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Nuclear Androgen Receptors in the Prostate of Male Praomys (Mastomys) Natalensis

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Summary. The binding of dihydrotestosterone (DHT) within the nuclear fraction of the prostate of male Praomys (Mastomys) Natalensis has been investigated. Using in vivo or in vitro labelling with ^{3}H-DHT, the presence of a receptor protein having a sedimentation coefficient of 3.0 S was demonstrated. The binding was shown to be specific towards DHT and could not be found in control tissue. Analysis of radiometabolites associated with the steroid receptor complex demonstrated that the majority of the bound steroid was DHT. The similarity between this steroid receptor complex and that of rat ventral prostate, together with the potential use of this experimental model are discussed.

Key words: Prostate, Androgen receptors, Mastomys, dihydrotestosterone, Nuclear receptor, Androgen metabolism.

INTRODUCTION

In previous communications from this Laboratory, the uptake and distribution of androgens by the ventral prostate of male Mastomys was studied and the presence of a cytoplasmic receptor which bound preferentially to dihydrotestosterone was demonstrated (5, 11). It was found that the distribution of ^{3}H-testosterone in the prostate and non-target tissue was comparable to that of the rat. Furthermore, the dihydrotestosterone receptor protein from the prostatic cytosol was similar to the specific dihydrotestosterone binding protein in the rat prostate.

Mastomys are a distinct subgenus of rodent intermediate in size between rat and mouse. All females of this species have been shown to possess a well developed prostate, which can selectively take up and retain androgens (4) through receptor proteins which preferentially bind to dihydrotestosterone (6).

The use of this animal for studying prostatic disease was suggested following several reports of spontaneous tumours in the urogenital tract, as well as other organs, in this animal (7, 13, 14, 15).

In recent studies we have found remarkable similarities between the prostate of both male and female animals in respect of androgen retention (4, 5) and binding within the cytoplasmic fraction of the cytosol (6, 11). The present investigation was carried out to identify and characterise androgen binding protein from the prostatic nuclei in order to have a better understanding of the mechanism of hormone action in the prostate of Mastomys and to compare these findings with those of the rat.

MATERIALS AND METHODS

Animals

Male Mastomys (5-9 months old) were bred in our colony and fed with standard laboratory diet. When required bilateral orchidectomy was performed under fluothane anaesthesia, either 24 or 72 h prior to the experiment.

Radioactive Steroids

(1α, 2α-^{3}H)-testosterone, Sp. activity 49 Ci/mmol (^{3}H-T) and (1, 2, 4, 5, 6, 7-^{3}H)-5α-dihydrotestosterone, Sp. activity 130 Ci/mmol (^{3}H-DHT) (Radiochemical Centre). The purity of the compounds using t.l.c. was >97%.

Steroids

The following unlabelled steroids were used: T = testosterone (17β-hydroxyandrost-4-en-3-one) and 5α-DHT = 5α-dihydrotestosterone (17β-hydroxy-

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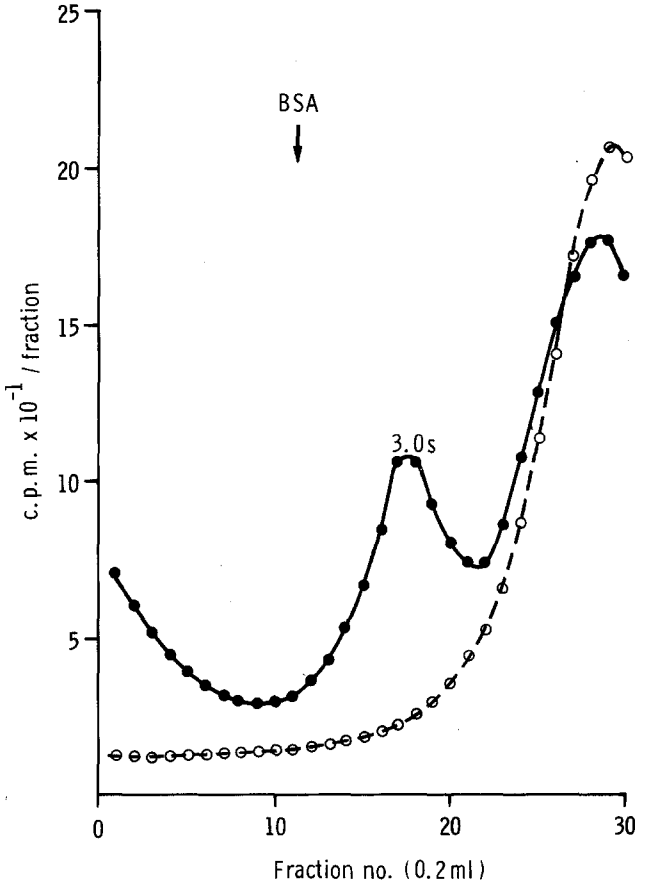

