Reaction of Mouse Ovary to Opioid Receptor Block during Early Postnatal Ontogenesis

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Prolonged administration of naloxone (5 mg/kg body weight) during the juvenile period stimulated ovarian folliculogenesis and pubescence in mice; the effect was absent in animals of other ages, when a single injection was given, or when the dose of preparation was decreased.

Key Words: *ovary; pubescence; naloxone; opioid peptides*

Prepubertal changes in the secretion of luteinizing hormone (LH), expressed in specific daytime "peaks" of high amplitude, figure prominently in the complicated mosaic of events attending the pubescence of mammalian females [11]. A central genesis of this phenomenon, also peculiar to ovariectomized animals, is obvious but its mechanisms are not clear. In recent years the role of endogenous opioids in the modulation of gonadotropin secretion has been widely discussed [1,3]. It has been demonstrated that the morphine-induced inhibition of ovulation caused by the blockade of the preovulatory elevation of the LH level in the bloodflow is arrested by opioid peptide antagonists including naloxone [4,13]. The ability of the latter to hasten the pubescence of female rats was used to show that the brain opioid system acts as an inhibitor of puberty [5,14]. However, the degree of universality of this mechanism (in a number of mammals) is still not clear and there is no consensus on the estimated age limits of the effect of opiate antagonists [9,10,14].

In the present investigation the effect of naloxone on the rate of pubescence and on the morphogenesis of mouse ovary was studied as a function of the time and scheme of drug administration.

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MATERIALS AND METHODS

Experiments were carried out on female CBA mice. In the first series the animals used were of different ages corresponding to periods of maturation as follows: newborn, infant (14- and 21-day), juvenile (26- and 28-day), and peripubertal (32-day) females. Naloxone injections (5 mg/kg body weight) were performed 4 times a day at 2-hour intervals during 4 days; 0.1 ml of saline was introduced to control animals according to the same scheme. The times of vagina opening and of the 1st ovulation were recorded according to the vaginal smear. For the assessment of ovarian folliculogenesis and ovulation productivity some animals were decapitated on the day of vagina opening and the others on the 1st day of estrus. On serial sections of the ovaries stained with hematoxylin and eosin the composition of the follicular population, classified as described elsewhere [12], was analyzed morphometrically and the number of corpora lutea was calculated in the ovaries of "estrous" females. In an individual group of animals (naloxone injections from the 28th day of life, n=16) the serum concentration of estradiol-17β and progesterone was measured (on the day of vagina opening) by radioimmunological assay. In the 2nd and 3rd experimental series 28-day-old females were used as follows: in the first case the effect of a decrease of a one-time dose (0.5 mg/kg body weight) was estimated under the conditions of chronic administration described above, while in the second the reaction to a single

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injection of naloxone in a dose of 10 mg/kg was assessed; the results were tested at the times of vagina opening and of the 1st estrus. The influence of the initial state of ovarian folliculogenesis and, as a result, of steroidogenesis on the aftereffects of pharmacotherapy was assessed in an additional (4th) series of tests. For this purpose the following drug combination was tested: 10 IU of serum gonadotropin (pregnant mare serum, PMS) was administered to 24-day-old mice (induction of anthral follicle growth) followed by (after 48 h) injections of naloxone according to the scheme for the 1st series. The same volume of saline was injected to control females (n=12) of the same age. The Student t test was used for the statistical treatment of the results.

RESULTS

According to the data of the 1st experimental series the realization of the effect of opioid receptor blockage has age limitations. Acceleration of pubescence in mice (according to the date of the first ovulation) occurs only in the case of prolonged naloxone administration in the juvenile period (Table 1). The desired effect was absent in experiments with newborn and infant females, contrary to observations reported previously [14]. Injections of the preparation from the 26th to 28th day of life induced a premature (<34 days) onset of the first estrus (ovulation) in 38 and 44% of females, respectively. In parallel, an earlier (<30 days) vagina opening was noted as well as a shortening of the interval between this external sign

