CHANGES IN TESTOSTERONE CONCENTRATION IN THE FETAL RABBIT TESTIS AFTER REMOVAL OF THE HYPOTHALAMUS (ENCEPHALECTOMY)

E. V. Proshlyakova, O. N. Rumyantseva, and M. S. Mitskevich

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Sex steroids secreted by the testes of fetuses and newborn animals are responsible for masculinization of the genital tract, just as of the CNS. Synthesis and secretion of testotesterone by the testes in rabbits begin between the 18th and 19th days of intrauterine life [6]. This precedes differentiation of the genital tract in males, which occurs on the 20th through the 25th day of prenatal development [8].

The morphofunctional state of the testes in the fetus is under the control of the fetal adenohypophysis, which is affected through gonadotrophins. The gonadotrophic factor in relation to testosterone synthesis and secretion is found in the pituitary of 19-day rabbit fetuses [12] and, starting with the 19th day, the testes of the rabbit fetus can respond by increased synthesis and secretion of testosterone to the action of exogenous luteinizing hormone (LH). Immunoreactive LH can be detected in the fetal pituitary gland after the 19th, and in the blood after the 20th day of pregnancy [14]. In rabbit fetuses hypophysectomized by decapitation, and also in rabbits whose pituitary anlage has been destroyed by x-rays, development of the genital apparatus is retarded because of the deficiency of gonadotrophins and of testicular androgen function which develops [9].

Luteinizing hormone releasing hormone (LHRH) is found in extracts of the hypothalamus of rabbit fetuses on the 20th day of development [5]. Neurovascular connections between the pituitary and hypothalamus are estalished from the 21st through the 23rd day of prenatal life [4]. It has also been shown that at the end of pregnancy the pituitary–testes system can respond to exogenous LHRH [11]. However, the question of the functional role of endogenous LHRH in rabbits has not yet been answered. There is evidence that the fetal hypothalamus in rats participates in the regulation of function of the pituitary–testicular system [2, 7]. The aim of this investigation was to obtain direct data on the role of the hypothalamus in regulation of the adrogen function of the testes in rabbit fetuses.

EXPERIMENTAL METHODS

Experiments were carried out on 139 fetuses from 30 pregnant rabbits. Pregnancy was timed from the moment of copulation. For encephalectomy on the fetuses, pregnant rabbits were subjected to laparotomy under pentobarbital anesthesia (50 mg/kg, intraperitoneally); surgical encephalectomy was performed on the fetuses in utero by the method described previously on the 21st–23rd day of pregnancy [1].

In the experiments of series I after-effects of blocking hypothalamic influences on the testicular testosterone concentration were studied. Pregnant animals were anesthesized with pentobarbital, the fetuses removed, and their testes isolated 7, 4, and 2 days after encephalectomy, i.e., on the 29th, 25th, and 23rd days of intrauterine development. Intact fetuses from 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 determination of their testosterone concentration.

In the experiments of series II the effect of blocking the hypothalamus was abolished by injecting LHRH into the encephalectomized fetuses. In this series 25-day-old fetuses,

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TABLE 1. Changes in Testosterone Concentration in Rabbit Fetal Testis after Encephalectomy and after Injection of LHRH into Encephalectomized Fetuses (M ± m)

Series of experiments	Group of animals	Treatment of fetuses	Age of fetuses, days		Weight of testes, mg	Testosterone concentration in testicular tissue, pg/mg
			at operation	at fixation		
I	1-	Control	22	29	6,5±0,3 (17)	28,16±3,1 (16)
	2-	Encephalectomy			6,7±0,4 (19)	10,96±3,4*** (18)
	3-	Control	21	25	4,2±0,3 (11)	40,30±8,6 (16)
	4-	Encephalectomy			4,5±0,5 (9)	13,33±2,6** (17)
	5-	Control	21	23	2,1±0,1 (7)	84,23±11,2 (12)
	6-	Encephalectomy			2,1±0,1 (7)	72,08±9,5 (12)
II	7-	Control			3,2±0,7 (4)	44,72±9,8 (9)
	8-	Encephalectomy + injection of physiological saline	23	23	3,7±0,5 (8)	6,33±2,2*** (15)
	9-	Encephalectomy + injection of LHRH			3,9±0,2 (15)	16,02±3,2* (24)