Fig. 1. Density gradient centrifugation of nuclear prostatic extract. Tissues were incubated with (^{3}H) dihydrotestosterone and the nuclear fractions prepared. The extracts (0.4 ml) were centrifuged on linear, 5-20% (w/v) sucrose gradients containing 0.4 M KCl at 2°C in an MSE 65 ultracentrifuge (3 x 6.5 ml Swing-out rotor) for 16 h at 60,000 rpm. Bovine serum albumin (4.6 s) migrated to the position shown. Sedimentation was from right to left. The distributions of radioactivity are shown from samples incubated at 37°C for 1 h (●) and 0°C for 3 h (- -o-).

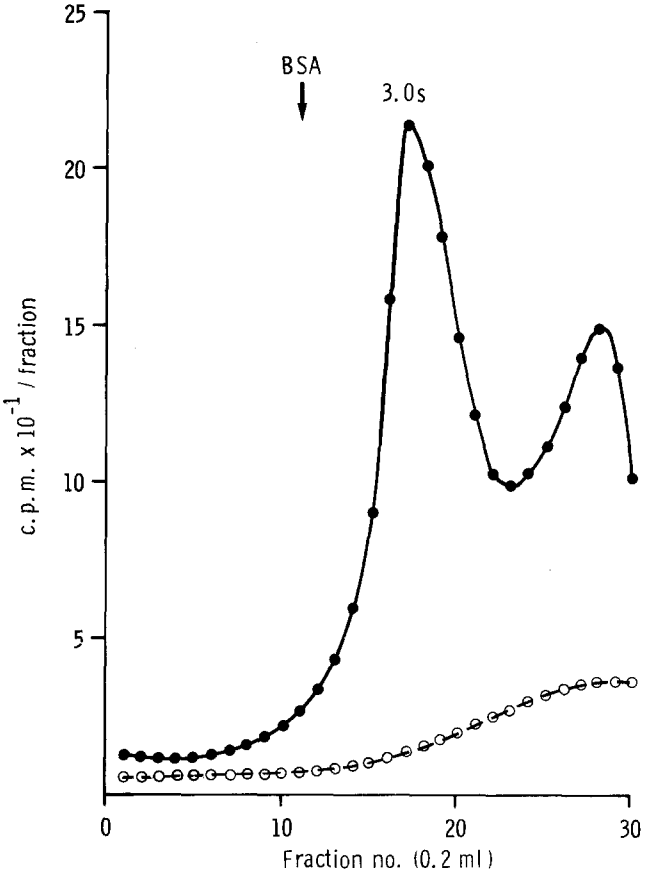

Fig. 2. Analysis of prostatic nuclear extract on linear, 5-20% (w/v) sucrose density gradients. Extracts from both prostatic tissue and liver were prepared following administration of (^{3}H) testosterone (1 μCi/10 g body weight). Samples were centrifuged at 2°C for 16 h at 60,000 rpm in the presence of 0.4 M KCl. Sedimentation was from right to left. Bovine serum albumin migrated to the position shown. Radioactivity distributions are shown for prostatic nuclear extract (●) and liver nuclear extract (- -o-).

