CHANGES IN THE TESTOSTERONE CONCENTRATION IN RAT FETAL TESTES AFTER ENCEPHALECTOMY

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UDC 612.616.31.018-06:612.826.4

KEY WORDS: rat fetuses; encephalectomy; testes; testosterone.

Sex steroids secreted by the testes of fetal and neonatal rats are known to be responsible for masculinization of the genital tract and also of the CNS. The period during which sex steroids can exert their organizing action in rats extends from the last week of embryonic life to the 5th-10th day after birth.

It has also been shown that the morphological and physiological state of the testes in fetuses is under the control of the fetal adenohypophysis, effected through gonadotrophins. In rat fetuses gonadotrophins can be detected radioimmunologically as early as on the 16th-17th day in the adenohypophysis [2, 3] and also in the blood [3]. Removal of the pituitary by decapitation of 18-20-day rat fetuses *in utero* leads to a reduction in the total volume of the Leydig cells in the testes [6] and to a sharp reduction in their testosterone content [11].

Luteinizing hormone releasing hormone (LHRH) is found in the rat fetal hypothalamus, both radioimmunologically [8] and immunohistochemically [10] after the 18th day of development. According to other data [2], small quantities of LHRH can be found as early as on the 16th-17th day of fetal development. However, the functional role of LHRH has not yet been settled. It has been suggested that this neurohormone may take part in differentiation of the pituitary gonadotrophic hormones [4]. In anencephalic human fetuses the testes are in a hypoplastic state, the number of Leydig cells is significantly reduced, and there are certain features of underdevelopment and reduced activity of structures dependent on the testes [13]. Although a pituitary is present, the gonadotrophin level in the plasma of newborn anencephalic infants is sharply reduced. Injection of LHRH into newborn anencephalic infants does not lead to an increase in the blood gonadotrophin level, possibly because of the need for prolonged LHRH stimulation to obtain a response of the adenohypophyseal gonadotrophic hormones in human anencephalics [9]. In rat fetuses injury to the hypothalamus as a result of intracranial injections of paraffin leads to a reduction in the total volume of Leydig cells in the testes [6, 7]. The data described above are indirect evidence of a possible functional role of the fetal hypothalamus in control of testicular hormonal function.

The object of this investigation was to obtain direct data on the role of the hypothalamus in the control of the androgenic function of the testis in rat fetuses.

EXPERIMENTAL METHOD

Wistar rat fetuses were used. Female rats weighing about 200 g were mated with males at 6 p.m. and separated next morning. If spermatozoa were found in the films the day after copulation was taken as the first day of pregnancy.

To carry out encephalectomy on the fetuses, laparotomy was performed on the pregnant rats under ether anesthesia at the stage of 18.5 days of pregnancy, and surgical encephalectomy was performed on the fetuses *in utero* by the method described previously [1].

In the experiments of series I the effects of removal of the influence of the hypothalamus on the testosterone concentration in the testes was studied. On the 3rd day after encephalectomy, i.e., at the age of 21.5 days of intrauterine development, the mother rat was anesthetized with pentobarbital, the fetuses were removed from the uterus, and their testes iso-

Laboratory of Hormonal Regulation, N. K. Kol'tsov Institute of Developmental Biology, Academy of Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Kraevskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 95, No. 3, pp. 93-95, March, 1983. Original article submitted June 1, 1982.

 0007-4888/83/9503-0366$07.50

TABLE 1. Changes in Testosterone Concentration in Testes of Rat Fetuses after Encephalectomy and after Injection of LHRH into Encephalectomized Fetuses

Series of experiments	Treatment of fetuses	Weight of testes, mg	Testosterone concentration in testicular tissue, pg/mg
I	Control	3,33±0,14 (28)	44,33±8,63 (14)
	Encephalectomy	3,14±0,14 (38)	13,40±2,48* (15)
II	Encephalectomy and injection of LHRH	3,32±0,09 (33)	51,41±6,85† (10)
	Encephalectomy and injection of physiological saline	3,41±0,05 (22)	12,4±2,11 (10)

Legend. 1) Number of fetuses shown in parentheses. 2) Level of significance of differences compared with corresponding control: *$P < 0.01$, †$P < 0.001$. 3) Age of fetuses at operation 18.5 days, at sacrifice 21.5 days.

lated. Intact fetuses of the same litter served as the control. The isolated testes were weighed with an accuracy of 0.05 mg and kept at −70°C until testosterone determination.

