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ANDROGEN RECEPTORS IN THE RAT ADRENAL GLAND

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SUMMARY

Cytosol fractions from rat adrenal glands contain specific androgen binding proteins showing high affinity ($K_D \sim 3.9 \times 10^{-9}$ M) and limited capacity (22 fmol/mg protein) for testosterone and with physicochemical properties identical to those of androgen receptors in the prostate, epididymis and testis. The androgen receptor complexes have a sedimentation coefficient of 7–8 *S* by sucrose density gradient centrifugation, and a mobility relative (R_F) to bromophenol blue of 0.5 by electrophoresis in 3.25% polyacrylamide gels containing 0.5% agarose. Isoelectric focusing in agarose-polyacrylamide gels indicated a pI of 5.9. Androgen binding to these receptors is destroyed by heating at 45°C for 30 min, by incubation with *p*-chloromercuriphenyl sulphonate (2 mM) or protease, but not by RNase. Testosterone (T) and 5α-dihydrotestosterone (DHT) were the most potent androgens in displacing [^{3}H]-T binding. However, 4-androsten-3,17-dione (A) and 5α-androstan-3α,17β-diol (Adiol) also significantly displaced [^{3}H]-T binding from the receptor. The reasons for this are the high activities of 17β-OH steroid dehydrogenase (converting A into T) and 3α-oxido-reductase (converting Adiol into DHT) in the cytosol fractions. These enzymes are active even at 0°C. Estradiol-17β in high concentrations (100-fold excess) also reduced the [^{3}H]-T binding, by approximately 60%, whereas corticosterone was without effect. The androgen receptors were found in similar concentrations in the adrenal cytosol of male and female rats, but were virtually absent in cytosol fractions obtained from rats with testicular feminization syndrome (*tfm* rats). The presence of specific androgen receptors in the adrenal gland of normal rats indicates that androgens may affect adrenal steroid production by a direct action.

INTRODUCTION

It is well known that testicular hormones affect the function of the adrenal gland both in male and female rats. This effect may be partly mediated through the hypothalamus-anterior pituitary [1], hepatic steroid metabolism [2], the levels of corticosteroid binding globulin [3] or directly on the adrenal gland [4, 5]. Androgen insensitive (*tfm*) rats show a marked adrenal hyperplasia [6]. However, greatly elevated CBG and normal or subnormal plasma levels of corticosterone (Purvis, K. unpublished) indicate that the peripheral androgen insensitivity may be associated with abnormal function of the adrenals.

Since many, if not all, of the effects of androgens are believed to be mediated by specific intracellular receptors, the possibility exists that such a receptor system may be operating also in the adrenal gland.

The purpose of the present study was to determine whether or not specific androgen receptors are present in the adrenal gland of normal and *tfm* rats and to compare their properties to those of androgen receptors in classical androgen target tissues like the prostate, epididymis and testis.

MATERIALS AND METHODS

Chemicals

[1,2,4,5,6,7-^{3}H]-5α-dihydrotestosterone (100 Ci/mmol: [^{3}H]-DHT), [1,2,6,7-^{3}H]-testosterone (91 Ci/mmol: [^{3}H]-T), and [1,2-^{3}H]-5α-androstan-3α,17β-diol (50 Ci/mmol: [^{3}H]-Adiol) were purchased from New England Nuclear Corporation (U.S.A.). [1,2,6,7-^{3}H]-androstenedione (87 Ci/mmol: [^{3}H]-A), and [1,2,6,7-^{3}H]-progesterone (84 Ci/mmol: [^{3}H]-P) were obtained from the Radiochemical Centre, Amersham (England). The solvents (benzene and ethanol) were evaporated under nitrogen at 50°C and the hormones were dissolved in ethanol. The purity of the hormones, tested by thin layer chromatography, was greater than 98%. Unlabelled steroid, Tris (hydroxymethyl)-aminomethane (Tris), *p*-chloromercuriphenylsulphonate (PCMPS), pronase, RNase, bovine serum albumin (BSA) and glycine were obtained from Sigma Chemical Company (USA). Human gamma globulin (IgG) was obtained from A. B. Kabi (Sweden) and ferritin from E. Merck (West Germany). Agarose was purchased from L'Industrie Biologique Francaise S.A. (France), Acrylamide, N,N-methylenebisacrylamide, and N,N,N,N-tetramethylene-diamine (TEMED) from Eastman Kodak Company (U.S.A.). Ethylenediamine tetracetic acid tetrasodium salt (EDTA) was from Fluka AG (Buchs SG, Switzerland) and silica gel plates, F 1500 LS 254, were purchased from Schleicher & Schüll (West Germany).

