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A COMPARATIVE STUDY OF ANDROGEN BINDING IN RAT UTERUS AND PROSTATE

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SUMMARY

The preferential accumulation of DHT in rat prostate and of T in rat uterus, is not based on the presence of androgen receptors with different properties, since these proteins have about the same concentration and specificity in both organs. Indeed, the apparently lower binding of DHT in uterus cytosol, is due to extensive metabolism of this steroid during incubation at 0°C. Very marked differences exist, on the other hand, with regard to non-receptor binding. In uterus cytosol this is due to a remarkably high concentration of serum albumin, whereas prostate cytosol contains a Prostatic Binding Protein, whose properties are described in this study. This protein exists also in rat prostatic fluid.

INTRODUCTION

Androgen-dependent organs, such as rat prostate, contain low concentrations of androgen-binding proteins with a high affinity for these steroids and there is increasing evidence that these proteins have a "receptor" function [1]. More recently, similar binding proteins were detected in a number of other organs, which are influenced to some degree by androgens, such as kidney [2], muscle [3], uterus [4] and submaxillary gland [5]. In some of these organs, however, the androgen "receptors" may have a different steroid-binding specificity, with preferential testosterone (T) binding, in the uterus [4, 6] for instance, as opposed to preferential dihydrotestosterone (DHT) binding in the prostate. The possible existence of DHT and T receptors with a different specificity led us to make a detailed comparison of the androgen receptors in rat uterus and rat prostate, since these organs accumulate respectively T and DHT during *in vivo* infusion of testosterone [7]. The results of this study, however, favour the hypothesis that the androgen receptors in both organs have intrinsically the same specificity. The observed differences of *in vivo* accumulation and *in vitro* binding may then be due to major differences in androgen metabolism and non-receptor binding [8]. With regard to the latter, the presence of a characteristic "prostate binding protein" (PBP) in prostate cytosol and prostatic fluid will be described in this study.

EXPERIMENTAL

Animals

Adult male and female Wistar rats were used in this study. For receptor studies male rats were castrated approx. 17 h before death.

Techniques

For most techniques we refer to the Materials and Methods section of a previous paper [7]. The following methods, however, were not described.

Measurement of "non-receptor" binding in prostate cytosol

After incubation of the samples for 3 h at 0°C with a high concentration (1.5 μM) of labeled steroid, an equal vol. of a suspension of Dextran-coated charcoal (40 mg/ml charcoal; 2 mg/ml Dextran T 70) was added. Twenty min later the samples were centrifuged at 0°C for 10 min at 4000 rev/min and the radioactivity in the supernatant was measured. In samples with a low concentration of protein the charcoal concentration was diminished to 10 mg/ml in order to reduce protein adsorption.

Ultracentrifugation was performed on linear 6 to 20% sucrose gradients in Tris–HCl buffer (50 mM, pH 7.4) or in 0.2 M KCl in the same buffer. The samples (0.2 ml) contained [^{14}C]-BSA (9) and were adjusted to the salt concentration of the gradient. The tubes were centrifuged in an MSE 6 × 5 ml Titanium Swing-Out rotor at 50,000 rev./min (240,000 g) at 4°C for 15 h.

Sephadex G-100 gel filtration

The dimensions of the gel bed were 84 × 1.5 cm., and the elution was performed with Tris–HCl buffer (50 mM; pH 7.4). A 1 ml sample was applied and fractions of 2 ml were collected.

Polyacrylamide gel electrophoresis on 5% and 10% acrylamide gels (5% cross linking) was executed in Tris–glycine buffer as described by Rodbard and Chrambach [10], with omission of the stacking gels.

Ion exchange chromatography

The sample (1 ml) in Tris–HCl buffer (50 mM, pH 7.4) was applied on a column (bed size 5 × 0.9 cm.) of DEAE-cellulose (Whatman DE 52) equilibrated with the same buffer and eluted in 40 ml of a linear 0 to 0.4 M KCl gradient.

