SENSITIVITY OF FETAL RABBIT LEYDIG CELLS TO THE ACTION OF VARIOUS PITUITARY HORMONES

O. I. Brindak, V. B. Aleshin,
D. Csaba, and O. Dobozy

UDC 612.647:612.617.014.2].
014.467:[577.175.328+
577.175.324

KEY WORDS: Leydig cells, fetus, receptor

For a hormone to induce a definite effect in a target cell the latter must possess specific receptors for binding the hormone and must be able, as a result of interaction between them, to realize a specific program of biochemical and physiological changes and to achieve the final effect [7, 15]. The time of appearance of ability of endocrine cells to respond to the corresponding hormone in ontogeny is thus not only of theoretical, but also of definite practical importance, for hormones can create a basis for the appearance of developmental anomalies of the fetus, of certain of its organs and, in particular, of its endocrine system. Investigations into the formation of sensitivity of the Leydig cells of the embryonic testis are particularly important, for differentiation of organs and systems according to male type is largely dependent on the hormone which they produce [1, 2, 5-7. 12, 13, 15].

The aim of this investigation was to study sensitivity of Leydig cells to chorionic gonadotrophin, thyrotrophin, and prolactin in rabbit fetuses.

EXPERIMENTAL METHOD

Experiments were carried out on 30 rabbit fetuses. Thryotrophin (Ambinon, from "Organon Oss," The Netherlands) was injected in a dose of 5 U/kg, chorionic gonadotrophin (from Moscow Endocrine Factory) in a dose of 500 U/kg, and prolactin ("Laktin," from Kaunas Endocrine Preparations Factory) in a dose of 3.5 U/200 g body weight. The hormones were injected once only into the fetus at the presumed stage of differentiation of their undifferentiated gonads according to the male type, and before birth, intraperitoneally, and also over a period of 5 days at the specified times into the pregnant females. To inject the hormones into the fetuses a midline laparotomy was performed on the mother, and the uterine cornua with the fetuses were brought up into the wound. Under a binocular microscope, the hormones were injected through the wall of the uterine tube into the peritoneal cavity of the fetuses. The animals were killed 24 h after the injection. The fetuses were removed and their urogenital complexes fixed in Carnoy's fluid and in an 8-10% solution of neutral formalin. Blood was taken for radioimmunoassay of testosterone, using Testok and Testok M kits ("CEA Sorin," France). The urogenital complexes or separate testes were taken through a series of alcohols of increasing concentration and then embedded in paraffin wax blocks, by the usual method. Paraffin sections were cut and stained with Mayer's hematoxylin and eosin and also with acridine orange for microspectral luminescence analysis by Karnaukhov's method [3]. The volume of the nuclei was determined by Tashke's method [8]. The numerical results were subjected to statistical analysis by "Konsul" computer.

EXPERIMENTAL RESULTS

At the stage of differentiation of the undifferentiated gonad according to the male type, chorionic gonadotrophin, thyrotrophin, and prolactin had a similar action on the Leydig cells, namely stimulation of their differentiation and synthetic activity. In the gonads more mature cells, with a larger volume of their nucleus, predominated compared with the control (Table 1). In the cytoplasm of these cells RNA synthesis was increased, as shown by high values of the

---

Laboratory of Pathomorphology, Khar'kov Research Institute of Endocrinology and Hormone Chemistry, Ministry of Health of the Ukrainian SSR. Department of Biology, Semmelweiss University of Medicine, Budapest, Hungary. (Presented by Academician of the Academy of Medical Sciences of the USSR L. T. Malaya.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 107, No. 1, pp. 91-93, January, 1989. Original article submitted December 18, 1987.

