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DIFFERENCES IN THE STEROID-BINDING SITE SPECIFICITIES OF RAT PROSTATE ANDROGEN RECEPTOR AND EPIDIDYMAL ANDROGEN-BINDING PROTEIN (ABP)*

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SUMMARY

The steroid specificity of androgen-binding proteins is crucial in determining their physiological functions and is important to consider for designing synthetic hormones and anti-hormones. Two proteins which bind testosterone and dihydrotestosterone with high affinity in the rat have been studied: the intracellular androgen receptor from ventral prostate and the epididymal androgen-binding protein (ABP). The binding sites were characterized in castrated animals by charcoal assay and gel electrophoresis, based on the specific binding of [^{3}H]-methyltrienolone in prostate cytosol and of [^{3}H]-dihydrotestosterone in epididymal extracts. In both tissues, kinetic and equilibrium data were compatible with a homogeneous class of sites having the expected physico-chemical properties. Competition experiments were then performed in order to assess the ability of both proteins to bind 86 steroids including androstane, pregnane and C-21 hydroxypregnane derivatives. Double-reciprocal plots suggested that all active compounds bound to the same site as the radioactive ligand. A comparison of their apparent affinity constants indicated that the binding sites on the receptor and on ABP clearly differ in their steroid specificity. The differences involve the interaction of these sites with both extremities of the testosterone molecule since substituents either on the A-ring (e.g. 1-CH_3) or on the D-ring (e.g. 17α-CH_3) may have opposite effects on ABP and receptor binding. Also, the presence of a pregnane side-chain is less favourable for the binding to ABP than to the androgen receptor. Thus, some compounds are very specific for the androgen receptor, while others have an affinity for ABP one order of magnitude higher than for the androgen receptor. These data may lead to a better understanding of the mechanism of the interaction of androgens and anti-androgens with their binding proteins. They also suggest that steroid analogues with selective high affinity for either the androgen receptor or ABP could be developed.

I. INTRODUCTION

A variety of synthetic compounds are capable of interacting with the steroid binding site on the intracellular receptors for steroid hormones. Some compounds mimic the naturally occurring hormone and are called agonists. Others do not activate the receptor and their binding does not elicit a hormonal effect. These antagonists are therefore capable of competing with natural or synthetic agonists and of inhibiting their actions. Thus, anti-mineralocorticoids, anti-estrogens and anti-androgens which act at the receptor level have become available. Development of such synthetic hormones or anti-hormones, and understanding their possible side-effects requires a knowledge of both the steroid specificity of the receptor and the affinities of antagonists relative to the agonists [1].

Androgens control at least three distinct physiological processes perhaps via different androgen-binding proteins: development and maintenance of secondary sex organs, spermatogenesis and sperm maturation, and skeletal muscle metabolism. Apart from the sex steroid-binding proteins in plasma, two androgen-binding proteins have been characterized: the intracellular androgen receptor such as that in rat prostate and the androgen-binding protein (ABP) found in the testis and epididymis [2, 3]. ABP is produced by the Sertoli cells upon stimulation by FSH and secreted into the seminiferous tubular fluid [4]. It is transported to the epididymis by the efferent duct fluid where it seems to be degraded or reabsorbed since the level of ABP is highest in the caput epididymis and can no longer be detected beyond the cauda [5]. In mammals spermatozoa acquire fertilizing ability when passing through the epididymis. In rabbits and guinea pigs sperm maturation requires androgens acting via the epididymal tissue [6, 7]. It has been suggested that ABP because of its high androgen binding capacity (60 ng testosterone/ml tubular fluid) might serve as an androgen-concentrating factor essential for sperm maturation in the epidi-

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dymis [4]. High local concentrations of testosterone are also required for spermatogenesis in the seminiferous tubules [8]. The presence of ABP in close proximity to these androgen target cells would make testosterone available for uptake, conversion to the active metabolite 5α-dihydrotestosterone (DHT) and binding to intracellular receptors [4].

Thus, it is important to determine the steroid-binding specificities of the androgen-receptor and of ABP. Differences between the two might allow one to use steroid analogues as specific probes for further studying the physiology of ABP. Should the formation of an ABP-testosterone complex prove to be an absolute requirement for sperm maturation, preventing androgens from binding to ABP might provide a means of reversibly inducing infertility in the male. This could be achieved by competitive inhibition provided the competing drug does not bind to intracellular androgen receptors which would lead to unacceptable side effects.

In the present report we have approached these problems by comparing the affinities of 86 steroids for rat prostate androgen receptor and epididymal ABP. We show that, although both proteins bind natural androgens with similar affinities, they differ clearly in their binding properties. We also discuss how the specificity for either protein critically depends on some substituents of the steroid molecule.

II. MATERIALS AND METHODS

(a) *Chemicals*

TEG-buffer contained 20 mM Tris, 1.5 mM EDTA and 10% (v/v) glycerol, pH 7.4 with or without 0.25 mM dithiothreitol. [^{3}H]-DHT [1,2 ^{3}H]-17β-hydroxy-5α-androstan-3-one), S.A. 56 or 60 Ci/mmol, was purchased from the Radiochemical Centre, Amersham, U.K. [^{3}H]-Methyltrienolone (R 1881) [6,7 ^{3}H]-17β-hydroxy-17α-methyl-estra-4,9,11-triene-3-one), S.A. 55.5 Ci/mmol was generously supplied by Dr. J. P. Raynaud, Roussel-UCLAF, Romainville, France. Unlabelled steroids were either purchased from Steraloids, Pawling, N.Y., U.S.A. and Sigma Co. St Louis, Missouri, U.S.A., or were gifts from Roussel-UCLAF, Romainville, France, Shering A.G., Berlin, Germany, Glaxo Research Ltd., Greenford, Middlesex, U.K., Merck, Sharp and Dohme, Rahway, N.J., U.S.A. and Ayerst Lab. McKenna and Harrison Ltd. Montreal, Canada.

Steroids were dissolved in absolute ethanol to a concentration (2–4 mM) which was verified by spectrophotometry on the basis of molar extinction coefficients, when available. Final dilutions were made in TEG-buffer. All other chemicals were of analytic grade.