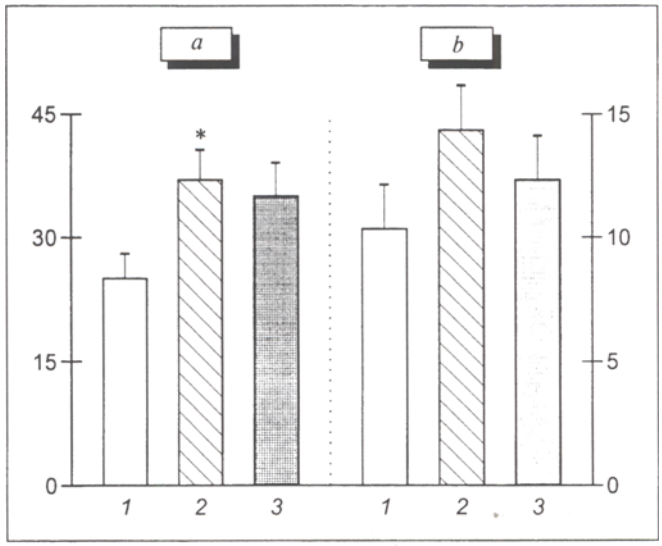

Fig. 1. Estradiol (*a*) and progesterone (*b*) concentration in mouse plasma (on the day of vagina opening) for chronic administration of naloxone (5 mg/kg) from the 28th day of life. *1*) control; *2*) premature pubescence; *3*) no effect. Ordinate: left - estradiol-17β concentration, pg/ml; right - progesterone, ng/ml. *p<0.05 compared with the control.

TABLE 1. Effect of Naloxone on the Tempo of Pubescence in Mice under Different Experimental Conditions

Experimental series, initial age			Effect[1]	Number of mice	Date of first ovulation (age, days)
1st series:					
	5 days	control	-	5	36.1±1.3
		experiment	-	6	35.9±0.9
	14 days	control	-	6	35.5±1.5
		experiment	-	6	36.4±0.8
			+/-	3	37.9±0.9*
	21 days	control	-	7	35.8±1.6
		experiment	-	7	36.7±0.8
	26 days	control	-	7	36.6±1.6
		experiment	-	8	37.1±0.9
			+/-	2	35.4±1.8
			+	6	33.5±0.4*
	28 days	control	-	8	35.9±1.7
		experiment	-	6	36.3±1.5
			+/-	3	35.4±1.2
			+	7	32.3±1.1*
	32 days	control	-	6	38.4±1.9
		experiment	-	6	39.1±1.7
2nd series:					
	28 days	control	-	6	37.1±0.6
		experiment	-	7	36.7±1.4
			+/-	1	36.0
3rd series:					
	28 days	control	-	7	36.9±1.3
		experiment	-	7	37.1±0.8

Note. [1] "-" - no effect, "+" - acceleration of pubescence according to date of first ovulation, "+/-" time of vagina opening, but not of the first estrus brought forward. Here and in Table 2: *p<0.05 as compared to the control.

TABLE 2. Characteristics of Ovarian Folliculogenesis in Mice Chronically Administered Naloxone in a Dose of 5 mg/kg Body Weight (1st Experimental Series, Indexes Recorded on Day of Vagina Opening)

Age, days (start of injection)	Effect	Number of mice	Number of follicles according to stages of development:				Atretic index, %
			1-3a	3b-5a	5b-6	7-8	
Control	-	30	$(39\pm4)\times10^2$	$(34\pm7)\times10$	56±8	5±2	29±3
5	-	7	$(41\pm3)\times10^2$	$(32\pm3)\times10$	57±9	7±2	26±4
14	+	2	$(42\pm2)\times10^2$	$(31\pm4)\times10$	52±7	5±2	42±5*
	-	4	$(40\pm3)\times10^2$	$(30\pm5)\times10$	55±6	7±3	28±4
21	-	5	$(39\pm4)\times10^2$	$(33\pm4)\times10$	58±5	6±3	30±6
26, 28	+	6	$(37\pm5)\times10^2$	$(34\pm6)\times10$	50±8	10±2*	34±2
	-	8	$(40\pm2)\times10^2$	$(35\pm5)\times10$	56±4	8±4	27±4
32	-	4	$(36\pm3)\times10^2$	$(36\pm4)\times10$	59±7	7±3	32±6

Note. Total values are given due to similarity of indexes in groups. "+" - premature opening of the vagina, "-" - absence of an effect.

of puberty and the first ovulation. In some animals (12-18%) the effect was limited to an earlier vagina opening without a reliable decrease of the age of the first estrus. Such an imitation of earlier puberty but with the first ovulation delayed by 2-3 days was recorded in the group of females injected with naloxone from the 2-week age. The animals aged 32 days proved virtually naloxone-tolerant in that pubescence was delayed (the vagina is already open in 85-90% of CBA mice of this age). Individual variations in the rates of pubescence, that is, the establishment of hypothalamic-hypophyseal-gonadal interactions, may be one of causes of the absence of a 100% effect in the "sensitive" group of females (naloxone injections in the juvenile period). This possibility is indirectly supported by the results of the 4th experimental series, in which early juvenile females were premedicated with PMS. Under these conditions a certain synchronization of the initial ovarian status led to a significant increase in the number of animals sensitive to the treatment; thus, for instance, acceleration of pubescence was noted in 66% of mice (39% after administration of naloxone alone). This situation can be correctly interpreted as a naloxone potentiation of the effect of estrogens [8], the secretion of which was stimulated by follicle-stimulating hormone (FSH).