Animals

Male and female Sprague–Dawley rats weighing 220–320 g and 3–4 months old were used. Rats with testicular feminization syndrome (*tfm*) and their normal littermate male (NL) rats were obtained from the colony established by Stanley and Gumbreck, Univer-

sity of Oklahoma. At the time of the experiments these animals were 11 months old. The experimental animals were kept under standardized conditions of temperature (23°C) and light (12 h light/12 h dark) on a diet consisting of commercial pellets and water *ad libitum*. Certain rats of both sexes were castrated one day before the experiments.

Tissue sampling and preparation of cytosol

The rats were killed by decapitation. The adrenals and in some cases the anterior pituitary, spleen or muscle tissue were immediately removed, cooled on ice and weighed after being carefully dissected free from adherent fat and connective tissue. Following mincing, the pooled tissues were transferred to 3 vol. of 0.05 M Tris–HCl buffer, pH 7.4, containing 0.25 M sucrose and 0.001 M EDTA. Homogenization was carried out in an ice bath using roundbottomed glass homogenizers with Teflon pestles for the adrenals and the anterior pituitary gland. The spleen and muscle tissue were homogenized with a X-1020 homogenizer (Intern. Laborat. App. GmbH, 7801, Gottingen, West Germany) using 3 pulses of 5 s and with at least one min between each of the pulses. The cytosol fractions were prepared by centrifugation of the homogenates at 105000 *g* for 60 min at 0°C. Protein concentrations in the cytosol fraction were 7–9 mg/ml.

Incubation procedures

Aliquots of the cytosol fractions were incubated with 2 nM [^{3}H]-T, [^{3}H]-DHT, [^{3}H]-Adiol, [^{3}H]-A or [^{3}H]-P for 16 h at 0°C. In addition, aliquots of the cytosol fractions were incubated with 2 nM [^{3}H]-T or [^{3}H]-DHT in the absence or presence of (a) unlabelled T, DHT, estradiol-17β, A, Adiol or corticosterone at a concentration of 10 and 100 fold excess over the radioligand, (b) pronase (100 μg/ml), RNase (100 μg/ml), or PCMPS (2 mM) for 2 h at 0°C, (c) heating for 30 min at 45°C. Bound radioactivity was determined either by sucrose density gradient centrifugation or polyacrylamide gel electrophoresis.

Characterization of radioactive steroids

Steroids in the incubation. In the studies with [^{3}H]-T, [^{3}H]-DHT and [^{3}H]-A aliquots of the incubates were taken when the incubation was terminated, for examination of the degree of metabolism of the radioligands. Aliquots of the labelled cytosol were extracted 3 times with 2 vol of diethyl ether. The ether phase was evaporated to dryness and the residues were subjected to thin layer chromatography (t.l.c.) on silica gel plates which were developed in methylene dichloride–diethyl ether (7:1, v/v). Derivative formation by reduction, oxidation or acetylation was carried out for some of the steroids according to earlier descriptions [7].

Protein bound steroids. The steroids bound by the proteins sedimenting in the 7–8 *S* area of the sucrose density gradients were determined by t.l.c. after ether extraction of the fractions of this area pooled from 5 gradients.

