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BINDING OF ANDROGENS IN DOG PROSTATE CYTOSOL AND IN PLASMA

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SUMMARY

When dog prostate cytosol is incubated with the synthetic androgen, methyltrienolone (R1881), specific binding is observed in the 9S region of sucrose gradients. This binding shows the classical features of androgen receptors: specificity, affinity and sedimentation coefficient. Under identical experimental conditions, the natural hormone 5α-dihydrotestosterone (DHT) exhibits an additional saturable binding peak in the 3–4S region. This binding is different from the 9S receptor and is saturated by very low concentrations of diethylstilbestrol. Chromatographic analysis reveals that the 4S peak radioactivity consists mainly of 5α-androstane-3β, 17β-diol while DHT is predominant in the 9S peak. When tritiated 5α-androstane-3α, 17β-diol is incubated with prostate cytosol, some 9S binding is observed along with binding to slower sedimenting components. The 9S binding is attributed to a conversion of 5α-androstane-3α, 17β-diol into DHT during the *in vitro* incubation and not to a different androgen receptor. Tritiated 5α-androstane-3β, 17β-diol sediments exclusively in the 4S region of sucrose gradients. [^{3}H]-Testosterone (T), which is not metabolized during *in vitro* incubations, is associated with both 9S and 4S components saturable by low concentrations of DHT and R1881. Thus, there seems to be a single androgen receptor which is specific for DHT and T. The level of androgen receptors was measured with the use of tritiated R1881 in immature, mature and hypertrophic dog prostates. No apparent differences could be found between the different groups. The mean concentration of binding sites in 22 prostates was 83 ± 7 (S.E.M.) fmol/mg prot. and the association constant (K_A) at 0°C was 3.2 ± 0.4 × 10^9 M^{-1}.

An androgen binding protein is also present in male and female dog plasma. It is different from the prostate receptor in many respects: sedimentation coefficient, steroid specificity and affinity. It is concluded that dog plasma binding protein is either present only in low amounts in dog prostate cytosol or does not intefere with androgen receptor determination by usual charcoal assays.

INTRODUCTION

Elevated levels of 5α-dihydrotestosterone (DHT) have been measured by several workers [1, 2] in the dog prostate. This finding suggested that high affinity binding proteins for androgens were present in the prostate cytosol of this animal. In that respect, Boesel *et al.*[3] were the first to demonstrate the existence of a DHT receptor in the prostate of intact and castrated adult dogs. Previous studies by Evans and Pierrepoint[4] have also shown the existence of a different binding component for 5α-androstane-3α, 17α-diol. Furthermore, it has been shown that the administration of 5α-androstane-3α, 17β-diol leads to prostatic hypertrophy in the castrate dog [5]. To evaluate the possible role of the latter steroid in the development of this hypertrophy, it was of interest to determine whether it was also bound to a specific receptor.

In man the measurement of specific androgen receptors in target tissues is hampered by the contamination of a plasma sex steroid binding protein (SSBP). In homogenized tissues such as the prostate, the usual ligand, [^{3}H]-5α-dihydrotestosterone (DHT), is thus bound to 8–9S receptors on sucrose gradients and also to 4–5S component which corresponds mainly to SSBP and various non saturable binding proteins. The existence in dog plasma of a protein analogous to SSBP is suggested by the experiments of Carstensen *et al.*[6], although a previous report [7] indicated that testosterone binding could not be demonstrated by polyacrylamide gel electrophoresis. We have determined some of the physicochemical characteristics of dog plasma SSBP in order to evaluate its possible interference in the determination of androgen receptors.

Terminology: 5α-dihydrotestosterone, DHT = 17β-hydroxy-5α-androstan-3-one; methyltrienolone, R1881 = 17β-hydroxy-17α-methyl-4,9,11-estra-trien-3-one; 19-nortestosterone = 17β-hydroxy-4-estren-3-one; 7α-methyl-19-nortestosterone = 7α-methyl-17β-hydroxy-4-estren-3-one; 3α,17β-diol = 5α-androstane-3α,17β-diol; 3β,17β-diol = 5α-androstane-3β,17β-diol; 3α,17α-diol = 5α-androstane-3α,17α-diol; cyproterone acetate = 6-chloro-17α-acetoxy-1,2α-methylene-4,6-pregnadiene-3, 20-dione; estradiol = 1,3,5(10)-estratrien-3,17β-diol; DES = diethylstilbestrol; R5020 = 17,21-dimethyl-19-norpregna-4,9-diene-3,20-dione; T = testosterone.

MATERIALS AND METHODS

Steroids. [6,7-^{3}H]-R1881 (55.5 Ci/mmol) and radioinert R1881 were generous gifts from Roussel-Uclaf, Romainville, France. [1,2-^{3}H]-Dihydrotesto-

sterone (44 Ci/mmol), [1,2-^{3}H]-testosterone (40 Ci/mmol), [1,2-^{3}H]-cortisol (42 Ci/mmol) and [1,2-^{3}H]-5α-androstane-3α, 17β-diol (40 Ci/mmol) were purchased from New England Nuclear Corp. and [1,2-^{3}H]-5α-androstane-3β, 17β-diol (43 Ci/mmol) from Amersham/Searle Corp. These radioactive steroids were periodically purified by paper chromatography (Bush B_3 system). Cyproterone acetate was given by Schering AG, Berlin. 5α-Androstane-3α, 17α-diol was obtained from the Medical Research Council Steroid Reference Collection, England. Other radioinert steroids were bought from Sigma or Steraloids.

Animals. Immature and adult dogs, of no specific breed, were selected for this study. They were obtained from Le Centre de Biomédecine, Laval University, Québec. The exact age of the dogs was unknown. They were castrated 24 h before use by a scrotal incision under nembutal anesthesia. Following sacrifice, the prostate was quickly dissected out. Adhering fat and urethra were removed. Portions of the tissues were used freshly for sucrose density gradient analysis. The rest of the tissues was immediately frozen at −80°C and kept for future analysis. Even after several months of freezing, prostate tissues behaved similarly as fresh tissues in regard to concentration of binding sites and affinity constant as measured by charcoal assay.