TABLE 1. Volume of Nuclei of Leydig Cells in Rabbit Fetal Testes under the Influence of Chorionic Gonadotrophin, Thyrotrophin, and Prolactin

Age of fetus (in days), hormone	Leydig's cells, hormones injected into	
	mother	fetus
19-th		
Chorionic gonadotrophin	186,4±19,97	256,3±29,91
Thyrotrophin	186,1±24,58	326,2±28,01
Prolactin*	149,8±21,40	269,7±25,17
Control	110,4±14,82	109,4±4,33
31st		
Chorionic gonadotrophin	354,1±29,3	381,2±19,53
Thyrotrophin	200,4±26,25	309,0±19,97
Prolactin*	146,9±7,71	292,2±19,72
	141,0±10,08	202,6±17,43

*p< 0.05.

**p) Not significant; for all other values p < 0.001.

TABLE 2. Parameters of RNA Synthesis in Cytoplasm of Leydig Cells of Rabbit Fetal Testes

Age of fetus (in days), hormone	Mean values of parameter after injection of hormones into	
	mother	fetus
19-th		
Chorionic gonadotrophin	1,86±0,20	1,22±0,28
Thyrotrophin	1,78±0,18	1,06±0,02
Prolactin*	0,92±0,28	0,64±0,04
Control	0,82±0,08	0,52±0,06
31-st		
Chronic gonadotrophin	0,50±0,07	0,59±0,08
Thyrotrophin	0,43±0,01	0,38±0,03
Prolactin*	0,22±0,01	0,20±0,03
Control	0,22±0,01	0,25±0,02

*p) Not significant; for all other values p < 0.001.

TABLE 3. Plasma Testosterone Levels in Pooled Rabbit Fetal Blood under the Influence of Chorionic Gonadotrophin, Thyrotrophin, and Prolactin (in nmoles/liter)

Age of fetus (in days), hormone	Testosterone levels after injection of hormones into	
	mother	fetus
19-th		
Chorionic gonadotrophin	4,03±0,08	1,86±0,02
Thyrotropin	3,42±0,06	1,83±0,02
Prolactin	1,86±0,02	2,34±0,10
Control	1,28±0,02	1,08±0,09
31-st		
Chronic gonadotrophin	3,34±0,08	3,58±0,03
Thyrotrophin	2,31±0,05	3,07±0,03
Prolactin*	2,20±0,08	2,55±0,04
Control	1,74±0,02	1,25±0,02

*p<0.05, for other values p<0.001.

parameter α during microspectral luminescence analysis (Table 2), and the blood testosterone level was raised (Table 3). As we showed previously [4], correlation exists between the value of the parameter α in the Leydig cells and testosterone synthesis by them.

The effect of prolactin on differentiation and synthetic activity of the Leydig cells at this stage of development was rather different from the effect of chorionic gonadotrophin and thyrotrophin, whereas the action of the latter on them hardly differed at all.

Before birth of the fetuses, despite the continuing tendency for all three hormones to have a stimulating effect on the Leydig cells, some differences were observed in the intensity of their stimulating action, especially of thyrotrophin. For instance, whereas at the stage of differentiation of the undifferentiated gonad in accordance with the male type, as was mentioned above, thyrotrophin and chorionic gonadotrophin had almost the same action on the Leydig cells, before birth of the fetus, the effect of thyrotrophin on these cells was much weaker than that of chorionic gonadotrophin. Under the influence of prolactin, at this stage of fetal development, RNA synthesis was reduced in the Leydig cells, as shown by the low values of the parameter α. However, the plasma testosterone level in pooled blood of fetuses of this age period was actually a little higher than in the controls. This fact can evidently be explained as follows: prolactin probably does not stimulate testosterone synthesis by the Leydig cells of fetuses, but merely facilitates its release; the possibility likewise cannot be ruled out that the blood testosterone level in the fetus is maintained by the adrenals.