The steroids bound by the proteins in the peak

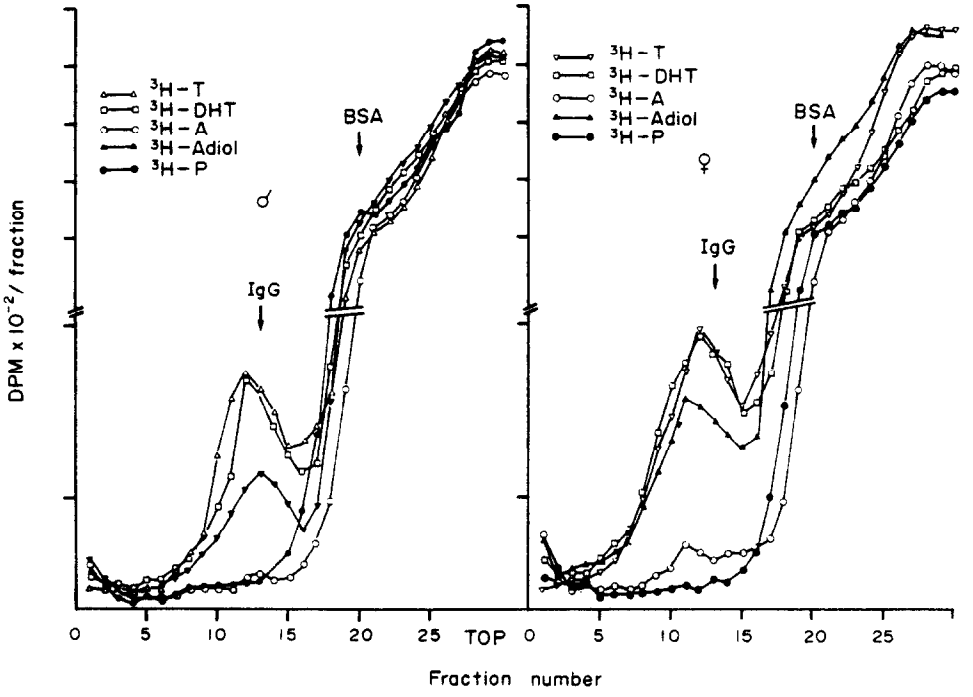

Fig. 1. Sucrose gradient centrifugation of cytosol fractions, male (left) and female (right) adrenals incubated with 2 nM of [^{3}H]-T, [^{3}H]-DHT, [^{3}H]-Adiol, [^{3}H]-P for 16 hr at 0°C. Aliquots of 250 μl with equal protein concentrations were layered on the top of the gradients and centrifuged at 265.000 *g* (av) for 20 h at 0°C. Human gamma globulin (IgG: 7 *S*) and bovine serum albumin (BSA: 4.6 *S*) were used as markers.

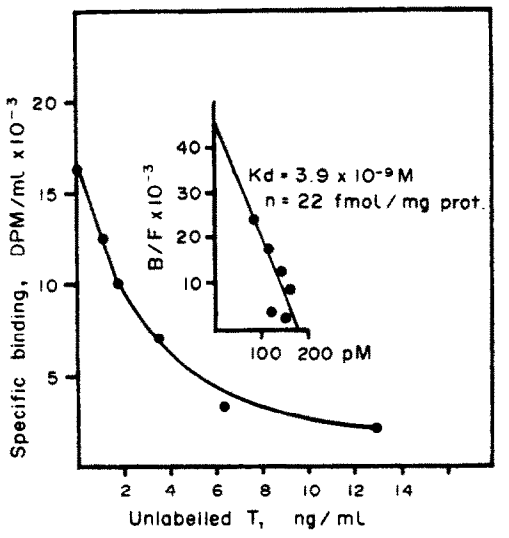

Fig. 2. Effect of increasing doses of unlabelled T (0.88–13.0 ng) on [^{3}H]-T binding to androgen receptors and Scatchard plot analysis of data (insert). Cytosol fractions from male rat adrenals (8.1 mg protein/ml) were incubated at 0–4°C for 16 h with [^{3}H]-T (3.1 nM) and increasing concentration of unlabelled T (3.08–45.1 nM) in a total vol. of 0.315 ml. After sucrose gradient centrifugation (see methods), [^{3}H]-T binding and total T binding in the 7–8 *S* peak were calculated. The estimated K_D (3.9×10^{-9}) is at best an approximation, since metabolism of trace and standard occurred during the incubation (Tables, 2 and 3). The number of binding sites (22 fmol/mg protein) is also underestimated because equilibrium with the endogenous T may not have been achieved.

area obtained by polyacrylamide gel electrophoresis were determined by extraction of the gel segments corresponding to this area with toluene overnight at room temperature. Subsequent to evaporation of the toluene at 50°C the residues were analysed by t.l.c. and derivative formation.

Sucrose density gradient centrifugation (SGC). Polyacrylamide gel electrophoresis (PAGE). Isoelectric focusing

Sucrose density gradient centrifugation was carried out according to Martin and Ames[8]. PAGE was performed as described by Tindall *et al.*[9] using gels containing 3.25% acrylamide and 0.5% agarose. The isoelectric focusing was performed with gels containing 3.5% acrylamide and 0.5% agarose as described by Naess *et al.*[10].

Radioimmunoassay of endogenous androgens

Androstenedione, T and DHT were measured in adrenals of adult male and female rats and in 50 day castrated male rats using the technique described by Purvis *et al.*[11]. Pooled adrenals were weighed and homogenized in acetone and the androgens partitioned stepwise across ether–water (10:1, v/v) and 90% methanol-water–hexane (1:2, v/v). An additional partition of 50% methanol-water–methylene dichloride (1:4, v/v) was also found to be necessary. The methylene dichloride was then evaporated: the residues were dissolved in isooctane (1.0 ml) and chromatographed on Celite microcolumns. The three androgens were finally determined by radioimmunoassay [11].

Other analytical methods

Protein was measured by the method of Lowry *et al.*[12] using BSA as reference standard.

