The appearance of extrasynaptic sensitivity to acetylcholine in the presence of the tripeptide in a larger number of fibers than in the control can be attributed to the fact that the tripeptide prevents manifestation of the trophic effect of the corresponding opioid molecules present in the incubation medium. The presence of such molecules in the incubation medium cannot be ruled out. Their source may be either the embryonic calf serum added to the medium or the muscle itself.

It can thus be concluded that neurotrophic control of the properties of the chemosensitive membrane of skeletal muscle fibers may be exerted through the participation of a neurogenic peptide which possesses opiate properties. A possible candidate for this role may be β-endorphin.

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FEATURES OF CERTAIN FORMS OF GOAL-DIRECTED BEHAVIOR AFTER INDUCED CHANGES IN THE ENDOGENOUS β-ENDORPHIN LEVEL IN RATS

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β-endorphin is an endogenous peptide factor which is widely distributed in the CNS and gastrointestinal tract, is present in the blood serum and cerebrospinal fluid of animals and man, and can pass through the blood-brain barrier. It has been shown that β-endorphin is a powerful analgesic, it modifies activity of the cardiovascular and respiratory systems and the body temperature, and induces variously directed changes in hormone secretion and mediator metabolism, and it also exerts a considerable influence on various forms of behavior (feeding, sexual, defensive, etc.) and on the motor activity of animals [5, 6, 9, 10]. However, modern views on the physiological role of β-endorphin are still insufficiently complete. In order to explain the physiological role of the peptide factor, it is necessary to study the effects of selective blocking of its formation or action by binding with appropriate antibodies [2, 11]. It must be pointed out that complex forms of animal behavior after administration of antiserum or immunization against oligopeptides have not been adequately studied [1, 7, 8, 12].

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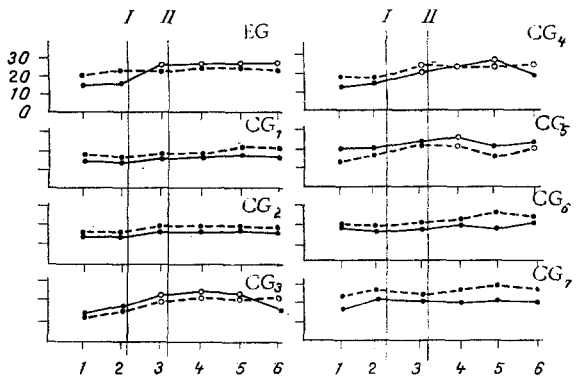

Fig. 1

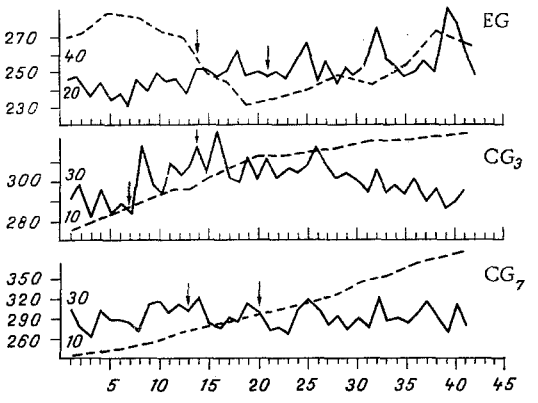

Fig. 2

Fig. 1. Time course of average daily consumption of food (continuous line) and water (broken line) in rats of EG and CG. Abscissa, time of observation (in weeks); ordinate, quantity of food (in g) and water (in ml) consumed. I) First immunization; II) 2nd immunization. Circles — $p < 0.05$ compared with background.

Fig. 2. Examples of individual time course of change in body weight (broken line) and food consumption (continuous line) for rats of EG and of some CG. Abscissa, time of observation (in days); ordinate, quantity of food consumed (in g) and body weight (in g). Arrows indicate 1st and 2nd immunizations.

Accordingly, in the present investigation the time course of various forms of goal-directed behavior was studied in rats immunized with a conjugate of β-endorphin and bovine serum albumin (BSA) in order to lower the endogenous β-endorphin level.