Note. Number of fetuses shown in parentheses. *P < 0.05, **P < 0.01, ***P < 0.001 compared with corresponding control.

encephalectomized at the age of 23 days, were used. After laparotomy the uterus was opened and the fetuses removed, while maintaining the connection between fetus and placenta; synthetic LHRH (2 μg in 0.05 ml physiological saline per fetus; from Calbiochem, USA) was injected intraperitoneally into the encephalectomized fetuses from one uterine cornu. An equal volume of physiological saline was injected into the encephalectomized fetuses of the other cornu. All the viable fetuses (including those on which no operations were done) were taken for study 30 min after injection of the substances.

To determine the testosterone concentration, the testicular tissue was homogenized in distilled water. Testosterone was extracted from the homogenate with ether, and aliquots of ether were evaporated to dryness. Testosterone was determined by radioimmunoassay using the "Testok" kit from CEA-Sorin (France). The significance of differences was estimated by Student's test.

RESULTS

The testosterone concentration in the rabbit fetal testes in the control series decreased by about 2.5 times during the period from the 23rd through the 29th day of development, whereas the weight of the testes increased from 2.0 to 6.5 mg (Table 1).

To discover the time when the hypothalamus acts in regulating androgen function, different times were used between the surgical operation and fixation (Table 1). A significant decrease in the testicular testosterone concentration was observed in 29-day-old fetuses encephalectomized on the 22nd day of development (a decrease of 60%, $P < 0.001$). A similar result was observed in 25-day-old fetuses encephalectomized at the age of 21 days (a fall of 67%, $P < 0.01$). When the hypothalamus was removed on the 21st day and the testosterone concentration measured on the 23rd day, the decrease in the testosterone concentration in the gland was not significant (by 14%, $P > 0.05$).

These results suggest that functional dependence of the pituitary–gonadal axis in male fetuses on hypothalamic control is most marked between the 23rd and 25th days of development of the rabbits. The experiments confirmed this hypothesis. In fact, the degree of lowering of the testosterone concentration in the testes when hypothalamic influences were blocked was maximal in 25-day-old fetuses, encephalectomized at the age of 23 days (a fall of 86%, $P < 0.001$). The results of the experiments of series II confirmed the validity of the view that the decrease in testicular hormonal activity observed after encephalectomy is linked with blocking of the hypothalmic stage of regulation. The testosterone concentration in their testes 30 min after injection of LHRH into 25-day-old encephalectomized fetuses was 253% higher than in encephalectomized fetuses from the same litter receiving an injection of physiological saline.

The results are thus evidence that the hypothalamus, pituitary, and testes in the rabbit aged 23-25 days of prenatal development constitute a single functional system.

It is interesting to compare these data on the establishment of hypothalamic control over androgen function with the dynamics of the blood LH concentration in rabbit fetuses, studied by other workers [14]. Involvement of the hypothalamus in the regulation of pituitary—testicular function is reflected in a marked rise of the LH concentration in the fetal blood at the 23rd-25th day of development. Experiments with decapitation of rabbit fetuses also showed that the testes are maximally dependent on the fetal pituitary between the 22nd and 24th days of development [9].

It has been suggested that feedback control of LH secretion by androgens is effected through the hypothalamus [10]. Involvement of a feedback mechanism is evidence that the individual components are united into a single physiological system. According to some authors [13], the mechanism of feedback by circulating androgens begins to act between the 24th and 25th days of prenatal development.

On the basis of data in the literature [5] and of the results of the present investigation, it can be concluded that both rabbit [7] and hog [3] fetuses, the hypothalamus begins to regulate pituitary gonadotrophic activity after LHRH can be detected in the hypothalamus itself.

Data on the fall of the testosterone concentration in the testes after encephalectomy and the possibility of abolishing this effect by injection of LHRH into encephalectomized fetuses, obtained in the present investigation, and also data in the literature cited above are evidence that gonadal function in male rabbits is already under the control of the fetal hypothalamus by the 23rd-25th day of development.

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