The radioactivity of the fractions obtained by sucrose density gradient centrifugation was determined after adding 5 ml of Bray's solution [13] while the gel segments after PAGE were extracted overnight with 5 ml Instafluor (Packard Instrument Company Inc., U.S.A.) and radioactivity measured in a Nuclear Chicago, Mark II liquid scintillation spectrometer with a [^{3}H]-counting efficiency of 44% and 55% respectively.

RESULTS AND DISCUSSION

The cytosol fractions from intact adult male and female adrenal homogenates were found to contain a specific binding protein for T and DHT. Figure 1 shows the sedimentation profile of cytosol fractions incubated with 2 nM [^{3}H]-T and subjected to sucrose density gradient centrifugation. An androgen protein complex with a sedimentation coefficient of 7–8 *S* was present. This complex was seen after incubation with similar concentrations of [^{3}H]-DHT and [^{3}H]-Adiol. In some experiments, much lower, but still significant binding was found after incubation with [^{3}H]-A, whereas no binding could be detected after incubation with [^{3}H]-P (Fig. 1). Once again no obvious sex difference was apparent. Thus, the sedimentation coefficient of 7–8 *S* is very similar to that of the androgen receptors in other androgen responsive tissues. [10, 14, 15].

In order to obtain information about binding affinity (K_D) and capacity (n) of available androgen receptors, cytosol fractions from male rat adrenals were incubated with varying doses of T and binding to receptors analyzed by sucrose gradient centrifugation. As seen from Fig. 2, addition of increasing amounts of unlabelled T, decreased the specific binding in the 7–8 *S* region of the gradient. When the data were plotted according to Scatchard[19], an apparent K_D of 3.9×10^{-9} M and a capacity of available binding sites of 22 fmol/mg protein were detected. The affinity for T may be slightly underestimated due to metabolism of the [^{3}H]-T to 4-androstene-3,17-dione(A) during the binding assay (Table 2 and 3).

Previously we have successfully characterized androgen receptors in a variety of tissues by polyacrylamide gel electrophoresis [9, 10, 15]. This technique was therefore used for further characterization of the adrenal androgen protein complexes. The cytosol fractions from both male and female rat adrenal glands were found to contain specific androgen bind-

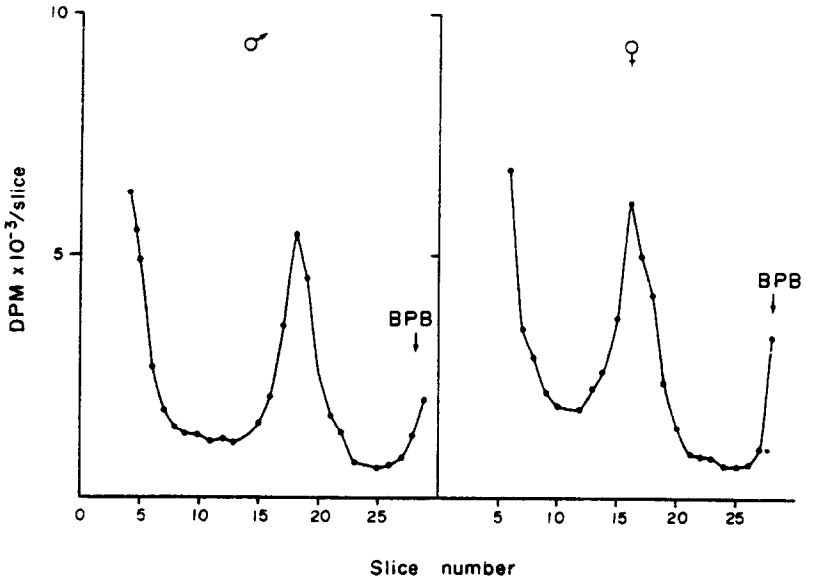

Fig. 3. Polyacrylamide gel electrophoresis of male (left) and female (right) adrenal cytosol fractions incubated with 2 nM of [^{3}H]-DHT for 16 h at 0°C. Aliquots of 200 μl with equal amounts of protein (2 mg) were layered on the top of the gels. Electrophoresis was run at 2 mA/tube at 0°C. Following electrophoresis the gels were cut into 2.3 mm segments and the radioactivity was measured in each of the slices as described in Materials and Methods. BPB = Bromophenol blue.

