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A Soluble Androgen Receptor in the Cytoplasm of the Male Mastomys Prostate

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Summary. A steroid receptor protein was isolated from the cytoplasmic fraction of Mastomys prostate. Following in vivo and in vitro labelling of the tissue with tritiated testosterone or dihydrotestosterone, samples were analysed by gel exclusion chromatography or sucrose density gradient centrifugation. A steroid receptor complex was isolated on Sephadex G-200. Analysis of the steroids associated with this complex showed that the major part of the bound radioactivity was 5 α-dihydrotestosterone. The binding was inhibited by unlabelled testosterone and could not be demonstrated in the liver cytosol. Using sucrose density gradient centrifugation, the dihydrotestosterone receptor complex sedimented at 5.6 s together with heavier aggregates. In the presence of 0.4 M KCl a single complex was sedimented at 4.6 s. The results demonstrate a receptor protein in the cytosol of the Mastomys prostate which binds to dihydrotestosterone and is comparable to that of rat prostate.

Key words: Androgen receptor - Prostate - Praomys (Mastomys) Natalensis - Dihydrotestosterone - Androgen metabolism.

Praomys (Mastomys) Natalensis is a subgenus of rodent intermediate in size between rats and mice. The uptake and distribution of radiolabelled androgen in the male has recently been reported from this laboratory (4). It was found that the ability of the prostate to take up and retain androgen was similar to that of the rat ventral prostate. The androgen levels in the prostatic tissue and circulating blood were also measured (7). Based on these findings, further studies were planned to identify and characterise the receptor protein for androgen in this animal and to compare it with that of the rat ventral prostate.

MATERIALS

Animals

Male Praomys (Mastomys) Natalensis were bred in our colony and fed with standard laboratory diet. The ages of the animals used varied from 5-6 months for in vivo studies, to 8-9 months for in vitro experiments. Bilateral orchidectomy was performed under fluothane anaesthesia, either 24 or 72 h prior to the experiment.

Radioactive Steroids

(1 α, 2 α-^{3}H) testosterone, Sp. activity 49 Ci/mmol and (1, 2, 4, 5, 6, 7-^{3}H) 5 α-dihydrotestosterone, Sp. activity 130 Ci/mmol were purchased from the Radiochemical Centre Amersham, U.K. The purity of these compounds on t.l.c. was over 97%.

Steroids

The following unlabelled steroids were used:

Testosterone (17 β-hydroxyandrost-4-en-3-one) and 5 α-dihydrotestosterone (17 β-hydroxy-5α androstan-3-one) (Sigma Chemicals Ltd). Androsterone (3 α-hydroxy-5 α-androstan-17-one), androstanediol (5 α-androstane-3 β, 17 β-diol), androstenediol (androst-5-ene-3 β, 17 β-diol), androstanedione (5 α-androstane-3, 17-dione),

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and androstenedione (androst-4-ene-3, 17-dione) (Koch-Light Laboratories).

Buffers

1. Buffer A consisted of 20 mM Tris-HCl containing 320 mM sucrose and 3 mM magnesium chloride at pH 7.4.
2. Buffer B was 20 mM Tris-HCl containing 1.5 mM Ethylene-diaminetetra-acetic acid, disodium salt with 2 mM 2-mercaptoethanol at pH 7.4.

All reagents were of Analar grade and obtained from British Drug Houses (B. D. H.).

Solvents

Redistilled chloroform, acetone, diethyl ether (peroxide free), Triton X-100 and Toluene were all of Analar grade and supplied by B. D. H.

Scintillation Fluid

Toluene: Triton X-100 (2 : 1) containing 0.4% 2, 5-diphenyloxazole (P. P. O) (Koch-Light Laboratories) was used for samples containing aqueous media. Toluene containing 0.4% w/v P. P. O. was used for non-aqueous samples.

Chromatographic Materials

Sephadex G-200 (40-120 μ) and Sephadex G-25 (50-150μ) (Pharmacia Fine Chemicals) were used for column chromatography. Thin layer chromatography (t. l. c.) was performed on aluminium backed silica gel plates F254 (Merck).