Acetone precipitation and ether extraction were executed as described by Ichii [11].

RESULTS

1. *Androgen receptors in prostate and uterus cytosol*

Demonstration of high affinity testosterone binding in uterus cytosol. By incubation of uterus cytosol with various concentrations of testosterone, the presence of a binding protein with a high affinity for testosterone could be demonstrated, using different techniques for the separation of bound and unbound cytosol: gel filtration, Dextran-coated charcoal and precipitation of the receptor with protamine sulfate. The concentration of this testosterone receptor was estimated to be 66.5 ± 19.4 (S.D.) fmol/mg protein while the K_D was 1.1 ± 0.3 nM. In prostate cytosol the corresponding values were 43.3 fmol/mg protein and 1.2 nM.

Competition of various steroids with androgen binding in uterus and prostate cytosol. As shown in Table 1 a similar competition pattern exists for T binding in uterus cytosol and DHT binding in prostate cytosol. With regard to natural steroids the best competition is observed with DHT and T, followed by 5α-androstane-3β, 17β-diol and estradiol. 5α-Androstane-3α, 17β-diol is less competitive. Whereas progesterone is an intermediate competitor, 17α-hydroxyprogesterone has very little competitive effect; after acetylation at C_{17}, however, competition becomes very marked. The same phenomenon is observed with cyproterone acetate and medroxyprogesterone acetate.

Comparison of DHT and T binding in uterus cytosol. In prostate cytosol DHT binds better to the androgen receptor than testosterone [1]. In uterus cytosol, on the other hand, the binding of DHT appears to be weaker and more variable than the binding of T. This apparent difference in specificity is probably due, however, to an underestimation of DHT binding in uterus cytosol. The observation, that the dissociation of bound T occurs at an approx. 10 times faster rate (0.029 vs 0.0024 min^{-1} at 25°) than that of bound DHT forms an indirect argument for stronger DHT binding and probably explains why DHT gives better results in ultracentrifugation experiments.

Table 1. Competition of various steroids with T-binding in uterus cytosol (I) and DHT-binding (II) in prostate cytosol

Steroid	Concentration for 50% competition	
	I	II
testosterone	1.1	3.3
5α-dihydrotestosterone	4.3	2.0
5α-androstane-3α, 17β-diol	39	35
5α-androstane-3β, 17β-diol	7.2	9.9
estradiol	6.1	12
17α-OH-progesterone	140	250
17-acetoxyprogesterone	5.4	7.2
cyproterone	22	31
cyproterone acetate	2.6	4.1
medroxyprogesterone acetate	2.2	1.4

A more direct proof of intrinsically stronger DHT binding is found in the effect of charcoal pretreatment of cytosol, which produces a weak increase in T binding (K_D from 0.9 to 0.8 nM) but a marked increase of DHT binding (K_D from 2.1 to 0.4 nM).

Two factors probably play a role in the relative underestimation of DHT binding in uterus cytosol, since they influence the availability of the steroid for the receptor: the pronounced metabolism of DHT in uterus cytosol during incubation at 0°C and the effect of non-receptor binding. The significance of these factors will be discussed in other sections of this study.

Other physico-chemical properties of the androgen "receptors" in uterus and prostate. For the study of these parameters, uterus or prostate cytosol was incubated for 3 h at 0°C with a low concentration (approx. 0.2 nM) of [^{3}H]DHT. Thereafter unbound or weakly bound radioactivity was removed by charcoal treatment; the supernatant "bound fraction" of this technique was then submitted to various procedures.

Ammonium sulfate precipitation. In uterus cytosol the largest part of the "bound fraction" is precipitated at 40% saturation with ammonium sulfate. In prostatic cytosol, on the other hand, a biphasic precipitation curve is obtained, with a first precipitation zone, similar to the one seen in uterus cytosol, followed by a second one at higher salt concentrations (>50% saturation). The latter is probably due to non-receptor binding, as will be discussed in a following section.

