EFFECT OF ENDOGENOUS OPIOIDS ON FORMATION OF THE REPRODUCTIVE FUNCTION IN RATS

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In recent studies neurophysiologists have devoted great attention to neuropeptides, hypothalamic releasing hormones and statins, and certain hormones of the pituitary gland and gastrointestinal tract. These are all biologically active substances which play the role of neurotransmitters or neuromodulators in the nervous system and have an action on the course of various physiological processes, one of which is the formation of the neuroendocrine reproductive function of the body.

It has long been known that the reproductive function of persons taking narcotics is disturbed, at the level both of the peripheral organs and the CNS [8, 10]. Numerous recent investigations have shown that opioid peptides exert their damaging action at the hypothalamic level, and disturbances at the periphery are secondary [13, 14].

Facts confirming that the regulation of reproductive function likewise is under hypothalamic control have been obtained in our laboratory, and it has been shown that this process is mediated through receptor proteins specific for sex hormones [2, 5]. It has also been shown that during the neonatal period of life, during formation of the type of regulation of pituitary gonadotropic function in rats, a steroid-receptor system characteristic of the male or female type of regulation is formed, and depends on the sex hormone level during this period [3].

Accordingly the question arises of the possible effect of opioid peptides in the neonatal period of development on formation of the steroid-receptor system in male and female rats. To shed light on this problem, the investigation described below was carried out.

EXPERIMENTAL METHOD

Experiments were carried out on male and female albino rats of a mixed population, which were given intramuscular injections of β-endorphin in a dose of 1 μg/g body weight from the 1st through the 5th day of postnatal life. Control animals received physiological saline. The animals were divided into two groups. In group 1, consisting of females who had reached the age of 26 days, estradiol benzoate was injected at 10 a.m. in a dose of 2.5 μg/100 g body weight, followed 48 h later by progesterone (P) in a dose of 2.5 mg/100 g body weight. The males were castrated at the age of 26 days. The animals were killed at the age of 28 days and their serum levels of sex hormones and luteinizing hormone (LH) were determined. Animals of group 2, which received β-endorphin during the first 5 days of life, were killed at the age of 90 days: females at 6 p.m. on the day of proestrus. Nuclear receptors for sex hormones were determined in the pituitary and in two regions of the hypothalamus: in the preoptic region and region of the anterior hypothalamus (PR), and in the region of the medial-basal hypothalamus (MBH). For each determination material from 30 animals was pooled. The tissue was homogenized in Tris-EDTA buffer and the homogenate centrifuged for 10 min at 800 g. The nuclei were isolated from the residue thus obtained by Chauveau's method in the modification in [4]. 1,2,6,7-^{3}H-testosterone (^{3}H-T) and 2,4,6,7-^{3}H-estradiol, with specific activity of 85-120 Ci/mmole, were used as labeled hormones. The conditions for incubation of the labeled steroids with the nuclear fraction of the pituitary and hypothalamus and calculation of the number of specific binding sites

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TABLE 1. Receptor Binding of ^3H-E_2 and ^3H-T-Nuclear Fraction of Hypothalamic Regions and Pituitary Gland of Male and Female Rats Receiving β-Endorphin from 1st through 5th Days of Postnatal Life

Receptors	Tissue	Group of animals			
		females		males	
		intact	intact + β-endorphin	intact	intact + β-endorphin
Nuclear E_2 receptors, fmoles/mg DNA	PR (6)	789,5±61,3	848,1±79,5	373,4±41,1	632,3±59,2
	p	<0,05		<0,01	<0,05
	MBH (7)	265,3±30,4	380,7±57,0	797,1±65,3	380,0±47,7
Nuclear T receptors, fmoles/mg DNA	Pituitary (7)	227,1±33,4	196,0±21,1	238,4±36,6	187,0±22,3
	PR (6)	125,1±20,3	154,3±24,5*	415,6±55,4*	155,1±20,9*
	p			<0,05	
	MBH (6)	156,3±31,2	139,1±19,0	912,3±88,9*	215,5±38,8*
	Pituitary (8)	124,9±15,3	153,7±25,5	1179,1±111,3*	390,5±61,4*

Legend. *$p < 0.001$ for comparison of intact animals with those receiving β-endorphin. Here and in Tables 2 and 3, number of animals given in parentheses.

were described previously [1]. The concentrations of sex hormones were determined with the aid of kits for radioimmunoassay of estradiol (E_2) — RIN-E_2-^3H — and testosterone (T) — sterone T-^3H, and the LH level also was determined by radioimmunoassay.

EXPERIMENTAL RESULTS

The results are shown in Tables 1-3. The number of nuclear estradiol receptors in PR of intact female rats was 2.5 times higher than in MBH (Table 1). Injection of β-endorphin during the first 5 days of life had no effect on the concentration of E_2 receptors in the hypothalamus or pituitary, or on their ratio in the two hypothalamic regions.

A different picture was observed in male rats. In intact animals the concentration of receptors in MBH was twice as high as in PR. As a result of early chronic administration of β-endorphin the number of E_2 receptors in PR rose by more than 1.5 times, whereas in MBH it fell by half. The concentration of estrogenic receptors in the male pituitary gland remained unchanged.

