In vitro STUDY OF FUNCTIONAL MATURATION OF THE CRF-ACTH AXIS IN MAN IN THE INTRAUTERINE PERIOD

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The ability of the embryonic human pituitary gland to secrete polypeptide hormones and of the hypothalamus to store releasing factors has been established in the early stages of development [9]. Maturation of the CNS in respect of regulation of the hypothalamic-hypophyseal system is observed later. Little is known of the time sequence of appearance of polypeptide hormones in the pituitary and the appearance of the corresponding releasing hormones in the hypothalamus. It has been shown by an immunocytochemical method that ACTH cells are detectable for the first time in the human pituitary after the 7th week of embryonic development [1, 6]. ACTH has been found at this same time in the gland by a radioimmunologic method [1]. However biologically active ACTH appears in the human pituitary after the 9th-10th weeks of prenatal development [4].

It was shown previously that the human fetal adenohypophysis at the beginning of the 2nd trimester of pregnancy can secrete biologically active ACTH for a long time *in vitro* [14]. This investigation was followed by a number of others which demonstrated the ability of the human fetal pituitary to produce other trophic hormones [2, 3, 7, 8, 13]. Meanwhile evidence has been obtained that immunoreactive thyrotrophin-releasing and luteinizing hormone releasing factors and somatostatin can be detected with effect from the 10th week of embryonic life. However, at this stage of development the activity of these hormones is quite low and their detection often unreliable. By the 13th-15th weeks of development a considerable increase in their activity is observed.

Information on the appearance of corticotrophin-releasing factor (CRF) in the human fetal hypothalamus cannot be found in the literature. Accordingly the investigation described below was undertaken to study whether CRF is present in the human fetal hypothalamus, and at what stage of embryonic development it begins to exert its physiological action. Since it was impossible to obtain a specific antiserum against CRF, tissue culture *in vitro* was used as the model for this task.

EXPERIMENTAL METHOD

Pituitary glands were obtained from 26 human fetuses from the 6th to the 30th weeks of development, and organ and/or monolayer cultures were prepared from them. ACTH secretion into the medium after incubation of the cultures for 2 h was determined at different times of culture by a radioimmunologic method [12]. The sensitivity of the culture to CRF was judged from the response of the human fetal pituitary cells in culture to extracts of the pituitary stalk and median eminence (PSME) of adult rats. Basal and stimulated ACTH secretion in the culture was expressed as a function of the age of the fetus and duration of culture *in vitro*. The pituitary cultures from adult rats were prepared as described previously [11]. ACTH secretion after addition of human fetal hypothalamic extracts was determined by a radioimmunologic method.

The hypothalamus was removed from 13 human fetuses from the 3rd to the 32nd weeks of development, obtained after medical abortions or as a result of spontaneous abortion. Hypo-

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TABLE 1. Basal and Stimulated ACTH Secretion *in vitro* by Pituitary Glands from 6-13 Week Human Fetuses (in °/₀₀/pituitary gland in 2 h)

Time of culture, days	Age of fetuses, weeks							
	6—7	6—7	6—7	6—7	10	10	13	13
2	0,18	0,14	0,12	0,14	0,72	0,72	0,69	0,75
	0,13*	0,16*	0,20*	0,12*	1,30*	1,13*	1,01*	0,71*
4	0,37	0,21	0,13	0,21	0,92	0,87	0,60	0,60
6	0,27	0,28	0,27	0,28	1,25	0,72	0,60	0,40
	0,30*	0,45*	0,32*	0,70*	—	—	—	—
8	0,32	0,56	0,20	0,56	0,82	0,57	0,38	0,30
	0,35*	0,60*	0,59*	0,45*	1,55*	1,28*	0,99*	0,98*
10	0,22	0,23	0,25	0,23	0,85	0,65	0,56	0,38
12	0,30	0,30	0,28	0,30	1,00	0,60	0,70	0,59
	0,70	—	—	—	1,60*	1,20*	1,40*	0,87*
14	—	0,42	0,25	0,42	—	—	—	—
	—	0,70*	0,65*	0,80*	—	—	—	—

*ACTH secretion stimulated by addition of 0.5 PSME of adult rats.

TABLE 2. Basal and Stimulated ACTH Secretion *in vitro* by Human Fetal Pituitary Gland during 2nd and 3rd Trimesters of Pregnancy (in °/₀₀/pituitary gland in 2 h)

Time of culture, days	Age of fetuses, weeks						
	16	18	19	20	22	28	30
3	0,85	0,33	N.d.	1,00	1,15	0,62	0,80
	4,00*	—	2,47*	—	5,36*	—	4,34*
4	0,85	0,43	0,74	0,90	1,25	0,50	0,99
7	1,20	0,50	0,96	0,90	1,32	0,70	1,00
	—	—	—	5,30*	—	4,96*	—
8	0,99	0,39	0,76	0,90	0,89	0,80	0,80
	—	1,35*	—	—	—	—	—
9	1,20	0,74	1,11	—	1,13	—	—
10	0,50	0,88	0,91	1,00	0,90	0,60	0,85
	2,00*	—	2,30*	—	1,43*	—	—
12	0,45	0,90	0,90	1,20	0,90	0,50	0,80
14	0,20	0,75	0,65	0,50	0,60	0,60	0,65
	—	1,53*	—	—	—	3,00*	2,80*
24	0,25	0,30	0,41	—	0,29	—	—
	0,70*	1,20*	1,15*	—	1,31*	—	—