Protamine sulfate precipitation. The "bound fraction" is precipitated from uterus cytosol at a concentration of protamine sulfate of approx. 50 μg/ml. In prostate cytosol somewhat more protamine sulfate is needed for precipitation. In both cases the necessary amount of protamine sulfate increases proportionally with the concentration of cytosol.

Ultracentrifugation. In low salt gradients the "bound fraction" of uterus cytosol shows a narrow peak coinciding with BSA (4.6 S) and a wide peak with a maximum at approx. 8 S. In prostate cytosol two similar peaks are found at respectively, 3.8 S and 9 S. In addition there is a large zone of radioactivity expanding from the origin to the 3.8 S peak. When the same experiments are performed on 0.2 M KCl gradients, the 8 and 9 S peaks disappear in favour of the peaks with lower S-values.

2. *Androgen metabolism in uterus and prostate*

Very marked differences exist in androgen metabolism in rat uterus and prostate. Whereas the prostate contains a high 5α-reductase activity situated primarily in the particulate fraction [12], this activity is low in the uterus [13]. Uterus cytosol, on the other hand contains a high 3α-hydroxysteroid dehydrogenase activity, which produces more than 50% reduction of DHT after 30 min of incubation at 0°C without addition of coenzyme [7]. This activity increases approx. 80-fold upon addition of NADPH. Enzymatic characteristics measured at 0°C give a K_M of 6.5 × 10^{-8} M

and a V_{max} of 10.9 nmol/g protein/min. At 37°C the corresponding values are 1.9×10^{-7} M and 222 nmol/g protein/min.

3. *Non-receptor binding in uterus cytosol*

In this organ the non-receptor binding of androgens is primarily due to contamination of cytosol with serum albumin. Indeed, uterus cytosol contains a marked electrophoretic band coinciding with serum albumin on 5, 7.5 and 10% acrylamide gels. Measurement of serum albumin by radial immonodiffusion [14] gives a value of 2.3 mg/ml for a total protein concentration of 7.9 mg/ml. This concentration does not change markedly by perfusing the tissues with saline before homogenisation. Androgens bound to albumin dissociate almost completely during charcoal treatment, in contrast to non-receptor binding in rat prostate.

4. *Non-receptor binding in prostate cytosol: a "Prostatic Binding Protein" (PBP)*

Rat prostate cytosol contains a small amount of serum albumin (approx. 0.2 mg/ml) but the non-receptor binding in this organ is primarily due to a protein with characteristic binding properties. Since we found this protein only in rat prostate we call it "prostatic binding protein" (PBP) in this study.

(*a*) *Steroid binding properties of Prostatic Binding Protein (PBP)*

Charcoal resistant binding. In contrast to androgen binding to albumin, the non-receptor binding of DHT and various other steroids in prostate cytosol resists charcoal treatment, when this procedure is performed at 0°C. Indeed, when non-receptor binding of DHT is measured by the charcoal technique (see Experimental) 10–20% of the radioactivity remains in the supernatant. This is not due to an inefficient removal of unbound or loosely bound steroid, but to a special form of protein-bound steroid. Indeed, when the charcoal procedure is repeated on the supernatant, 70 to 80% of the ratioactivity remains in the bound fraction. Prolongation of the contact time with charcoal results in a very slow decrease of bound radioactivity. At higher temperatures (22°C), however, this dissociation occurs much more rapidly.

Steroid specificity. Such charcoal-resistant binding is not only observed with DHT, but several other steroids (testosterone, estradiol, progesterone) are bound to a similar degree (10–20% binding), whereas the binding is about twice as high for androstenedione and pregnenolone. The binding is negligible (< 1%) for 5α-androstane-3β, 17β-diol and 17α-hydroxyprogesterone.

Tissue specificity. The presence of similar charcoal-resistant binding, observed at high steroid concentrations was investigated in various other tissues. Whereas in prostate cytosol 23.1% of androstenedione was bound, this value varied from 0.1 to 0.9% in cytosol from kidney, uterus, liver, seminal vesicles, submaxillary gland and in diluted hemolysed rat blood or plasma at similar protein concentrations (13 to 22 mg/ml).

