EFFECT OF BLOCKERS OF PROTEIN AND OLIGOPEPTIDE SYNTHESIS ON SELF STIMULATION

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Self stimulation is formed on an emotional basis of subjective sensations. An important role in the formation of self-stimulation behavior, based on ascending activating influences of the emotiogenic structures of the brain, has been shown to be played by endogenous oligopeptides [1, 3, 4]. It can accordingly be postulated that self-stimulation behavior, aimed at achieving a positive emotional state in the subject, is determined by expression of the genes of endogenous oligopeptides.

The investigation described below was undertaken to test this hypothesis experimentally.

EXPERIMENTAL METHODS

Experiments were carried out on 34 male Chinchilla rabbits weighing 2.5-3.5 kg. A few days before the experiment began, a bipolar needle electrode (diameter of tip 0.12 mm) was implanted at various points of the lateral hypothalamus of each animal after preliminary scalping. The electrodes were fixed at points in the brain whose electrical stimulation led to the appearance of self stimulation in the rabbits. The freely moving rabbits, on touching a metal lever with their nose or lips, closed the circuit of an electric current stimulating their brain (strength of current 20-60 μA, pulse duration 1.4 msec, duration of stimulation 0.3 sec). Cannulas 0.8 mm in diameter were inserted into the lateral ventricles. By means of a microinjection syringe inhibitors of protein synthesis actinomycin D (Serva, West Germany), which disturbs the template function of DNA (dose of 0.4, 1.6, 3, 25, and 50 μg/kg), cycloheximide (Serva), a translation inhibitor (15-100 μg/kg), the oligopeptides Leu-enkephalin and Met-enkephalin (100 ng/kg), cholecystokinin (0.1 μg/kg), pentagastrin (25-50 μg/kg), and $ACTH_{4-10}$ (30 μg/kg, in a volume of 10 μl of 0.9% physiological saline) were injected through the cannulas into the experimental animals. Control animals received an injection of isotonic 0.9% physiological saline (10 μl). After the experiments the location of the electrode tip (in the lateral hypothalamus) and of the cannula (in the lateral ventricle) was verified histologically. The spontaneous frequency of self stimulation was calculated during successive 5-min intervals during the 15 min before injection of the blockers of protein synthesis, and again throughout the duration of the experiment. Absolute values of the spontaneous frequency of self stimulation were taken as 100%. Changes in the frequency of self stimulation were examined relative to the spontaneous level.

EXPERIMENTAL RESULTS

After injection of actinomycin D, which disturbs the template function of DNA, and of cycloheximide, which inhibits translation, into the lateral ventricles of the animals, depending on the dose, a complete block of self stimulation or marked inhibition was observed for 60-230 min. The action of the inhibitors of protein synthesis developed and continued in the course of 24 h or more (Fig. 1). Self stimulation was not completely restored even after 7 days of observation. After injection of actinomycin D in one of the five rabbits, and after injection of cycloheximide in two of the 18 rabbits, self-stimulation

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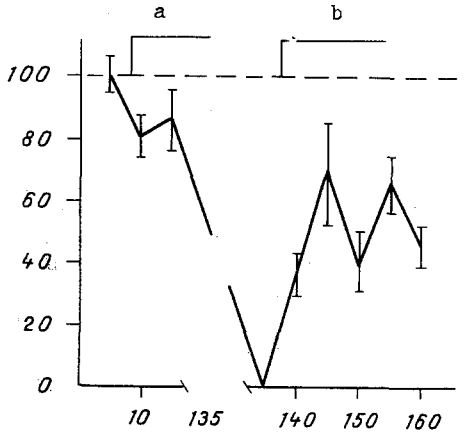

Fig. 1. Time course of frequency of self stimulation after injections of cycloheximide (a) and $ACTH_{4-10}$ (b) into lateral ventricles of rabbits. Abscissa, time (in min); ordinate, frequency of self stimulation (in % of spontaneous level).

behavior was transformed into defensive behavior. Under these circumstances the animals touched the lever with their lips or nose and closed the circuit of the electric current, after which they performed impetuous panic motor responses, curled into a ball, hid in the corner of the experimental chamber, and characteristically struck the floor with their hind limbs. The intensity of the observed effect increased during the 2, 3, 4, and 5 days after injection of the inhibitors of protein synthesis, and then decreased a little on the 7th day. The remaining experimental rabbits, like animals of the control group, gave no passive defensive responses.

