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The Androgen Receptor in the Fetal Epididymis Is Similar to That in the Mature Rabbit[1] (42768)

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Abstract. To provide insight into the mechanism of wolffian duct virilization the androgen receptor has been characterized in pooled epididymal tissue from Day 27 rabbit embryos. Although the receptor is present at levels about a tenth that of other androgen target tissues in rabbit embryos, it appears to be a typical androgen receptor in regard to sedimentation characteristics and preferential binding of 5α-dihydrotestosterone over testosterone. Thus, the mechanism by which testosterone virilizes the wolffian duct cannot be explained by unique binding characteristics of the androgen receptor in tissues derived from the wolffian ducts. © 1988 Society for Experimental Biology and Medicine.

Virilization of the male embryo requires two androgens, testosterone and its 5α-reduced metabolite, 5α-dihydrotestosterone (17β-hydroxy-5α-androstan-3-one). Testosterone is responsible for differentiation of the wolffian duct into the seminal vesicle, epididymis, and vas deferens, whereas virilization of the external genitalia of the male (formation of penis, scrotum, male urethra, and prostate) is mediated by 5α-dihydrotestosterone. This dual-hormone formulation of embryonic virilization was originally based on studies of androgen metabolism in embryos in which it was demonstrated that anlage of the external genitalia (urogenital sinus and genital tubercle) synthesize 5α-dihydrotestosterone from testosterone, whereas the wolffian ducts (anlage of the internal urogenital tract) do not form 5α-dihydrotestosterone prior to male differentiation (1, 2). Confirmation of this theory is provided by studies of 5α-reductase deficiency in humans (3). Males with 5α-reductase deficiency do not synthesize 5α-dihydrotestosterone in normal amounts and consequently have failure of external virilization (the external phenotype at birth is female). Virilization of the wolffian ducts (into epididymis, vas deferens, and seminal vesicle) is, however, normal (3). Likewise, the administration of 5α-reductase inhibitors to pregnant rats selectively impairs the virilization of the external genitalia of male embryos, but does not prevent wolffian duct differentiation, thus providing a phenocopy of 5α-reductase deficiency (4, 5).

The molecular mechanism by which the two androgens exert distinct actions in different tissues during embryogenesis is not clear. To provide insight into androgen action during embryogenesis, we previously compared the characteristics of fetal androgen receptors from the anlage of the external genitalia of the rabbit to those of adult androgen-dependent tissues (seminal vesicle, epididymis, and prostate) (6). No major differences in the binding characteristics of fetal vs adult androgen-receptor preparations were noted. However, we were unable to measure receptor in embryonic wolffian duct derivatives and hence were unable to determine whether the receptor system in wolffian ducts has unique binding characteristics that allow it to respond to testosterone better than in other androgen target tissues. We here report that the fetal rabbit epididymis (derived from wolffian duct) contains androgen receptors with characteristics similar to those described for other androgen-dependent tissues of the fetal and mature rabbit.

Materials and Methods. *Animals and tissue preparation.* Adult male and pregnant female New Zealand White rabbits were obtained from Hickory Hill Farms (Flint, TX). The day of mating was considered Day 0 of

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pregnancy. On the 27th day of gestation, pregnant does were killed with an overdose of nembutol. The embryos were killed by decapitation, and the urogenital tissues were dissected, pooled, and stored frozen in liquid nitrogen. The Day 27 fetal rabbit epididymis is well developed and has a recognizable head and tail. In the course of these experiments more than 250 male rabbit embryos were used as tissue donors.

Fetal tissue pools (~300 mg) were thawed and homogenized (1 g tissue/3 ml buffer) at 0–4°C in 10 m*M* Tris (pH 7.4) containing 1.5 m*M* EDTA, 0.1% (v/v) monothioglycerol, 10 m*M* Na_2MoO_4, and 10% glycerol (TEGMSH) in a Dounce homogenizer (10 strokes of a loose pestle, followed by 20 strokes of a tight pestle). Adult tissues were homogenized at 0–4°C in the same buffer with three 10-sec bursts of a Polytron homogenizer (Brinkmann Instruments) at 80% maximum setting. Homogenates were centrifuged at 200,000*g* for 30 min to prepare low-salt extracts. The pellet from the high-speed centrifugation was homogenized by Dounce followed by sonication in TEGMSH containing 0.5 *M* KCl. This second homogenate was centrifuged at 200,000*g* for 30 min; the supernatant was designated high-salt extract.

Sucrose density gradients. Aliquots (200 μl) of low-salt and high-salt extracts that had been equilibrated for 2 hr with 5 n*M* [^{3}H]5α-dihydrotestosterone ± 500 n*M* cold 5α-dihydrotestosterone were layered on 5–20% sucrose gradients (made in the corresponding low- or high-salt buffer) containing concentrations of radioactive and nonradioactive 5α-dihydrotestosterone identical to those in the initial incubation period. The gradients were centrifuged at 250,000*g* for 18 hr and then fractionated. Each fraction was treated with hydroxylapatite to separate bound from free hormone and assayed for radioactivity as described (6).