Table 2. Binding characteristics of PBP in prostate cytosol and prostatic fluid*

Conditions Sample	Steroid	Concentration μM	K_A 10^6 M^{-1}
prostate cytosol			
nl	pregnenolone	1.0	0.6
acetone-treated	pregnenolone	17.2	2.1
nl	androstenedione	2.5	0.1
acetone-treated	androstenedione	33.0	1.1
prostatic fluid			
nl	pregnenolone	28.0	0.5
acetone-treated	pregnenolone	101.0	6.1

* Measured by the charcoal technique after incubation for 3 h at 0°C with different concentrations of steroid.

Effect of temperature. An increase of temperature results in a marked increase of dissociation. For this reason the charcoal step has to be performed at 0°C. Binding, however, increases markedly when the incubation with steroid is performed at higher temperatures. DHT binding, for instance, increased from 10.9% at 0°C to 39.0% at 37°C. Finally, the binding is relatively resistant to heating up to 60°C.

Effect of acetone precipitation. It has been proposed [11] that acetone precipitation increases androgen-receptor binding in prostate cytosol. The observed increase in binding is most probably not due to receptor binding, which in our hands disappears during acetone treatment, but to an increased binding to PBP. Indeed, this procedure increases as well as the apparent concentration and the apparent affinity of PBP (Table 2).

(*b*) *Other physico-chemical characteristics*

Ammonium sulfate precipitation. As opposed to receptor-bound steroid, the precipitation of PBP-bound steroid occurs at salt concentrations higher than 50% saturation.

Gel filtration on Sephadex G-100. After gel filtration of prostate cytosol, the non-receptor binding activity is detected in the eluate as a single peak, following 4 fractions (8 ml) behind added [^{14}C]-BSA. The position of this peak corresponds to an estimated molecular weight of 50,000.

Ultracentrifugation. When sucrose gradient ultracentrifugation is performed on cytosol incubated with labeled steroid (1.5 μM), PBP does not show up as a clearcut peak with androstenedione or DHT, but, rather as a trail of radioactivity from the origin to the 4 S-region; with pregnenolone, however a 3.7 S peak is observed. When the same procedure is executed with acetone-extracted cytosol, a single and marked 3.7 S peak is observed with all three steroids. A single peak at 3.7 S is also observed when binding activity is measured in all fractions after ultracentrifugation of untreated prostate cytosol.

Ion exchange chromatography. When prostate cytosol is applied on a DEAE-cellulose column PBP is

retained on the column and eluted as a single major peak of binding activity at approx. 0.2 M KCl; concomitantly there is a parallel change of optical density at 280 nM.

Polyacrylamide gel electrophoresis. On 5% acrylamide gels in Tris–glycine [10] PBP coincides with rat serum albumin, but it precedes albumin on more concentrated gels. On 10% gels, for instance, the relative mobility is 1.3.

(c) *PBP in prostatic fluid*

Since rat prostate has a very acinar structure, filled with secretory material [15] the presence of PBP in rat prostatic fluid obtained by the method of Levy and Fair [16] was investigated. This fluid contained a protein band coinciding with PBP on 5, 7.5 and 10% acrylamide gels. Furthermore, the typical non-specific binding, increasing after acetone treatment was present at high concentration (Table 2).

DISCUSSION

The data of the present study favour the hypothesis that the androgen receptors of rat uterus and prostate have a similar specificity. Indeed, the preferential binding of T, observed in some studies [4, 6] is probably the result of intensive DHT metabolism occurring during the incubation *in vitro* [7]. This metabolism of DHT in the uterus, and the presence of 5α-reductase activity in the prostate [12] but not in the uterus [13] may also explain the selective accumulation of T in the uterus and of DHT in the prostate observed during *in vivo* infusion of T [7].