(b) *Animals and tissues*

Epididymis and ventral prostate were obtained from Wistar rats (8–14 weeks) 20–24 h after bilateral orchidectomy via the scrotal route under nembutal anaesthesia. Epididymal tissue was minced with scissors and homogenized in 3 vol. (w/v) of TEG-buffer without dithiothreitol using a motor-driven Teflon–glass homogenizer. Likewise prostate homogenates were prepared in TEG buffer containing dithiothreitol. All preparations procedures were carried out at 0°C. Homogenates were centrifuged at 200,000 *g* for 1 h at 0°C to obtain the cytosol. Rat prostate cytosol was used immediately, whereas epididymis cytosol was usually stored at −20° and incubated before use for 30 min at 50°C [9] (in later experiments 30°C, see below) to inactivate androgen receptors.

(c) *Binding studies by charcoal assay*

1. ABP. Aliquots of cytosol (0.1 ml) were incubated in a total volume of 0.4 ml with [^{3}H]-DHT (0.5–5 nM) in TEG buffer at 0°C. Nonspecific binding was assessed in parallel incubations containing, in addition, 10^{-5} M unlabelled DHT. After incubation (3 h for equilibrium studies) hormone was removed by adding 0.2 ml of activated charcoal suspension (Norit A, 50 mg/ml, dextran 5 mg/ml). Tubes were briefly vortexed (3 sec) and the charcoal sedimented immediately by centrifugation (5 min, 2000 g). Bound radioactivity was counted in 0.3 ml of the resulting supernatant.

2. Androgen receptor. The accurate assessment of [^{3}H]-DHT binding to prostate androgen receptors was complicated by high levels of nonspecific binding. We therefore employed the synthetic androgen methyltrienolone (MT) which has a higher affinity for the androgen receptor than the natural hormones testosterone and DHT and also exhibits lower nonspecific binding [10]. Incubations for receptor binding of [^{3}H]-MT(0.5–5 nM) were prepared at 0°C as described for ABP. After 18–20 h (in equilibrium studies) 0.15 ml of charcoal suspension was added and the tubes vortexed briefly. After 10 min 0.05 ml of ethanol was added [11]. The tubes were vortexed immediately and incubated for a further 20 min. The charcoal was then sedimented and aliquots of the supernatant (0.3 ml) taken for counting radioactivity. Nonspecific binding of [^{3}H]-MT was determined in parallel incubations containing, in addition, 10^{-5} M unlabelled MT

3. Affinity of competitors. This was determined at equilibrium using a constant concentration of unlabelled competitor against four different concentrations of radioactive ligand. Binding data were plotted according to Lineweaver–Burk and the apparent equilibrium dissociation constants of [^{3}H]-DHT or [^{3}H]-MT in the absence and presence of test compounds were determined. The equilibrium dissociation constant (K_i) of competitors was calculated using the formula

$$K_i = \frac{K_D \times F_i}{\text{Kapp} - K_D} \times \frac{K_D}{K_D}$$

where K_D is the dissociation constant of [^{3}H]-DHT or [^{3}H]-MT respectively, Kapp is the apparent affinity constant of [^{3}H]-DHT or [^{3}H]-MT in the

presence of unlabelled competitor, F_i is the concentration of free competitor and $\overline{K}_D$ was the mean value of the dissociation constant of the respective radioactive ligands from all our experiments. F_i was taken as the total concentration of inhibitor added as the concentration of bound inhibitor is negligible as compared to the former. Scatchard plots of specific binding data of [^{3}H]-DHT and [^{3}H]-MT were routinely done and they also demonstrated the presence of only one class of high affinity binding sites.

(d) *Polyacrylamide gel electrophoresis (PAGE)*

Five percent Polyacrylamide gels (6 × 50 mm) containing 10% glycerol were prepared according to Tindall *et al.*[9]. Where indicated hormones were added in glycerol to the gel solution prior to polymerisation to give a uniform concentration of hormone in the gel.

Aliquots of cytosol (0.1 ml) were layered directly over the gel and electrophoresis was run at 1–2 mA per tube with tubes immersed in the lower buffer cooled to 2°C. Upper and lower buffers were 0.6% Tris, 2.9% glycine in water (pH 8.6) and bromophenol blue was added to the upper buffer as an electrophoretic marker. Electrophoresis was stopped when the bromophenol blue-band had reached the bottom of the tubes and gels were sliced into 1 mm transverse sections. The sections were placed in counting vials, 10 ml of scintillation fluid added and left overnight at room temperature before counting. Relative mobilities (R_x) of proteins were expressed as the ratio of distances covered by the protein and the bromophenol blue band, respectively. Gels containing marker proteins were fixed and stained in a solution of 45% methanol and 9.2% acetic acid containing 0.25% Coomassie blue.

(e) *Other assays*

Protein was determined by the method of Lowry *et al.*[12] using bovine serum albumin as a standard. Radioactivity was counted by liquid scintillation spectrometry in 10 or 5 ml of scintillation fluid (Aqualuma, Lumac Systems A.G., Basel, Switzerland). Counts were corrected for quenching and systematically converted to mol of steroid using an external standard system of calibration.

III. RESULTS AND DISCUSSION

(a) *Characterization of the binding proteins*

Screening a large number of compounds for androgen receptor and ABP binding called for a rapid and reproducible assay. Also, we wanted to determine whether these compounds bound to the same binding site as the natural steroid and, if so, what was their affinity constant.

Firstly, the kinetics of the binding of [^{3}H]-DHT to ABP and of [^{3}H]-MT to the androgen receptor were studied to establish optimal assay conditions. Rates of association were determined by incubating for various time-periods prostate or epididymis cytosol containing a known concentration of androgen receptor or ABP with a single concentration of their respective radioactive ligand. Dissociation rates were studied by determining the concentration of bound radioactive hormone at various times after the addition of a large excess of unlabelled hormone. Confirming earlier work [9] it was found that the androgen receptor and ABP differ greatly in their dissociation rate constant, with [^{3}H]-DHT-ABP complexes having a half-time for dissociation of about 4 min as compared to 23 h for [^{3}H]-MT-androgen receptor complex. The charcoal adsorption of free hormone in assays for ABP binding is therefore a critical step and the time of incubations with charcoal has to be the shortest to allow for efficient removal of free steroid without substantial dissociation of [^{3}H]-DHT-ABP complexes. Consistent with the second-order association rates and the first-order dissociation rates, Scatchard plots of the equilibrium binding of [^{3}H]-MT in prostate cytosol and [^{3}H]-DHT in epididymis cytosol were compatible with the presence of only one species of non-interacting binding sites showing high affinity and low capacity (Fig. 1). Mean values for the equilibrium dissociation constants were 0.78 nM ± 0.07 S.E.M. and 2.32 nM ± 0.24 S.E.M. for the MT-androgen receptor complex and the DHT–ABP complex, respectively. The mean concentrations of binding sites were 42 ± 4.0 S.E.M. and 850 ± 170 S.E.M. fmol/mg protein, respectively.