METHODS

Preparation of Tissue Extracts

a) Labelling of tissue in vivo. Groups of ten male Mastomys castrated 72 h previously received an intraperitoneal injection of 1μ Ci/10 g body weight of (^{3}H) testosterone in 20% (v/v) ethanol/saline. The animals were killed after one hour by cervical dislocation. The ventral prostates were dissected out together with samples of liver. The tissues were minced, blotted and weighed.

b) Labelling of tissue in vitro. Ventral prostates pooled from 24 h castrated male Mastomys were minced, washed in buffer A and incubated with 0.25μ Ci of tritiated steroid per 2 ml of buffer A at either 37° C for 1 h or 0° C for 3 h. After incubation the tissue was washed for three 10 minute intervals with ice cold buffer A. The tissue was blotted, weighed and used for cell fractionation.

c) Cell fractionation. Labelled tissue was homogenised with 3 vols of buffer B using a teflon-glass motor driven homogeniser for four 20 s periods, with 20 s cooling intervals. The homogenate was centrifuged at 105,000 g for 1 h at 2° C. The cytosol fraction (supernatant) was removed and stored at -20° C for subsequent analysis. The protein concentration of the cytosol was 5-7 mg/ml.

Density Gradient Centrifugation

Linear sucrose gradients (5.6 ml, 5-20%) in buffer B either with or without 0.4 M KCl were prepared by the method of Martin and Ames (11). Centrifugation was performed at 60,000 rpm in an MSE 3 x 6.5 ml Titanium Swing-out Rotor at 2° C for 12 to 16 h. Fractions (0.2 ml) were collected from the bottom of the tubes and the radioactivity measured.

Determination of the sedimentation coefficients were made by the method of Martin and Ames (11). Bovine serum albumin ($S_{20,w}$ = 4.6) was used as a standard marker protein (Sigma Chemicals).

Other Procedures

Thin layer chromatography was carried out according to the procedures described previously (5). Protein was estimated by the method of Lowry (9).

RESULTS

Isolation of a Receptor Protein from the Cytosol by Column Chromatography

Pooled prostatic tissue obtained from 10 animals castrated 24 h prior to the experiments was incubated with (^{3}H) steroids at 0° C for 3 h or at 37° C for 1 h. The cytoplasmic fractions were prepared and subjected to Sephadex G-200 column chromatography. No significant differences between incubations at 0° C or 37° C were observed. When a 100-fold excess of unlabelled testosterone was introduced, the radioactivity associated with proteins eluted at the void volume was totally abolished. These results are shown in Figure 1.

Characterisation of the Receptor Protein by Gradient Centrifugation

a) In vitro studies. Following incubation of prostatic tissue with (^{3}H) dihydrotestosterone at either 30° C for 1 h or 0° C for 3 h, the cytoplasmic fractions were prepared and subjected to sucrose density gradient centrifugation as described in the method. In the presence of

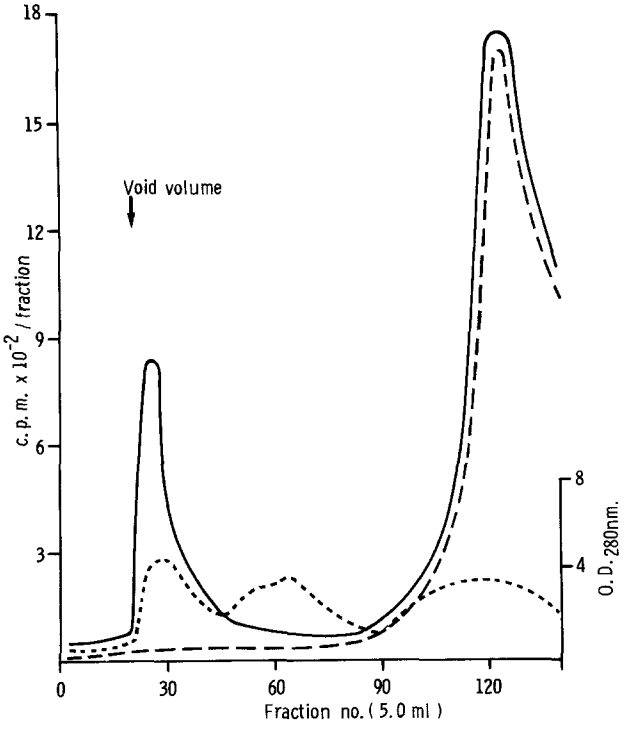

Fig. 1. Separation of labelled prostatic cytosol on Sephadex G-200 columns. The extracts were obtained from tissue incubated with (^{3}H) steroids (0.25 Ci/2 ml) at 37° C for 1 h. The radioactivity profile is shown following incubation with (^{3}H) testosterone either alone (——) or in the presence of 100-fold excess unlabelled testosterone (— —), together with the elution profile (- - -), E_{280}