ing proteins with an electrophoretic mobility of 0.55 (R_F) relative to BPB as illustrated in Fig. 3. The electrophoretic mobility of these DHT–protein complexes was identical to that of androgen receptors in other tissues [9, 15]. Similar to other androgen receptors these complexes were destroyed after heating at 45°C for 30 min, by pronase and *p*-chloromercuriphenyl sulphonate (PCMPS), but not by RNase (Fig. 4). The fact that these androgen-binding proteins were undetectable in the cytosol fractions of rats having the syndrome of testicular feminization adds further support to the conclusion that these proteins are intracellular androgen receptors (Fig. 5)

There was no obvious sex difference in [^{3}H]-DHT binding to adrenal androgen receptors as demonstrated both by SGC and PAGE (Figs 1 and 3). However, since these studies were performed on intact rats, this finding does not necessarily reflect the relative concentrations of androgen receptors in male and female rats. It is well known that androgen receptor complexes dissociate very slowly at low temperature [15]. This is also the case with the adrenal androgen receptors. When adrenal cytosol fractions were equilibrated with 2 nM of [^{3}H]-DHT overnight at 0°C and subsequently exposed to 1000-fold excess of non-labelled DHT to displace the bound [^{3}H]-DHT, less than 30% of the bound [^{3}H]-DHT dissociated during 24 h at 0°C (not shown).

Androgen supply to the adrenal is partly provided by circulating testosterone and partly by local production. We therefore found it important to quantitate the endogenous concentrations of T, DHT and A in adrenal tissues from male and female rats (Fig. 6). Furthermore, the change in adrenal androgens after orchiectomy, may indicate the proportion of the adrenal androgens which are of testicular origin. Surprisingly, the concentrations of T and DHT were very

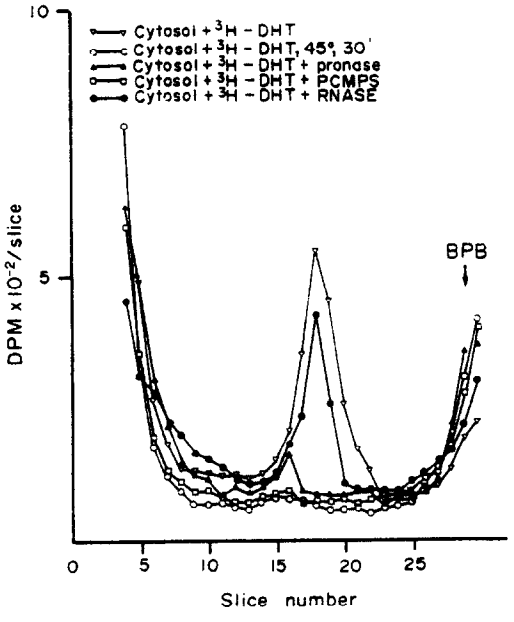

Fig. 4. Certain properties of the adrenal androgen binding protein. Aliquots of adrenal cytosol incubated with 2 nM of [^{3}H]-DHT for 16 h at 0°C were subjected to treatment with (1) pronase (100 μg/ml), RNase (100 μg/ml), or *p*-chloromercuriphenyl sulphonate (PCMPS: 2 mM) for 2 h at 0°C, or to (2) heating at 45°C for 30 min. The samples thereafter were examined by polyacrylamide gel electrophoresis as described in Fig. 3.

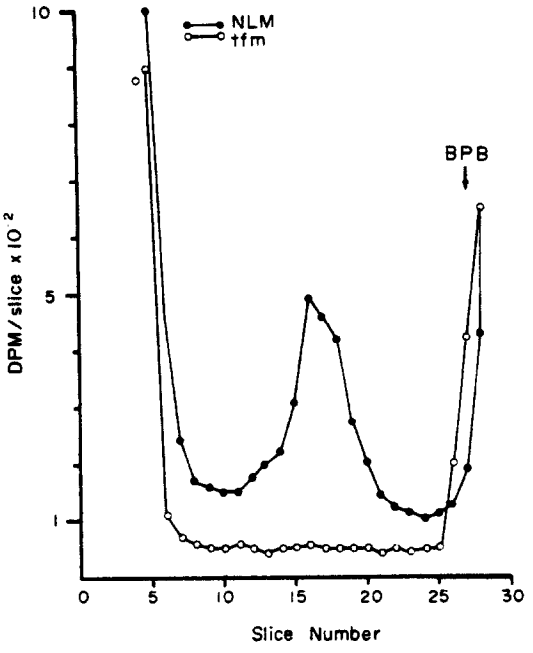

Fig. 5. Polyacrylamide gel electrophoresis of adrenal cytosol fractions from androgen insensitive (*tfm*) rats and normal littermates (NLM) incubated with 2 nM of [^{3}H]-DHT for 16 h at 0 C.