Although the other characteristics of the androgen receptors, such as protamine sulfate precipitation curves and ultracentrifugation patterns are not completely identical. This probably does not indicate that the androgen receptors of both organs are different proteins. Indeed, it seems more likely that this reflects differences in environment, such as the concentration of other proteins, which interact with protamine sulfate or which favour the formation of aggregates. With this respect, it is of interest to note that addition of prostate cytosol, immediately before ultracentrifugation results in a shift of the 8 S peak in uterus cytosol labeled with DHT to the 9 S position.

With regard to non-receptor binding, we found a surprisingly high concentration of serum albumin in this organ. This protein is probably localized extravascularly, as shown by autoradiographic localization of [^{131}I]-albumin [17]. This extravascular pool of plasma protein in the uterus may be of some relevance also with regard to the presence of other plasma steroid-binding proteins in the uterus [19].

In prostate cytosol, on the other hand, the non-receptor binding is due to a characteristic Prostatic Binding Protein (PBP). The concentration of the latter is surprisingly high (Table 2). Indeed, assuming 1 site per molecule and a molecular weight of 50,000 the concentration of PBP, measured after acetone extraction corresponds to a value of 0.8 to 1.6 mg/ml on approx. 10% of cytosolic protein. The concentration of PBP is even higher in prostatic fluid (Table 2) but the total protein concentration also is more elevated. The steroid binding properties of PBP are remarkable. Indeed, although the apparently equilibrium constant of dissociation is rather high the dissociation rate is low, when measured at 0°C. Furthermore, binding increases at higher temperature, as opposed to binding to plasma steroid binding proteins. It is conceivable that this uncommon binding behaviour and also the marked increase in binding after acetone treatment are due to occupation of the binding sites of the protein by unknown material.

With regard to its other characteristics, PBP is a protein with a 3.7 S mobility on ultracentrifugation, a molecular weight estimated by gel filtration of 50,000 and the electrophoretic mobility of serum albumin on PAGE (5% acrylamide gels); it is eluted at 0.2 M KCl from DEAE-cellulose and precipitated by high concentrations of ammonium sulfate. Some of these properties are very similar to those described for the "pregnenolone receptor" by Karsznia *et al.* [19] and for the α-protein or complex I of Liao and Fang [20], who may have studied the same protein.

The significance of PBP is unknown. It is conceivable, in view of the steroid-binding properties, that PBP plays a role in the uptake, accumulation or storage of androgens and other steroids by the prostate. The presence of a high concentration of PBP in prostatic fluid, on the other hand, suggests that PBP is primarily a quantitatively important secretory product of the prostate, with a possible function in reproductive physiology.

REFERENCES

1. King R. J. B. and Mainwaring W. I. P.: *Steroid–Cell Interactions.* Butterworth, London (1974).
2. Bullock L. P. and Bardin C. W.: *Endocrinology* **94** (1974) 746–756.
3. Jung I. and Baulieu E. E.: *Nature New Biol.* **237** (1972) 24–26.
4. Giannopoulos G.: *J. biol. Chem.* **248** (1973) 1004–1010.
5. Verhoeven G. and Wilson J. D.: *Endocrinology* **98** (1976) 79–92.
6. Rochefort H. and Lignon F.: *Eur. J. Biochem.* **48** (1974) 503–512.
7. Heyns W., Verhoeven G. and De Moor P.: *J. steroid Biochem.* **7** (1976) 335–343.
8. Verhoeven G., Heyns W. and De Moor P.: *Vit. Horm.* **33** (1975) 265–280.
9. Rice R. H. and Means G. E.: *J. biol. Chem.* **246** (1971) 831–832.
10. Rodbard D. and Chrambach A.: *Analyt. Biochem.* **40** (1971) 95–134.
11. Ichii S.: *Endocr. japon.* **22** (1975) 433–438.
12. Wilson J. D. and Gloyna R. E.: *Recent Prog. Horm. Res.* **26** (1970) 309–336.
13. Hoffmann U., Maass H. and Lisboa B. P.: *Eur. J. Biochem.* **59** (1975) 305–312.
14. Mancini G., Carbonara A. O. and Heremans J. F.: *Immunochemistry* **2** (1965) 235–254.