Secondly, we verified that binding to either the androgen receptor or ABP was destroyed after preincubation of prostate or epididymis cytosol with trypsin (50 μg/ml at 4°C for 5 h). Blocking of thiol groups by preincubation with *p*-chloromercuribenzoic acid (PCMB 0.5 mM) prevented the binding of [^{3}H]-MT to androgen receptors whereas binding of [^{3}H]-DHT to ABP in epididymis cytosol was only slightly reduced. At concentrations over 0.1 mM, dithiotreitol inhibited ABP binding, a decrease of 70% being observed at 10 mM. Binding activity of prostate cytosol was completely abolished after preincubation at 30°C, whereas only a slight reduction of the binding activity in epididymis cytosol was observed at this temperature. However, binding of [^{3}H]-DHT in epididymis cytosol was reduced at temperatures over 30°C, being only 6% of control levels after preincubation at 50°C.

Thirdly, by PAGE, the androgen-binding component from epididymis cytosol had a relative mobility of R_x = 0.49–0.53 (n = 6). In the presence of an excess of unlabelled DHT in the gel this peak was reduced to baseline levels of radioactivity (Fig. 2). An excess of unlabelled cyproterone acetate (1 μM) or preincubation with PCMB had no effect. Specifically bound radioactivity in 0.1 ml epididymis cytosol was 15,700 d.p.m. determined by charcoal assay as compared to 16,260 d.p.m. after subtraction of baseline radioactivity under the peak with R_x = 0.53 (slice

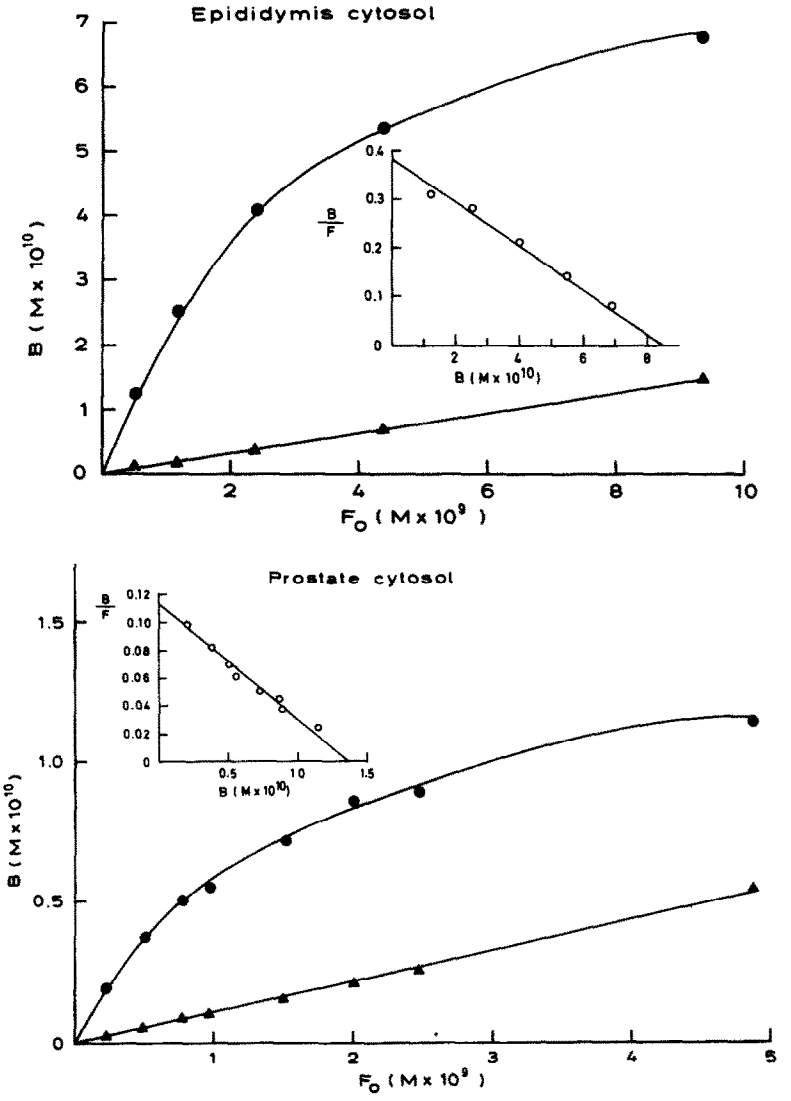

Fig. 1. Equilibrium binding of [^{3}H]-DHT in epididymis cytosol and of [^{3}H]-methyltrienolone in rat prostate cytosol. Nonspecific binding (▲) was subtracted from total binding to yield specific binding (●) to ABP and androgen receptor, respectively. Insets show Scatchard plots of specific binding data. Fo, F and B refer to total free, and receptor-bound steroid concentrations.

Nos 22–28, Fig. 2). Only under steady-state PAGE conditions a second peak of radioactivity ($R_x = 0.71$), undetected by charcoal assay, was observed which comigrated with albumin marker proteins and was unaffected by an excess of unlabelled DHT in the gel. A peak was occasionally observed at $R_x = 0.3$ which may correspond to the androgen receptor described by others in rat epididymis cytosol [9]. When prostate cytosol prelabelled with [^{3}H]-MT was analysed by PAGE only one peak of radioactivity with a relative mobility of $R_x = 0.28$ was resolved. Binding was completely abolished in the presence of a 200-fold excess of unlabelled cyproterone acetate or by preincubation with PCMB.

Thus, in epididymal extracts and prostate cytosol, the binding sites detected by our charcoal assay procedure exhibited the characteristics assigned respectively to ABP and to the androgen receptor by other investigators [5, 9, 13, 14].

