BIOLOGICAL ACTIVITY OF FOLLICLE-STIMULATING HORMONE IN THE HUMAN FETAL PITUITARY

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Changes in the follicle-stimulating hormone (FSH) level in the human fetal pituitary were studied by biological methods. FSH was detected in the fetal pituitary throughout the period of investigation: from the 8th to the 34th week. From the 17th to the 34th week of intrauterine development considerable sex differences were found both in the total and the relative content of the hormone in the gland.

KEY WORDS: pituitary; follicle-stimulating hormone; human fetuses.

Several studies of the formation of the follicle-stimulating function of the human adenohypophysis in the prenatal period of development have recently been published [1, 5, 8, 11, 15]. By means of the radioimmunological method used in most of these investigations immunoreactive follicle-stimulating hormone (FSH) could be detected. There is information in the literature that immunological and biological activity in the same preparation often do not coincide, and sometimes one may be high whereas the other is completely absent [9, 12]. However, before the physiological role of a particular hormone in embryogenesis is assessed, its biological activity must first be established.

The object of this investigation was to determine the FSH level in the human pituitary gland during the period of intrauterine development by means of modern biological methods.

EXPERIMENTAL METHOD

Altogether 366 human fetuses of both sexes, 288 of which were between the 8th and 11th-13th weeks of prenatal development and 78 between the 17th and 34th weeks, were used. The fetuses were obtained from gynecological hospitals in Moscow from mothers free from endocrine diseases. The age of the fetus was determined from the estimated ovulation time, the gynecologist's opinion, and the length of the fetus.

Pituitary glands from fetuses at the 8th-13th weeks of development were removed under the MBS-2 microscope 3-4 h after death, weighed on torsion scales with an accuracy of 0.05 mg, and glands from fetuses of the same age were pooled and kept at −15°C until required for investigation.

Pituitary glands from fetuses aged 17-34 weeks were removed under the MBS-2 microscope 2-18 h after death, the posterior lobe was detached, and the anterior lobe was weighed on torsion scales with an accuracy of 0.1 mg, immersed in 1 ml physiological saline, and kept at −15°C until required for investigation.

Follicle-stimulating activity in the pituitary of 8-13-week fetuses was determined in female CBA mice weighing 7.5-10 g by the method of Igarashi and McCann [6]. On the day of investigation the pituitary glands were frozen and homogenized in a glass homogenizer. Chorionic gonadotropin (CG) and physiological saline were added in the proportion of 2 ml of a mixture containing 0.25 units CG and 2-3 mg of pituitary tissue or standard FSH (0.68, 2, 6, and 18 μg NIH-FSH-S_4) to each recipient. Mice receiving only 0.25 unit CG in 2 ml physiological saline served as the control. Each group contained at least 4-8 mice. In the course of 3 days the animals received five subcutaneous injections each of 0.4 ml. On the 4th day the mice were killed and the uterus was weighed with an accuracy of 0.1 mg. To express the results quantitatively, using the international NIH-FSH-S_4 standard, a calibration curve was plotted each time and used to determine the FSH concentration in the sample. The minimal sensitive dose was 0.68 μg NIH-FSH-S_4.

Follicle-stimulating activity in the adenohypophyses of 17-34-week fetuses was determined in female Wistar rats weighing 40-50 g, using the method of Steelman and Pohley [14] in the modification of Johnson and

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TABLE 1. Weight of Pituitary and Total and Relative FSH Content in Pituitary of Human Fetuses at 8-13 Weeks of Intrauterine Development

Age of fetuses, weeks	Weight of pituitary, mg*	Total FSH content in pituitary, μg	Relative FSH content in pituitary, μg/mg†
8	0.38±0.01 (132)	1.41	3.7 (3)
9	0.65±0.01 (106)	3.77	5.8 (3)
10	0.99±0.02 (35)	6.93	7.0 (2)
11—13	2.36±0.09 (15)	16.40	6.95 (2)

*Number of fetuses in parentheses.
†Number of determinations in parentheses.