Receptors to T were found in small numbers in both groups of female rats, in both the hypothalamus and the pituitary; injection of β-endorphin, moreover, did not affect their level or distribution in the hypothalamus. In the intact male rats, a high concentration of T receptors was observed both in the pituitary and in the hypothalamus; in MBH their level was twice as high as in PR. Injection of β-endorphin in the neonatal period led to a three- to fourfold decrease in the number of androgenic receptors in all the tissues studied; a significant difference in the concentration of T-binding sites was not observed moreover between the hypothalamic regions in the animals of this group.

It will be clear from Table 2 that injection of β-endorphin in the neonatal period into mature female and male rats did not affect the concentrations of sex hormones and LH.

In the second series of experiments the serum concentrations of sex hormones and LH were determined in 28-day-old rats in order to discover the possible action of β-endorphin on the formation of tonic and cyclic types of regulation of gonadotrophin secretion. Table 3 shows that the E_2 concentration in 28-day-old female rats with preliminary treatment with EB and P did not differ from that in intact 28-day-old females. Early injection of β-endorphin likewise had no effect on the E_2 concentration, however, the LH level in animals receiving and not receiving β-endorphin but treated with EB and P, was 3-4 times higher than in intact females at this age. In males receiving and not receiving β-endorphin and castrated at the age of 26 days, the T concentration was 12-13 times lower, whereas the LH level was 3-4 times higher than in intact 28-day-old male rats.

The results of these experiments showed that chronic administration of β-endorphin in the first days of life did not affect the formation of the estradiol- and testosterone-receptor systems in female rats, but caused feminization of these systems in males.

TABLE 2. Serum Concentrations of E_2, T, and LH in Rats Receiving β-Endorphin in Neonatal Life

Group of animals	E_2, pg/ml	T, pg/ml	LH, ng/ml
Females (9):			
intact	38,3±3,1	65,0±5,9	654,0±39,6
intact + β-endorphin	35,9±2,7	71,2±6,7	515,7±33,8
Males (9):			
intact	15,3±2,2	342,2±43,1	63,8±4,1
intact + β-endorphin	12,7±2,9	298,3±21,2	60,1±6,6

TABLE 3. Effect of β-Endorphin, Injected during First 5 Days of Life, on Differentiation of Tonic and Cyclic Centers of Gonadotrophin Regulation

Group of animals	E_2, pg/ml	T, pg/ml	LH, ng/ml
Females 28 days old			
intact	10,5±1,9	—	33,1±5,3
intact + EB + P (8)	11,1±1,2	—	135,3±19,1
intact + β-endorphin + EB + P (7)	7,8±0,9	—	105,3±10,8
Males aged 28 days			
intact	—	77,4±7,1	33,5±3,3
castrated + TP	—	4,5±0,3	88,4±9,9
intact + β-endorphin + TP	—	5,5±0,8	101,1±11,5

Opioid peptides capable of affecting reproductive function are known to be present at different levels of the hypothalamo-hypophyseo-gonads system [11]. However, the chief effect of opiates in this system, namely regulation of pituitary gonadotrophic function, is realized at the level of the hypothalamus and pituitary [7], and is expressed in the form of an inhibitory action on gonadotrophin secretion [6, 15].

Immediately after reduction of the gonadotrophin concentration there is a decrease in the level of sex hormones, which in the neonatal period are inducers of sexual differentiation of the brain.

In the present experiments the blood level of sex hormones evidently fell to a level which could not sustain the normal process of sexual differentiation of the hypothalamus according to the male type, as shown by the feminized distribution of estrogenic receptors in the regions of the hypothalamus and a sharp fall in the level of T receptors in all tissues.

When the concentrations of receptors thus obtained were compared with the blood levels of sex hormones and LH it was noted that chronic treatment of the animals with β-endorphin did not affect hormone levels in mature rats, possible evidence that in females and males negative feedback is present, but in females positive feedback also is present.

As has already been stated, in rats in the "critical" period sexual differentiation of the brain and its neuroendocrine structures takes place, and these processes are regulated and controlled in adult animals not only by hormones, but also by monoamines and opioids. The action of opioids is mediated through several types of receptors, the most important of which in the mechanism controlling gonadotrophic secretion are the mu-receptors [15]. According to some workers, the concentration of mu-receptors depends on the blood levels of sex hormones [9, 12, 16]. It can be tentatively suggested that the fall of the E_2 and T levels during the first days of life under the influence of β-endorphin could reflect the damaging action on the opioid receptor system of the brain. The result of that action could be reduction of the number or effective structure of the mu-receptors, leading to the formation of unstable bonds in the mechanism controlling gonadotrophin secretion and, as a result, a disturbance of cyclic function and ovulation.

Thus the result of this investigation indicates that chronic administration of β-endorphin in the neonatal period, leading to a deficiency of sex hormones in the blood, feminizes the number and distribution of E_2 and T receptors in male rats, and while not changing the direction of sexual differentiation of the hypothalamic structures, it leads to the establishment of unstable connections in the mechanism controlling pituitary gonadotrophic function.

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