Venous blood was withdrawn from 16 intact dogs (8 males and 8 females) into heparinized tubes. The blood was centrifuged shortly after and plasma collected and immediately frozen at −20°C until analysis.

Binding assay. Prostatic tissues were finely minced with surgical scissors and were homogenized with a Polytron PT-10 homogenizer in 2–5 vol. of 0.01 M Tris–HCl buffer at pH 7.4 containing 1.5 mM EDTA. The homogenates were centrifuged at 30,000 *g* for 30 minutes in a Sorvall centrifuge. Aliquots of the supernatant (cytosol) were incubated at 0°C for 3–4 h in the presence of [^{3}H]-R1881, [^{3}H]-testosterone, [^{3}H]-DHT or [^{3}H]-3α-androstanediol. Scatchard analysis and competition experiments were done with charcoal assay (5% charcoal, 1% bovine gamma globulin and 0.5% dextran T-70). Ten-fold diluted plasma samples were similarly processed except that the charcoal mixture was diluted 5-fold. The time of contact of the cytosol and plasma with charcoal was respectively 10 minutes and 1 minute unless otherwise indicated. Non specific binding was determined by parallel incubation with 100-fold excess of radioinert R1881 or DHT. After centrifugation at 4°C and at 2000 rev./min for 5 minutes, the supernatants were counted by liquid scintillation.

In some experiments, incubated plasma was mixed with an equivalent volume of a saturated ammonium sulfate solution and kept at 0°C for 10 minutes. The mixture was then centrifuged at 2000 rev./min for 10 minutes and the supernatant was counted by liquid scintillation.

Sucrose density gradients. Aliquots of 0.4 ml of cytosol or of diluted plasma were layered on 5–20% linear sucrose gradients containing 10% glycerol. The tubes were centrifuged for 18 h at 45,000 rev./min (cytosol) or 52,000 rev./min (plasma) in an SW-56 or SW 60 Ti rotor using an L2-65B Beckman ultracentrifuge. The fractions (three drops) were collected from the bottom of the tube, dispersed in toluene PPO-POPOP scintillation solution and counted. Bovine serum albumin and gamma globulin were used as sedimentation markers.

Metabolism. Androgen metabolism during *in vitro* incubations with tritiated androgens was determined after 3–4 h of incubation at 0°C in the charcoal treated cytosol (bound fraction) and in the 9S and 4S fractions collected after 18 h of centrifugation on sucrose density gradients. The reaction was stopped by extraction with anhydrous ether; the extraction procedure was repeated three times. Radioactive DHT and androstanediol were separated by thin layer chromatography on precoated silica gel plates (60-F-254-E, Merck) using chloroform–methanol (196.5:2.5, V/V) system. Unlabeled standard steroids were chromatographed along with the samples and the spots were visualized with iodine vapors. The silica gel in the areas corresponding to DHT and 5α-androstanediols was aspirated under vacuum in small glass columns and eluted with chloroform–methanol (2:1, V/V) directly into scintillation vials. The radioactivity was determined after evaporation of the eluate. In some other cases, the extracts were analyzed by paper chromatography (Bush B_3 system). The paper was divided into 1 cm strips and counted by liquid scintillation.

RESULTS

In a first series of experiments, the prostate cytosol binding of 4 natural androgens (5α-dihydrotestosterone, 5α-androstane-3α, 17β-diol, 5α-androstane-3β, 17β-diol and testosterone) and 1 synthetic androgen (R1881) was studied by the technique of sucrose density gradient centrifugation. The results are shown in Figs 1 and 2. Saturable binding of [^{3}H]-DHT could be seen both in the 9S and 3–4S regions of sucrose gradients. By contrast the synthetic hormone, [^{3}H]-R1881, showed only the 9S component. However, if only the binding is taken into consideration, DHT and R1881 show comparable levels of binding. The differences observed are entirely explainable by the difference in specific activity of both tracers (44 vs 55.5 Ci/mmol). 5α-Androstane-3β, 17β-diol exhibited only the 3–4S binding component. Unexpectedly, some binding was observed in the 9S region when the cytosol was incubated with 5α-androstane-3α, 17β-diol. These sedimentation patterns shown in Fig. 1 were reproducibly obtained for all the other dog prostates which were examined by this technique although the level of binding varied with each prostate. In similar experimental conditions,

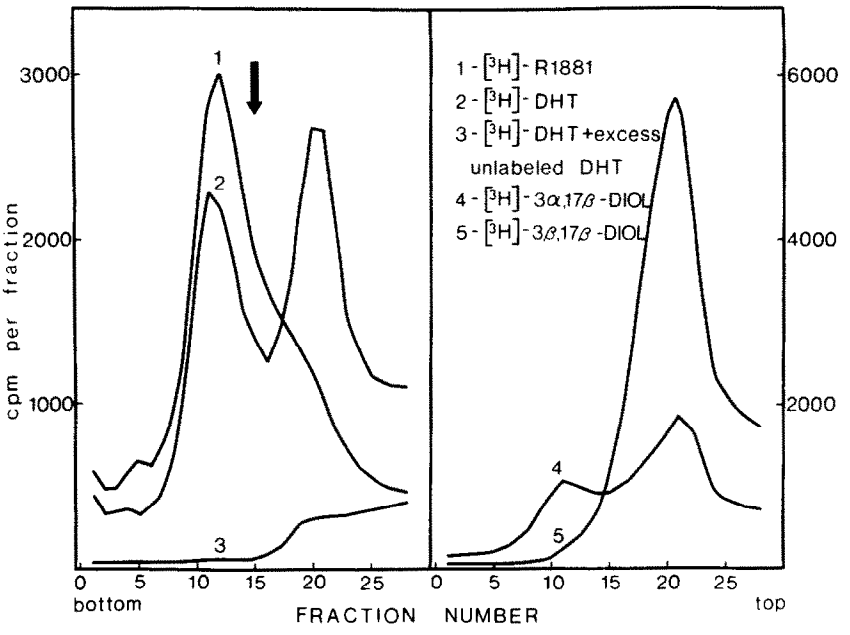

Fig. 1. Sucrose density gradient profiles of the binding of 10.4 nM [^{3}H]-R1881 (dark triangles), of 12.5 nM [^{3}H]-5α-androstane-3α, 17β-diol (dark squares), of 18.4 nM [^{3}H]-5α-androstane-3β, 17β-diol (open squares) and of 15.9 nM [^{3}H]-DHT alone (dark circles) or in the presence of 1.7×10^{-6} M unlabeled DHT (open circles) in dog prostate cytosol. The arrow indicates the position of bovine γ-globuline marker (7S). The amount of protein applied on the gradients was 7.9 mg. Unbound steroids were removed by charcoal treatment before applying the cytosol on the gradients.