Naqvi [7]. Standard dilutions (10, 20, 40, and 80 μg NIH-FSH-S_4) or extracts of human fetal adenohypophyses (4-5 mg tissue) were mixed with 50 units CG in 1.5 ml physiological saline. At 9 a.m. 0.8 ml of the mixture was injected subcutaneously into recipient rats, and 0.7 ml of the mixture was injected at 4:30 p.m. on the same day. Physiological saline containing 50 units CG was injected into the rats of the control group. Each group consisted of at least five animals. The rats were killed 54 h after the first injection and the ovaries were weighed. Follicle-stimulating activity was judged from the change in weight of the ovaries in the experimental animals compared with the controls. The minimal threshold of sensitivity was 10 μg NIH-FSH-S_4. The numerical results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

The method of Steelman and Pohley in the modification of Johnson and Naqvi, which was used to determine the FSH content in the pituitary of 17-34-week human fetuses is specific and sufficiently accurate, although it is not highly sensitive. To increase the sensitivity and accuracy of the method, the suitability of Fau, Wistar, and August rats was investigated, and from these, Wistar rats were chosen. However, when testing the pituitary glands from human fetuses during the first third of embryonic development, because of the inadequate sensitivity of Johnson and Naqvi's method, no follicle-stimulating activity could be detected in them. The more sensitive biological method of Igarashi and McCann [6] had accordingly to be used. There are conflicting data in the literature on the specificity and accuracy of this method. On the one hand, Uberoi and Meyer [17] reported the low specificity of Igarashi and McCann's method, whereas on the other hand, Shiraishi Masato [16] showed that only large, unphysiological doses of other pituitary hormones affect the specificity of this method. However, the present writers showed that the addition of 5 μg of growth hormone to the various standard dilutions did not change the response of the recipient mice. Since the available information indicates that the sensitivity and accuracy of Igarashi and McCann's method [6] depend on the strain of mice, CBA mice were chosen as most suitable for this test.

The results of determination of follicle-stimulating activity in the pituitary of the human fetuses are given in Tables 1 and 2 and Figs. 1 and 2. FSH was detected in the pituitary of the human fetuses after the 8th week of embryonic development. This agrees with the results of histochemical investigations [2, 3, 10], which showed that cells containing mucoproteins, whose presence is usually associated with gonadotrophins, begin to appear in the adenohypophysis of fetuses aged 7-8 weeks. With an increase in the age of the fetus from 8 to 13 weeks, the relative FSH content in the pituitary increased from 3.7 to 6.95 μg/mg and its total content from 1.41 to 16.4 μg.

After the 17th-18th week of development sex differences were found in both the total and relative content of hormone in the gland. These differences persisted throughout the period of investigation (until the 34th week; Figs. 1 and 2). It will be noted that no FSH was found in 16 of 39 male fetuses (Table 2). The relative FSH content in the pituitary of the female fetuses from the 17th to the 26th week increased from 2.12 to 8.84 ± 1.99 μg/mg, and thereafter until the 34th week varied only slightly, between 5.5 and 7.34 μg/mg. The FSH content in the pituitary glands of female fetuses increased throughout the period of investigation (except between the 29th and 31st weeks). This exception may perhaps be explained by the insufficient number of fetuses (two) studied at this stage of development.

TABLE 2. Weight of Pituitary and Total and Relative FSH Content of Pituitary in Human Fetuses at 17-34 Weeks of Intrauterine Development

Age of fetuses, weeks	Weight of adenohypophysis, mg*		Number of determinations in which FSH < 10 μg		Total FSH content in adenohypophysis of fetuses, μg			Relative FSH content in adenohypophysis of fetuses, μg/mg		
	female	male	female	male	female	male	p	female	male	p
17—18	12,1±1,1 (3)	10,9 (2)	1	1	31,8±19,3	3,1		2,12	0,25	
19—20	13,8±1,3 (2)	13,8±1,6 (8)	—	3	71,1±28,4	18,1±12,7		4,10±0,82	1,12±0,73	<0,05
21—22	19,3±3,2 (6)	19,2±2,3 (6)	—	2	142,6±31,4	49,3±22,2	<0,05	7,44±1,37	2,0±1,5	<0,05
23—24	26,4±1,5 (10)	21,6±1,0 (6)	—	4	195,3±36,4	61,3±25,8	<0,01	7,83±1,40	2,94±1,21	<0,02
25—26	29,7±3,6 (8)	26,4±1,7 (6)	2	2	257,9±76,9	59,8±27,3	<0,05	8,84±1,99	2,4±0,97	<0,02
27—28	35,5±3,2 (6)	37,2±2,7 (4)	—	1	296,6±81,5	95,0±40,4	<0,05	7,34±1,08	2,67±1,17	<0,02
29—31	39,7 (2)	43,0±6,4 (3)	—	3	201,5	0		5,5	0	
32—34	54,7 (2)	—	—	—	371,4	—		7,0	—	