Against the background of the action of inhibitors of protein synthesis, additional injections of oligopeptides (pentagastrin, Met- and Leu-enkephalin, cholecystokinin) into the lateral ventricles did not restore self stimulation. Self stimulation was restored only after injection of the ACTH fragment $ACTH_{4-10}$ (Fig. 1). Injection of $ACTH_{4-10}$ in a dose of 30 μg/kg restored self stimulation even when totally blocked, when neither stimulation of the lateral hypothalamus, artificially imposed by the experimenter, nor an increase in the strength of the stimulating current induced self stimulation. The period of recovery of self stimulation varied from 25 sec to 6 min after injection $ACTH_{4-10}$. Against the background of complete blockade by inhibitors of protein synthesis the longest time during which restoration of self stimulation was observed following intraventricular injection of $ACTH_{4-10}$ was 58 min and the shortest time 12 min.

The experiments show that excitation arising during stimulation of emotiogenic structures of the hypothalamus is aimed at the genetic apparatus of the nerve cells. This leads to expression of the genes of special oligopeptides that organize self-stimulation behavior. One such neuropeptide, as our experiments showed, is $ACTH_{4-10}$, which restores self stimulation in rabbits when completely blocked by inhibitors of protein synthesis. The mechanism of formation of self-stimulation behavior which we discovered differs from the molecular mechanism of realization of food motivation into goal-directed behavior, found in Sudakov's experiments [2], which showed that food behavior, when blocked by cycloheximide, is restored by pentagastrin, although in the present experiments this substance was ineffective. These data, and also the modification of self-stimulation behavior into defensive behavior observed after injection of inhibitors of protein synthesis, point to a definite and specific character of the molecular mechanisms of motivations differing in their biological quality.

The role of $ACTH_{4-10}$ which we found does not rule out the possible participation of other as yet unstudied oligopeptides in the mechanisms of gene expression during self stimulation.

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SOME BIOCHEMICAL CHARACTERISTICS OF MUSCLE PERFUSATE AND ITS EFFECT ON TRANSMITTER RELEASE IN THE NEUROMUSCULAR SYNPASE

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It was suggested previously that the muscle metabolite histidine may participate directly or indirectly in antidromic regulation of motor nerve ending function [8, 10]. Exogeneous histidine significantly increases the quantum composition of end-plate potentials (EPP) through an increase in the reserves of release-ready quanta and it reduces the frequency of miniature EPP (MEPP) [12]. Meanwhile it has been shown that resting and, in particular, synaptically activated skeletal muscles secrete into the incubation medium a substance giving a positive diazo reaction, and presumed to contain in its composition a histidine residue [10, 13], but not identical with free histidine or the histidine-containing muscle dipeptide carnosine [4]. Data have been obtained which suggest that carnosine may be the source of this substance [10].

As long ago as in 1935, Kibyakov [6, 7] found that muscle perfusate abolishes fatigue in a nerve–muscle preparation, and, as is now known, this fatigue is presynaptic in nature [11]. The active principle of the perfusate was found to have motor nerve endings as its target, and it is evidently neither a protein nor a catecholamine [6, 7]. Incidentally, fatigue in the nerve–muscle preparation is also abolished by exogenous histidine and carnosine [1, 4].

The aim of this investigation was to attempt to solve two problems: 1) are histidine-containing substances released into the medium during perfusion of the vascular bed of skeletal muscles; 2) does the perfusate have a presynaptic action similar to the action of histidine?

EXPERIMENTAL METHODS

The hind limbs of a donor frog were perfused in situ through the dorsal aorta and perfusate was collected from the abdominal vein. Ringer's solution, with the addition of 12 mM $MgCl_2$ (normal concentrations of Na^+, K^+, and Ca^{++}) was used as the perfusion medium. This solution was identical with that in which the control parameters of synaptic transmission of the recipient preparation (the dermo-pectoralis muscle of another frog) were recorded; Mg^{++} was used to reduce the quantum composition of EPP. A working portion of perfusate (about 50 ml without blood) was collected over 30-40 min. Before and after perfusion the pH of the solution was determined and its K^+ ion concentration measured with the aid of a K^+-selective valinomycin electrode, with a sensitivity of 28-30 mV to a tenfold change in K^+

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