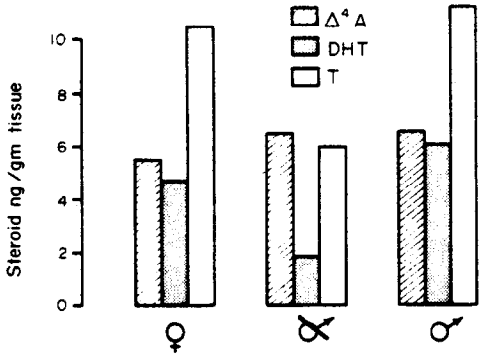

Fig. 6. Endogenous androgens in adrenal tissue (ng/g of tissue) from intact male and female rats and from castrated male rats. 4-Androstene-3,17-dione(Δ4-A), 5α-dihydrotestosterone(DHT) and testosterone(T) were measured by radioimmunoassay. Each group consisted of 10 animals, and the steroids were analyzed in triplicate. ♀ female, ♂ castrated male, ♂ male rats.

similar in normal male and female rats. The concentration of T was approximately 10 ng/g of tissue, whereas that of DHT was approximately half of this value. This indicates that local androgen production in the female can compensate for the lower plasma concentration of androgens, and that plasma testosterone is of minor importance in determining the endogenous tissue concentration of androgens in the female adrenals. Figure 6 also shows that the concentrations of T and DHT in the male adrenal gland after orchiectomy are only the half of that in intact male and female rats, whereas the concentration of A did not change. The reduction in the adrenal concentration of T and DHT after orchiectomy probably reflects the testicular contribution of these androgens to the male adrenal. Thus, approximately half of the T and DHT in the adrenal of male rats appears to be of testicular origin, whereas all of the A is produced in the adrenal itself. The adrenal androgen concentration (T + DHT ~ 15 ng/g ~ 50 nM) is sufficient to occupy most of the adrenal androgen recep-

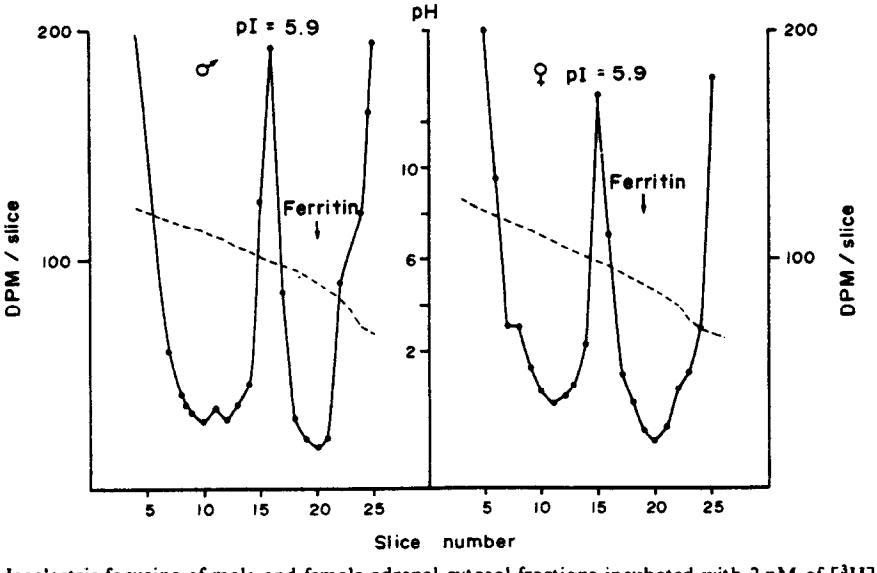

Fig. 7. Isoelectric focusing of male and female adrenal cytosol fractions incubated with 2 nM of [^{3}H]-T for 16 h at 0 C. Aliquots of 100 μl were examined in 3.5% acrylamide gels containing 0.5% agarose and 2% ampholines as described in the Materials and Methods. Following electrofocusing the gels were cut into 2.3 mm segments and the pH and radioactivity were measured in each of the segments. —— d.p.m./segment, - - - - - pH.