*Number of fetuses in parentheses.

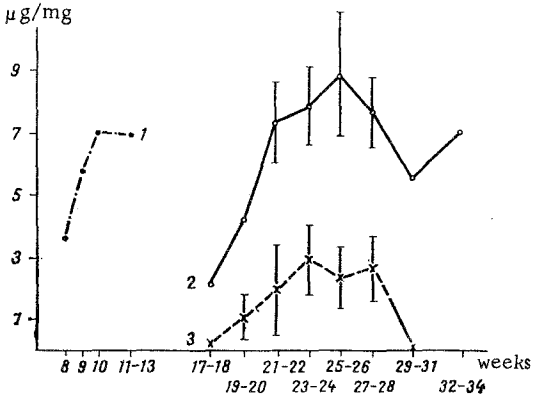

Fig. 1

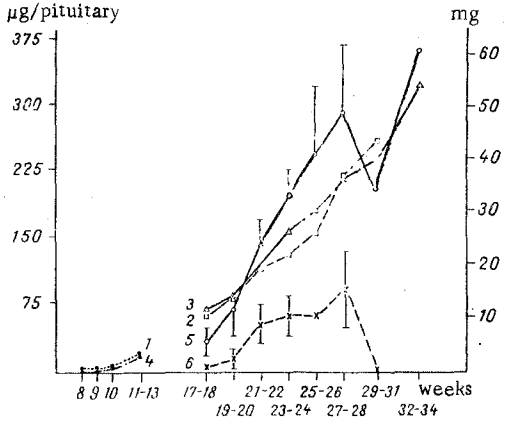

Fig. 2

Fig. 1. Relative FSH content in human fetal pituitary (in μg/mg): 1) pituitary glands of fetuses of both sexes; 2) adenohypophyses of female fetuses; 3) adenohypophyses of male fetuses.

Fig. 2. Weight of pituitary and its total FSH content in human fetuses. 1) Weigh of pituitary in fetuses of both sexes (in mg); 2) weight of adenohypophysis in female fetuses (in mg); 3) weight of adenohypophysis in male fetuses (in mg); 4) total FSH content in pituitary of fetuses of both sexes (in μg); 5) total FSH content in adenohypophysis of female fetuses (in μg); 6) total FSH content in adenohypophysis of male fetuses (in μg).

Throughout the period of investigation both the total and the relative FSH content in male fetuses was significantly lower than in female fetuses. A similar rule was observed by Levina and Ivanova [1] who, using Brown's method, detected biological FSH activity in the pituitary of a female human fetus after the 13th-14th week and in the pituitary of a male fetus after the 20th week of development. However, when determining biological FSH activity in acetone-extracted pituitary glands of human fetuses, Parlow [11] found no sex differences. These disagreements between the results can evidently be explained by the use of acetone to dry the pituitary tissue, and this procedure is known to reduce FSH activity.

The significant sex differences in the FSH level in the fetal pituitary gland discovered in the present experiments can perhaps be explained on the grounds that the fetal testes at an early stage of embryonic development (about 8 weeks) acquire hormonal activity [4, 13] and, probably by the negative feedback principle, inhibit gonadotrophin (especially FSH) formation in the fetal pituitary. There is also evidence that in the prenatal period the human hypothalamus contains a releasing hormone which can regulate the synthesis and secretion of FSH by the human pituitary.

It was also intended in these investigations to determine the ability of the human fetal pituitary to secrete FSH. However, neither of the biological methods used was sufficiently sensitive to determine the hormone in the blood of the human fetuses.

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