Legend. N.d. not determined.

thalamic tissue from 12-32 week fetuses was divied into two parts: the fragment of the anterior hypothalamus (AH) included the preoptic, paraventricular, and periventricular nuclei, whereas the fragment of posterior hypothalamus (PH) contained the dorsomedial, ventromedial, and arcuate nuclei, and the median eminence. Hypothalamic tissue was homogenized with 0.1 N hydrochloric acid (in the proportion of 1 ml to 10 mg) and centrifuged after 2-4 h at 2000g for 10 min. The resulting supernatant was lyophilized. Before use, the lyophilized material was dissolved in Krebs' solution and centrifuged at 2000g. The resulting supernatant was tested in three dilutions.

EXPERIMENTAL RESULTS

The results of determination of basal and stimulated ACTH secretion by cultured pituitary glands from 6-13 week human fetuses (Table 1) showed that secretion of ACTH into the medium by fetal pituitary glands at 6-7 weeks of development can be detected, but after incubation for 2 h it is low. With an increase in the time of culture the basal secretion increased a little. Addition of extract of 0.5 PSME from adult rats to the culture after 1 week did not change the ACTH level in the medium, but in the 2nd week of culture it doubled.

The fact that pituitary glands from 6-7 week human fetuses do not respond during the first week in culture to addition of PSME from adult rats is evidence that the ability of embryonic ACTH-cells to respond to their specific releasing factor appears during a later stage of development. In the 2nd week of culture the pituitary glands increased their basal ACTH secretion after addition of hypothalamic extract, when clear morphological differentiation was observed *in vitro*. By contrast, in cultures of 10-13-week human fetuses high basal secretion of ACTH into the medium was found. The response of the pituitary glands to extract of PSME, detected after the 2nd day of culture, remained virtually unchanged throughout the period of culture. These results showed that from the 10th week of prenatal development

TABLE 3. ACTH Secretion *in vitro* by Adult Rat Pituitary Glands Stimulated by Human Fetal Hypothalamic Extracts (in °/oo/ml)

Dose of extract, ml	Age of fetuses, weeks																					
	6	7	8	12		16		17—18		21		21—22		22		23—24		28		30—31		31—32	
		H		AH	PH	AH	PH	AH	PH	AH	PH	AH	PH	AH	PH	AH	PH	AH	PH	AH	PH	AH	PH
	1,50	1,78	1,89	0,30	0,22	0,30	2,42	0,16	1,07	0,42	1,02	0,32	1,43	0,38	0,31	0,39	0,45	0,40	0,46	0,40	1,08	0,46	0,34
1,00	0,47*	1,88*	2,06*	1,20*	0,90*	2,62*	10,68*	2,88*	10,80*	1,07*	v.h.	1,91*	3,41*	1,35*	3,71*	3,74*	2,85*	3,16*	1,63*	4,64*	7,28*	1,97*	3,89*
	1,20	1,67	1,31	0,37	0,30	0,50	0,59	0,40	0,67	0,41	0,63	0,36	0,75	0,38	0,47	0,33	0,38	0,31	0,21	0,32	0,54	0,38	0,33
0,50	1,32*	1,54*	1,46*	1,22*	0,73*	2,75*	2,43*	1,33*	2,09*	3,80*	11,39*	1,37*	2,60*	2,46*	3,16*	3,22*	2,07*	2,81*	2,35*	4,39*	2,25*	2,82*	2,45*
	—	—	—	0,27	0,30	0,24	0,87	0,30	0,64	0,45	0,61	0,36	0,72	0,38	0,40	0,34	0,37	0,35	0,18	0,49	0,10	0,40	0,30
0,25	—	—	—	1,00*	0,49*	0,83*	0,39*	1,19*	0,52*	1,34*	2,50*	1,97*	0,40*	2,11*	1,63*	1,35*	1,66*	2,20*	1,97*	1,76*	0,88*	1,50*	2,14*

*ACTH secretion.

Legend. H) hypothalamus; v.h.) very high level.

the human pituitary can respond *in vitro* to CRF. Basal ACTH secretion in the culture obtained from fetal pituitary glands at the 2nd and 3rd trimesters of pregnancy did not exceed basal ACTH secretion by pituitary glands from 10-13-week fetuses, but the response to addition of extract of 0.5 PSME of adult rats in the course of incubation for 2 h was 3-5 times higher than the control ACTH level and remained unchanged throughout the period of culture (Table 2). The maximal response was observed 30 min after addition of PSME extracts irrespective of the age of the culture.

It will be clear from Table 3 that hypothalamic extracts from 6-8 week fetuses did not increase the basal ACTH level in a culture of adult rat pituitary glands. Hypothalamic extracts at the 12th week of development increased ACTH secretion into the culture, but this effect was independent of dilution. Possibly CRF appears in the human hypothalamus as early as the 12th week of embryonic development.