Table 1. Competition for [^{3}H]-Testosterone binding by unlabelled steroids

[^{3}H]-Steroid	Unlabelled steroid	% Binding
[^{3}H]-Testosterone +	none	100
[^{3}H]-Testosterone +	Testosterone × 10	31
[^{3}H]-Testosterone +	Testosterone × 100	0
[^{3}H]-Testosterone +	5α-Dihydrotestosterone × 100	5
[^{3}H]-Testosterone +	17β-Estradiol × 100	42
[^{3}H]-Testosterone +	Adiol × 10	94
[^{3}H]-Testosterone +	Adiol × 100	72
[^{3}H]-Testosterone +	Androstenedione × 10	59
[^{3}H]-Testosterone +	Androstenedione × 100	23
[^{3}H]-Testosterone +	Corticosterone × 100	103

Aliquots of adrenal cytosol were incubated with 2 nM of [^{3}H]-testosterone with and without unlabelled steroids at varying excess concentrations above that of the radioligand and analysed by sucrose gradient centrifugation. Bound radioactivity in the 7–8 S area of the gradients (c.p.m. in fractions 4–14) is expressed as per cent of the control.

tors. Thus, in our *in vitro* studies only a minor portion of the total androgen receptors may have been labelled. However, since the binding peaks observed after incubation with [^{3}H]-T and [^{3}H]-DHT as well as the endogenous concentration of these steroids were identical in adrenals from both sexes, the receptor concentration is probably also similar.

Since androgen receptors in target tissues like the prostate, epididymis, testis and anterior pituitary all have the same isoelectric point (pI ~ 5.8) [9, 10, 15], we also examined the pI of the adrenal androgen receptor complexes by isoelectric focusing. Figure 7 shows that the adrenal cytosol fraction of both the male and female rat contained a distinct androgen-receptor complex having a pI = 5.9. Thus, the androgen receptors in the rat adrenal appear to be very similar to those in other classical target tissues with regard to sedimentation rate, electrophoretic mobility, isoelectric point and stability. However, Adiol and in some experiments A had a surprisingly high affinity for the receptors as illustrated in Fig. 1 and Table 1. In addition, estradiol-17β in high concentration did compete with [^{3}H]-T binding to the androgen receptors.

The binding of Adiol and A to the adrenal androgen receptors could indicate that the steroid specificity of these receptors is different from those in the peripheral sex tissues. However, this is probably not the case. The data presented in Table 2 suggest that the cytosol contains an active 17β-OH steroid dehydrogenase which converts A into T (and DHT into 5α-androstane-3,17-dione). Thus, this metabolism may explain the apparent high affinity of A for the receptors. Rat adrenal cytosol also has an active 3α-oxido-reductase (DHT ↔ Adiol) which would encourage the formation of DHT after incubation with [^{3}H]-Adiol *in vitro*. Table 3 shows that, although some 50% of the added [^{3}H]-T is metabolized to A during the 16 h of incubation at 0°C, only [^{3}H]-T is bound to the androgen receptors. Thus, the steroid specificity must be interpreted in the light of metabolism proceeding during the incubation. From these studies we conclude that there is no reason to believe that the steroid specificity of the adrenal androgen receptor is different from that in other androgenic target tissues.

The physiological role of these androgen receptors remains to be established. Androgen administration to rats causes a dose-dependent decrease in circulating corticosterone as well as CBG [16]. However, testosterone administered to rats that are hypophysectomized and gonadectomized stimulates adrenal corticosterone production *in vitro* [17]. This effect may partly be mediated through a testosterone-induced inhibition of an adrenal 5α-reductase [18]. In addition, the finding that adrenal tissue devoid of androgen receptors (as is the case in *tfm* rats) exhibits marked hyperplasia [6] but reduced levels of free circulating corticosterone [16] also strengthens the view

Table 2. Metabolism of different [^{3}H]-androgens during incubation of adrenal cytosol for 16 h at 0 C

[^{3}H]-Metabolites (%)	[^{3}H]-T cytosol	[^{3}H]-A cytosol	[^{3}H]-DHT cytosol
Polar compounds*	0	1.8	0
Adiol	0	0	16.6
Testosterone	56.3	25.4	0
Androsterone	0	0	25.1
5α-Dihydrotestosterone	0	0	32.1
Androstenedione	43.6	72.5	0
5α-Androstanedione	0	0	26.2
Unidentified	0.1	0.3	0

Adrenal cytosol from intact male rats was incubated with 2 nM [^{3}H]-T, [^{3}H]-A or [^{3}H]-DHT for 16 h at 0 C. Samples were extracted with diethyl ether and processed by thin layer chromatography and derivative formation. All values are corrected for blanks and presented as per cent of total radioactivity counted.

* Polar compounds: compounds more polar than Adiol on thin layer chromatography.

Table 3. Free and bound radioactive androgens in adrenal cytosol incubated with [^{3}H]-testosterone

	[^{3}H]-Testosterone	
[^{3}H]-Metabolites %	Free	Bound
Polar compounds	7.7	0
Adiol	0	0
Testosterone	40.2	93.2
Androsterone	0	0
5α-Dihydrotestosterone	0	0
Androstenedione	52.1	3.5
5α-Androstanedione	0	0
Unidentified	0	4.0

Adrenal cytosol was incubated with 2 nM of [^{3}H]-testosterone for 16 h at 0°C and subjected to sucrose gradient centrifugation. The fractions corresponding to the 7–8 S area of bound androgens and the area of free steroids were pooled from 5 gradients, extracted with diethyl ether and processed by thin layer chromatography.

that androgens under normal circumstances exert a direct positive influence on the adrenal gland.