Direct hydroxylapatite assay for binding. After equilibration of low- and high-salt extracts with 5 n*M* [^{3}H]5α-dihydrotestosterone in the presence or absence of excess (50 or 500 n*M*) nonradioactive 5α-dihydrotestosterone or testosterone for 3 hr at 0–4°C, the extracts were treated with hydroxylapatite to separate bound from free hormone (6) and assayed for radioactivity in a liquid scintillation counter.

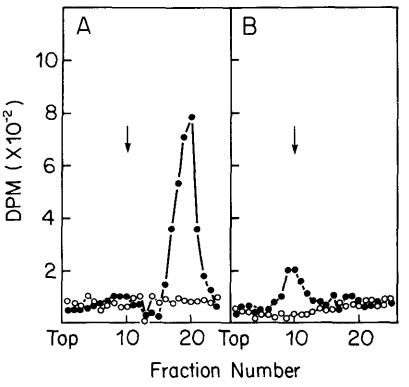

FIG. 1. Sucrose density gradient analysis of 5 n*M* [^{3}H]5α-dihydrotestosterone binding in Day 27 fetal rabbit epididymis. The procedure is described in the text. ●, 5 n*M* [^{3}H]5α-dihydrotestosterone; ○, 5 n*M* [^{3}H]5α-dihydrotestosterone plus 500 n*M* cold 5α-dihydrotestosterone. (A) Low-salt extract, 11.7 fmole/mg protein; (B) high-salt extract, 6.4 fmole/mg protein. Total specific binding was 86 fmole/g tissue. The arrows represent the approximate level of sedimentation of bovine serum albumin in our gradients.

Results. Figure 1 depicts representative (one of three experiments) sucrose density gradient profiles of androgen receptors ([^{3}H]5α-dihydrotestosterone binding) in low-salt and high-salt extracts of Day 27 fetal rabbit epididymis. Although most of the androgen receptor was extracted in the initial low-salt homogenization buffer, specific peaks of binding were detected in both extracts. The total amount of binding was approximately 86 fmole/g tissue. The sedimentation pattern in the fetal epididymis resembled that in extracts of adult rabbit epididymis and of Day 27 fetal rabbit urogenital tubercle (Fig. 2). In both cases, all of the detectable receptor was extracted in the low-salt buffers, but the amount of androgen receptor in extracts of both tissues (401 fmole/g tissue in adult rabbit epididymis and 875 fmole/g tissue in fetal rabbit urogenital tubercle) was greater than the binding in extracts of fetal epididymis.

Radioactive testosterone was a poor ligand for the fetal epididymal androgen receptor, and a clearcut sedimentation profile could not be demonstrated even when [^{3}H]-

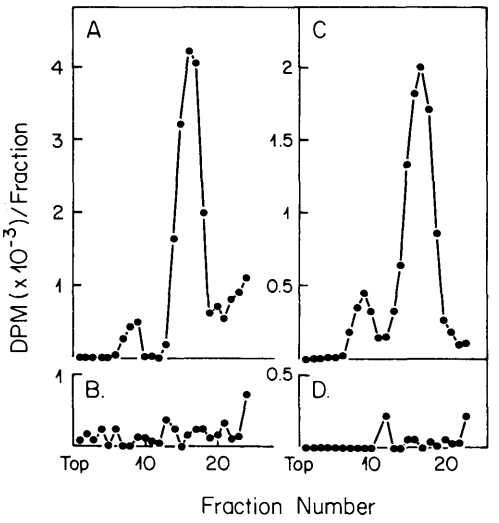

FIG. 2. Sucrose density gradient analysis of 5 n*M* [^{3}H]5α-dihydrotestosterone binding in castrate adult epididymis (A, B) and Day 27 fetal male urogenital tubercle (C, D). Sucrose density gradient experiments were performed as described in the text. The panels depict specific binding for low-salt (A, C) and high-salt (B, D) extracts. The total specific binding in the low-salt extract of adult epididymis was 22 fmole/mg protein or 401 fmole/g tissue, and the total specific binding in the low-salt extract of Day 27 fetal male urogenital tubercle was 35 fmole/mg protein or 875 fmole/g tissue. As indicated in Fig. 1, bovine serum albumin (4.6 S) sediments approximately to the level of fraction 10 in our gradients.

testosterone was used at a 5-fold higher concentration than [^{3}H]5α-dihydrotestosterone (25 vs 5 n*M*) (results not shown). Failure to demonstrate testosterone binding in epididymal tissue was not due to metabolism of the ligand since greater than 90% of the [^{3}H]-testosterone was unmetabolized in incubations of both nuclear and cytosolic extracts. Therefore, an experiment was designed to assess indirectly the relative binding affinities of the two androgens to the androgen receptor. A 10-fold excess of nonradioactive 5α-dihydrotestosterone competed for 75–80% of 5 n*M* [^{3}H]5α-dihydrotestosterone binding in both low- and high-salt extracts of Day 29 fetal rabbit epididymis, whereas 50 n*M* nonradioactive testosterone was less effective, resulting in only 10 and 40% competition with 5 n*M* [^{3}H]5α-dihydrotestosterone binding in low- and high-salt extracts, respectively (Fig. 3). A 100-fold excess of either nonradioactive testosterone or nonradioactive 5α-dihydrotestosterone was equally effective in competing for specific androgen binding in the two extracts (Fig. 3). These results suggest that 5α-dihydrotestosterone binds preferentially to the androgen receptor in fetal rabbit epididymis, as is the case in other androgen-dependent tissues of the fetal and adult rabbit (6).