EXPERIMENTAL METHOD

Experiments were carried out on 66 male Wistar rats(12 experimental and 54 control). The period of the experiments as a whole was divided into the following states: I) background observation lasting 2 weeks; II) the first immunization or injection of the control solution, followed 1 week later by the second immunization or injection of control solution; III) the postimmunization period lasting 3 weeks. The quantity of food and water consumed per diem, and the body weight and temperature were recorded in all the animals. Three times during the period of the experiments all the animals were placed in an "open field" for 5 min (10th-12th, 17th-18th, and 32nd-34th days).

β-Endorphin was isolated from bovine pituitary glands [3, 4]. The method of isolation consisted of extraction of the pituitary glands with acid acetone, precipitation of the hormones with sodium chloride, desalting on Sephadex G-22; ion-exchange chromatography on CM-cellulose, and gel-filtration through Sephadex G-50.

To conjugate β-endorphin with BSA, bis-diazotized benzidine, obtained before conjugation by treatment of benzidine with sodium nitrate in the presence of an excess of acid, was used as the binding agent. To obtain the conjugate, the initial molar ratio of β-endorphin, bis-diazotized benzidine, and BSA was 10:10:1. The efficiency of conjugation, determined in a model experiment with ^{125}I-β-endorphin was 60%, i.e., 6 moles of β-endorphin was bound with 1 mole BSA in the conjugate.

The 12 animals of the experimental group (EG) were immunized with an emulsion of the conjugate of β-endorphin and BSA with Freund's complete adjuvant, which was injected subcutaneously in a dose of 0.1 ml into the upper third of the rats' hind limbs. Under these circumstances the dose of β-endorphin injected as the conjugate into one animal was 75 μg. In control group No. 1 (CG_1) 12 animals were immunized with emulsion of a conjugate of BSA with tyrosine (1 mg BSA + 0.24 mg tyrosine), as in EG. Each animal received 140 μg BSA in the composition of the conjugate. Animals of CG_2 (n = 12) received an injection of 140 μg BSA in 0.2 ml of physiological saline (0.1 ml into each limb subcutaneously). The rats of CG_3 (n = 6) were given a subcutaneous injection of 0.2 ml of the mixture of β-endorphin, not chemically bound with BSA, in a dose of 75 μg per animal, BSA, and Freund's adjuvant. Animals of CG_4 (n = 6) received a subcutaneous injection of 0.2 ml of physiological saline containing 75 μg of β-endorphin. Rats of CG_5 (n = 6) were given an injection of 0.2 ml of Freund's adjuvant

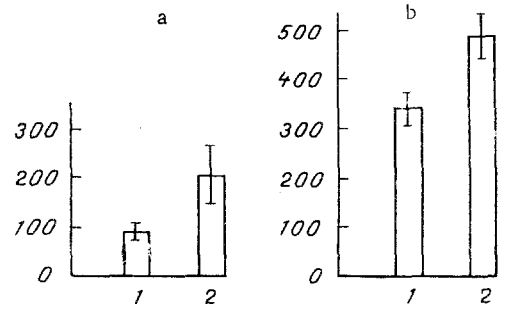

Fig. 3. β-Endorphin concentration in hypothalamus and pituitary glands of rats of EG and CG_3. a) β-Endorphin concentration in hypothalamus (in 10^{-15} mole/mg tissue); b) β-endorphin concentration in pituitary gland (in 10^{-12} mole/mg tissue). 1) EG; 2) CG_3.

only. Rats of CG_6 (n = 6) and CG_7 (n = 6) received injections of 0.2 ml of 0.1 M Na-phosphate buffer (pH 7.4) and of physiological saline respectively. Some of the animals (six rats of EG and six rats of CG_3) were decapitated 2 months after immunization, the pituitary gland and hypothalamus were quickly isolated, and with the aid of standard kits (Immuno Nuclear Corp., USA) the β-endorphin concentration in them was determined, using specific antiserum to endorphin in a dilution of 1:1500. Peptides were extracted with 0.2 M acetic acid solution at 100°C for 10 min, and after homogenization, they were centrifuged at 10,000g for 10 min. The supernatant was frozen and lyophilized. The residue was resuspended in BSA-borate buffer, the pH was adjusted to 8.0, and centrifugation was then repeated. The supernatant was kept at −20°C.

Nonparametric difference tests were used for statistical analysis of the results. The time course of food and water consumption was evaluated individually with respect to average values of the daily food and water consumption during each week of the experiment.