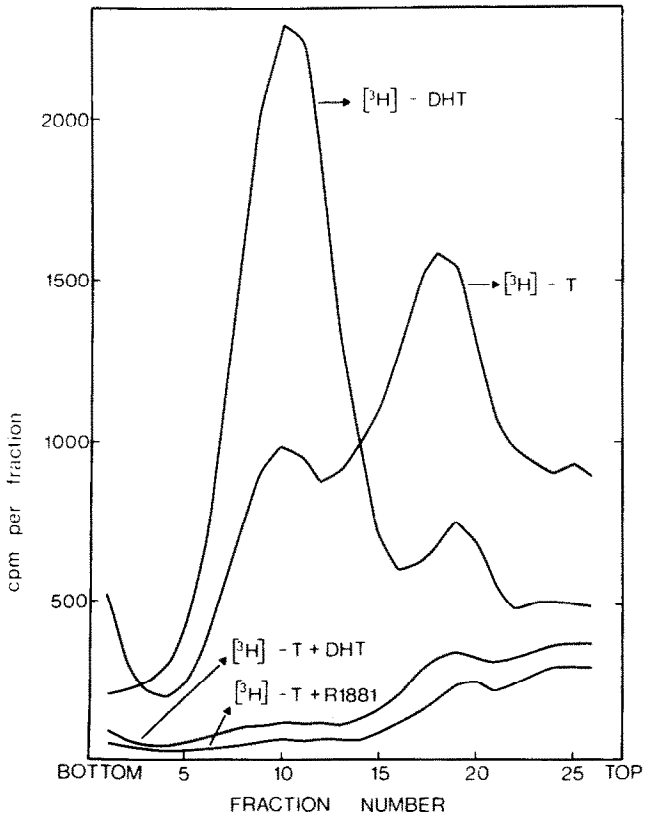

Fig. 2. Sucrose density gradient profiles of the binding of 14.9 nM [^{3}H]-DHT and of 15.5 nM [^{3}H]-testosterone alone or in the presence of 69 nM unlabeled DHT and R1881 in dog prostate cytosol. The other experimental conditions are similar to those described under Fig. 1.

[^{3}H]-testosterone was associated with both 9S and 4S components saturable by low concentrations of DHT and R1881 (Fig. 2). In this experiment, the sum of specific 9S and 4S binding was equivalent to the 9S peak of [^{3}H]-DHT binding. The poor resolution of 9S and 4S peaks of [^{3}H]-testosterone binding suggests that this steroid is dissociated from the 9S receptor during centrifugation.

In view of the binding of 5α-androstane-3α, 17β-diol in the 9S region and taking into consideration the extensive metabolism of natural androgens occurring in many androgen target tissues even at 0°C (8–10), the steroid composition in 9S and 3–4S fractions after incubation of the cytosol with the four natural androgens was determined by thin-layer chromatography. This system does not separate the 3α and 3β epimers of 5α-androstanediol. This experiment showed that DHT represents more than 75% of total radioactivity in 9S peak whether the cytosol is incubated with DHT or 5α-androstane-3α, 17β-diol. By contrast 5α-androstanediols are predominant in the 3–4S peak even when the cytosol is incubated with tritiated DHT. 5α-Androstane-3β, 17β-diol is not reconverted into DHT and remains unchanged. Similarly, [^{3}H]-testosterone is not metabolized during *in vitro* incubations and both 9S and 4S peaks are constituted exclusively by testosterone. In a further experiment, the charcoal treated cytosols previously incubated with the natural androgens were analyzed by a paper chromatographic system which resolves the 3α and 3β epimers. The charcoal treated cytosol represents the bound fraction and thus is similar to total radioactivity which was previously applied on sucrose gradients. This experiment confirmed the previous results and showed that 5α-androstane-3β, 17β-diol is the principal metabolite of DHT. In fact, after 3.5 h of incubation at 0°C of the cytosol with [^{3}H]-DHT, the 3α and 3β epimers of 5α-androstanediol represented respectively 9.3 and 46% of total extracted radioactivity. When [^{3}H]-5α-androstane-3α, 17β-diol was incubated in similar conditions, 16% of the radioactivity was recovered in 5α-androstane-3β, 17β-diol spot and 18% in DHT spot.

Since after incubation of the cytosol with [^{3}H]-DHT, unconverted DHT is bound in the 9S fraction and its metabolite 5α-androstane-3β, 17β-diol in the 3–4S fraction, and because both fractions (9S and 3–4S) are saturated by adding a large excess of unlabelled DHT (Fig. 1), we then studied the binding specificity of both fractions on sucrose gradients (Fig. 3). The 9S and 3–4S fractions appear to have completely different specificities. The 9S peak behaves as classical androgen receptors. It is saturable by low concentrations of DHT but not by diethylstilbestrol and progesterone. On the contrary, the 3–4S peak is almost completely abolished by diethylstilbestrol but only incompletely by DHT.