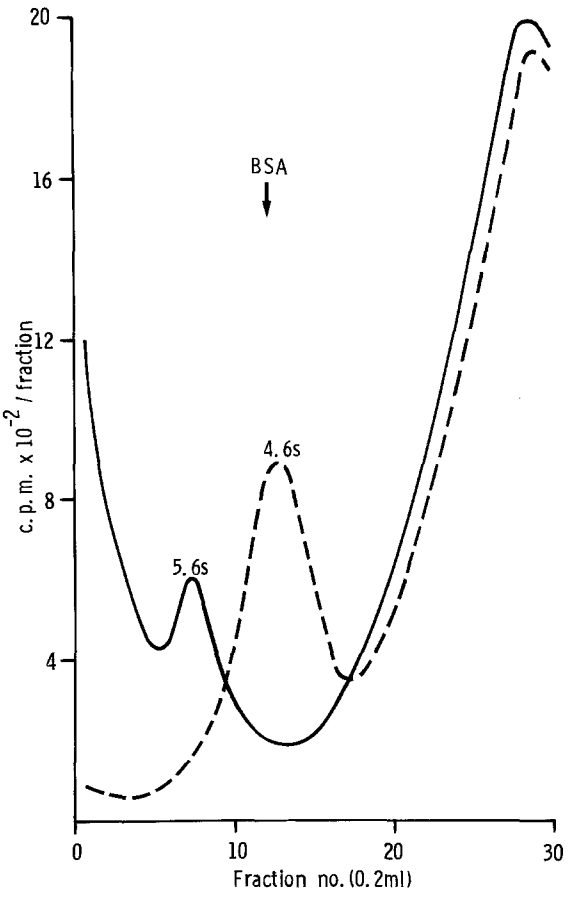

Fig. 2. Density gradient centrifugation of prostatic cytosol. The tissue was incubated with (^{3}H) dihydrotestosterone at 37° C for 1 h. The cytosol was centrifuged on a linear 5-20% (w/v) sucrose gradient at 2° C in an MSE 65 Mark II ultracentrifuge (3 x 6.5 ml Swing-out rotor). Bovine serum albumin (BSA, 4.6 s) migrated to the position shown. Sedimentation was from right to left. The radioactivity distributions are shown following either centrifugation alone (——) or in the presence of 0.4 M KCl for 16 h (- - -)

0.4 M KCl a single peak corresponding to a sedimentation coefficient at 4.6 s was observed. These results are shown in Figure 2.

b) In vivo studies. After the injection of (^{3}H) testosterone to 72 h castrated animals for 1 h the cytoplasmic fractions of the prostate and liver were prepared and subjected to sucrose gradient centrifugation as described in the method. The receptor proteins were characterised in the presence or absence of 0.4 M KCl. No significant binding was observed in cytoplasmic fractions obtained from liver tissue. The prostatic cytosol showed a similar binding pattern to that observed in vitro. The results for 12 h centrifugation are shown in Figure 3.

Preliminary Identification of (^{3}H) Steroids Associated with the Receptor Complex

Radioactive steroids, associated with the cytoplasmic binding protein isolated by Sephadex chromatography from tissue previously labelled with (^{3}H) testosterone, were extracted with diethyl ether. These extracts were subjected to thin layer chromatography. The results expressed as a percentage of the total recovered for each steroid are shown in Table 1.

DISCUSSION

In the present investigation when the cytoplasmic extract of labelled prostate was subjected to gel exclusion chromatography on Sephadex G-200, a peak of radioactivity was found to be associated with proteins eluted in the void volume. This binding could be totally abolished by a 100-fold excess of unlabelled testosterone. Extraction and analysis of the (^{3}H) steroid protein