5α-androstan-3-one). Oestradiol-17β (1,3,5 (10) oestratriene-3, 17βdiol) and Progesterone (Pregn-4-ene-3, 20 dione). (Sigma Chemical Co.). Androsterone (3α-hydroxy-5α-androstan-17-one), androstanediol (5α-androstane-3α, 17β-diol), androstenediol (androst-5-ene-3β, 17β-diol), androstanedione (5α-androstane-3, 17-dione), 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' contained 20 mM Tris-HCl with 1.5 mM EDTA (disodium salt) and 2 mM 2-mercaptoethanol at pH 7.4.

All reagents were of 'Analar' grade and obtained from BDH.

Solvents

Redistilled chloroform, acetone, diethyl ether (peroxide free), Triton X-100 and toluene were all of 'Analar' grade (BDH).

Scintillation Fluid

Toluene: Triton X-100 (2:1) containing 0.4% 2,5-diphenyloxazole (P.P.O.) (Koch-Light Laborato-

ries) was used for samples containing aqueous media. Toluene containing 0.4% (w/v) P. P. O. was used for non-aqueous samples.

Chromatographic Materials

Column chromatography was performed on Sephadex G-200 (40-120 μ) and Sephadex G-25 (50-150 μ) (Pharmacia Fine Chemicals). 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 were injected intraperitoneally with ^{3}H-testosterone in 0.2 ml of 0.9% normal saline containing 20% (v/v) ethanol. Each animal received a dose of 1 μCi/10 g body weight. The animals were killed one h after injection by cervical dislocation.

The ventral prostates, dissected free of capsule, and samples of liver were removed and transferred immediately to ice cold buffer A. The tissues were minced, blotted, weighed and used for the preparation of subcellular fractions.

b. Labelling of Tissue in vitro. Ventral prostates pooled from ten Mastomys castrated 24 h prior to the experiment were minced, washed in buffer A and blotted. The tissue was transferred to incubation flasks containing 0.25 μCi of tritiated steroid per 2 ml of buffer A. Incubations were carried out at either 37°C for 1 h or at 0°C for 3 h. After incubation the tritiated media was removed and the tissue washed for three 10 minute intervals with ice cold buffer A. The tissue was blotted, weighed and used for cell fractionation.

c. Cell Fractionation. Tissue labelled with (^{3}H) steroids was homogenised with 3 vols of buffer B. Homogenisation was carried out at 2°C with a teflon-glass motor-driven homogeniser for four 20 sec periods, with 20 sec cooling intervals. The homogenate was filtered through a double layer of 100 gauge nylon gauze and the filtrate centrifuged at 800 x g for 10 min at 2°C. The crude nuclear pellet was washed three times with 5 ml of buffer A containing 0.1% Triton X-100. The suspension was centrifuged at 600 x g for 10 min at 2°C each time. Light microscopy of the nuclear preparation revealed, clean, intact nuclei.

The purified nuclear pellet was disrupted by 0.4 M KCl in buffer B and the sample was finally centrifuged at 40,000 x g for 30 min at 2°C. The supernatant was removed and stored at -20°C for subsequent analysis. The protein concentration of the nuclear extract was 1.5-2.0 mg/ml.

