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Androgen Receptors in the Prostate of the Rhesus Monkey

R. Ghanadian, G. Auf, C. B. Smith, G. D. Chisholm, and N. J. Blacklock[1]

Prostate Research Laboratory, Urological Unit, Royal Postgraduate Medical School, Hammersmith Hospital, London, and [1]Royal Naval Hospital, Haslar, U.K.

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Summary. The presence of an androgen receptor protein in the supernatant preparation of the prostate from Rhesus monkey (macaca mulatta) is described. The molecular weight of this receptor protein was found to be $2.8\text{-}2.9 \times 10^5$ daltons. The levels of free and bound androgen receptors were measured in the caudal and cranial lobes of the prostate by an exchange assay using methyltrienolone (R1881). The concentration of the free binding sites in the caudal lobe ranged between 3.7-23.7 fmol/mg protein. The value in the cranial lobe was 2.0-7.7 fmol/mg protein. The total binding sites in the caudal lobe ranged between 36.0-112.7 and in the cranial between 21.2-55.0 fmol/mg. The bound receptor ranged between 43.3-109.0 and 19.1-47.3 fmol/mg protein for caudal and cranial lobes respectively. The level of both bound and free receptors was found to be significantly higher in the caudal lobe. This data suggests that the two lobes of the prostate in the Rhesus monkey can be equated with the two zones of the human prostate in respect of androgen responsiveness.

Key words: Androgen receptors - Rhesus monkey - Prostate - Receptor assay.

The existence of a receptor protein which binds specifically to dihydrotestosterone has been demonstrated in the prostate of several species (3). In a preliminary study from this laboratory it was demonstrated that the caudal and cranial lobes of the prostate in the Rhesus monkey (macaca mulatta) were able to take up and retain androgen and that the uptake of testosterone by the caudal lobe of the prostate was significantly higher than any other target or non-target tissue (4). Both in the relationship to surrounding structures and in their differential histological characteristics the cranial lobe of the prostate of the monkey appears to be homologous with the central zone of the human and the caudal lobe with the peripheral zone (2).

The present study was carried out to characterise androgen receptors in the cytosol of Rhesus monkey prostate and to determine the concentration of these receptors in the cranial and caudal lobes.

MATERIALS AND METHODS

Monkeys

Rhesus monkeys (macaca mulatta) aged between 2 and 10 years, were used in this study. Prostate and other tissues were dissected out whilst the animals were under general anaesthesia. Samples were kept in containers on ice and transported to the laboratory. All the operative procedures were performed between 11 and 15 h.

Steroids

(1α, 2α, ^{3}H) 5α-dihydrostestosterone (^{3}H)DHT (specific activity 58 Ci/mmol; Radiochemical Centre, Amersham, U.K.). Unlabelled 5α-dihydrotestosterone (DHT) (Sigma Chemicals). (6,7-^{3}H) methyltrienolone (^{3}H) R1881 (specific activity 55.5 Ci/mmol) and unlabelled R1881 were kindly supplied by Roussel Uclaf, France.

Buffers

Buffer A: Tris buffer (20 mM) containing 1.5 mM EDTA and 2 mM mercaptoethanol adjusted to pH 7.4.

Buffer B: Tris buffer (10 mM) containing 1.5 mM EDTA and 250 mM sucrose adjusted to pH 7.4.

Both buffers were freshly prepared and stored at 4° C.

Two solutions (1 and 2) of dextran coated charcoal were prepared in buffer B. Solution 1 consisted of 5% water washed Norit GSX charcoal (Hopkins and Williams) and 0.5% dextran T-70 (Pharmacia). Solution 2 consisted of 1.25% water washed Norit GSX charcoal and 0.625% dextran T-70. Both solutions were stored at 4° C.

Solvents

Ethanol, Methanol, Toluene and Triton X-100 were all of Analar grade (British Drug Houses).

Enzymes

DNase and RNase from bovine pancreas and pronase from streptomyces griseus (Sigma Chemicals).

Column Chromatography

Sepharose 6B (Pharmacia Fine Chemicals).

Scintillation Fluid

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

Preparation of Cytosol

Sections of either caudal or cranial lobes of the prostate were sliced, minced, blotted with filter paper and weighed. The minced tissue was placed in a pre-cooled B24 tube and homogenised in buffer (1 : 6 w/v). Homogenisation was carried out using an Ultra Turrax type TP18-10 (Junks and Kunkle AG) using 5 strokes each of 10 seconds with 30 seconds cooling intervals. The homogenate was then centrifuged at 105,000 g for 1 h at 4° C. The resulting supernatant (cytosol fraction) was cleanly pipetted taking special care to avoid contamination from the lipid layer on the top. All the above procedures were carried out at 0-4° C.

Free endogenous hormone was removed from the cytosol fraction by incubating the cytosol with charcoal buffer (solution 1) in a ratio of 10 : 1 v/v at 0-4° C for 10 mins. The steroid bound to charcoal was precipitated by centrifugation at 3700 g at 4° C for 20 mins. Aliquots of 100 µl of the cytosol were then used for protein estimations (8). The remaining cytosol was used for the characterisation and the assay of both unoccupied and total binding sites.