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REFERENCES

1. Coyne M. D. and Kitay J. I.: Effect of orchiectomy on pituitary secretion of ACTH. *Endocrinology* **89** (1971) 1024–1028.
2. Yates F. E., Herbst A. L. and Urquhart J.: Sex difference in rate of ring A reduction of Δ^4-3-ketosteroids *in vitro* by rat liver. *Endocrinology* **63** (1958) 887–902.
3. Gala R. R. and Westphal U.: Corticosteroid-binding globulin in the rat: Studies on the sex difference. *Endocrinology* **77** (1965) 841–851.
4. Zizine L.: Action comparative de la testosterone et de la 5α-dihydrotestosterone sur la cortico-surrenale du rat hypophysectomisé. *C.r. Soc. Biol. (Paris)* **168** (1974) 1182–1185.
5. Sharma D. C., E. Forchielli and Dorfman R. I.: Inhibition of enzymatic steroid 11β-hydroxylation by androgens. *J. biol. Chem.* **238** (1963) 572–575.
6. Stanley A. J., Gumbreck L. G., Allison J. E. and Easley R. B.: Male pseudo-hermaphroditism in the laboratory Norway rat. *Rec. Progr. horm. Res.* **29** (1973) 43–64.
7. Djöseland O., Hansson V. and Haugen H. N.: Androgen metabolism in rat epididymis. *Steroids* **21** (1973) 773–783.
8. Martin R. G. and Ames N. A.: A method for determining the sedimentation behaviour of enzymes: Application to protein mixtures. *J. biol. Chem.* **236** (1961) 1372–1379.
9. Tindall D. J., Hansson V., McLean N. S., Ritzén E. M., Nayfeh S. N. and French F. S.: Androgen binding proteins in the rat epididymis: Properties of a cytoplasmic receptor for androgen similar to the androgen receptor in the ventral prostate and different from androgen-binding protein (ABP). *Molec. cell. Endocr.* **3** (1975) 83–101.
10. Naess O., Hansson V., Djöseland and Attramadal A.: Characterization of the androgen receptor in the anterior pituitary of the rat. *Endocrinology* **97** (1975) 1355–1363.
11. Purvis K., Clausen O. P. F., Haug E. and Hansson V.: Decreased Leydig cell responsiveness in the testicular feminized male rat. *Endocrinology* **102** (1978) 1053–1060.
12. Lowry O. H., Rosebrough N. J., Farr A. L. and Randall R. J.: Protein measurements with the folin phenol reagent. *J. biol. Chem.* **193** (1951) 254–275.
13. Bray G. A.: A simple efficient liquid scintillator for counting aqueous solutions in a liquid scintillation counter. *Analyt. Biochem.* **1** (1960) 279–285.
14. Mulder E., van Beurden-Lamers W. M. O., De Boer W., Brinkmann A. O. and van der Molen H. J.: Testicular estradiol receptors in the rat. In *Hormone Binding and Target Cell Activation in the Testis* (Edited by Dufau M. L. and Menas A.). Plenum Press. New York/London (1974) pp. 343–355.
15. Hansson V., Ritzén E. M., French F. S. and Nayfeh S.: Androgen transport and receptor mechanisms in the testis and epididymis. In *Handbook of Physiology*, Vol. V. (Edited by Hamilton D. W. and Greep R. O.). American Physiological Society, Washington D. C. (1975) pp. 173–210.
16. Purvis K., Calandra R. C. and Hansson V.: Adrenal secretion and plasma CBG levels in the immature male rats: Effects of 5α-reduced androgens and antiandrogens. *J. steroid. Biochem.* **8** (1977) 1121–1124.
17. Kitay J. I.: Depression of adrenal corticosterone production in oophorectomized rats. *Endocrinology* **77** (1965) 1048–1052.
18. Kitay J. I., Coyne M. D. and Swygert N.: Influence of gonadectomy and replacement with estradiol or testosterone on formation of 5α-reduced metabolites of corticosterone by the adrenal gland of the rat. *Endocrinology* **87** (1970) 1237–1265.
19. Scatchard G.: The attractions of proteins for small molecules and ions. *Ann. N.Y. Acad. Sci.* **51** (1949) 660–672.