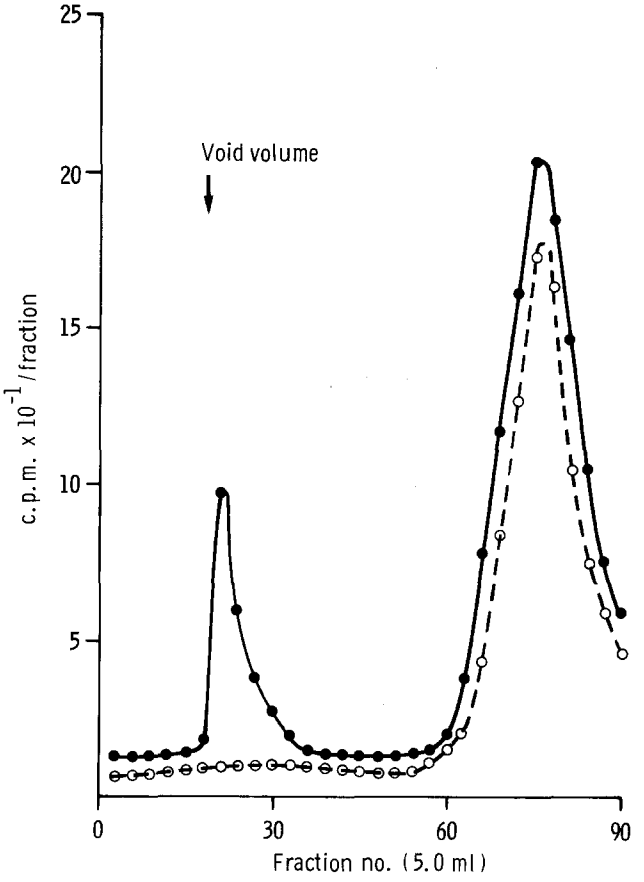

Fig. 3. Separation of (^{3}H) labelled prostatic nuclear extract on Sephadex G-200 columns. Extracts were obtained from tissue incubated with (^{3}H) testosterone for 1 h at 37°C. The radioactivity profiles are shown following incubations with (^{3}H) testosterone either alone (-●-) or in the presence of a 100-fold excess of unlabelled testosterone (- -o-)

Density Gradient Centrifugation

Linear sucrose gradients (5.6 ml, 5-20%) in buffer B containing 0.4 M KCl were prepared by the method of Martin and Ames (10). A sample (0.4 ml) was layered on the surface of the gradient and centrifuged at 60,000 rpm in an MSE 3 x 6.5 ml Titanium Swing-out rotor for 16 h at 2°C. Fractions (0.2 ml) were collected from the bottom of the tubes and the radioactivity measured.

Determination of the sedimentation constant was made by the method of Martin and Ames (10). Bovine serum albumin ($S20,_w$ = 4.6) was used as a standard marker protein (Sigma Chemicals).

Column and Thin Layer Chromatography

Column chromatography of the nuclear extract was carried out using columns of Sephadex G-200 and G-25 (6).

Table 1. In vitro radiometabolites of ^{3}H-testosterone recovered from the nuclear steroid receptor complex of the Mastomys prostate isolated on Sephadex G-25 following the in vitro incubation of tissues for 1 h at 37°C. 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 steroid was expressed as a percentage of the total radioactivity recovered

Steroids recovered	% Steroid recovered (Mean ± S.E.M.)	Rf value
Dihydrotestosterone	93.9 ± 1.0	0.47
Androstanedione	1.8 ± 0.6	0.76
Testosterone	1.4 ± 0.1	0.30
Androstanediol) Androstenediol)	1.3 ± 0.1	0.21
Androstenedione	ND	0.62
Androsterone	ND	0.39
Others	1.5 ± 0.5	-

Radioactive steroids associated with receptor fractions eluted in the void volume of Sephadex columns, were extracted with diethyl ether and subjected to thin layer chromatography as described previously (6).

Specificity Studies

Nuclear extract was prepared from prostatic tissue (250 mg) from intact animals and subjected to protamine sulphate precipitation followed by an exchange assay using ^{3}H-DHT.

The extract was diluted with buffer B to a KCl concentration of 0.2 M. Aliquots (0.2 ml) of the nuclear extract were taken and an equal volume of protamine sulphate solution (Sigma Chemicals) in buffer B (0.1%) was added to each tube. The contents were mixed and allowed to stand for 15 min and centrifuged at 800 g for 10 min at 2°C. The resulting precipitates were washed 3 times with ice cold buffer B. The washed precipitates were incubated for 16 h at 15°C in 0.2 ml of buffer B containing 20 nM ^{3}H-DHT either alone or together with a 100-fold excess of either unlabelled DHT, progesterone or oestradiol. The precipitates were then sedimented at 800 g for 10 min, washed six times with buffer B and finally extracted twice with ethanol (1 ml). The combined extracts were evaporated in scintillation vial and the radioactivity was counted. Radioactivity was counted using an Intertechnique SL40 liquid scintillation spectrometer.

