SELECTIVE UPTAKE AND METABOLISM OF ANDROGENS BY RAT EPIDIDYMIS. THE PRESENCE OF A CYTOPLASMIC RECEPTOR

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SUMMARY: Epididymis actively concentrated androgens with a predominant intranuclear localization. Spermatozoa did not concentrate androgens. Macromolecular complexes were formed in both nuclei and cytoplasm. Density gradient centrifugation showed two binding zones in the cytosol. One had a sedimentation coefficient of 8.5± 0.3 and seemed to be highly specific for DHT. The second, approximately 3.5S, corresponded with the bulk of protein applied to the gradient. Testosterone was ineffective as a competitor of the formation of the 8.5S-DHT complex. Cyproterone acetate was inhibitory when administered *in vivo*, but less effective *in vitro*. Blockade of the SH groups prevented the formation of the 8.5S-DHT complex, which could be destroyed by digestion with Pronase.

Although the epididymis has been known to be an androgen dependent organ (1), it has not yet been fully characterized as a target tissue for these hormones. Preliminary results on androgen uptake and formation of macromolecular complexes by this organ has been published (2-4).

In keeping with current concepts on the mechanism of steroid hormone action, a cytoplasmic receptor should be present to enable the tissue to retain the active hormones at the nuclear site of action (5).

The present paper reports evidence on the uptake and metabolism of androgens by epididymal tissue and the presence of a cytoplasmic receptor.

This set of data, coupled with our recent finding that the presence of androgens is an absolute requirement for epididymal function, as far as sperm maturation is concerned (6), provides a comprehensive picture of the epididymis as an androgen target tissue.

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MATERIALS AND METHODS

Male rats, with a body weight of 150-200g were orchidectomized on the right side, avoiding damage to the epididymis, and placed with females for 3 weeks. This procedure yielded a well preserved organ completely free of spermatozoa. Uptake and metabolism experiments were performed as previously described (2). Either 1-2 H^3 testosterone (S.A. 50Ci/mM) or 1-2 H^3 dihydrotestosterone (S.A. 44Ci/mM) were injected intravenously (30uCi) into eviscerated rats that had been castrated for 3 days.

Tissues were homogenized with 0.6M sucrose in 0.01M Tris-HCl buffer, pH 7.4, containing 1.5mM $CaCl_2$. The use of a plastic homogenizer with Teflon pestle was necessary to avoid rupture of epididymal nuclei. Nuclei were purified by centrifugation on a double gradient made of equal volumes (10 ml) of 1.6 and 2.0M sucrose in the same buffer at 23.500rpm for 90' with an SW25 rotor in a Spinco ultracentrifuge.

Nuclei thus obtained were extracted with the procedure described by Bruchovsky et al.(7). Complete extraction of intranuclear radioactivity and chromatin was achieved by using a 0.9M NaCl solution in 0.01 M Tris-HCl, pH 7.4, containing 1.5mM EDTA.

Steroids were extracted and fractionated as previously described (2).

When desired, spermatozoa were collected from the left epididymis by washing a fine mince over a Nitex net.

In vitro labeling was performed by incubation of either minced epididymis or 105.000 x g supernatant (cytosol) of homogenates with tritiated steroid for 3-5 hs at 0°C . Tris-HCl buffer, 0.05M, pH 7.4 at 0°C, containing 1.5mM EDTA was used troughout these experiments. All procedures were carried out at 0-2°C in the cold room. Gel filtration was performed on Sephadex G-25 columns, 1 x 40 cm, at a flow rate of 2ml per min, collecting 3ml fractions.

Cytoplasmic receptors were investigated by means of density gradient ultracentrifugation of a 0.2-0.4ml aliquot of cytosol (1.3-2.5mg of protein) on linear 5 to 20% sucrose gradients, which were run at 50.000rpm for 12 hs in a SW65 rotor in a Spinco L65B ultracentrifuge at 0-2°C. Gradients were fractionated, from bottom to top, with a peristaltic pump and standardized to obtain 30 samples (0.16ml each). Beef heart catalase,

yeast alcohol dehydrogenase and bovine serum albumin were used as markers. Protein was determined, in aliquots of the samples, by the method of Lowry (8) or by UV absorption at 280 mu.

RESULTS

Table 1 contains the data on uptake, subcellular distribution and metabolism of radioactive testosterone (T) and dihydrotestosterone (DHT) 1 hour after its injection into rats castrated 3 days previously. The predominant intranuclear concentration of androgens is clearly shown by the large organ to muscle ratios obtained for the nuclear fraction. It must be noted that in the nuclear fraction T was almost totally metabolized to DHT, while this conversion was only partial in the cytoplasm. The main product of DHT metabolism seemed to be 5αandrostan-3α-17βdiol (diol).