The hypothesis that DHT binds to two different components is strengthened by the observation that DHT yields more binding sites than the synthetic

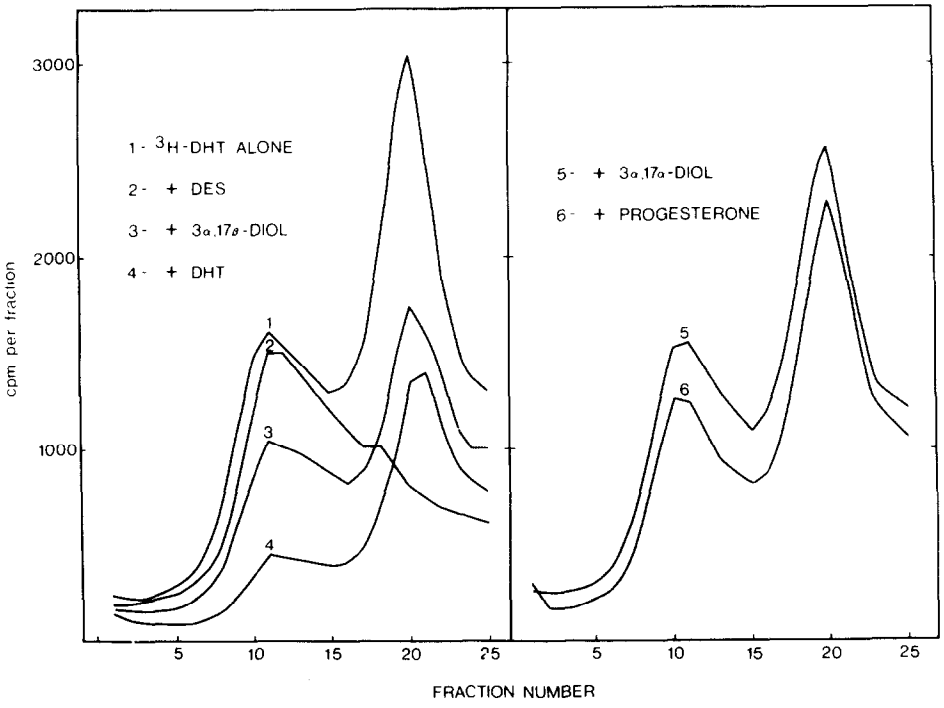

Fig. 3. Differential competition of various steroids for the prostate cytosol binding of [^{3}H]-DHT (and metabolites) to the 9S and 3–4S peaks in sucrose gradients. The competing unlabeled steroids were present at a concentration of 34 nM. The other experimental conditions are similar to those described under Fig. 1.

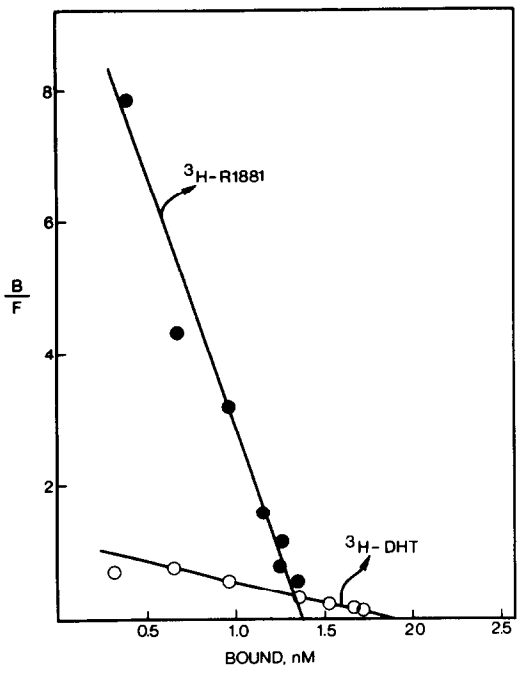

Fig. 4. Scatchard analysis of the binding of [^{3}H]-R1881 (dark circles) and [^{3}H]-DHT (open circles) at 0°C in the same dog prostate cytosol. The concentrations of labeled steroids ranged from 0.2 to 4 nM for [^{3}H]-R1881 and from 0.8 to 15 nM for [^{3}H]-DHT.

androgen R1881 when Scatchard analysis is performed (Fig. 4). This type of results was confirmed with three more dog prostates (results not shown).

In order to avoid the problems encountered with DHT, further characterization and determination of the androgen receptor was done with tritiated R1881 which is not metabolized during *in vitro* incubations with prostatic cytosol [11]. Competition experiments indicate that the receptor is highly specific for natural and synthetic active androgens (Table 1). The concentration of binding sites was then determined by Scatchard analysis in 22 dog prostates (Fig. 5). For this study, we have deliberately chosen to use a wide range of prostate wt. (immature: up to 5 g; mature: between 5 and 15 g; hypertrophic: 15 g and more). The maturity of the animal could be roughly estimated by the size of the testis. Hypertrophic prostates were obtained by screening several dogs by rectal examination. No apparent differences in androgen receptor levels could be found between the different groups. The mean receptor level in the whole group was 83 ± 7 (S.E.M.) fmol/mg prot. and the affinity constant (K_A) 3.2 ± 0.4 (S.E.M.) × 10^9 M^{-1}.

Table 1. Relative competition activity* of various steroids for the binding of [^{3}H]-R1881 in dog prostate cytosol

Competing steroids	Relative activity
R 1881	100
19-Nortestosterone	63
7α-Methyl-19-nortestosterone	51
5α-Dihydrotestosterone	28
Testosterone	25
5α-Androstane-3α, 17β-diol	6
Progesterone	4
Cyproterone acetate	4
5α-Androstane-3β, 17β-diol	3
Estradiol	3
R 5020	2
5α-Androstane-3α, 17α-diol	0.3
Cortisol	<0.1

* The amount of each steroid needed to decrease the binding of [^{3}H]-R1881 was determined from competition curves. Competition activity was then calculated relative to unlabeled R1881.

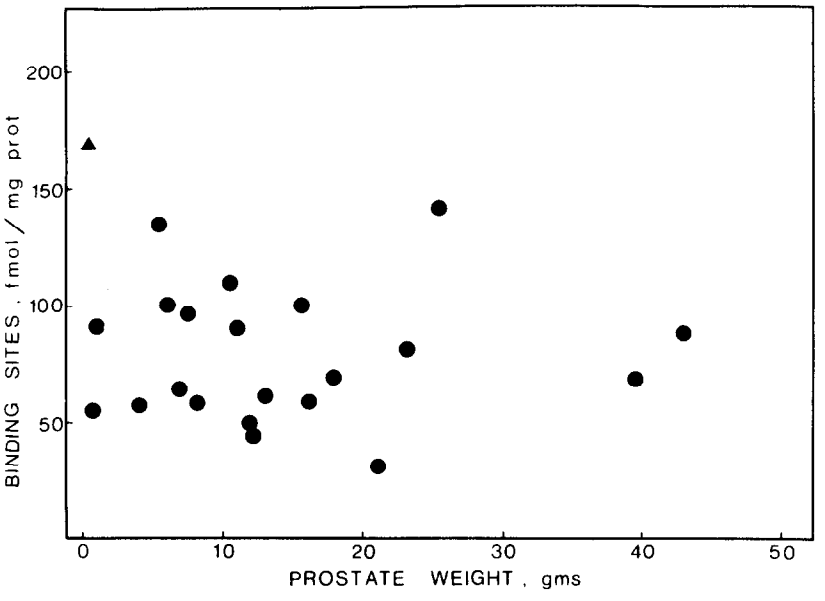

Fig. 5. The relation between prostate weight and androgen receptor content. All individual determinations were obtained by Scatchard plot using [^{3}H]-R1881. The triangle represents the result for a pool of 3 prostates from immature dogs.

