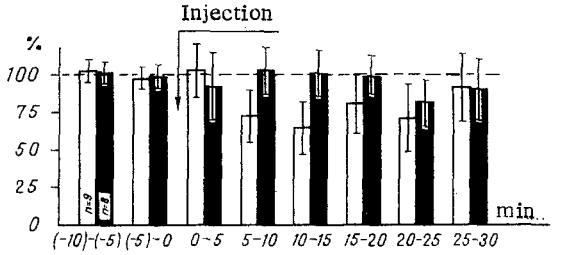

Fig. 2. Changes in self-stimulation reaction under the influence of intraventricular injections of physiological saline (unshaded columns) and $ACTH_{5-10}$ (shaded columns). Legend as to Fig. 1.

and 400, and the maximal frequency was 200-300. To eliminate differences in the individual "contribution" of single animals to the general picture of changes in this type of behavior under the influence of $ACTH_{5-10}$, absolute values of the frequency of self-stimulation were made equal to 100%. In each experiment the mean frequency of the animals' approaches to the lever during the 10-min period before injection was taken as this level. Intraperitoneal injections of $ACTH_{5-10}$ were given in 12 experiments (Fig. 1, shaded columns). The time course of the self-stimulation reaction in the control experiments (Fig. 1, unshaded columns) was represented by a steady decline in the frequency of approaches to the lever, with a fall of 20% being reached after 30 min of continuous behavior. $ACTH_{5-10}$, in a dose of 50 μg/kg, evoked a small ($P > 0.05$) decrease in the frequency of self-stimulation throughout the course of the experiment. Intraventricular injection of 5 μl of physiological saline inhibited the self-stimulation reaction in the period from 5 to 25 min after injection (Fig. 2, unshaded columns). This inhibition reached a maximum between 5 and 10 min after the injection. Injection of $ACTH_{5-10}$ in a dose of 50 pmoles/kg body weight in the same volume of physiological saline completely restored this inhibitory effect and actually reversed it a little. The potentiating action of intracerebral injection of the peptide reached the level of significance between the 10th and 15th minute after injection, i.e., in the period of maximal inhibitory action resulting from injection of physiological saline. In the same doses, and when administered in the same way as when learning improved in animals in experiments with drinking [4] and nocieptive [5] reinforcement, $ACTH_{5-10}$ had no significant effect on the mechanism of nonspecific positive reinforcement in the model of self-stimulation of the hypothalamic satisfaction centers. It is an interesting fact that in the form of whole molecules, ACTH, which possesses corticotrophic hormonal activity, similarly produces some weakening of the self-stimulation reaction [3]. The possibility cannot be ruled out that this effect may

be connected with the central action of steroid hormones. We know that $ACTH_{4-10}$ strengthens the binding of corticosterone with specific receptors in the hippocampus [12], whereas cortisol, one of the steroid hormones, according to our own data strongly inhibits the self-stimulation reaction in rabbits [3].

Intraventricular injection restored the intensity of the reinforcing effect of hypothalamic stimulation when weakened by an exogenous factor such as intraventricular injection of physiological saline.

The results are in agreement with those of experiments of Kamenskii et al. [6], who found that minimal doses of ACTH fragments causing facilitation of learning had no effect on autonomic and metabolic parameters in animals which, in turn, can be used as indicators of a change in the emotional state. The results also confirm those obtained on rats showing that $ACTH_{4-10}$ has no action on the self-stimulation reaction from the region of the lateral hypothalamus [13]. All these facts are proof that $ACTH_{4-10}$ and $ACTH_{5-10}$ have no effect indirectly through mechanisms of motivation and reinforcement on memory processes.

