THE SEPARATION OF ANDROGEN RECEPTOR AND 5α-REDUCTASE ACTIVITIES IN SUBCELLULAR FRACTIONS OF RAT PROSTATE.

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SUMMARY Measurements of 5α-reductase* and androgen receptor activities were performed on subnuclear fractions of rat prostate nuclei to establish whether these activities are conducted by a single nuclear-associated protein. Differences in intranuclear localization, extractability into KCl and in thermal stability suggest that these functions are associated with different nuclear proteins. Separation of 5α-reductase and androgen receptor activities was also achieved in the 105,000g supernatant fraction of rat prostate.

Specific androgen receptors have been identified in both the nuclear[1-3] and cytoplasmic[4-6] fractions of homogenates of rat prostate. Since the receptors have maximum binding affinity for 5α-dihydrotestosterone and the 5α-reductase responsible for the formation of this steroid is also present in both nuclear[7] and extranuclear[8,9] fractions, it was of considerable importance to establish whether these activities were performed by a single protein in these prostatic subcellular fractions.

MATERIALS AND METHODS

Experiments were performed on prostatic tissue obtained from adult Sprague-Dawley rats (300g) 24 hr. after bilateral orchidectomy, with 8-12 animals per experimental group. In studies on the receptor proteins, the animals were labelled for 30 min. *in vivo* by the subcutaneous injection of 25 μc [1,2-^{3}H] testosterone (28C/m-mole) in 0.2 ml. isotonic NaCl [3]. Animals for 5α-reductase assay received NaCl only. Pooled batches of radioactive and non-radioactive tissue were then processed and analysed separately,

* Abbreviations: 5α-reductase, Δ^4-3-oxo steroid 5α-reductase; 5α-dihydrotestosterone, 5α-androstan-17β-ol-3-one.

but in strict parallel.

Isolation procedures were conducted at 2-4°. Purified nuclei were prepared by centrifugation through 2.2M sucrose[3] and resuspended in 0.25M sucrose-25mM tris-HCl, pH7.4. Disruption of nuclei was accomplished by carefully controlled sonication[10] (20 sec; 20 K cycles/sec.) and differential centrifugation at 1,000, 10,000 and 200,000g for 15 min. separated the chromatin into heavy, medium and light fractions respectively. Salt extraction of nuclear suspensions was conducted in a Dubnoff metabolic shaker for 30 min. at 0° and the material sedimented by centrifugation at 105,000g for 30 min. was taken for further analysis. In the partial separation of nuclear 5α-reductase and receptor activities, nuclear lysates in 0.5M KCl (2 ml.) were layered directly onto discontinuous gradients composed of 1.5 ml. 1.7M sucrose and 1.5 ml. 1.0M sucrose-0.5M KCl and centrifuged at $75,000g_{(av)}$ for 1 hr. in a swing-out rotor. The clear upper layer (supernatant) combined interphase materials and sedimented chromatin (pellet) were taken for further analysis.

The preparation and analysis of the prostatic 105,000g supernatant fraction have been described in detail elsewhere[4].

For the assay of 5α-reductase, prostatic fractions were resuspended in 2.0 ml. 50mM tris-HCl, pH7.2, containing 5mM $MgCl_2$, 50mM NaCl and 2mM dithiothreitol and after the addition of $NADP^+$ (0.4 mg), glucose-6-phosphate (0.75 mg), glucose-6-phosphate dehydrogenase (20 μg) and [1,2-^{3}H] testosterone (30,000 c.p.m., radiochemical purity $\rightleftharpoons$ 98%), the suspensions (total volume 2.5 ml.) were incubated for 1 hr. at 37°. Non-radioactive testosterone and androstene-3,17-dione (20 μg each) were added as chromatographic markers and the steroids were extracted in $CHCL_3$-CH_3OH[7], separated by thin-layer chromatography[4] on Kieselgel GF_{254} and counted. Under

these conditions, 5α-dihydrotestosterone migrates midway between the chromatographic markers. Soluble fractions from sucrose density gradients, prepared in 50mM tris-HCl, pH7.4-2mM dithiothreitol, were supplemented with all the components of the standard assay system prior to analysis for 5α-reductase activity.

RESULTS AND DISCUSSION

All experiments have been performed at least in duplicate with reproducible results (variation of means ± 10%).

A. NUCLEI

When purified nuclei were disrupted by sonication and subjected to differential centrifugation, the androgen receptor and 5α-reductase activities were not recovered in the same chromatin fractions (Table 1). In agreement with the results of other investigators both on prostatic chromatin[10] and that from other androgen-dependent tissues[11], receptor activity was present in all the chromatin fractions but particularly in the light chromatin. In contrast, the 5α-reductase activity was essentially associated with the heavy chromatin. These differences were maintained whether the results were expressed with reference to either protein (as in Table 1) or DNA.

TABLE 1. Subnuclear distribution of receptor and 5α-reductase activities.

Chromatin fraction	Composition (mg) DNA	Protein	*Receptor (c.p.m./mg.protein)	5α-reductase (c.p.m./hr./mg.protein)
Heavy	1.0	3.6	1,040 ± 60	3,106 ± 190
Medium	0.9	3.4	1,176 ± 40	2,031 ± 160
Light	0.3	2.0	1,475 ± 100	361 ± 50

* Animals labelled in vivo.
Nuclei from 12 animals (2.3 mg. DNA) as starting material.

