BINDING OF TESTOSTERONE BY TISSUES OF THE PRIMORDIAL HUMAN FETAL PROSTATE

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The biological action of steroid hormones on target cells is realized through the participation of corresponding cytoplasmic and nuclear receptors [5]. The ability of primordial tissues of the embryonic reproductive tract of laboratory animals to selectively absorb labeled sex steroids at critical periods of morphogenesis has been reported [1-4]. No analogous investigations have been conducted on tissues of human fetuses. Yet the study of this problem is important both to reveal the mechanisms of hormonal control of development of the human reproductive system and to elucidate the pathogenesis of androgen-dependent diseases and, in particular, of tumors of the prostate gland.

In this paper we present data on specific binding of testosterone (TS) in tissues of the urogenital sinus (UGS) of human fetuses during the period corresponding to the beginning of differentiation of the prostate.

EXPERIMENTAL METHOD

Experiments were carried out on material obtained from 25 human fetuses aged 10-12 weeks, obtained at therapeutic abortions. The age of the fetuses was determined from the length of the foot, and the sex from the presence or absence of Y-chromatin in interphase nuclei of skin cells.

The UGS was isolated under a stereoscopic microscope and divided sagittally into two equal parts, which were incubated simultaneously. The urinary bladder (UB) of the same fetus, which is not an androgen-dependent organ, was used as the control.

Binding of ^{3}H-TS was studied in the tissue of UGS and UV and also in the isolated mesenchyme of these anlagen, separated beforehand from the epithelium in a solution of EDTA at room temperature. The tissues for testing were incubated in medium RPMI-1640 ("Serva," West Germany) for 40 min at 37°C in an atmosphere with 5% CO_2. Before incubation began, 1,2,6,7-^{3}H-TS (specific activity 3.9 TBq/mmole) was added to the medium in an amount of 111 kBq/ml. Specific uptake of ^{3}H-TS was determined in parallel samples which, together with labeled hormone, contained a 1000-fold excess of unlabeled. After incubation the tissues were washed in medium RPMI-1640 3 times, for 10 min each time, at 4°C and, after drying on filter paper, they were weighed. Before radiometry the tissues were solubilized in hyamine for 48 h at 56°C. Radioactivity was measured in a Mark III liquid scintillation counter ("Trakor Europe").

EXPERIMENTAL RESULTS

Starting with the 10th week of development the formation of prostatic bands was observed in fetuses of both sexes on microscopic examination. Toward the end of the 12th week, they showed signs of degradation in female fetuses. Within this age period the tissues of UGS of fetuses of both sexes actively absorbed labeled TS (Fig. 1). Total uptake of ^{3}H-TS in the tissues of UGS was 5.5 times greater than in the tissues of UB ($p < 0.001$). Values of specific binding of ^{3}H-TS, defined as the difference between total uptake of the hormone and radioactivity of samples containing an excess of unlabeled TS, amounted to 50-91% of total binding in UGS, but to tens of times less in UB (Fig. 2). In 10- to 12-week male fetuses uptake of ^{3}H-TS in the region of UGS was similar to its uptake in female fetuses. Although in the latter

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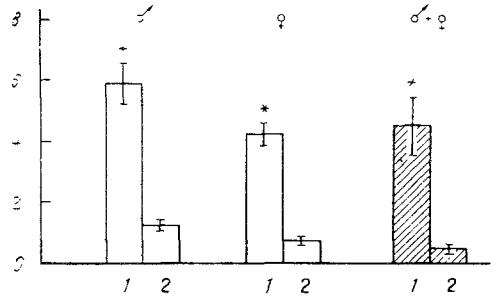

Fig. 1. Total uptake of ^{3}H-TS by tissues of UGS and UB of human fetuses at 10-12 weeks. Abscissa: 1) UGS, 2) UB. Shaded columns indicate isolated mesenchyme; ordinate, radioactivity (in cpm·mg·10^3). *p < 0.001 compared with UB.

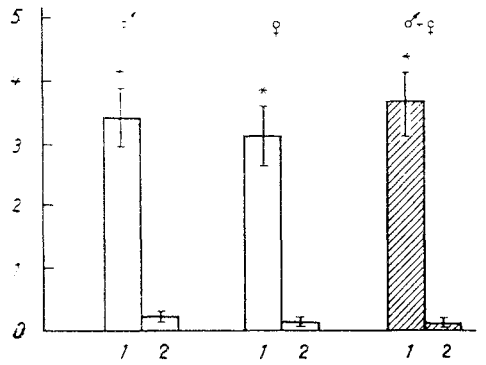

Fig. 2. Specific binding of ^{3}H-TS by tissues of UGS and UB of 10- to 12-week human fetuses. Legend as to Fig. 1.

the mean values of total and specific binding of ^{3}H-TS were rather lower than in male fetuses, the difference between them was not significant.