RESULTS

Characterisation of Nuclear Receptor Protein by Gradient Centrifugation

a. In Vitro Studies. Following the incubation of prostatic tissue with ^{3}H-DHT at either 37°C for 1 h or 0°C for 3 h, the nuclear extracts were prepared and subjected to sucrose density gradient centrifugation as described in the method. The results for 16 h centrifugation are shown in Figure 1. No binding was observed in nuclei obtained from tissue incubated at 0°C.

b. In Vivo Studies. ^{3}H-T was injected into 72 h castrated animals 1 h prior to sacrifice, the nuclear fractions of prostate and liver were then prepared and subjected to sucrose gradient centrifugation as described in the method. Prostatic nuclei contained a receptor which sedimented at 3.0 S when analysed in 0.4 M KCl gradients for 16 h. Similar binding was not observed in the nuclear extract obtained from liver tissue.

Isolation of the Receptor Complex by Column Chromatography

When the nuclear extract from prostatic tissue incubated with ^{3}H-T at 37°C was prepared and applied to columns of either Sephadex G-200 or Sephadex G-25 eluted with media B, the receptor was eluted in the void volume of the column. A 100 fold-excess of unlabelled testosterone added to the incubation media, totally abolished binding in this region. These results are shown in Figure 3.

Preliminary Identification of (^{3}H) Steroid Metabolites Associated with the Nuclear Receptor

The steroid receptor complex eluted in the void volume on columns of Sephadex G-25 from tissue previously labelled with ^{3}H-T was extracted with diethyl ether. The extracts were subjected to thin layer chromatography as described in the method. The results expressed as percentage of the total activity recovered for each steroid are shown in Table 1, together with their Rf values.

Specificity of the Nuclear Receptor

The specificity study of the nuclear receptor was performed as described in the methods. The results showed that the receptor protein had a very high specificity towards dihydrotestosterone, whilst progesterone and oestradiol were weak competitors competing only by 23 and 14 percent respectively.

DISCUSSION

The results demonstrated the presence of a nuclear receptor protein which binds preferentially to DHT. This receptor was initially isolated using gel exclusion chromatography and subsequently

characterised by gradient centrifugation. The receptor protein was found to have a sedimentation coefficient of 3.0 S and could not be found in the liver control tissue. Furthermore, this binding could not be demonstrated following the incubation of prostatic tissue with tritiated DHT at 0°C. These observations are in keeping with similar findings reported for receptor proteins in the nuclei of rat ventral prostate (2, 8).

The DHT receptor complex isolated from rat ventral prostate nuclei, aggregates rapidly in the absence of KCl (3) and a similar effect was observed with the Mastomys nuclear receptor. This is demonstrated by the disparity between a sedimentation coefficient of 3.0 S in the presence of 0.4 M KCl and the observation that the complex is eluted in the void volume of the sephadex G-200 column in the absence of KCl.

Analysis of the radiometabolites revealed that approximately 94% of the radioactivity associated with the isolated receptor was dihydrotestosterone. This finding is again similar to that reported for the nuclear receptor isolated from the rat ventral prostate (1, 9). Using protamine sulphate precipitation combined with an exchange assay with ^{3}H-DHT the receptor was shown to have a very high specificity towards DHT. The advantages of this technique for specificity determination, i.e. both in the ability to use a long term exchange assay and in removing the enzyme interference of 5α-reductase.

The present study demonstrates the similarity between the binding of DHT to the nuclear receptor protein of Mastomys to those reported for the rat. This, in combination with the presence of cytoplasmic receptor for dihydrotestosterone in this animal, suggests that the mechanism of action of androgens in the prostate of this species is very similar to that of the rat prostate and gives further support for the suggestion that this animal could be used as an experimental model for studying hormone effects on the prostate.

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