It thus follows from the above description that the sensitivity of Leydig cells to thyrotrophin, chorionic gonadotrophin, and prolactin is established in the early stages of rabbit embryogenesis. In the initial stages of development, moreover, the interstitial cells of Leydig react equally to thyrotrophin and chorionic gonadotrophin, and not until just before birth

are differences observed in the action of these hormones on the Leydig cells. This is evidently due to the immaturity of the receptor system of the Leydig's cells in the early stages of embryogenesis. As a result, hormones similar in structure (thyrotrophin and chorionic gonadotrophin are both glycoproteins and have a similar molecular structure) may give rise to the same effect. The results are in agreement with those in [9, 11, 14], which demonstrated the crossed effect of thyrotrophin and gonadotrophins (FSH and LH) on the developing testes of the chick embryo.

However, in our opinion, it is not a question of immaturity of the receptor system in general, but of immaturity of a particular receptor system to the corresponding group of hormones. This view is supported by the fact that in the early stages of embryogenesis the Leydig's cells react equally to chorionic gonadotrophin and thyrotrophin, which are representatives of one group of hormones [7], and their response to prolactin, belonging to another group, differs. If the receptor system were immature in general, the Leydig's cells would react to all the hormones studied equally. It can be tentatively suggested that in the early stages of embryogenesis the receptor system has a simpler structure than in the late stages. Initially it is constructed so that it reacts to a group of related hormones, for example, two glycoproteins, but later it undergoes finer specialization, i.e., a brief repetition of phylogeny is observed in the ontogeny of this system.

As regards the character of action of the three hormones studied (direct or indirect) on the Leydig's cells, in our opinion their direct action predominates in this case. This suggestion is based, first, on the almost identical reaction of the Leydig cells to chorionic gonadotrophin, for which they are the target [12], and to thyrotrophin; second, receptors are found in these cells to all three hormones; third, those workers [9, 10, 11] who studied the effect of gonadotrophins and thyrotrophin in vitro, and Davies et al. [10], who also studied the effect of prolactin on the testes of chick embryos and of laboratory animals, found that they have a direct action on differentiation of the structures of the gland. In other words thyrotrophin, like all the other hormones, acted in vitro on the testes directly. There is no doubt that other indirect (especially for thyrotrophin) mechanisms of influence cannot be completely ruled out, but in the embryonic period they evidently play a less important role.

LITERATURE CITED

1. B. V. Aleshin and O. I. Brindak, Ontogenez, No. 3, 324 (1983).
2. V. N. Babichev, Neurohormonal Regulation of the Ovarian Cycle [in Russian], Moscow (1984).
3. V. N. Karnaukhov, Luminescence Spectral Analysis of the Cell [in Russian], Moscow (1978).
4. N. A. Karnaukhova, O. A. Brindak, and B. V. Aleshin, Probl. Éndokrinol., No. 5, 76 (1986).
5. M. S. Mitskevich, Hormonal Regulation in Animal Ontogeny [in Russian], Moscow (1978).
6. A. G. Reznikov, Sex Hormones and Brain Differentiation [in Russian], Kiev (1982).
7. V. B. Rozen, Principles of Endocrinology [in Russian], Moscow (1983).
8. K. Tashke, Introduction to Quantitative Cyto-Histologic Morphology [in Russian], Bucharest (1980).
9. G. Csaba, M. A. Shanin, and O. Dobozy, Arch. Anat. Histol. Embriol., 63, 31 (1980).
10. T. F. Davies, B. R. Smith, and R. Hall, Endocrinology, 102, 6 (1978).
11. O. Dobozy, F. Sudar, M. A. Shanin, and G. Csaba, Acta Morphol. Acta Morphol. Acad. Sci. Hung., 30, 11 (1982).
12. A. Jost, J. Physiol. (Paris), 73, 877 (1977).
13. A. Jost and S. Magre, Ann. Endocrinol. (Copenhagen), 4, 303 (1980).
14. M. A. Shanin, G. Csaba, and O. Dobozy, Wilhelm Roux's Arch., 172, 58 (1981).
15. J. D. Wilson, J. E. Criffer, F. W. George, and M. Leshin, Aust. J. Biol. Sci., 36, 101 (1983).