(b) *Steroid-binding specificity of androgen receptor and ABP*

Unlabelled compounds were first tested at a single concentration (10 μM) for their ability to inhibit, under equilibrium conditions, the binding of [^{3}H]-DHT or [^{3}H]-MT to ABP or androgen receptor, respectively. The affinities (K_i) or active inhibitors for the two binding proteins were then determined at an appropriate concentration by competitive equilibrium binding assay. All Lineweaver-Burk plots were compatible with competitive inhibition of binding to the same binding site. Representative experiments are shown in Figure 3. Androstane, pregnane and C-21 hydroxypregnane derivatives were tested. Their affini-

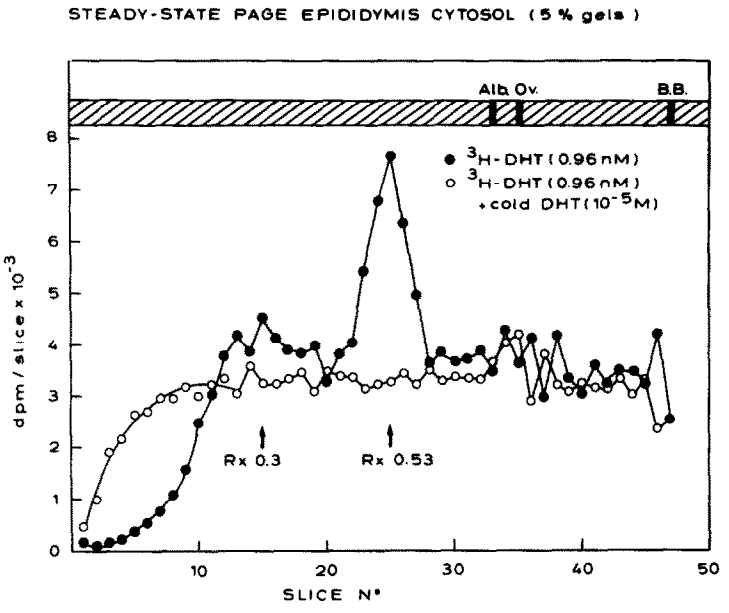

Fig. 2. Steady-state polyacrylamide gel electrophoretic profile of [^{3}H]-DHT binding in epididymis cytosol. 0.1 ml aliquots of cytosol (400 μg protein) were layered over 5% polyacrylamide gels containing 0.96 nM [^{3}H]-DHT without (●——●) or with (○——○) 10 μM unlabelled DHT. Electrophoresis was performed as described in Methods. R_x = mobility of bound radioactivity relative to bromophenol blue (B.B.). The electrophoretic mobility of albumin (alb.) and ovalbumin (Ov.) run on parallel gels is indicated in the shaded area.

ties for ABP, in order of decreasing importance, and for the androgen receptor are listed in Tables 1–3.

Consistent with other work [2, 3, 15] we find that ABP, which reportedly is a testosterone-binding protein *in vivo*, has a higher affinity for DHT than for testosterone (compound 1.1. vs 1.6 in Table 1). In all cases of 5α-reduction (1.6 vs 1.1, 1.7 vs 1.3, 1.8 vs 1.2, 1.20 vs 1.19) the affinity for ABP was improved. The only androst-4-ene derivative which bound better than testosterone was 9-dehydrotestosterone (1.5). It is interesting that 9-dehydroandrostenedione (1.13) also binds better to ABP than its reduced analogue (1.20). The 12 compounds with the highest affinity for ABP are all 17β-OH androstane derivatives (1.1–1.12). Then comes progesterone (2.1), showing that high affinity binding to ABP is compatible with the addition of a pregnane side-chain on the testosterone molecule. However, all the 21 pregnane derivatives tested (2.2–2.22) have a lower affinity than progesterone. Thus, Δ_1 (2.1 vs 2.3), 6α-CH_3 (2.1 vs 2.10, 2.5 vs 2.13, 2.11 vs 2.16), 11α-OH (2.1 vs 2.17), 11β-OH (2.1 vs 2.12), 16α-CH_3 (2.1 vs 2.11, 2.5 vs 2.21, 2.10 vs 2.16), 16β-CH_3 (2.1 vs 2.15) and 17α-OH (2.1 vs 2.5, 2.10 vs 2.13, 2.11 vs 2.21) substitutions all decrease the affinity. Yet, some additional substituents improve somewhat the binding of pregnane derivatives. It is the case for th 9α-fluoro (2.14 vs 2.6) and the 16α-OH (2.2 vs 2.5) groups. Hydroxylation of progesterone at C-21 (deoxycorticosterone, 3.11) provokes a 12-fold decrease in the binding of progesterone, unless additional substituents are also present. Thus, a 5β-configuration (3.1) or a 17 (or 16) α-OH substitution (3.2 and 3.3) increase the affinity of deoxycorticosterone 7-fold and 3-fold respectively. In the androstane series, we find that a 17α-CH_3 substitution has little effect on the binding of testosterone (1.7 vs 1.6, 1.9 vs 1.8) or of DHT (1.3 vs 1.1). A C1–2 unsaturation decreases the affinity of testosterone derivatives (1.7 vs 1.8, 1.6 vs 1.9) while it increases the affinity of DHT and its analogues (1.14 vs 1.10, 1.3 vs 1.2). Addition of a 11α-OH (1.6 vs 1.16) or of a 11β-OH group (1.6 vs 1.12, 1.20 vs 1.25) decreases the affinity 5–20-fold. A 11β-OH group also inhibits binding in the pregnane series (2.1 vs 2.12) while it stimulates it in the C-21 hydroxypregnane series (3.11 vs 3.5). ABP binding of androstane derivatives is reduced by the presence of a 1-CH_3 group whether in quasi-equatorial (1.8 vs 1.15, 1.4 vs 1.22) or in β-configuration (1.1 vs 1.14). Finally, oxidation of the 17β-OH group into a ketone (1.1 vs 1.19, 1.5 vs 1.13, 1.6 vs 1.20, 1.12 vs 1.25) or loss of either the 3-ketone (1.17 vs 1.1) or the 17β-OH (1.6 vs 1.18) lead to a 50–200-fold decrease in affinity for ABP.