The nuclear receptor and 5α-reductase activities also differed in their extractability into KCl solutions (Table 2). As found in earlier work[3,12], the nuclear receptor was extracted on raising the ionic strength of the suspending medium. The 5α-reductase was also extracted with KCl, but at a slower rate than the androgen receptor. At low KCl concentrations, the specific enzyme activity was apparently increased although the variable effects of residual KCl in these samples cannot be ruled out. The release of enzyme by NaCl treatment had been reported previously[7]. When nuclear extracts in 0.5M KCl were fractionated in discontinuous sucrose gradients, the highest receptor and 5α-reductase activities were recovered in different fractions (Table 3).

TABLE 2. Differential extraction of nuclear-associated activities.

KCl (molarity)	*Receptor (c.p.m./mg.protein)	5α-reductase (c.p.m./hr./mg.protein)
Zero	1,707 ± 110	1,400 ± 106
0.1	1,583 ± 125	1,550 ± 146
0.2	1,230 ± 90	2,670 ± 230
0.4	852 ± 41	2,401 ± 119
0.75	460 ± 30	2,000 ± 170

* Animals labelled in vivo. After extraction with KCl, the sediments from centrifugation at 105,000g were analyzed.

TABLE 3. Partial separation of nuclear receptor and 5α-reductase activities in discontinuous sucrose gradients.

Fraction	*Receptor (c.p.m./mg.protein)	5α-reductase (c.p.m./hr./mg.protein)
Supernatant	5,280 ± 410	600 ± 61
Interphase	890 ± 110	741 ± 70
Sediment	695 ± 70	1,260 ± 112

*Tissue labelled in vivo. Nuclei lysed in 0.5M KCl prior to centrifugation.

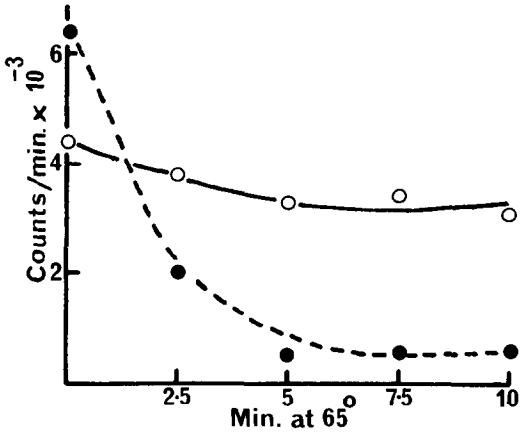

Fig. 1. Effect of heat on nuclear receptor and 5α-reductase activities. Aliquots of nuclei were incubated at 65° and returned to a 37° bath for 1 hr. Components for reductase assay added on return to 37°; receptor measured in 105,000g sediment after incubation at 37°. Reductase (c.p.m. x 10^{-3}) —●—; receptor (c.p.m. x 10^{-3}) —o— (after labelling in vivo).

The nuclear androgen receptor was also more stable than the 5α-reductase to heating at 65° (Fig.1). The thermal stability of the nuclear receptor was also reported by other investigators[12], but not in previously sonicated nuclei[1]. This result, in itself, is not definitive evidence that the 5α-reductase and receptor activities are associated with different proteins, but taken in conjunction with the other experimental findings, it adds indirect support for such a conclusion.

B. CYTOPLASMIC FRACTION

Compared with other prostatic fractions, the 105,000g supernatant is relatively low in 5α-reductase activity (Table 4). Despite the low specific activity, when samples of prostatic supernatant fraction were separated in linear sucrose density gradients, the 5α-reductase activity did not sediment with the receptor activity of sedimentation coefficient 8S (Fig.2). This is conclusive evidence that these cytoplasmic activities are performed by different proteins.

TABLE 4. 5α-reductase activity in prostatic subcellular fractions.

Fraction*	Total protein (mg).	5α-reductase (c.p.m./hr./mg.protein)	% Total activity
6,000g sediment	13.6	4,230 ± 390	73
100,000g sediment	6.8	4,050 ± 276	19
100,000g supernatant	27.6	460 ± 50	8

* Obtained by differential centrifugation of homogenates in 0.25M sucrose (pH7.4).

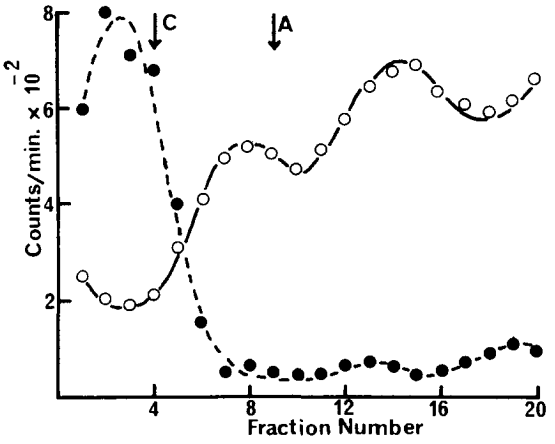

Fig. 2. Analysis of 105,000g supernatant in 5-20% sucrose density gradients[4], 12 mg.protein/gradient. Sedimentation markers C, catalase (11.4S); A, alcohol dehydrogenase (7.4S). Reductase (c.p.m. x 10^{-2}) from 2 pooled gradients, —●—; receptor (c.p.m. x 10^{-2}) —○— (after labelling in vivo).

The separation of these biochemical activities suggests that metabolism of testosterone in vivo precedes binding to the receptor proteins. This speculation is substantiated by the fact that the receptors have a low affinity for testosterone[1-6] and that protein-bound steroids are generally considered to be protected from enzymic action[13].

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