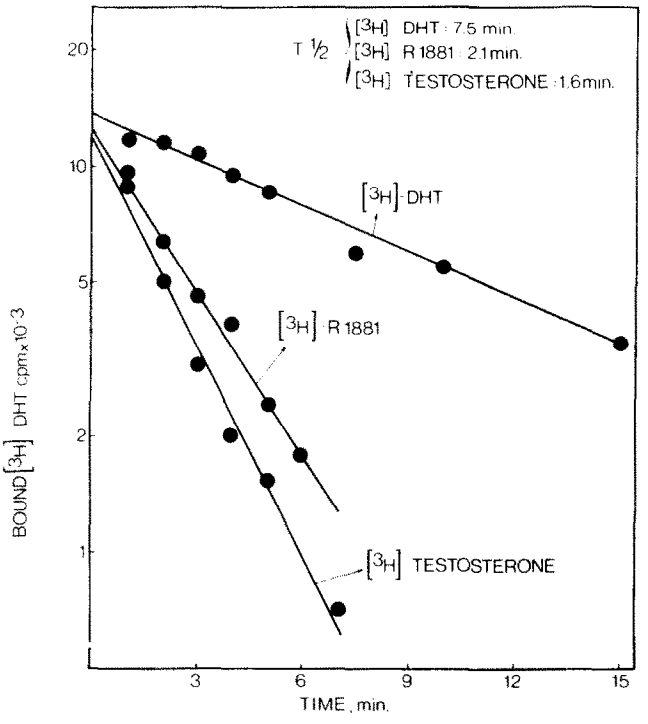

Fig. 6. Effect of 0.5% charcoal on the rate of dissociation of [^{3}H]-DHT, [^{3}H]-R1881 and [^{3}H]-testosterone from dog plasma binding protein.

Preliminary studies indicating that saturable DHT binding was also present in dog plasma, the next experiments were designed to find suitable conditions for charcoal assay of the plasma binding component. Thus, the rate of dissociation of specific DHT binding was studied. It was found to be rapid in the presence of 0.5% charcoal with a half life of 7.5 minutes (Fig. 6). The addition of 20% glycerol in the plasma did not change the rate of dissociation. For further experiments, the following conditions were chosen: 1 min of contact of the diluted plasma with charcoal. Under these conditions, there is an underestimation of about 10% of the number of binding sites. By comparison, [^{3}H]-R1881 and [^{3}H]-testosterone are dissociated at a much faster rate than [^{3}H]-DHT (Fig. 6).

The DHT binding protein can be separated from transcortin (CBG) by ammonium sulfate precipitation (Fig. 7). One can see that 85% of the ^{3}H-DHT is precipitated at 50% saturation. Remaining binding (probably due to albumin) is entirely precipitated at 80% saturation. By contrast, cortisol is little affected by 50% saturation and precipitates at only 60 to 80% saturation of ammonium sulfate. This precipitation technique (50% saturation) was used for the determination of affinity constant (K_A) and concentration of binding sites.

The thermal stability of the binding protein was then studied by incubating diluted plasma for 10 minutes at various temperatures. The binding protein appears to be unusually heat labile since it is

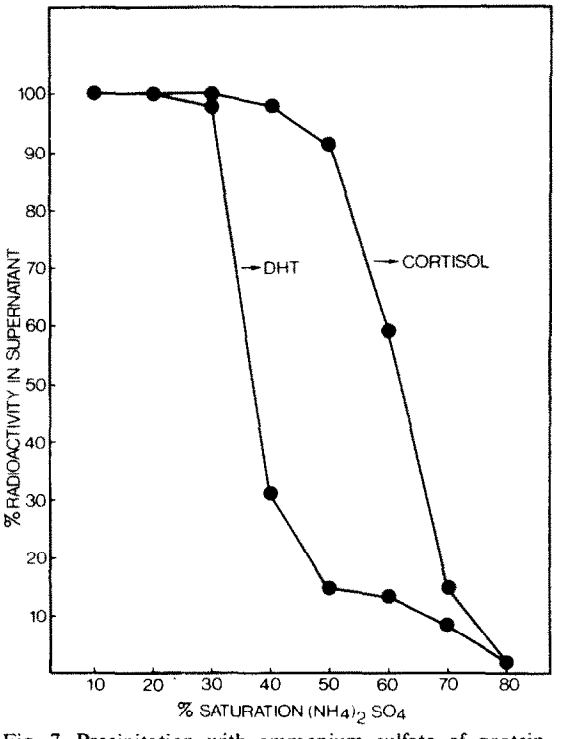

Fig. 7. Precipitation with ammonium sulfate of protein bound [^{3}H]-DHT and [^{3}H]-cortisol. Aliquots of 0.1 ml of undiluted dog plasma were incubated with 100,000 c.p.m. of tritiated steroid at 0°C for 30 minutes. Ammonium sulfate in a total volume of 0.9 ml at the appropriate concentration was added to each tube. The tubes were centrifuged and the supernatant counted by liquid scintillation.

completely inactivated at 45°C. Half maximal binding was seen at about 32°C. The addition of unlabeled DHT to the diluted plasma at a concentration of 10 ng/ml affords some protection from denaturation. By contrast, undiluted plasma appears to be stable over the range of temperatures tested (0–45°C). Instability of diluted plasma was also observed in 0°C incubations with a 27% decrease of activity after 6 h. However, no appreciable loss of activity occurs when whole plasma is kept frozen for at least 2–3 weeks.