As to the methyltrienolone-binding component in prostate cytosol, its properties are similar to those of the androgen receptor described by others in this tissue [10, 16]. Of all the steroids tested, only methyltrienolone had a higher affinity than DHT for the androgen receptor. Testosterone had a slightly lower affinity than DHT. This is in agreement with other work using similar techniques [17]. The 11 steroids with the highest affinities are found in the series of androstane derivatives. As a rule, 5α-reduction increases the affinity (1.6 vs 1.1, 1.7 vs 1.3, 1.8 vs 1.2,

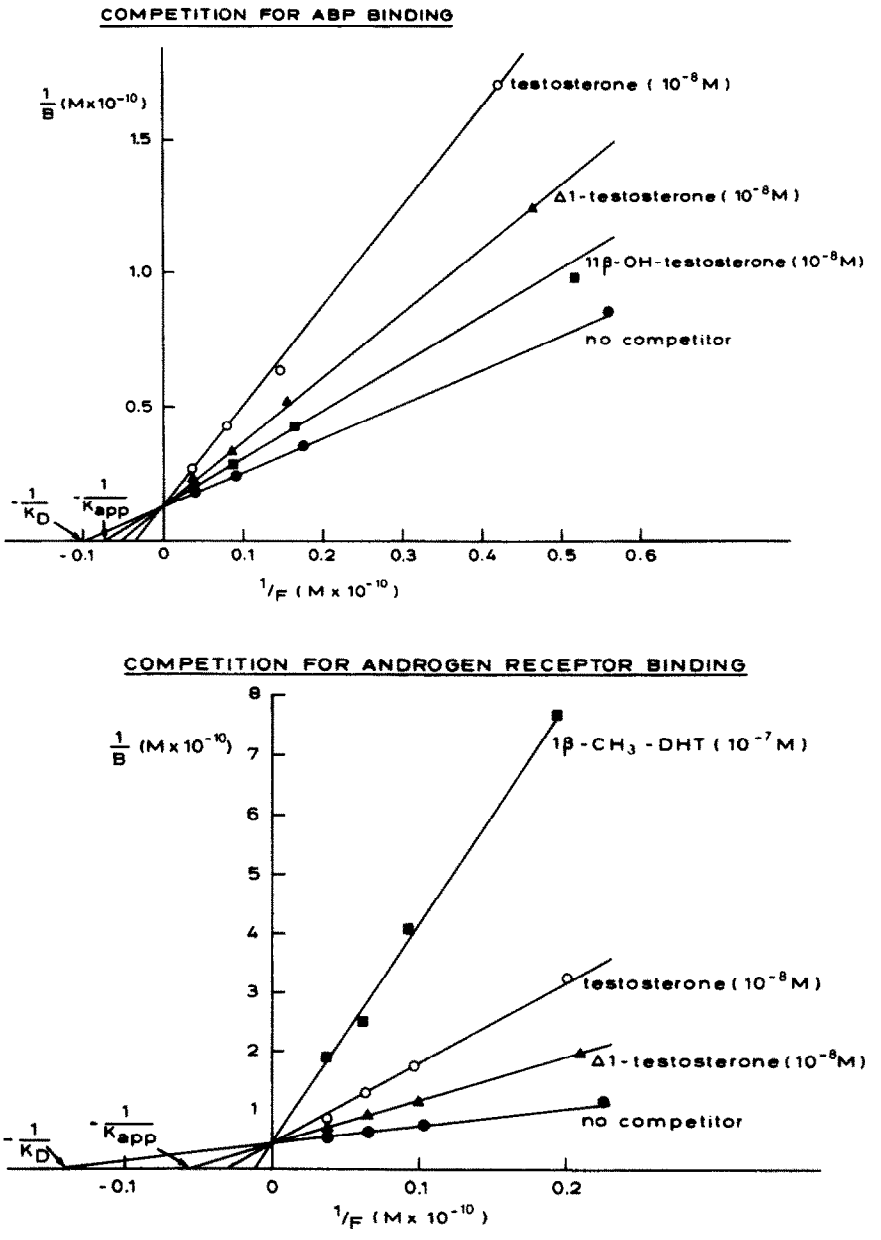

Fig. 3. Competitive inhibition of [^{3}H]-DHT binding to ABP and of [^{3}H]-methyltrienolone binding to the androgen receptor by various steroids. Specific binding of the radioactive steroids was determined in absence and presence of a constant concentration of nonradioactive competitors as indicated on the figure.

1.20 vs 1.19). However, progesterone (2.1) and four of its analogues (2.10, 2.7, 2.8, and 2.13) also bind with high affinity. Unfavourable substitutions on progesterone are 11β-OH (2.1 vs 2.12), 16α-OH (2.2 vs 2.5), 17α-OH (2.1 vs 2.5, 2.10 vs 2.13, 2.11 vs 2.21), 16α-CH_3 (2.1 vs 2.11, 2.10 vs 2.16, 2.5 vs 2.21) and 16β-CH_3 (2.1 vs 2.15) groups as well as a C1–2 unsaturation (2.1 vs 2.3). Transition to the C21-hydroxypregnane series leads to a 3-fold decrease in affinity (3.11 vs 2.1). Desoximethasone (3.10) has a surprisingly high affinity as compared to the negligible binding of other C-21 hydroxypregnane derivatives. Addition of a 17α-OH group on desoximethasone (3.32) virtually inhibits the binding. In the androstane derivatives, an interesting finding is the unfavourable effect of the 17α-CH_3 (1.6 vs 1.7, 1.9 vs 1.8, 1.1 vs 1.3). The C1–2 unsaturation either decreases (1.6 vs 1.9, 1.7 vs 1.8), does not change (1.3 vs 1.2) or increases (1.14 vs 1.10) the affinity. An equatorial- (1.8 vs 1.15, 1.4 vs 1.22) or a β-methyl group (1.1 vs 1.14) on C1 reduces the affinity considerably. Thus, one would predict that Δ1-DHT (not tested) binds with

Table 1. Affinity (equilibrium dissociation constant, nM) of androstane derivatives for the androgen-binding protein (ABP) and the androgen receptor (AR)