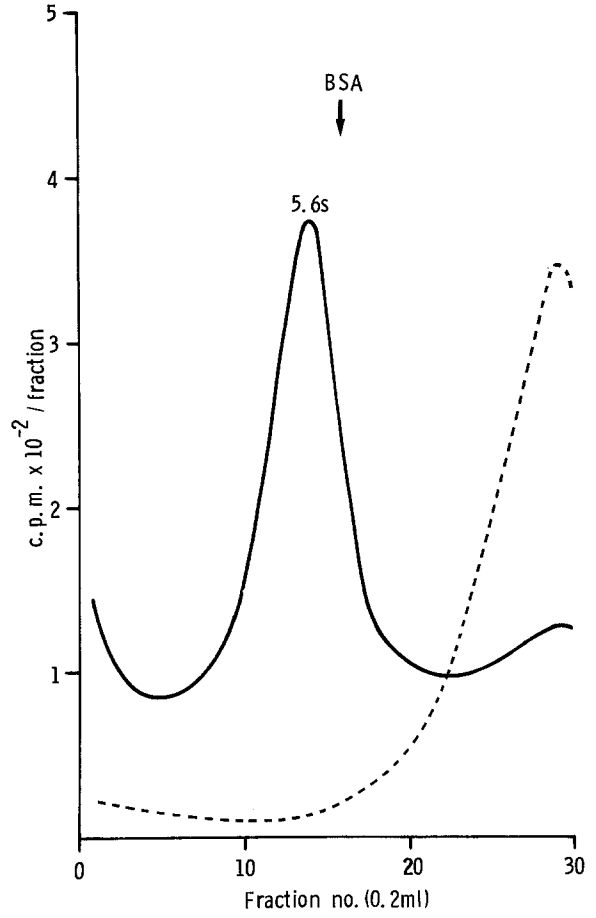

Fig. 3. Analysis of prostatic cytosol on sucrose density gradient 5-20% (w/v). Cytoplasmic extracts from both prostatic tissue and liver were prepared following administration of (^{3}H) testosterone (1μ Ci/10 g body weight) into 72 h castrated animals. Samples were centrifuged at 2° C for 12 h at 60,000 rpm. Sedimentation was from right to left. Bovine serum albumin (B. S. A.) migrated to the position shown. Prostatic cytosol (——) and liver cytosol (- - -)

complex by t. l. c. showed that 68-88% of the recovered radioactivity migrated to the same position as (^{3}H) dihydrotestosterone. Similar results have been obtained by several research groups when these experimental conditions were applied to rat ventral prostate (10, 12, 18, 19).

Using sucrose density gradient centrifugation the results suggest that in the presence of a high salt solution (0.4 M KCl) the steroid receptor complex has a sedimentation coefficient of 4.6 s. These results are similar to those reported by Baulieu and Jung (1) who found a 4-5 s receptor in the cytosol fraction of the rat prostate in the presence of a high salt solution. The results also demonstrate that in the absence of 0.4 M KCl the steroid receptor complex

Table 1. In vitro metabolites of (^{3}H) testosterone recovered from steroid receptor complex isolated from the cytoplasmic fraction on Sephadex G-200. In 5 experiments the (^{3}H) steroids associated with the receptor complex were extracted with diethyl ether and analysed by thin layer chromatography. The radioactivity recovered corresponding to each authentic steroid was expressed as the % of the total radioactivity recovered.

Steroids recovered	% Steroid recovered (mean ± S. E. M.)	Rf value
Dihydrotestosterone	76.90 ± 5.92	0.47
Androstanedione	7.50 ± 2.65	0.76
Testosterone	7.00 ± 2.14	0.30
Androstenedione	2.10 ± 1.16	0.62
Androstanediol Androstenediol	0.87 ± 0.38	0.21
Androsterone	0.83 ± 0.15	0.39
Others	4.77 ± 1.19	-
Total recovered	97.43 ± 0.18	-

sediments preferentially at 5.6 s. Under these conditions the binding pattern was polydisperse and some of the aggregates sedimented at the bottom of the tube. This interesting observation is similar to the behaviour of the 8-10 s cytoplasmic receptor of the rat ventral prostate. Baulieu, Jung, Blondeau and Robel (2), Liao, Fang, Tymoczko and Liang (8) and Ghanadian (3) have reported that in the rat ventral prostate the 8-10 s receptor complex and associated aggregated material disappeared in the presence of 0.4 M KCl with the apparent generation of a 3-5 s complex. In the cytoplasmic fraction of the male Mastomys the 5.6 s receptor and associated aggregates can be dispersed by 0.4 M KCl with the production of a single steroid receptor complex which sediments at 4.6 s. When samples of liver cytosol from Mastomys labelled in vivo with (^{3}H) testosterone were analysed by sucrose gradient centrifugation no significant binding was observed. This study clearly demonstrated the presence of a receptor which binds to dihydrotestosterone in the cytoplasmic fraction of the male Mastomys prostate.

Spontaneous tumours of several organs have been reported in this animal (6, 13, 15, 16, 17). It is of interest that the female of this species has a well developed prostate which

rarely develops spontaneous adenocarcinoma (14). In our own studies we have not observed any tumours in the prostate of either male or female Mastomys though tumours have been evident in other organs. This present study has shown that the androgen receptor in the male prostate is almost identical to that seen in the rat; the female prostate has similar androgen receptor characteristics (5, 7) but exists in a low androgen environment and therefore could be preferred to the male as a model in the study of hormone effects on the prostate.

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