EFFECT OF β-ENDORPHIN ON UNIT ACTIVITY IN THE PREOPTIC REGION AS THE REGULATING CENTER FOR GONADOTROPHIC FUNCTION

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Endogenous oligopeptides giving a morphine-like effect [5, 15] and also specific receptors for them [4] have been found in different parts of the CNS. Morphine, if injected in the critical period, prevents the development of ovulation in rats [13] and blocks preovulatory secretion of luteinizing hormone (LH) into the blood stream [11]. It has also been shown that endogenous morphine-like peptides block liberation of luteinizing hormone releasing factor (LRH) from the hypothalamus [14].

It was therefore decided to study the sensitivity of single neurons in the preoptic region, as the center for cyclic regulation of pituitary gonadotrophic function, to the opiate oligopeptide β-endorphin, in order to study the mechanism of action of morphine-like substances on reproductive function.

EXPERIMENTAL METHOD

Experiments were carried out on 12 female rats weighing 200-220 g, kept under standard conditions of lighting (10 h of daylight, 14 h of darkness) and feeding. Animals with a stable 4-day estrous cycle were used. The experiments were carried out in the evening during the stage of proestrus (from 5 to 7 p.m.), the time of development of the preovulatory wave of LH in the blood in rats. The animals, anesthetized with urethane (1 g/kg body weight), immobilized with tubocurarine, and artificially ventilated, were fixed in a stereotaxic apparatus. Multibarreled glass microelectrodes were used for extracellular recording and microelectrophoretic application of β-endorphin. The central barrel of the electrode, which was used to record single unit activity in the preoptic region of the diencephalon, was filled with 2% Pontamine Sky Blue in 2 M NaCl and its impedance was 3-10 mΩ. The side barrels were filled with a solution of β-endorphin (1 mg/ml, pH 7.5) and 0.15 M NaCl to monitor the effect of the electric current on the preoptic neuron and to apply a compensating potential during microelectrophoresis of β-endorphin. The strength of the current for electrophoresis was 100 nA and its duration 120 sec. The impedance of the side barrels was 30-100 mΩ. Details of the technique used to record unit activity were described previously [1]. Microelectrophoresis of β-endorphin was carried out by means of the multibarreled "Microionophoreometer" manufactured by the EPM-1 Experimental Instruments Workshop, Academy of Medical Sciences of the USSR. The duration of electrophoresis was very accurately fixed by means of a Va-G-120 quartz generator. Amplitude–frequency–time analysis of unit activity was carried out by means of the Nokia-LP-4840 multichannel analyzer. The microelectrodes were oriented in accordance with the atlas [9] at coordinates H = 00(+1.5), AP = 7.8, L = 0.4. After activity had been recorded through the central barrel filled with a 2% solution of Pontamine Sky Blue in 2 M NaCl, electrophoresis of the dye was carried out (10 μA, 20 min) in order to identify the location of the microelectrode tip. The label was identified in brain sections 30 μ thick, cut with a freezing microtome and stained with hematoxylin and eosin.

EXPERIMENTAL RESULTS

Activity of 74 neurons recorded in the proptic region of the diencephalon was recorded; in 34 cases sensitivity to microelectrophoretic application of β-endorphin was investigated. The spontaneous discharge frequency of 55.8 ± 5.8% of the neurons tested was about 10 spikes/sec and the mean value for the group was 4.6 ± 0.69 spikes/sec. Only 17.6 ± 4.4% of neurons discharged with a frequency of between 10 and 20 spikes/sec.

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The mean spontaneous firing rate in this interval was 14.5 ± 1.3 spikes/sec. The same number of neurons discharged with a frequency of 30 to 40 spikes/sec (mean 34.7 ± 1.2 spikes/sec). A very few neurons, 5.9 ± 2.7% and 2.9 ± 1.9%, discharged with frequencies of 20-30 and 40-50 spikes/sec, respectively. Analysis of the results showed that of the 34 neurons whose sensitivity to β-endorphin was studied, 15 cells, i.e., 44.1 ± 8.5% of the total number of neurons tested with the oligopeptide, responded by inhibition to electrophoretic application of β-endorphin. The mean depth of the inhibitory response was 54.9 ± 5.65%. In 13 cases it was stable in character and persisted for 60 sec or more after the end of electrophoretic application of β-endorphin, but in two neurons there was only an initial inhibitory response which soon disappeared. Of the neurons tested in the preoptic region only six, or 17.7 ± 6.5% of the number of neurons tested, responded to this opiate oligopeptide by activation. The mean intensity of the activation response was 225.8 ± 29.3%. This response was usually observed in nerve cells characterized by low-frequency activity and it was unstable in character, whereas in three cases the response consisted of infrequent bursts of high-frequency discharges. No response to β-endorphin was given by 13 neurons (38.2 ± 8.3%). Statistical analysis showed that an inhibitory type of response to β-endorphin was found significantly more often ($P < 0.01$) among neurons of the preoptic region of the diencephalon than an activation type of response. This suggests that the inhibitory action of morphine-like oligopeptides on liberation of gonadotrophins and their releasing factors is based on specific blocking of neurons in the cyclic center for regulation of gonadotrophic function, the role of which, in the modern view, is played by the preoptic region of the diencephalon. The activation effect of β-endorphin on some of the neurons tested may perhaps also have a definite functional significance.