Number	Trivial name	Systematic name	ABP	AR
1.1	5α-Dihydrotestosterone (DHT)	17β-Hydroxy-5α-androstan-3-one	2.3	1.5
1.2	17α-Methyl-1-dehydro-DHT	17α-Methyl-17β-hydroxy-5α-androst-1-en-3-one	4.0	3.0
1.3	17α-Methyl-DHT	17α-Methyl-17β-hydroxy-5α-androstan-3-one	5.2	3.3
1.4	1-Dehydro-DHT acetate	3-Oxo-5α-androst-1-en-17β-yl acetate	7.7	37
1.5	9-Dehydrotestosterone	17β-Hydroxy-4,9(11)-androstadien-3-one	8.6	3.9
1.6	Testosterone	17β-Hydroxy-4-androsten-3-one	12	2.3
1.7	17α-Methyltestosterone	17α-Methyl-17β-hydroxy-4-androsten-3-one	14	6.5
1.8	17α-Methyl-1-dehydrotestosterone (metandienone)	17α-Methyl-17β-hydroxy-1,4-androstadien-3-one	21	13
1.9	1-Dehydrotestosterone	17β-hydroxy-1,4-androstadien-3-one	27	6.5
1.10	1-Methyl-1-Dehydro-DHT	1-Methyl-17β-hydroxy-5α-androst-1-en-3-one	36	4.0
1.11	Methyltrienolone	17β-hydroxy-17α-methyl-4,9,11-estratrien-3-one	48	0.8
1.12	11β-Hydroxytestosterone	11β,17β-Dihydroxy-4-androsten-3-one	68	49
1.13	9-Dehydroandrostenedione	4,9(11)-Androstadien-3,17-dione	125	99
1.14	1β-Methyl-DHT	1β-Methyl-17β-hydroxy-5α-androstan-3-one	135	10
1.15	1,17α-Dimethyl-1-dehydrotestosterone	1,17α-Dimethyl-17β-hydroxy-1,4-androsta dien-3-one	155	625
1.16	11α-Hydroxytestosterone	11α,17β-Dihydroxy-4-androsten-3-one	260	18
1.17	17β-Hydroxyandrostane	17β-Hydroxy-5α-androstane	300	120
1.18	17-Deoxytestosterone	4-Androsten-3-one	450	2750
1.19	Androstanedione	5α-Androstan-3,17-dione	550	79
1.20	Androstenedione	4-Androsten-3,17-dione	1180	520
1.21	6-Dehydroandrostenedione	4,6-Androstadien-3,17-dione	1910	1310
1.22	1-Methyl-1-dehydro-DHT acetate	1-Methyl-3-oxo-5α-androst-1-en-17β-YL acetate	1980	235
1.23	Aldactone	3 Potassium(3-oxo-17β-hydroxy-4,6-androstadien 17α-yl) propionate	2190	3880
1.24	1-Dehydro-11-ketoandrostene-dione	1,4-Androstadien 3,11,17-trione	7370	>10,000
1.25	11β-Hydroxyandrostenedione	11β-Hydroxy-4-androstene-3,17-dione	>10,000	3660

Table 2. Affinity (equilibrium dissociation constant, nM) of pregnane derivatives for the androgen-binding protein (ABP) and the androgen receptor (AR)

Number	Trivial name	Systematic name	ABP	AR
2.1	Progesterone	4-Pregnene-3,20-dione	120	90
2.2	16α,17α-Dihydroxyprogesterone	16α,17-Dihydroxy-4-pregnene-3,20-dione	425	>10,000
2.3	1-Dehydroprogesterone	1,4-Pregnadiene-3,20-dione	600	430
2.4	20α-Hydroxyprogesterone	20α-Hydroxy-4-pregnen-3-one	645	535
2.5	17α-Hydroxyprogesterone	17-Hydroxy-4-pregnene-3,20-dione	645	1590
2.6	6α,9α-Difluoro-11β-Hydroxy-progesterone acetonide	6α,9α-Difluoro-11β-hydroxy16α-17-isopropylidene-dioxy-4-pregnene-3,20-dione	800	2570
2.7	Chlormadinone acetate	6-Chloro-4,6-pregnadiene-3,20-dione-17α-yl acetate	990	25
2.8	Cyproterone acetate	6-Chloro-1α,2α-methylene-4,6-pregnadiene-3,20-dione-17α-yl acetate	1060	37
2.9	6,17α-Dimethyl-6-dehydroprogesterone (Medrogestone)	6,17α-Dimethyl-1,6-pregnadiene-3,20-dione	1380	330
2.10	6α-Methylprogesterone	6α-Methyl-4-pregnene-3,20-dione	1470	19
2.11	16α-Methylprogesterone	16α-Methyl-4-pregnene-3,20-dione	2310	600
2.12	11β-Hydroxyprogesterone	11β-Hydroxy-4-pregnene-3,20-dione	2340	350
2.13	6α-Methyl-17-hydroxyprogesterone	6α-Methyl-17-hydroxy-4-pregnene-3,20-dione	2340	115
2.14	6α-Fluoro-11β-hydroxyprogesterone acetonide	6α-Fluoro-11β-hydroxy-16α,17-iso-propyl idene-dioxy-4-pregnene-3,20-dione	2680	4940
2.15	16β-Methylprogesterone	16β-Methyl-4-pregnene-3,20-dione	2720	4320
2.16	6α,16α-Dimethylprogesterone	6α,16α-Dimethyl-4-Pregnene-3,20-dione	3920	220
2.17	11α-Hydroxyprogesterone	11α-Hydroxy-4-pregnene-3,20-dione	5590	790
2.18	6α-Methyl-16-dehydroprogesterone	6α-Methyl-4,16-pregnadiene-3,20-dione	6610	1630
2.19	Alphaxalone	3α-Hydroxy-5α-pregnane-11,20-dione	7250	>10,000
2.20	6α-Fluoro-11α-Hydroxyprogesterone acetonide	6α-Fluoro-11α-hydroxy-16α,17-isopropyl idene-dioxy-4-pregnene-3,20-dione	>10,000	>10,000
2.21	16α-Methyl-17-hydroxyprogesterone	16α-Methyl-17-hydroxy-4-pregnene-3,20-dione	>10,000	4810
2.22	6α-Progesterone Ethylcarbamate	6α-Ethylcarbamate-4-Pregnene-3,20-dione	>10,000	>10,000