Sucrose density gradient analysis (Fig. 8) revealed that the [^{3}H]-DHT binding peak was localized in the same region as bovine serum albumin (4.6S). This binding peak was partly saturable by the addition of a 6-fold excess of unlabeled DHT. No further decrease of binding was observed by the addition of 150-fold excess of unlabeled DHT (results not shown). We think that the poor saturability and poor sedimentation profile observed in this experiment is a reflection of the lability of the binding protein in long experiments (18 h) and also of the high propensity of DHT to bind non-specifically to serum albumin. No metabolism of [^{3}H]-DHT could be detected after plasma incubations at 0°C. When the plasma was incubated with [^{3}H]-R1881, the radioactivity remained on top of the gradient and no specific peak could be observed.

The binding specificity was studied by incubating a fixed concentration of [^{3}H]-DHT in the presence of seven concentrations (4.3 nM to 4.3 μM) of various unlabeled steroids (Table 2). The following order of competition was noted: DHT > R1881 > testosterone > 5α-androstane-3β, 17β-diol > 5α-androstane-3α, 17β-diol > 19-nortestosterone > estradiol. Estrone, androstenedione, progesterone and cortisol showed no competition at the concentrations used.

The affinity constant (K_A) at 0°C and the concentration of DHT binding sites were determined for the 16 dogs that were studied. The K_A value was found to be higher by the ammonium sulfate precipitation procedure than by charcoal assay but the number of binding sites was similar. All the results reported below were obtained by the ammonium sulfate procedure. K_A values of 2.5 ± 0.4 (S.E.M.) $\times 10^8\,M^{-1}$ and 1.9 ± 0.3 (S.E.M.) $\times 10^8\,M^{-1}$ were found respectively for male and female dog plasma. The difference between these values was not statistically significant. However the concentration of binding sites was significantly ($P < 0.05$) higher in females than in males [4.6 ± 0.3 (S.E.M.) vs 3.3 ± 0.4 (S.E.M.) μg DHT bound/100 ml].

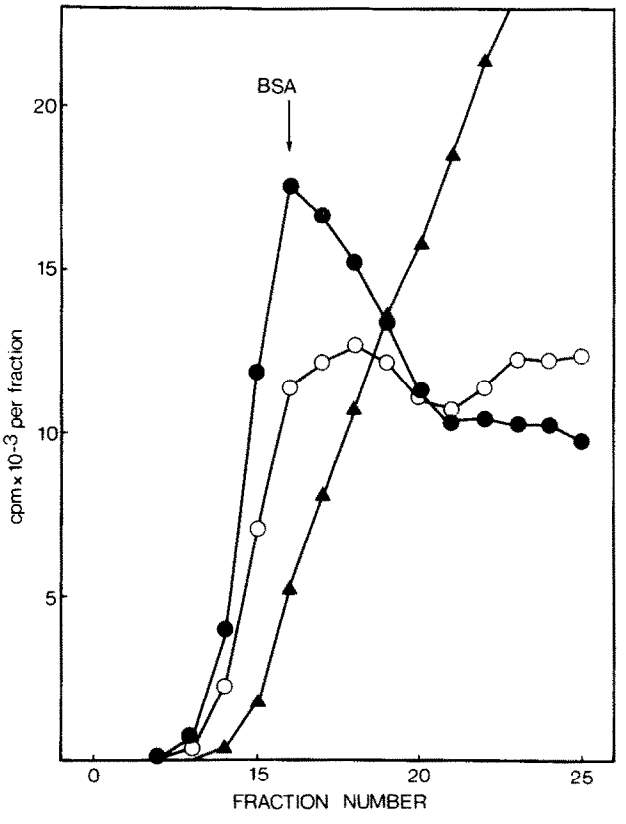

Fig. 8. Sucrose density gradient analysis of the binding of [^{3}H]-R1881 9.2 nM (dark triangles) and of [^{3}H]-DHT 11.4 nM alone (dark circles) or in the presence of a 6 fold excess of unlabeled DHT (open circles) in 10 fold diluted dog plasma. The arrow indicates the position of bovine serum albumin (BSA) marker (4.6S).

Table 2. Relative competition activity of various steroids for the binding of [^{3}H]-DHT to dog plasma DHT binding protein

Competing steroids	Relative activity*
17β-Hydroxy-5α-androstane-3-one (DHT)	100
17β-Hydroxy-17α-methyl-estra-4,9,11-trien-3-one (R1881)	88
Testosterone	42
5α-Androstane-3β, 17β-diol	35
5α-Androstane-3α, 17β-diol	13
17β-Hydroxy-4-estren-3-one (19-nortestosterone)	7.0
Estradiol-17β	2.5
Estrone	< 1.0
4-Androstene-3, 17-dione	< 1.0
Progesterone	< 1.0
Cortisol	< 1.0
Diethylstilbestrol	< 1.0

* Diluted plasma was incubated with [^{3}H]-DHT in the presence of increasing concentrations (3.4 nM to 3.4 μM) of competing steroids. The amount of each steroid needed to decrease the binding of [^{3}H]-DHT by 50% was determined from competition curves. Bound [^{3}H]-DHT was measured by charcoal assay.

DISCUSSION

Our study confirms and extends the previous results of Boesel *et al.*[3] on dog prostate androgen receptors and those of Carstensen *et al.*[6] on the plasma androgen binding protein. The properties of the two binding components are quite different in many respects. The prostate receptor appears to sediment exclusively in the 8–9S region of sucrose gradients and the plasma component in the 4S region. Their specificity towards various steroids is also markedly different. The dog plasma binding protein resembles analogous proteins of rabbit [12–15] and human [16–18] plasma although it has a lower affinity for DHT and testosterone which could explain the failure to detect it on polyacrylamide gel electrophoresis [7] and the absence of effect on the "metabolic clearance" rate of testosterone [19].