EXPERIMENTAL RESULTS

In the rats of EG, after immunization with the conjugate of β-endorphin and BSA, the daily food consumption increased significantly ($p < 0.05$; Fig. 1). This change was found after the first immunization and it lasted throughout the subsequent period of the experiment. A similar effect also was found in the animals of CG_3 and CG_4. Meanwhile, unlike in the animals of EG, in the rats of these groups an increase in water consumption was recorded ($p < 0.05$; Fig. 1). An increase in water consumption also was observed in the animals of CG_5 (Fig. 1). In animals of the remaining CG, no significant changes were observed in the consumption of food and water (Fig. 1).

Analysis of changes in the body weight of the experimental animals with time showed that in the rats of EG, unlike those of all the CG, the body weight did not increase throughout the period of the experiments. Examples of the individual time course of body weight, compared with that of food consumption in the animals of EG and of some CG (CG_3 and CG_7) are given in Fig. 2.

A study of the animals' motor activity in the open field test showed that compared with the background, after the 1st and 2nd immunization of the rats of EG, and also of CG_3 and CG_4, motor activity as reflected in the number of crossings between squares decreased sharply, but at the same time grooming intensified and obsessive motor stererotypes (chewing, shaking, etc.) appeared. In this case the animals of CG_5 showed an increase of motor activity.

No significant changes in body temperature were found at different stages of the experiments in the animals of EG and of all the CG.

In rats of EG, unlike those of all the CG, on the 10th-12th day after the 1st immunization with the conjugate of β-endorphin and BSA the response to handling decreased sharply, and then virtually disappeared. In this case the animals quietly sat on the palm of the experimenter's hand without exhibiting any orienting-investigative reaction, and responded only minimally to the sudden switching on of a bright light or to a loud sound. In the absence of any experimental procedures these animals likewise gave no aggressive reaction or autonomic manifestations (quickening of respiration, defecation, urination).

In the experiments with radioimmunoassay of β-endorphin in the hypothalamus and pituitary glands of the rats of EG and CG_3, the β-endorphin concentration in both structures of the rats was significantly lower ($p < 0.05$) by 59.6 and 30%, respectively, in the rats of EG than in those of CG_3, although 2 months had elapsed after immunization (Fig. 3).

Thus the rats of EG, after immunization with the conjugate of β-endorphin and BSA, developed definite changes in their feeding and aggressive-defensive behavior and motor activity, and these changes were only partially similar to the effect of injection of exogenous β-endorphin. The β-endorphin concentration was lowered in certain brain structures. Only in animals immunized with the conjugate of β-endorphin and BSA, by contrast with all rats of CT, was a prolonged increase in food consumption without any corresponding increase in body weight observed together with changes in the water intake, and also loss of the response to handling. It can be tentatively suggested that the changes described above are the result of modifications to the processes of synthesis, secretion, and degradation of β-endorphin in the animal under these conditions.

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EFFECT OF POTENTIATION OF ELECTROMYOGRAM–FORCE RELATIONSHIP

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KEY WORDS: potentiation of muscular contraction; electromyography

Electromyography is widely used in the physiology of movement, in clinical practice, and in sport medicine. It can be used to determine which muscles participate, and in what order, in the performance of a studied movement, and the intensity and duration of their excitation. The possibility of estimating the force developed by a muscle on the basis of parameters of its electrical activity is very attractive, but to do so it is necessary to have information on the relationship between the electromyogram (EMG) and force. Lippold [6] first discovered the linear relationship between the rectified integrated EMG of the gastrocnemius muscle and the force developed by the muscle. Subsequent investigation [4, 8, 9, 11] on various muscles revealed both a linear and a nonlinear relationship between these parameters. Very simple theoretical analysis predicts that the rectified integrated EMG ought to rise as a root of force, but there are virtually no relevant experimental data. The composition of the muscle fibers [11], the method of deriving the EMG (monopolar or bipolar) [11], and the length of the muscle [4, 9] are known to influence the EMG – force relationship. The view has been expressed [9] that this relatinship also is affected by factors such as the frequency characteristics of the motor units, the content and local distribution of fast and slow fibers, the signal from neighboring muscles, the work of antagonists and agonists, and the viscoelastic properties of the muscle. Yet another factor is evidently potentiation, which leads to an increase of force, and this must alter the EMG–force relationship

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