Table 3. Affinity (equilibrium dissociation constant, nM) of C-21 hydroxypregnane derivatives for the androgen-binding protein (ABP) and the androgen receptor (AR)

Number	Trivial name	Systematic name	ABP	AR
3.1	5β-Dihydrodeoxycorticosterone	21-Hydroxy-5β-pregnane-3,20-dione	225	1470
3.2	Cortexolone	17,21-Dihydroxy-4-pregnene-3,20-dione	520	2920
3.3	16α-Hydroxydeoxycorticosterone acetate	16α,21-Dihydroxy-4-pregnene-3,20-dione 21-acetate	610	7800
3.4	Betamethasone valerate	9α-Fluoro-16β-methyl-11β,17,21-trihydroxy-1,4-pregnadiene-3,20-dione 17-valerate	670	4740
3.5	Corticosterone	11β,21-Dihydroxy-4-pregnene-3,20-dione	930	805
3.6	Clobetasone butyrate	9α-Fluoro-21-chloro-16β-methyl-1,4-pregnadiene-3,11,20-trione 17α-butyrate	970	2160
3.7	16-Dehydrodeoxycorticosterone acetate	21-Hydroxy-4,16-pregnadiene-3,20-dione 21-acetate	1100	1020
3.8	Clobetasol propionate	9α-Fluoro-21-chloro-16β-methyl-11β-hydroxy-1,4-pregnadiene-3,20-dione	1210	5470
3.9	Beclomethasone	9α-Chloro-16β-methyl-11β,17α,21-trihydroxy-1,4-pregnadiene-3,20-dione	1270	5470
3.10	Desoximethasone	9α-Fluoro-16α-methyl-11β,21-dihydroxy-1,4-pregnadiene-3,20-dione	1450	190
3.11	11-Deoxycorticosterone	21-Hydroxy-4-pregnene-3,20-dione	1520	250
3.12	Triamcinolone	9α-Fluoro-11β,16α,17,21-tetrahydroxy-1,4-pregnadiene-3,20-dione	1700	> 10,000
3.13	Clobetasone	9α-Fluoro-21-chloro-16β-methyl-17α-hydroxy-1,4-pregnadiene-3,11,20-dione	1720	> 10,000
3.14	14α-Hydroxycortexolone	14α,17,21-Trihydroxy-4-pregnene-3,20-dione	2060	5740
3.15	Betamethasone	9α-Fluoro-16β-methyl-11β,17,21-trihydroxy-1,4-pregnadiene-3,20-dione	2080	951
3.16	9α-Fluoro-6α-methylprednisolone	9α-Fluoro-6α-methyl-11β,17,21-trihydroxy-1,4-pregnadiene-3,20-dione	2110	2990
3.17	Cortisone	17,21-Dihydroxy-4-pregnene-3,11,20-trione	2360	> 10,000
3.18	5α-Dihydrocortisol acetate	11β,17,21-Trihydroxy-5α-pregnane-3,20-dione 21-acetate	2410	1240
3.19	9α-Fluoroprednisolone	9α-Fluoro-11β,17,21-Trihydroxy-1,4-pregnadiene-3,20-dione	2420	5570
3.20	1-Methylprednisolone	1-Methyl-11β,17,21-Trihydroxy-1,4-pregnadiene-3,20-dione	2480	> 10,000
3.21	16α-Hydroxycortisol	11β,16α,17,21-Tetrahydroxy-4-pregnene-3,20-dione	2640	> 10,000
3.22	6α-Methylcortisol	6α-Methyl-11β,17,21-Trihydroxy-4-pregnane-3,20-dione	2850	2400
3.23	16α-Methylprednisolone	16α-Methyl-11β,17,21-trihydroxy-1,4-pregnadiene-3,20-dione	3090	> 10,000
3.24	16α-Methylcortexolone	16α-Methyl-17,21-dihydroxy-4-pregnene-3,20-dione	3150	2650
3.25	Clobetasol	9α-Fluoro-21-chloro-16β-methyl-11β,17-dihydroxy-1,4-pregnadiene-3,20-dione	3150	> 10,000
3.26	6-Dehydrocortisol	11β,17,21-Trihydroxy-4,6-pregnadiene-3,20-dione	3560	> 10,000
3.27	Cortisol	11β,17,21-Trihydroxy-4-pregnene-3,20-dione	3830	> 10,000
3.28	Prednisolone	11β,17,21-Trihydroxy-1,4-pregnadiene-3,20-dione	3870	9400
3.29	9α-Fluorocortisol	9α-Fluoro-11β,17,21-trihydroxy-4-pregnene-3,20-dione	4230	6960
3.30	11-Dehydrocorticosterone	21-Hydroxy-4-pregnene-3,11,20-trione	5140	4190
3.31	Prednisone	17,21-Dihydroxy-1,4-pregnadiene-3,11,20-trione	5270	> 10,000
3.32	Dexamethasone	9α-Fluoro-16α-methyl-11β,17,21-trihydroxy-1,4-pregnadiene-3,20-dione	7490	5780
3.33	7-Dehydroprednisolone	11β,17,21-Trihydroxy-1,4,7-pregnatriene-3,20-dione	> 10,000	> 10,000
3.34	Triamcinolone acetonide	9α-Fluoro-11β,21-dihydroxy-16α,17-isopropylidene-dioxy-1,4-pregnadiene-3,20-dione	> 10,000	> 10,000
3.35	Aldosterone	11β,21-Dihydroxy-18-AL-4-pregnene-3,20-dione	> 10,000	> 10,000
3.36	6α-Methylprednisolone	6α-Methyl-11β,17,21-trihydroxy-1,4-pregnadiene-3,20-dione	> 10,000	> 10,000
3.37	16α-Hydroxyprednisolone	11β,16α,17,21-Tetrahydroxy-1,4-pregnadiene-3,20-dione	> 10,000	> 10,000
3.38	5β-Dihydrocortisol acetate	11β,17,21-Trihydroxy-5β-pregnane-3,20-dione 21-acetate	> 10,000	> 10,000
3.39	6α-Hydroxyprogesterone acetonide	6α-Hydroxy-17,21-isopropylidene-dioxy-4-pregnene-3,20-dione	> 10,000	> 10,000
3.40	Tetrahydrocorticosterone	3α,11β,21-Trihydroxy-5β-pregnane-20-one	> 10,000	> 10,000

