We believe that our results can be used for a subsequent study of the pathogenesis both of male and of female infertility.

LITERATURE CITED


NEW DATA ON THE PARTICIPATION OF ß-ENDORPHIN IN THE NEUROENDOCRINE CONTROL OF THE RELEASE OF LUTEINIZING HORMONE

V. N. Babichev, V. Ya. Ignatkov, I. L. Kofman, and Yu. A. Pankov

Morphine, administered at the proestrus stage before the critical period, blocks the development of ovulation in cycling animals [1], and such blockage can be eliminated by electrical stimulation of the medial eminence of the hypothalamus [2]. On this basis it was suggested that morphine blocks the hypothalamic mechanisms providing for the triggering of the preovulatory wave of release of gonadotropins from the pituitary. Subsequent investigations using intravenous injections of morphine confirmed this hypothesis. It was shown that morphine inhibits the development of the preovulatory waves of luteinizing (LH) and follicle-stimulating hormones in the blood [3]. These data were of definite practical importance. However, after specific opiate receptors were detected in the central nervous system [4] and oligopeptides giving a morphine-like effect were detected in various structures of the brain [5], it was suggested that the endogenous opioids participate in the hypothalamic control of pituitary function [6]. The greatest concentration of ß-endorphin in a comparison of various regions of the brain is noted in the region of the arcuate nucleus and medial eminence of the hypothalamus [7]. And yet, there are data that opioid peptides are present in relatively large quantities in the preoptic-anterior hypothalamic regions of the brain [8] and that terminals from neurons that contain endogenous opioids end close to the estrogen-accumulating nerve cells of this region of the hypothalamus. As is well known, the preoptic region is a center of cyclic regulation of LH release by the pituitary [9] and the region through which endogenous triggering of the release of luteinizing hormone is accomplished [10].

An investigation of the dynamics in the content of opioid peptides in the preoptic-anterior hypothalamic region of the brain in the course of the estrus cycle in rats showed that their high level in the morning in the proestrus stage falls substantially during the evening hours of this key stage of the estrus cycle [11].

On the basis of the literature data enumerated above, we suggested that the endogenous peptides, exerting an influence on various neurons of the hypothalamic center of cyclic regulation of gonadotropic function, are included in the mechanisms of the formation of the preovulatory wave of LH. To resolve this question we studied the sensitivity of single neurons of the preoptic region (PR) of the hypothalamus to ß-endorphin under conditions of its microiontophoretic delivery, evaluating the influence of this opioid on the level of LH in the blood and pituitary under the indicated experimental conditions.

Laboratory of the Physiology of the Endocrine System and Laboratory of the Biochemistry of Protein Hormones, Institute of Experimental Endocrinology and Hormone Chemistry, Academy of Medical Sciences of the USSR, Moscow. Translated from Problemy Endokrinologii, Vol. 28, No. 2, pp. 45-49, March-April, 1982. Original article submitted June 10, 1981.
Fig. 1. Level of LH in the blood (A) and pituitary (B) (in ng per mg tissue) in animals of the first (1) and second (2) controls, as well as after microiontophoretic delivery of β-endorphin to the PR of the hypothalamus (3).

MATERIALS AND METHODS

The investigations were conducted on 23 control and 12 experimental female rats weighing 200-220 g, kept under standard conditions of illumination (14 h of light, 10 h of darkness) and nutrition. In the experiments we used animals with a stable four-day estrous cycle. The experiments were conducted in the evening hours of the proestrus stage (from 1700 to 1900 h), which are characterized by the development of a preovulatory wave of LH in the blood of the rats. A highly purified preparation of β-endorphin was used; it was isolated from pulverized cattle pituitaries according to a method including extraction with acidified aqueous acetone, precipitation in acetone, fractionation with NaCl, and ion-exchange chromatography on a column with carboxymethylcellulose. The concluding stage of purification of β-endorphin was filtration through Sephadex G-50 [12]. Under conditions of urethane anesthesia (1 g per kg of body weight), animals immobilized with tubarin and transferred to artificial respiration were fixed in a stereotaxic instrument. Extracellular recording of the activity of single neurons and microiontophoretic delivery of β-endorphin to them were performed with the aid of multichannel glass microelectrodes. The central stem of the electrode that was used to record the pulsed activity of single neurons of the PR was filled with a 2% solution of pontamine azure in 2 M NaCl. It had a resistance of 3-10 MΩ. The side stems were filled with a solution of β-endorphin (1 mg/ml, pH 7.5) and 0.15 M NaCl to monitor the effect of electric current on the investigated structure and to apply a compensating potential during microiontophoresis of β-endorphin. The strength of the iontophoretic current was 100 nA, duration 120 sec. Microiontophoresis was carried out with the aid of a Mikroionoforeometr instrument, developed at the laboratory of the physiology of the endocrine system of the Institute of Experimental Endocrinology and Hormone Chemistry, Academy of Medical Sciences of the USSR, in collaboration with the experiment industrial shops of the Academy of Medical Sciences of the USSR. The time of iontophoresis was set with high accuracy by a VA-G-120 generator. The activity of single neurons was recorded with the aid of a standard setup for electrophysiological investigations, including a UB2-03 amplifier of biopotentials, a CI-16 oscillograph, a FOR-2 photorecorder, and a NO-36 seven-channel magnetograph. Amplitude-frequency-time analysis of the pulsed activity was performed with the aid of an NOKIA LP-4840 multichannel pulse analyzer. The microelectrodes were oriented according to the Atlas [13] according to the coordinates H = 00 (+1.5), AP = 7.8, L = 0.4. The animals were decapitated 20 min after the end of the iontophoretic delivery of β-endorphin, and blood and the pituitary were collected for the determination of the LH level by a radioimmunological method [14]. In the determination we used the antibodies for LH produced in our laboratory on guinea pigs. Purified rat LH NIH-LH-SI was used in the radioimmunological determination. The quantitative data were expressed in nanograms per ml of plasma or per mg of pituitary tissue, converted to a preparation of highly purified LH-NIH-LH-SI. There were two groups of animals in the control experiments. In the first control group the animals were decapitated and blood and the pituitary were collected, while in the second group decapitation was performed after all the manipulations of the experiment, with the exception of microiontophoretic delivery of β-endorphin. In the animals in which the accuracy of the positioning of the microelectrode in the medial PR of the hypothalamus was monitored, a dye, pontamine azure, was delivered through the central stem of the microelectrode for 20 min with the aid of anodic current with a strength of 10 μA. The localization of the tip of the microelec-