Information on the activation effect of opiates is being published more and more often at the present time. Morphine, when applied microelectrophoretically, is known to have the same effect on activity of some single neurons [6]. Injection of opiate oligopeptides also evokes activation or inhibition among neurons of the cortex and brain stem [7]. A certain number of neurons in the hippocampus have been bound to give an activation response to opiate peptides [10]. Meanwhile, investigations on the Renshaw cells of the spinal cord, through which recurrent inhibition of α-motoneurons is brought about, have shown that these cells, which take part in the mechanism of the inhibitory process, are activated by opiates [8]. A high proportion of neurons giving an activation response to leucine-encephalin when injected by the intraventricular route has been found in the cerebral cortex. Characteristically in these investigations the activation effect was abolished by injection of naloxone, a specific opiate competitor [3]. It can be tentatively suggested that cells of the preoptic region giving this response to β-endorphin among the nerve cells of the preoptic region in the diencephalon is evidence that sensitivity to opiate is selective in character, and to some extent it confirms the functional specificity of the responding neurons.

It was shown previously that catecholamines, as CNS mediators, play an extremely important role in the transmission of information in the neuroendocrine system [1, 2]. The effect of β-endorphin in the system for hypothalamic regulation of pituitary gonadotrophic function may perhaps be realized at the level of the catecholamine receptors of the postsynaptic neuronal membrane as a result of competition between morphine-like peptides and catecholamines for receptor sites. There is information in the literature that competition does in fact exist between them [12]. Further evidence in support of this view is given by data showing that liberation of LRH from the mediobasal hypothalamus, which usually develops under the influence of dopamine, is blocked by opiates [14].

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CHANGES IN THE MICROCIRCULATION IN THE RAT MESENTERY AND SKELETAL MUSCLES CORRELATING WITH THE SYSTEMIC ARTERIAL PRESSURE DURING HYPOTHALAMIC STIMULATION

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No definite experimental data have yet been obtained on the role of the nervous system in the regulation of the microhemodynamics. The difficulty of the problem is that the microcirculatory component of the hemodynamic system is, on the one hand, a sufficiently autonomous system capable of self-regulation, whereas on the other hand, it includes in its structure the nerve fibers which connect the microcirculatory system with the central portions of the sympathetic and parasympathetic systems, and in this connection it can be assumed that the influence of the CNS is not restricted to mechanisms leading to changes in systemic arterial pressure (AP). Influences from the sympathetic nervous system leading to changes in capillary permeability and to local changes in the blood supply to individual regions of the microcirculatory system, and so giving rise to considerable pathological disturbances in those regions, also are interesting.

Since the diencephalon and, in particular, the hypothalamus is considered to be the center for cardiovascular regulation, it must be expected that electrical stimulation of the hypothalamic nuclei would evoke considerable responses of the peripheral blood vessels, not necessarily connected with changes in AP. On the basis of these hypotheses and the results of the first studies of nervous regulation of the microcirculation [4], it was decided to study the dynamics of microcirculatory changes in the mesentery and skeletal muscles of rats in response to stimulation of the hypothalamic nuclei, in correlation with the response of the systemic AP.

EXPERIMENTAL METHOD

Acute experiments were carried out on 20 Wistar rats weighing 250 ± 30 g anesthetized with pentobarbital (50 mg/kg intramuscularly). The hypothalamic nuclei were stimulated through bipolar nichrome electrodes (diameter 200 μ) by a series of square pulses (0.5 msec, 80 Hz, 10-25 V) for 10-45 sec, the electrodes being introduced into the brain structures parallel to one another, taking stereotaxic coordinates from an atlas of the rat's brain [5, 8]. By intravital microscopy in transmitted light, changes in the diameter of the blood vessels and the state of the blood flow were studied in blood vessels from 250 to 5 μ in diameter in the mesentery and the inferior part of the trapezius muscle in rats. The systemic AP was recorded through a catheter in the carotid artery. Synchronization of the stimulation marker and microfilming enabled the microcirculatory changes observed in response to hypothalamic stimulation to be correlated with the dynamics of AP and cardiac activity. The location of the electrodes in the brain was verified histologically after the experiment, using the atlas of the rat's brain as a guide.

EXPERIMENTAL RESULTS

Stimulation of the ventromedial hypothalamic nucleus gave rise, after a short latent period (0-1 sec) to a pressor response with maximal elevation of AP in the course of 3-10 sec; the systolic pressure rose by a greater degree than the diastolic during the first 5-16 sec of stimulation: ΔAP_s was 51 ± 11 mm Hg and ΔAP_d was 34 ± 10 mm Hg (Fig. 1A). The pressor response continued throughout the period of stimulation. Corre-

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