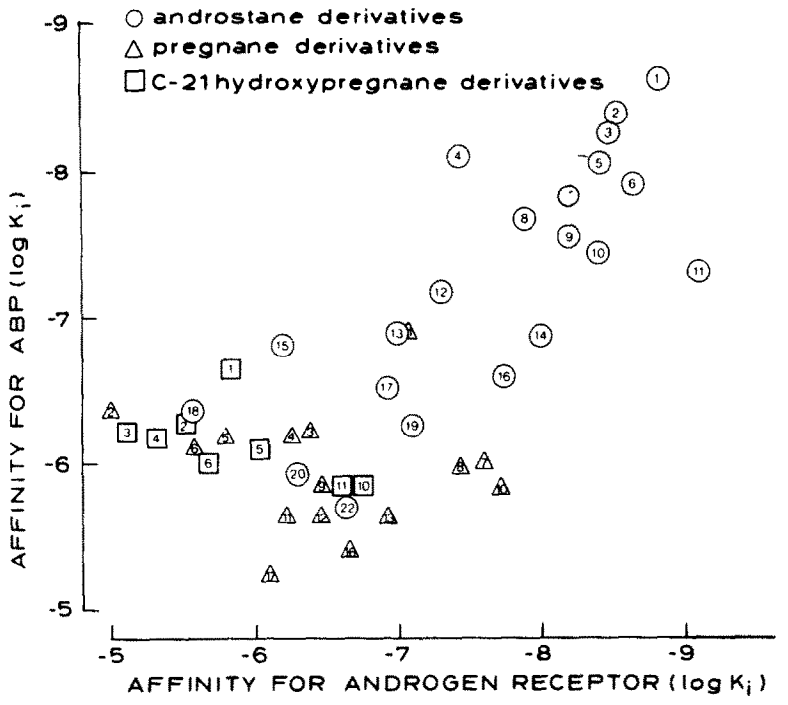

Fig. 4. Comparison of the affinity of various steroids for the androgen receptor and for ABP. Numbers in the symbols refer to compound numbers in Table 1 (○), Table 2 (△) and Table 3 (□). Compounds which have a negligible affinity ($K_D > 1\ \mu M$) for both binding sites are not shown.

very high affinity since its 1-CH_3 analogue (1.10) binds almost as well as testosterone itself. This was found to be the case by other investigators [18]. Finally, substitution of the 17β-OH by a ketone group (1.1 vs 1.19, 1.5 vs 1.13, 1.6 vs 1.20, 1.12 vs 1.25) decreases the affinity dramatically while loss of the 17β-OH group (1.6 vs 1.18) virtually abolishes binding. Loss of the 3-ketone (1.1 vs 1.17) is still compatible with binding. Addition of a 11β-OH (1.6 vs 1.12, 1.20 vs 1.25) or of a 11α-OH (1.6 vs 1.16) can reduce the affinity by 10–20-fold. The binding to rat prostate androgen receptor of a few of the steroids examined here has been studied by other investigators [17–22] who measured their relative inhibitory effect on the binding of radioactive DHT or MT. Although the "percentage of inhibition" data found in the literature are not always easy to compare, except in [19], with our K_i values, they are in general agreement.

If we now compare the steroid-binding specificity of the androgen receptor and of ABP, we find that both proteins have in common a high affinity for the natural androgens DHT and testosterone. However, the mechanisms involved in the binding must somehow differ for the two proteins, since the very fast off-rate of DHT from ABP is compatible with high affinity only because it is compensated by a very high rate of association. These rapid kinetics are in keeping with the proposed androgen-carrier function of ABP. By contrast, androgens associate with and dissociate from their intracellular receptor very slowly and this accounts for the selective retention of the hormone in target cells. Thus, it is not surprising that differences in the binding site properties become also apparent when discrimination between different steroid structures is at stake. This is illustrated in Fig. 4. It can be seen that several androstane derivatives have an affinity higher for ABP than for the androgen receptor. On the other hand, some compounds bind better to the androgen receptor than to ABP. The most striking example is methyltrienolone (1.11) with a ratio of 60-fold. Others have ratios of 7–14-fold. Here, substituents unfavourable to ABP are the 1-CH_3 (1.10, 1.14) and 11α-OH (1.16). The presence of a 17-ketone (1.19) is also tolerated better by the androgen receptor than by ABP. As to the pregnane derivatives tested, seventy percent bind better to the androgen receptor than to ABP. They include the known anti-androgens chlormadinone acetate (2.7), cyproterone acetate (2.8) and medrogestone (2.9). The anti-androgenic activity of spironolactones has been ascribed to their binding to the androgen receptor [23]. We find that aldactone (1.23) has a low affinity for this receptor and binds better to ABP. The 1-CH_3-Δ1-substitution appeared interesting because the corticoid derivative tested (3.20) bound neither to the glucocorticoid receptor [24] nor to the androgen receptor and yet it interacted somewhat with ABP. We thought that 1-CH_3-Δ1-androgens might bind with high affinity to ABP while losing

their affinity for intracellular receptors. Such compounds, however, not only bind with high affinity to ABP; they even bind better to the androgen receptor (1.10). Finally, the results obtained with compound 3.1 suggest that a 5β-configuration may selectively favor ABP binding.

In conclusion, we have shown that ABP and the androgen receptor clearly differ in their steroid specificity. Among the steroids tested, 35 compounds including pregnane and C-21 hydroxypregnane derivatives could bind to the androgen receptor at concentrations achievable *in vivo* ($K_D < 1\ \mu M$). Our data do not allow distinction between androgens and antiandrogens. However, they should lay the ground for a comprehensive study of the structural requirements for highly specific binding of steroid analogues to the androgen receptor. Some substitutions inhibit binding to ABP. Thus, androgen agonists or antagonists could be designed which would not bind to the circulating sex hormone binding globulin (TeBG) since in species, such as man, where this protein occurs it resembles [25] or may be identical to ABP [26]. On the other hand, twelve compounds among those which had a good affinity for ABP ($K_D < 1\ \mu M$) did bind better to this protein than to the androgen receptor. Derivatives of such compounds which would preferentially or exclusively bind to ABP might be helpful for further studying the role of this protein in physiology and in pathology.

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