As mentioned earlier for the human, prostate contamination by plasma SSBP seriously interferes with androgen receptor determination when DHT is used as ligand. In fact, serum contamination appears to represent 50% of the total protein present [20]. In dog prostate, the concentration of this protein is not known. Even though DHT and R1881 are rapidly dissociated from dog plasma SSBP in the presence of low concentrations of charcoal (0.5%), it is estimated that SSBP could cause some interference in prostate androgen receptor determinations particularly if high concentrations of plasma were present. In fact, when a final concentration of 2.5% charcoal was used for 10 minutes, 10% of the original DHT binding activity of the plasma remained (results not shown). These charcoal conditions are similar to those we currently use for receptor assays. When dog prostate cytosol is incubated with [^{3}H]-DHT, in addition to the 8–9S receptor, a saturable 3–4S peak can be observed on sucrose gradients (Fig. 2). However this peak has a steroid specificity which is strikingly different from SSBP with respect to diethyl-stilbestrol competition. This experiment suggests that either dog SSBP is present only in low amounts in dog prostate or is not measurable under these conditions. It thus appears that [^{3}H]-DHT could be used in dog prostate androgen receptor assays without fear from contamination by plasma binding proteins. A small amount of unlabelled diethylstilbestrol should then be added to saturate the 4S binding peak which is not attributable to the androgen receptor or to SSBP [25]. However because of extensive metabolism of DHT during *in vitro* incubations, a synthetic androgen such as R1881 should be preferred to DHT.

Our studies and those of Boesel *et al.*[3] are in agreement on most of the properties of dog prostate androgen receptors. The competition experiments and affinity constants in both studies are quite similar when [^{3}H]-R1881 is used as ligand probe. However some differences are also apparent. The concentration of binding sites in our study is higher. The use of 72 h instead of 24 h castrated dogs probably explains the lower concentration of binding sites obtained by the group of Boesel. In fact, several groups of workers have described a very substantial decrease of androgen receptors in rat prostate 3–4 days after castration [21–23]. *A priori*, a similar phenomenon could occur in dog prostate. However a major difference concerns the obtention of 3–4S binding peaks after incubation of the cytosol with [^{3}H]-DHT. We think that the relative absence of such a peak in the study of Boesel *et al.*[3] is due to the treatment of the cytosol with a charcoal mixture containing ethanol. In a previous paper [24], they have shown that the addition of ethanol eliminated the 4S binding peak in rat prostate cytosol. We then assume that such a treatment is susceptible to remove hormones bound with lower affinity to binding proteins and receptors. However the omission of ethanol in our case allowed us to clearly differentiate the binding of the commonest testosterone metabolites in dog prostate. The conclusions can be summarized as follows. Among the natural androgens, only DHT and T bind to the 9S receptor. The major metabolite of DHT during *in vitro* incubations, 5α-androstane-3β, 17β-diol is bound in the 3–4S region of sucrose gradients to a different component, the nature of which will be precised in the following paper [25]. Finally, 5α-androstane-3α, 17β-diol does not appear to bind to a significant extent to the 9S receptor. However, since it is readily interconvertible with DHT both *in vitro* and *in vivo*, it can contribute to the formation of 9S receptors. This interconversion could have important implications in androgen action in dog prostate in view of the recent findings of Walsh and Wilson[5] who have shown that 5α-androstane-3α, 17β-diol alone or in combination with estradiol can induce prostatic hypertrophy in the dog.

The finding of an important conversion of DHT into 5α-androstane-3β, 17β-diol in dog prostate is somewhat different from what one finds in other androgen target tissues (rat prostate, epididymis and muscles) where 5α-androstane-3α, 17β-diol is the major metabolite [26–28]. Preferential formation of the 3β epimer over the 3α epimer in dog prostate was also obtained after *in vivo* infusion of testosterone [29]. The binding of DHT in the 9S peak and of its metabolite, 5α-androstane-3β, 17β-diol, in the 3–4S peak is clearly different from the binding of 5α-androstane-3α, 17α-diol [4]. The latter steroid shows very little competition activity for the 9S or 3–4S binding (Fig. 3).

Our study further illustrates the usefulness of R1881 in androgen receptor studies as previously shown for rat prostate by Bonne and Raynaud[11]. In the case of the human prostate, the situation is different since R1881 binds almost entirely to a progestin binding component [30–33]. Fortunately, the castrated dog prostate seems to possess little of this component since competition experiments (Table 2) indicate that R1881 is highly specific for androgens and not for progesterone. However, in the second paper of this series [25], we will show that this progestin binding component also exists in dog prostate. It is present in low amounts under basal conditions and it does not interfere with androgen receptor determinations.

The examination of the concentration of androgen receptor sites in prostates of various sizes from castrated dogs reveals that there is no apparent differences between normal and hypertrophic prostates. While this manuscript was in preparation, similar results have been published by Shain and Boesel[34]. In conclusion, we can say that there is no pathologic changes in cytosolic androgen receptor concentrations in hypertrophic prostates. The causes of canine prostate hypertrophy will probably be explained by other factors such as increased production of some steroid metabolites, changes in androgen receptor processing or modifications in other hormone receptors.