The question of the character of action of the peptide when injected intraventricularly deserves special discussion. These injections were given in the immediate vicinity of the septal nuclei. According to histochemical data, exogenously injected fragments of ACTH are selectively bound by structures of the septohippocampal complex [15]. The fact that the peptide was injected close to the part of the brain where it is chiefly bound may lead to an excess of ACTH in the septum and, ultimately, to its stronger action than when administered systemically. Increased doses of behaviorally active ACTH fragments evoke increased motor activity of the animals, quicken their pulse, and increase their oxygen consumption [6]. However, there may be another explanation of the difference in our data that depends on the mode of administration of the peptide. According to other workers [13], $ACTH_{4-10}$ gives rise to some strengthening of the self-stimulation reaction from the septal region only if its frequency is low. If the intensity of stimulation was raised and the frequency of self-stimulation increased, injection of the peptide became ineffective. Admittedly these results were obtained when the level of self-stimulation was measured 1 h after injection of the peptide. Nevertheless, similar results were obtained in our own experiments. $ACTH_{5-10}$ increased the effectiveness of reinforcing stimuli only if the level of self-stimulation was lowered (as a result of intraventricular injection).

The action of memory peptide $ACTH_{5-10}$ on learning processes can evidently be attributed to fine influences on metabolism of neurons, and through functional changes in the cortico-subcortical system of connections based on selective incorporation of the individual elements of this system into the integrative functions of the brain. This view is supported by experimental data on the effect of ACTH fragments on brain protein metabolism [14] and on the character of redistribution of cortical electrical activity [7].

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PRINCIPLES OF INTERACTION BETWEEN BRADYKININ AND RECEPTORS OV VARIOUS BLOOD VESSELS

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Data now published indicate that interaction between bradykinin and smooth-muscle structures of the aorta and femoral artery of rabbits [10, 11], and of the guinea pig and cat ileum [10, 12] is described by the equation for the model of interaction between a substance and receptors suggested by Clark [9]:

$$P=\frac{P_M \cdot A}{K+A}, \quad (1)$$

where P is the effect produced by a given concentration of the agonist, P_M is the maximal effect, A the concentration of the agonist, and K the apparent dissociation constant of the agonist—receptor complex (1/K is the index of sensitivity of receptors).

However, for some vessels the character of the curves reflecting the relationship between effect and concentration of bradykinin differs considerably from those predicted theoretically by equation (1) [2]. This prevents the investigator from characterizing the principles governing the kininergic reaction quantitatively and from calculating the value of the apparent dissociation constant of the bradykinin—receptor complex, which is used to compare characteristics of receptor sensitivity [6], correctly in accordance with equation (1).

The object of this investigation was to study the possibility of using certain mathematical models to describe the kinetics of interaction between bradykinin and receptors of various blood vessels. The action of bradykinin was tested on arteries and veins of the hind limb, the peritoneal cavity, and lungs of dogs and also on the portal vein of guinea pigs.

EXPERIMENTAL METHOD

Experiments were carried out on isolated strips and segments of blood vessels. Spiral strips of arteries were prepared by Avakyan's method [1]. Preparations of the veins consisted of segments 10-15 mm long. Fragments of the vessels were placed in a constant temperature bath at 37°C with Krebs' solution of the following composition (in mM): NaCl 118.5; KCl 4.69; NaH_2PO_4 1.18; $NaHCO_3$ 25.88; $MgSO_4$ 1.16; $CaCl_2$ 2.52; glucose 5.5 (pH 7.4). The solution was made up immediately before the experiment. The contractile response was recorded by an apparatus for measuring tension in a thread (INN-3U), the transducer for which is a differential capacitive transducer. The magnitude of the contractile response to bradykinin was recorded 1-1.5 h after the vessel had been placed in the constant temperature bath. Different doses of bradykinin were added in a volume of 0.5 ml. Each subsequent addition was made 20-40 min after the vessel had been rinsed 5 or 6 times to remove the previous dose. The total number of tests of the different concentrations of bradykinin on one preparation was 6-7; the duration of work with one preparation was 3-5.5 h. The solution was oxygenated. To describe the kinetics of interaction between bradykinin and the receptors, concentration—effect curves were plotted. The magnitude of the response was calculated as a percentage of maximal. The bradykinin used in the experiments was from Reanal (Hungary).

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