We were unable to demonstrate an androgen receptor in the mesonephros (in which the wolffian duct forms) of the early (Day 18–20) rabbit embryo. We conclude that our methods for assessing the receptor are not adequate for demonstrating androgen receptors in this tissue and that other, more sensitive techniques, such as autoradiography, must be tried.

Discussion. We considered three possible explanations for our previous failure (6) to demonstrate androgen receptors in wolffian duct derivatives from the fetal rabbit: (i) that the homogenization techniques were inadequate in a tissue with such extensive stromal components, (ii) that the receptor is present in too low a concentration to be measured by the techniques we have developed for exam-

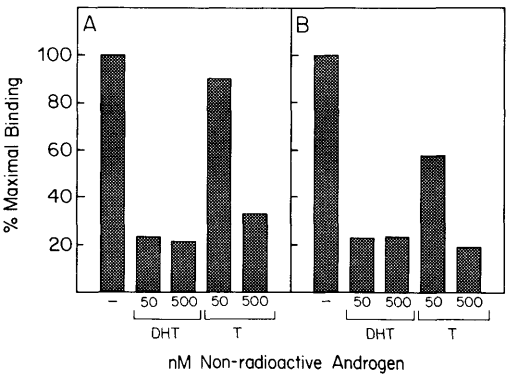

FIG. 3. Specificity of androgen binding to androgen receptors extracted from fetal rabbit epididymis. Direct hydroxylapatite binding assays were performed as described (6) on low-salt (A) and high-salt (B) extracts from 200 mg of Day 29 fetal rabbit epididymis. Aliquots of each extract were incubated with 5 n*M* [^{3}H]5α-dihydrotestosterone and the indicated n*M* concentrations of nonradioactive 5α-dihydrotestosterone (DHT) or testosterone (T). The original binding in the low-salt extract (without competing nonradioactive hormone, panel A) was 111 fmole/g tissue, and maximum binding in the high-salt extract (B) was 73 fmole/g tissue.

ining the receptor in other embryonic tissues (6), or (iii) that the receptor in this tissue has unique binding specificity and can only be recognized using radioactive testosterone as ligand (7). We therefore designed a series of experiments in which the embryonic tissues were pooled in larger aliquots, the tissue was extracted in high-salt as well as in conventional buffers so as to ensure better recovery of receptor, and both radioactive steroids were utilized as binding ligands. The findings in the present studies indicate that an androgen receptor is present in embryonic wolffian-derived epididymis, albeit at a relatively low level. It is not known whether the lower level of androgen receptor in fetal epididymis is due to high rate of degradation or is due to the fact that the androgen-responsive cells in the developing epididymis constitute only a fraction of the cells in the tissue (8). The fact that androgen-dependent elements (wolffian duct) constitute an even smaller fraction of the mesonephros may be the reason for failure to demonstrate androgen receptor in this tissue. It is also not known whether the androgen receptor of the wolffian duct derivatives is located in epithelial or stromal components of the tissue (9). Nevertheless, the receptor in the late fetal rabbit epididymis appears to resemble that in other androgen target tissues of the rabbit, namely in sedimentation characteristics and in preferential binding of 5α-dihydrotestosterone. Although we cannot exclude the possibility that a unique "embryonic" androgen receptor is present transiently in the wolffian ducts prior to Day 27 of embryonic development, these results suggest that testosterone-mediated differentiation of this tissue involves a typical androgen receptor.

Indeed, the fact that the androgen receptor of the developing epididymis binds 5α-dihydrotestosterone in preference to testosterone and resembles the receptor in other androgen target tissues of the fetal and adult rabbit is in keeping with the previous finding that 5α-dihydrotestesterone has the capacity to virilize the wolffian ducts of the rat (10). If our deduction is correct that this phenomenon cannot be explained at the level of the androgen receptor itself, then the mechanism will have to be sought at the level of hormone transport or metabolism within different tissues. It is possible that 5α-dihydrotestosterone serves to magnify the androgenic signal in androgen-dependent tissues but does not perform any unique function that testosterone itself cannot perform if testosterone is present in sufficient concentration within the cell. It is also possible that testosterone-mediated wolffian duct differentiation is caused by a testosterone-regulated factor produced at an extra-epididymal, androgen-response site.

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