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REFERENCES

1. Gloyna R. E., Siiteri P. K. and Wilson J. D.: Dihydrotestosterone in prostatic hypertrophy. II. The formation and content of dihydrotestosterone in the hypertrophic canine prostate and the effect of dihydrotestosterone on prostate growth in the dog. *J. clin. Invest.* **49** (1970) 1746–1753.
2. Lloyd J. W., Thomas J. A. and Mawhinney M. G.: Androgens and estrogens in the plasma and prostatic tissue of normal dogs and dogs with benign prostatic hypertrophy. *Invest. Urol.* **13** (1975) 220–222.
3. Boesel R. W., Klipper R. W. and Shain S. A.: Identification of limited capacity androgen binding components in nuclear and cytoplasmic fractions of canine prostate. *Endocr. Res. Commun.* **4** (1977) 71–84.
4. Evans C. R. and Pierrepoint C. G.: Demonstration of a specific cytosol receptor in the normal and hyperplastic canine prostate for 5α-androstane-3α, 17α-diol. *J. Endocr.* **64** (1975) 539–548.
5. Walsh P. C. and Wilson J. D.: The induction of prostatic hypertrophy in the dog with androstanediol. *J. clin. Invest.* **57** (1976) 1093–1097.
6. Carstensen H., Amer I., Södergard R. and Hietala S. O.: Testosterone binding capacity in relation to the production and metabolism of testosterone in dogs, experiences of a new method. *J. steroid Biochem.* **5** (1974) 757–766.
7. Corvol P. and Bardin C. W.: Species distribution of testosterone-binding globulin. *Biol. Reprod.* **8** (1973) 277–282.
8. DeMoor P., Verhoeven G. and Heyns W.: A comparative study of the androgen receptor apparatus in adult rodents. *J. steroid Biochem.* **6** (1975) 437–442.
9. Tremblay R. R., Dubé J. Y., Ho-Kim M. A. and Lesage R.: Determination of rat muscles androgen–receptor complexes with methyltrienolone. *Steroids* **29** (1977) 185–195.
10. Bullock L. P. and Bardin C. W.: Androgen receptors in mouse kidney: a study of male, female and androgen insensitive (*tfm/y*) mice. *Endocrinology* **94** (1974) 746–756.
11. Bonne C. and Raynaud J. P.: Methyltrienolone, a specific ligand for cellular androgen receptors. *Steroids* **26** (1975) 227–232.
12. Rosner W. and Darmstadt R. A.: Demonstration and partial characterization of a rabbit serum protein which binds testosterone and dihydrotestosterone. *Endocrinology* **92** (1973) 1700–1707.
13. Ho-Kim M. A., Dubé J. Y. and Tremblay R. R.: Caractérisation partielle et mesure d'une protéine sérique liant les androgènes chez le lapin. *L'Union médicale du Canada* **104** (1975) 352–355.
14. Hansson V., Ritzen E. M., French F. S., Weddington S. C., Nayfeh S. N., Reusch E. and Attramadal A.: Demonstration of a specific binding protein for testosterone and 5α-dihydrotestosterone in rabbit serum. Separation from the corticosteroid binding globulin (CBG). *Steroids* **22** (1973) 185–192.
15. Danzo B. J. and Eller B. C.: Steroid binding proteins in rabbit plasma: Separation of testosterone binding globulin (TeBG) from corticosteroid binding globulin (CBG), preliminary characterization of TeBG, and changes in TeBG concentration during sexual maturation. *Molec. cell. Endocr.* **2** (1975) 351–368.
16. Rosner W.: A simplified method for the quantitative determination of Testosterone-estradiol Binding Globulin activity in human plasma. *J. clin. Endocr. Metab.* **34** (1972) 983–988.
17. Hansson V., Larsen J. and Reusch E.: Physicochemical properties of the 5α-dihydrotestosterone binding protein in human male serum. *Steroids* **20** (1972) 555–574.
18. Vermeulen A. and Verdonck L.: Studies on the binding of testosterone to plasma. *Ann. Endocr. (Paris)* **29**, *Suppl.* **29** (1968) 1–149.
19. Chapdelaine A.: Sites of *in vitro* extraction and interconversion of testosterone and androstenedione in dogs. *J. clin. Invest.* **48** (1969) 2063–2073.
20. Wagner R. K., Characterization and assay of steroid hormone receptors and steroid-binding serum proteins by agargel electrophoresis at low temperature. *Hoppe-Seyler's Z. Physiol. Chem.* **353** (1972) 1235–1245.
21. Sullivan J. N. and Strott C. A.: Evidence for an androgen-independent mechanism regulating the levels of

receptor in target tissue. *J. biol. Chem.* **248** (1973) 3202–3208.
22. Bruchovsky N. and Craven S.: Prostatic involution: Effect on androgen receptors and intracellular androgen transport. *Biochem. biophys. Res. Commun.* **62** (1975) 837–843.
23. Blondeau J. P., Corpéchot C., Le Goascogne C., Baulieu E. E. and Robel P.: Androgen receptors in the rat ventral prostate and their hormonal control. *Vitam. Horm.* **33** (1975) 319–345.
24. Boesel R. W. and Shain S. A.: A rapid, specific protocol for determination of available androgen receptor sites in unfractionated rat ventral prostate cytosol preparations. *Biochem. biophys. Res. Commun.* **61** (1974) 1004–1011.
25. Dubé J. Y., Lesage R. and Tremblay R. R.: Estradiol and progesterone receptors in dog prostate cytosol. *J. steroid Biochem.* **10** (1979) 459–466.
26. Taurog J. D., Moore R. J. and Wilson J. D.: Partial characterization of the cytosol 3α-hydroxysteroid: NAD $(P)^+$ oxidoreductase of rat ventral prostate. *Biochemistry* **14** (1975) 810–817.
27. Djoseland O., Hansson V. and Haugen H. N.: Androgen metabolism by rat epididymis 2. Metabolic conversion of [^{3}H]-testosterone *in vitro*. *Steroids* **23** (1974) 397–410.
28. Dionne F. T., Dubé J. Y. and Tremblay R. R.: Apparent saturability of a 4S dihydrotestosterone-binding protein in rat muscle cytosol: role of 3α-hydroxysteroid dehydrogenase and albumin. *Can. J. Biochem.* **55** (1977) 995–1000.
29. Morfin R. F., Aliapoulios M. A., Chamberlain J. and Ofner P.: Metabolism of testosterone-4-^{14}C by the canine prostate and urinary bladder *in vivo*. *Endocrinology* **87** (1970) 394–405.
30. Asselin J., Labrie F., Gourdeau Y., Bonne C. and Raynaud J. P.: Binding of [^{3}H]-methyltrienolone (R1881) in rat prostate and human benign hypertrophy (BPH). *Steroids* **28** (1976) 449–459.
31. Dubé J. Y., Chapdelaine P., Tremblay R. R., Bonne C. and Raynaud J. P.: Comparative binding specificity of methyltrienolone in human and rat prostate. *Horm. Res.* **7** (1976) 341–347.
32. Cowan R. A., Cowan S. K. and Grant J. K.: Binding of methyltrienolone (R1881) to a progesterone-like component of human prostatic cytosol. *J. Endocr.* **74** (1977) 281–289.
33. Menon M., Tananis C. E., Hicks L., Hawkins E. F., McLaughlin M. C. and Walsh P. C.: Characterization of the binding of a potent synthetic androgen, methyltrienolone, to human tissues. *J. clin. Invest.* **61** (1978) 150–162.
34. Shain S. A. and Boesel R. W.: Androgen receptor content of normal and hyperplastic canine prostate. *J. clin. Invest.* **6** (1978) 654–660.