Spermatozoa were only lightly labeled, but were apparently active in metabolizing T to DHT. Uptake of both androgens was lower by epididymis which contained spermatozoa.

TABLE 1

Comparative uptake, subcellular distribution and metabolism of H^3 testosterone and dihydrotestosterone by rat epididymis

Fraction	Steroid injected	Uptake dpm/mg P[a]	Ratio O/M[b]	% of H^3 found as T[c]	DHT[c]	diol[c]
Nuclear R[d]	H^3T[c]	6445	52	3	93	-
Nuclear L[e]	"	5005	40	3	80	-
Cytoplasm	"	3392	1.8	12	55	8
Sperm	"	912	-	21	50	10
Plasma	"	2008	-	43	17	16
Nuclear R	H^3DHT[c]	5686	19	-	-	15
Nuclear L	"	4020	10	-	-	26
Cytoplasm	"	1449	2.4	-	-	30
Sperm	"	474	-	-	-	33
Plasma	"	1052	-	-	-	69

a = P = protein
b = ratio of dpm/mg P in the organ to dpm/mg P in skeletal muscle
c = T = testosterone, DHT = dihydrotestosterone, diol = 5αandrostan-3α17β diol
d = R = right epididymis, without spermatozoa
e = L = left epididymis, with spermatozoa

Intranuclear radioactivity and chromatin were extracted by increasing NaCl concentration. At 0.9M NaCl, 99.6% of the radioactivity was extracted together with 98% of the DNA.

Gel filtration of cytosol or nuclear extracts disclosed that the major radioactive peak was eluted bound to macromolecules in the void volume of the column (20ml) associated with the bulk of the protein applied to the column. A second, smaller peak appeared later (fraction N° 20, 60ml) and corresponded with standard free testosterone. The analysis of the bound radioactivity showed that only 16% of the injected H^3 testosterone remain ed as such in the nuclear extract, while the remaining 84% was present as DHT. The radioactivity of the cytosol fractions from these animals contain ed 19% of nonmetabolized T, while 44% was transformed to DHT and 18% to diol.

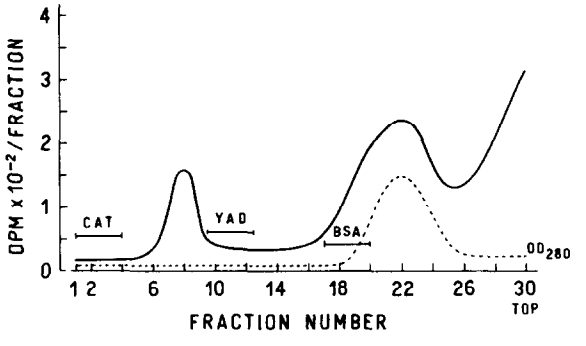

FIGURE 1 : Sedimentation profile of epididymal cytosol from castrated rats injected with H^3 testosterone. Samples were layered over 5 to 20% sucrose gradients and centrifuged at 50.000 rpm for 12 hs with an SW 65 rotor. Solid line : radioactivity. Broken line : E_{280} in arbitrary units. CAT : beef heart catalase; YAD : yeast alcohol dehydrogenase; BSA : bovine serum albumin.

Figure 1 shows the sedimentation profile, in sucrose gradients, of cytosol fractions of epididymis from H^3 testosterone injected animals. Two areas of radioactivity were found. The heavier peak appeared in fractions 7-10, with a calculated sedimentation coefficient of 8.5± 0.3 (mean ± SE) and an approximate molecular weight of 185.000 (9). The second peak comprised fractions 17 to 24, with an approximate sedimentation coefficient of 3.5 S.

While in the region of the 8.5S peak no protein was detected either by

E_{280} or Lowry's method, the second peak corresponded with the highest concentration of protein in the gradient.

Binding in these two areas was also achieved in vitro by incubation of minced epididymis or cytosol fraction with H^3 testosterone or H^3 DHT (2-6 x 10^{-9}M) at 0°C. Steroid analysis demonstrated that testosterone remained unchanged during incubation.

The incubation of cytosol simultaneously with H^3 DHT and nonradioactive testosterone, up to a concentration of 2.4 x 10^{-6}M, demonstrated the ineffectiveness of T as a competitor of DHT for the binding with the 8.5 S "receptor" (Figure 2).