Characterisation of Receptor

Aliquots of the cytosol stripped of endogenous hormone were incubated with 50 nM of (^{3}H) dihydrotestosterone in the presence and absence of unlabelled dihydrotestosterone at 15° C for 16 h. Unbound steroids were removed by incubating the cytosol with dextran charcoal solution 2 (2 : 1 v/v) at 0-4° C for 10 mins. Following centrifugation at 1000 g for 15 mins the supernatant was eluted on a precalibrated column (55 cm x 2.5 cm) of Sepharose 6B (7) at a flow rate of 40 ml/h with buffer A. Fractions (4 ml) were collected in an LKB 7000 fraction collector. Aliquots (2 ml) of each fraction were transferred to scintillation vials and scintillation fluid (15 ml) was added. The radioactivity was counted in a scintillation spectrometer. The results were expressed as counts per minute per fraction.

Measurement of the Receptors

a) Free Binding Sites. Unoccupied binding sites (free receptors) were measured in the cytosol fraction as follows:

For triplicate assay 6 test tubes were set up, each pair containing 0.1 pmol (^{3}H) R1881 with and without 500 pmol radioinert R1881 in ethanol. The solutions were evaporated to dryness and subsequently transferred to crushed ice. Aliquots of 0.1 ml cytosol were added to each tube and incubated at 0-4° C for 1 h. Unbound R1881 was removed by adding 50 µl dextran charoal (solution 2) which was then vortexed and incubated at 0-4° C for 10 mins. After centrifugation at 1000 g for 15 mins, aliquots of 100 µl of the resulting supernatant were transferred to 10 ml of scintillation fluid. The radioactivity was counted in a scintillation spectrometer (SL40 Intertechnique).

b) Total Binding Sites. In order to saturate all the DHT binding sites, cytosol stripped of free endogenous hormone was incubated with 10 nM DHT at 0-4° C for 1 h. The excess of DHT was then removed by adding charcoal buffer (solution 1) in a ratio of 1 : 10 v/v. Following centrifugation at 1000 g for 20 mins aliquots of 100 µl of the supernatant were removed for protein estimation (8). The remaining cytosol was then subjected to exchange assay as follows:

Portions (100 µl) of the saturated cytosol were incubated with (^{3}H) R1881 (20-50 nM) in the presence or absence of 5000 nM radioinert R1881 at 15° C for 16 h. Removal of free from bound (^{3}H) R1881 was achieved by adding 50 µl of dextran charcoal buffer (solution 2) at 0-4° C for 10 mins. After centrifugation at 1000 g for 15 mins aliquots of 100 µl of the supernatant were transferred to 10 ml scintillation fluid and the radioactivity counted in a scintillation spectrometer.

Analysis of the Results

The difference in counts/min between cytosol labelled with (^{3}H) R1881 and (^{3}H) R1881 + R1881 was taken as a measure of the quantity of DHT receptor in the cytosol. It was, there-

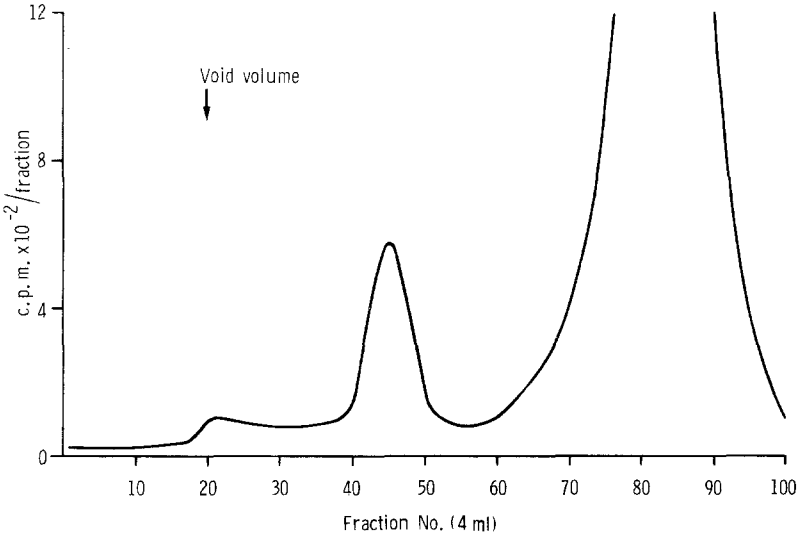

Fig. 1. Sepharose 6B gel exclusion chromatography of the steroid receptor complex from the caudal zone of the monkey prostate