Morphometric analysis of the ovary confirmed that induction of pulsed LH secretion stimulates folliculogenesis (or more precisely, the growth of anthral follicles) only in juvenile mice (Table 2). In this case a significant elevation of the number of follicles at the 7th stage and the appearance of the 8th stage of growth (preovulatory follicles) typical for proestrus were noted in females with premature pubescence. The strong correlation between the development of the follicular apparatus and the level of sex hormones in the blood, which was higher in mice sensitive to drug treatment (Fig. 1), is to be noted. Variations in the degree of ovary "maturation" at the time of vagina opening may determine fluctuations of the interval between this event and the time of the first ovulation. In an assessment of the informativeness of an external indication of puberty, the observations on its earlier appearance (with a subsequent delay of the first estrus) in mice injected from the 14th day of life are of interest. This phenomenon may be a result of boosted production of androgens capable of aromatization [11] under conditions of massive follicle atresia, which is probably provoked by an FSH/LH imbalance expressed at precisely this age.

A count of the corpora lutea in the ovaries from "estrous" females showed that acceleration of puberty did not affect the productivity of the first ovulation.

Thus, our findings provide evidence that the puberty-stimulating effect of opiate antagonists is limited to the juvenile period [10]. It is apparent that precisely at this age the stage is set for the development of a particular reaction, namely maximal sensitivity of the pituitary to LH and to gonadoliberin [7] and a proper level of ovarian folliculogenesis. The lack of an effect in the case of a decreased single dose of preparation (2nd series) may be due to the fact that different types of receptors interact with "low" and "high" naloxone doses [2]. The results of the 3rd series of experiments (Table 1) attest to a key role of prolongation of LH "peaks" in puberty onset.

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Activity of Nerve Growth Factor in Rat Regenerating Liver

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Two bioassays of specific activity in the presence and absence of specific antiserum, incubation with pheochromocytoma PC-12 cells, proteolytic digestion, and ultrafiltration demonstrate that nerve growth factor isolated from the liver exhibits the basic characteristics of classic mouse nerve growth factor. High activity of this factor is observed in the operated lobe during the first day (3-20 hours) and on days 3-10 of regeneration, i.e., before and after the phase of hepatocyte proliferation.

Key Words: *nerve growth factor; regeneration of the liver; pheochromocytoma; organ cultures*

A few published data [6,8-10] suggest that exogenously injected nerve growth factor (NGF) accelerates the initial stages of regeneration in organs and tissues. This effect is most likely due to an increase in the innervation of the damaged area and the development of so-called hyperneuria [1]. However, endogenous (synthesized in the tissue) NGF, which may be obtained during posttraumatic regeneration of the liver [4], has not yet been studied.

The aim of the present study was to perform a physiological and biochemical analysis of isolated hepatic NGF and to elucidate its possible effect on the repair processes in the liver.

MATERIALS AND METHODS

Random-bred albino rats were divided into several groups. In group I during the first few hours (3, 8, 15, and 20) and on days 1, 3, 5, 7, 10, 14, 21, and 30 of posttraumatic regeneration of the liver (50% resection of the left lobe) an NGF-like substance was isolated and a comparative analysis of NGF activity in the liver lobes and in the serum was carried out with the treatment of selected samples (control and days 3 and 7) with anti-NGF antiserum. In group II NGF activity was measured in a modified organ culture of the liver. In group III NGF isolated from the liver was tested in a culture of pheochromocytoma PC-12 cells. In group IV the obtained factor was treated with pronase to determine its nature and subjected to ultrafiltration on PM-10 membrane filters. Each group of rats had respective controls.

Hepatic NGF was isolated by chromatography on sorbents produced at the Institute of Biochemistry of the Academy of Sciences of the Republic of Uzbekistan (Patent No. 1172114 issued by the Research Institute of the State Patent Expert Commission, USSR, 1982). The activity of the end product was biologically tested by culturing the spinal ganglia of 8-day-old chick embryos in the presence of the dissolved test sub-

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