In the experiments of series II the effect of "blocking" the hypothalamus was abolished by injection of LHRH into the encephalectomized fetuses. Synthetic LHRH (from Calbiochem, 2 μg in 0.05 ml physiological saline per fetus) was injected intraperitoneally 2 h before sacrifice into 21.5-day-old encephalectomized fetuses from one uterine cornu. Encephalectomized fetuses from the other uterine cornu, into which physiological saline was injected, served as the control. The testes were processed as described above.

To determine testosterone in the testicular tissue a tissue homogenate was prepared in Tris-sucrose buffer, pH 7.5; testosterone was extracted from the homogenate with ether and aliquots of ether were evaporated to dryness. Testosterone was determined radioimmunologically by means of a kit from CEA-IRE-Sorin (France).

EXPERIMENTAL RESULTS

As Table 1 shows, removal of the hypothalamus from rat fetuses at the stage of 18.5 days of prenatal development caused no significant change in weight of the testes 3 days after the operation (3.33 ± 0.14 mg in the control, 3.14 ± 0.06 mg after encephalectomy).

Determination of testosterone in the testes of 21.5-day-old encephalectomized fetuses showed that the concentration of this hormone was reduced 3 days after the operation (44.33 ± 8.63 pg/mg in the control, 13.40 ± 2.48 pg/mg after encephalectomy; $P < 0.01$).

Testosterone is known to be synthesized in the testes by Leydig cells. The results now obtained showing changes in the testosterone concentration in the testes after encephalectomy agree with data in the literature on the decrease in the total volume of Leydig cells in the testes of encephalectomized rat fetuses [6, 7].

The validity of the hypothesis that the effect observed after encephalectomy was due to blocking of the hypothalamic stage of regulation itself is confirmed by the results of the experiments of series II. The testosterone concentration in the testes 2 h after injection of LHRH into 21.5-day-old encephalectomized fetuses had risen up to its level in intact fetuses; injection of an equal volume of physiological saline into encephalectomized fetuses had no such effect. These data are evidence also that 3 days after encephalectomy no profound morphological or functional changes have taken place in the steroid-producing cells of the testis. In this connection information in the literature that the testes of rat fetuses deprived of their hypothalamus and pituitary by decapitation *in utero* at the 18.5-day stage

of development, when investigated 3 days after the operation, remained capable of metabolizing [^{3}H]progesterone *in vitro* at the control level [12], is noteworthy. After decapitation no change was found in the weight of the testes, but their testosterone content was sharply reduced compared with the control (3.00 ± 0.36 pmoles/testis in the control; 0.63 ± 0.08 pmole/testis after decapitation [11]). Injection of luteinizing hormone into decapitated fetuses led to an increase in volume of the testis and in the number of Leydig cells up to the level of these parameters in control fetuses, whereas injection of LHRH into decapitated fetuses did not abolish the effect of decapitation [6, 7].

The data obtained in the present investigation, showing a decrease in the testosterone concentration in the testes after encephalectomy and the abolition of this effect by injection of LHRH into the encephalectomized fetuses, as well as data in the literature cited above, are evidence that function of the gonads in male rats is under the control of the fetal hypothalamus by the end of the prenatal period of development. This conclusion is in agreement with the recently published observations of Daikoku et al. [5], concerning the role of endogenous LHRH in the gonadotrophic function of the rat fetal pituitary gland.

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EFFECT OF A HELIUM–NEON LASER ON CELL ULTRASTRUCTURE AND PROLIFERATION OF THE EPITHELIUM OF THE DUODENAL MUCOSA

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UDC 616.342-018.25-02:615.849.19]-07

KEY WORDS: laser irradiation; duodenum; ultrastructure; cell proliferation.

The widespread use of various types of lasers in medicine has aroused interest in the study of morphological changes produced by coherent radiation in organs and tissues [1-3, 5]. Lasers generating low-power radiation and, in particular, helium–neon lasers, are known not to give rise to severe pathomorphological changes in the tissues, but under the influence of the low-energy radiation activity of metabolic processes is substantially modified [6]. Hence, there is a stimulating effect of helium-neon lasers on the course of repair processes, which is evidently primarily associated with an increase in proliferative activity of the cells under the influence of laser irradiation.

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(Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 95, No. 3, pp. 95-98, March, 1983. Original article submitted July 23, 1982.

0007-4888/83/9503- 0368$07.50