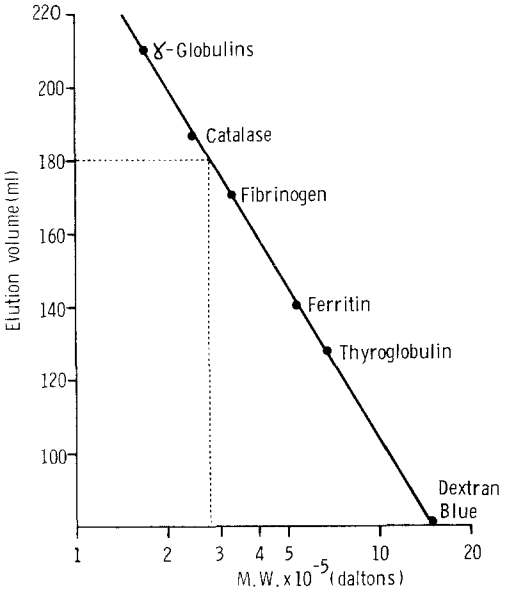

Fig. 2. Standard curve for molecular weights - Sepharose 6B

Table 1. Effect of enzymic digestion on receptor complex. Analysis on Sephadex G-25

	Percentage reduction in binding	
Enzyme	Caudal	Cranial
DNase	*ND	ND
RNase	ND	ND
Pronase	100%	96%

* Not detectable

Samples (100 µl) of cytosol previously labelled with (^{3}H) DHT as described were incubated alone or together with 20 µg of either pronase, deoxyribonuclease or ribonuclease at 37° C for 30 min. The samples were then subjected to chromatography on Sephadex G-25 column (20 x 1.5 cm) eluted with buffer A at 2° C. Radioactivity associated with material eluted with the void volume of the column was counted and compared to the control.

fore, possible to express the final results in terms of femtomole of specific receptor per mg of cytoplasmic protein. The difference between the total and unoccupied binding sites was considered as bound receptor (6).

RESULTS

1. Characterisation of the Receptor

The labelled cytosol prepared from caudal and cranial lobes revealed two distinct peaks of radioactivity (Fig. 1). The first peak, which was eluted between fractions 40 and 50, was totally abolished by an excess of unlabelled dihydrotestosterone. The second peak, which corresponded to free steroid, was not affected. The effect of enzymes on the elution profile showed that pronase completely abolished the first peak in the preparation from each lobe but RNase and DNase did not affect this peak (Table 1).

The molecular weight of the steroid receptor complex was estimated to be in the order of 2.8-2.9 x 10^5 daltons (Fig. 2).

Table 2. Concentration of androgen receptors in the cytosol of monkey prostate

Monkey		Cranial lobe fmol/mg protein			Caudal lobe fmol/mg protein		
No	Age (year)	total	free	bound	total	free	bound
1	9	39.7	2.0	37.9	112.7	3.7	109.0
2	9	41.5	2.6	38.9	101.3	6.6	94.7
3	6	55.0	7.7	47.3	67.0	23.7	43.3
4	2	21.2	2.1	19.1	36.0	6.3	29.7

2. Concentration of Androgen Receptors

The concentration for the total, free and bound receptors in 4 monkeys showed that the receptor content of the caudal zone was higher than in the cranial zone (Table 2). This difference was especially significant in the first two monkeys. The total receptor population in the third monkey was low but, comparatively, the free receptor was high. Although this monkey was a mature animal it was noted that the testes were small. The fourth monkey was immature and had a low level of receptors in both lobes. However, as with the mature monkey, the concentration of the receptor in the caudal lobe was higher than the cranial lobe.

DISCUSSION

These studies have demonstrated an androgen binding component in the caudal and cranial lobes of the monkey prostate.

The inhibitory effect of unlabelled dihydrotestosterone on the first peak and not the second suggests that the first peak is an androgen binding component. This, however, requires further investigation using other steroids to demonstrate the binding specificity. The effect of enzymes on this steroid binding component suggests that the nature of the "receptor" is a protein.

The results have shown that the behaviour of this receptor in the caudal and cranial lobes is similar. Furthermore, the molecular weight of this receptor is of the same order of magnitude as that of rat (9) and human prostate (unpublished results). Further studies are required to identify the labelled steroid bound to the receptor as well as the sedimentation constant of this steroid receptor complex. The procedure for the measurement of the free and bound receptors for androgens in this study was basically similar to that in our previous report (5) in which tritiated methyltrienolone, a synthetic androgen, was used in preference to labelled dihydrotestosterone. In the human prostate this compound specifically binds to dihydrotestosterone receptor protein and not to sex hormone binding globulin. Although in the present study the existence of SHBG in the monkey prostate has not been investigated, the fact that methyltrienolone unlike DHT does not undergo metabolism, makes this compound more advantageous for receptor measurement. It provides suitable conditions for an exchange assay which allow the maximum labelling of the receptor population. The specificity of R1881 for androgen receptors has been reported elsewhere (5).

In earlier studies from this laboratory it was demonstrated that the ability of the caudal lobe to take up and retain androgens was significantly higher than the cranial lobe (4). The present study supports our earlier findings and suggests that the caudal lobe contains more free and bound androgen receptors. These data indicate that the caudal lobe in the monkey can be equated with the peripheral zone of the human prostate. The data are also in keeping with the observation (1) that in development, the acinar structure of the peripheral zone is delayed until puberty compared with the central zone suggesting the greater dependence of the former on an androgen stimulus.

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R. Ghanadian, Ph.D.
Prostate Research Laboratory
Urological Unit
Royal Postgraduate Medical School
Hammersmith Hospital
London W12 OHS
U.K.