The isolated mesenchyme of UGS of fetuses of both sexes also exhibited high specific binding of ^{3}H-TS in the period of appearance of prostatic bands (10-12 weeks of development). Values of specific binding of the hormone averaged 79% of total uptake. This is significantly higher than binding of ^{3}H-TS in the mesenchyme of UB ($p < 0.001$; Fig. 2).

The beginning of morphogenesis of the prostate is preceded by active secretion of testicular androgens. In man between the 8th and 18th weeks of intrauterine development the TS concentration in the blood of male fetuses varies from 9.20 and 20.13 nmoles/liter, which is significantly higher than in female fetuses [6]. In processes of androgen-dependent differentiation of the mammalian prostate, the action of steroid androgens on the epithelium is mediated through the mesenchyme, which plays the role of inducer [7, 8]. Autoradiographic investigations on rodents showed that in early prenatal ontogeny receptors for androgens are expressed exclusively in the mesenchyme of UGS [9].

The ability of UGS of 10- to 12-week human fetuses and of its mesenchymal cells to carry out specific binding of ^{3}H-TS, which we discovered, characterizes the hormonal competence of these target tissues. Thus in the critical period of morphogenesis of the human prostate a receptor system exists which enables androgen-dependent differentiation of UGS to take place. Judging by the high level of binding of ^{3}H-TS in the UGS of female fetuses, the main cause of cessation of growth of prostatic bands and further formation of the prostate gland in them is evidently a deficiency of androgenic influences.

The absence of sexual dimorphism in the binding of TS by the tissues of UGS in human fetuses, while significant differences are found between circulating sex steroid levels can be regarded as one proof of the determined nature of the appearance of hormonal receptors in ontogeny.

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SIGMA RECEPTORS OF LOACH EMBRYOS CONTROL ORNITHINE DECARBOXYLASE ACTIVITY

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The sigma receptor agonist SKF 10.047 (N-allylnormethazocine), unlike certain other neurotransmitters and ligands of opioid, mu, delta, and kappa receptors, has a specific teratogenic action on embryos of the loach (*Misgurnus fossilis*) [1]. The primary mechanism of the morphogenic action of this compound is to potentiate aggregation of the embryonic shield cells during gastrulation, which in turn is brought about by clustering of the cell surface receptors. The action of SKF 10.047 has been shown to be mediated by specific binding centers, which are similar in their biochemical properties to the sigma receptors of the mammalian brain [9, 10] and of the adult female loach brain [2]. An important role in the maintenance of growth and differentiation of embryonic cells is played by endogenous polyamines, namely putrescine, spermine, and spermidine [3, 5], and this paper gives data indicating that the action of SKF 10.047 on embryonic cells may be linked with modulation of activity of the key enzyme involved in the biosynthesis of these compounds, i.e., ornithine decarboxylase (ODC).

EXPERIMENTAL METHOD

Ripe oocytes were obtained, fertilized, and incubated and the stages of their development were identified in accordance with the scheme described in [7]. The serial number of the stage of development corresponded to the number of hours of development at 21°C. Reagents (±)-SKF 10.047, (+)-SKF 10.047, and (−)-SKF 10.047 were generously provided by A. Hertz (Max Planck Institute of Psychiatry, West Germany). The L-1-^{14}C-ornithine was obtained from "Amersham International" (England) and the remaining reagents from firms in the USA. ODC activity was determined by the method suggested previously [8].

EXPERIMENTAL RESULTS

In the presence of (±)-SKF 10.047 (10^{-5} M), added immediately after fertilization, ODC activity increased more rapidly than in the control, and at stage 4 (eight blastomeres) it reached its highest value, more than $1^1/_2$ times greater than in the control (Fig. 1). Later, however, ODC activity declined, and by stage 8 (middle blastula) it had returned to the control level. Between stages 8 and 10 (late blastula to beginning of gastrulation) activation of ODC by the action of (±)-SKF 10.047 did not take place. However, ODC activity after stage 10 increased again in the presence of this substance and reached a maximum which was about 1.3 times higher than the ODC level in the control at stage 12 (formation of the embryonic shield). Activity of the enzyme then decreased and at stage 14 (separation of the notochord from the mesoderm) it returned to the control level. If (±)-SKF 10.047 was added at stage 7 to embryos developing under normal conditions, activation of ODC, just as with addition at stage 10 or with continuous incubation with this substance, did not begin until stage 10. Thus, the

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