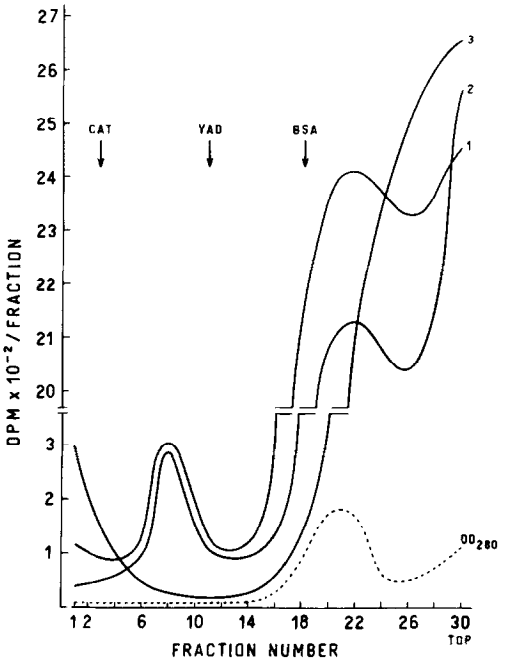

FIGURE 2 : Sedimentation profile of epididymal cytosol labeled in vitro with 2 x 10^{-9}M H^3DHT fro 3-5 hs at 0°C. Centrifugation as in figure 1. Solid line : radioactivity. Broken line : E_{280} in arbitrary units. Curve 1: cytosol incubated with H^3DHT ; Curve 2: cytosol incubated with H^3DHT and 2.4 x 10^{-6}M nonradioactive testosterone ; Curve 3: cytosol incubated with H^3DHT and 7 x 10^{-5}M cyproterone acetate.- CAT: beef heart catalase; YAD: yeast alcohol dehydrogenase; BSA: bovine serum albumin

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Cyproterone acetate, in vitro, had to be added at a concentration of 7 x 10^{-5}M to be effective as an inhibitor of the binding of H^3 DHT to the 8.5S androphilic molecules. On the other hand, pretreatment of the animals

with a single dose of 15mg of cyproterone acetate, 2 hs prior to the prepa ration of the cytosol, completely abolished the formation of the 8.5S-DHT complex.

Preincubation of the cytosol with p-mercuribenzoate, 2.5 x 10^{-3}M for 30' at 0°C, inhibited the formation of the receptor-steroid moiety. The labeled 8.5S - H^3 DHT complex was destroyed by incubation with 1.5mg Pronase for 2 hours at 0°C.

DISCUSSION

The selective uptake and intranuclear concentration of androgens by epid idymal tissue is clearly demonstrated by the high organ to muscle ratio obtained in our results. This is in agreement with current thoughts that steroid hormones have to reach the nucleus in order to exert their action (5, 10). Similar results have been obtained by several authors for other androgen target organs such as prostate and seminal vesicles (7, 10, 11-13).

Furthermore, we were also able to show that the intranuclear radio-activity originated from H^3 testosterone injection was predominantly in the form of DHT, thus concurring with the hypothesis that DHT, rather than T, is the active androgen in target tissues (7, 10, 12, 14, 15).

We also demonstrated that spermatozoa do not concentrate androgens. Important metabolization of both T and DHT is apparently achieved by sperm, although the possibility of metabolism of androgens by the epididymal tissue, prior to the passage of the hormones to the tubule lumen, cannot be excluded.

Gel filtration experiments demonstrated the formation of androgen-macro molecular complexes in both nuclei and cytoplasm, in a fashion similar to that recently reported by Hansson (4).

Finally, our gradient sedimentation analysis showed the presence of two regions of binding in the cytosol fraction of rat epididymis. The 8.5 S peak bears a close resemblance with the specific receptor described by other authors (16-18) in prostate. The specificity of the 8.5S receptor seems to be in favor of DHT, since an excess of nonradioactive testoster one did not prevent the formation of the 8.5-DHT complex. Nevertheless, testosterone, in the absence of any competitor, also binds to that zone.

The 3.5S binding area, associated with most of the protein applied to

the gradient, seems to be less specific, since testosterone diminished the binding of DHT.

The decrease in binding in the 8.5S zone produced by cyproterone acetate is probably related to the antiandrogenic properties of this compound. While a clear cut inhibition was observed *in vivo*, the *in vitro* competition, besides requiring a rather high concentration, led to the formation of agregates in the bottom of the tube and to a pattern in which it was not possible to differentiate between the H^3 bound to the 3.5S fraction and the free steroid on top of the tube.

The proteic nature of the androgen recptors in prostate has been described (16, 18, 19). The complete loss of binding in the 8.5S region when the SH groups of the cytosol had been blocked, and the destruction of the 8.5-DHT complex by Pronase digestion, suggest that the binding molecules of the 8.5 S region are also proteins.

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