Determination of the CRF-like substance in the fetal hypothalamus at the 2nd and 3rd trimesters of pregnancy clearly showed that the regions of AH and PH of the human fetus contain large quantities of CRF after the 16th week of intrauterine life. CRF activity, moreover, in extracts of PH was considerably higher than in extracts of AH. Activity of the PH extracts was shown to depend clearly on their dilution (Table 3).

More and more data are being published in the literature to show that hypothalamic hormones can influence differentiation of the pituitary in the prenatal period of development. However, there is very little information on the development of the hypothalamic-hypophyseal portal system [5]. According to Falin [5], the first signs of this differentiation are observed at about the 60th day of pregnancy, and consist of ingrowth of capillaries into the mesenchymal tissue surrounding Rathke's pouch and the diencephalon. Rapid vascularization and the parallel formation of the primary plexus of the primary portal system take place at about the 100th day of development. Further differentiation of the tuberal part of the median eminence is observed in the 17th week of pregnancy. Development of the primary and secondary plexuses of the portal system is complete by the 19th-21st weeks.

The following conclusions can be drawn from the data described above and our own observations: The human pituitary gland can synthesize and secrete *in vitro* an immunoreactive ACTH as early as on the 6th and 7th weeks of prenatal development. Since the first corticotrophic cells are observed in the gland at this same period [1, 6], it can be postulated that secretion of the hormone in the pituitary begins simultaneously with discovery of the first immunopositive ACTH cells. The human fetal hypothalamus in the 1st trimester of pregnancy probably does not yet contain CRF. Accordingly it can be postualted that in man the appearance of corticotrophic cells in the pituitary and secretion of ACTH precede maturation of corticotrophin releasing hormone in the hypothalamus. The writers showed previously that there is a period in the embryonic development of rats when the embryonic hypothalamus does not contain CRF, but corticotrophic cells capable of secreting ACTH spontaneously *in vitro* are already present in the pituitary. Since human fetal pituitary glands at the 2nd and 3rd trimesters of pregnancy had high basal ACTH secretion in culture and gave a marked response to hypothalamic CRF, and since there hypothalamus contained large quantities of CRF-like substance, it can be concluded that functional maturation of the CRF-ACTH system takes place at the end of the first third of the prenatal period of development. These observations confirm the concept [10] that in the period of early embryonic development relatively autonomous secretion of hypothalamic hypophyseotrophic releasing factors and pituitary hormones takes place, and that in the later period of development maturation of inhibitory or restricting components, effected through the CNS, modulates secretion of the hypothalamus and pituitary.

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EFFECT OF TESTOSTERONE AND ITS 5α-REDUCED METABOLITES ON THYROID FUNCTION AND PROTEIN SYNTHESIS

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KEY WORDS: thyroid gland; testosterone; 5α-dihydrotestosterone; 5α-androstane-3β, 17β-diol.

The results of many clinical and experimental investigations have demonstrated connections between the thyroid and sex glands [1, 6, 8]. The well-known fact that mainly women are prone to thyroid diseases [2] is evidence of the preventive role of male sex hormone in the pathogenesis of thyroid diseases. However, there is no general agreement in the literature either on the character of the effect of testosterone on function and proliferation of the throid gland or on the mechanisms of this effect.

This paper describes a comparative study of the action of testosterone and its active 5α-reduced metabolites on function and growth of the thyroid gland and on its protein synthesizing activity. The 5α-dihydrotestosterone and 5α-androstane-3β,17β-diol used in the experiments were synthesized at Khar'kov Research Institute of Endocrinology and Hormone Chemistry.

EXPERIMENTAL METHOD

Experiments were carried out on 120 male albino rats. There were three parallel series of experiments. The animals of each series were divided into four groups: one control and three experimental (10 rats in each group). The animals of the three experimental groups received testosterone propionate, dihydrotestosterone, or androstanediol, respectively, in the optimal dose established in preliminary experiments [4, 5], namely 0.5 mg/100 g body weight. All substances were injected subcutaneously in 0.2 ml persic oil daily for 14 days. Since the molecular weights of the compounds were similar, their administration in identical doses enabled their effects to be compared. The control group consisted of rats receiving the oil only. In the experiments of series I the percentage uptake of ^{131}I by the thyroid gland and the ratio between the content of iodinated amino acids in the gland were studied by ascending paper radiochromatography [10], and the plasma protein-bound iodine (PBI) level was studied by the method in [11]. In series II the structural reaction of the thyroid gland was studied in celloidin-paraffin sections stained with azan by Mallory's method. By using these methods it was possible to judge the state of thyroid function. In the experiments of series III activity of protein synthesis in the thyrocytes was determined by measuring β-radiation in total protein precipitated from rat thyroid gland homogenate 30 min after injection of ^{14}C-protein hydrolysate into the animals, on an SBS-2 scintillation counter. The results were subjected to statistical analysis by the Student—Fisher method.

EXPERIMENTAL RESULTS

Uptake of ^{131}I by the thyroid gland of the rats receiving testosterone propionate, expressed as a percentage, was higher than in the control (Table 1); intrathyroid hormone pro-

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