15. Neumann F., Richter K. D. and Senge Th.: *Vit. Horm.* **33** (1975) 103–132.
16. Levy B. J. and Fair W. R.: *Invest. Urol.* **11** (1973) 173–177.
17. Peterson R. P. and Spaziani E.: *Endocrinology* **89** (1971) 1280–1286.
18. Rosenthal H. E., Paul M. A. and Sandberg A. A.: *J. steroid Biochem.* **5**, (1974) 219–225.
19. Karsznia R., Wyss R. H., Heinrichs W. R. and Hermann W. L.: *Endocrinology* **84** (1969) 1238–1246.
20. Fang S. and Liao S.: *J. biol. Chem.* **246** (1971) 16–24.

DISCUSSION

O'Malley. Dr. Liao, would you agree that this molecule is similar to the alpha protein?

Liao. Yes, they are similar. We do not know their role in the target cells, but it is interesting to point out that, in the rat ventral prostate, the α-protein fraction can inhibit the nuclear retention of the DHT–receptor complex.

Jungblut. You did precipitate with acetone?

Heyns. It is just precipitation of the proteins with cold acetone, followed by ether extraction of the precipitate, which is then redissolved in buffer. This is a method described by Ichii (*Endocrinol. Jap.* **5** (1975), 433–437) in a paper on androgen-receptor binding in rat prostate.

O'Malley. Would you conclude that there is only one androgen receptor in the rat?

Heyns. Yes, I think that the testosterone receptor in the uterus and the DHT receptor in the prostate are the same protein.

O'Malley. This appears to be a contradiction to the Ohno theory in which he examined mutations of the *tfm* gene locus in mice and rats which is supposedly a single gene mutation. In these mutants androgen response was blocked simultaneously in all cells, whether it be a response to T or DHT, and whether it be a secondary sex tissue response or an anabolic response. Could I ask an additional question. Could you summarize the differences between this protein and ABP?

Heyns. The concentration of PBP is much higher; I don't know exactly the characteristics of ABP. Maybe someone else can give them, but it seems to be different. Indeed, ABP has another sedimentation coefficient and the affinity of PBP for steroids is much lower than that of ABP, which is reported to have an affinity of $10^8 M^{-1}$ to $10^9 M^{-1}$.

Krieg. I don't want to give the characterization of the ABP, but you have no chance to assay the ABP on polyacrylamide gel quantitatively if you did not use steady state polyacrylamide gel technique. This has been clearly shown in the paper by Ritzén *et al.* (*J. biol. Chem.* **249** (1974) 6597) where you can find also other characteristics of ABP.

Heyns. You see PBP-bound radioactivity on polacrylamide gel with acetone-treated cytosol. You also see the protein peak, since it is more than 10% of the protein in prostate cytosol. This protein peak has different mobilities at different concentrations of acrylamide.

Mainwaring. I am very interested, that you are labelling PBP with androstenedione and it would be interesting to compare the properties of your protein with that which has been recently described by Gustaffson in Sweden which was also labeled by androstenedione.

Heyns. That was in liver, but in liver I did not find much binding activity.

Mainwaring. But are the other physical properties of the proteins totally different? That's really my question.

Heyns. I do not know precisely the characteristics of this protein.

Krieg. Dr. Heyns, you have just reported that 5α-androstane-3β,17β-diol competes well with DHT for the cytostolic binding. How could you explain that, because we found in the prostate cytosol no specific binding with tritiated 5α-androstane-3β,17β-diol at all (*J. Endocr.* **64** (1975) 529).

Heyns. This was done with unlabeled 3β-diol in competition with DHT binding. One possibility is that the 3β is metabolized to DHT but I don't think that's the case. Maybe it's also a question of affinity.

Krieg. In our *in vivo* studies there is only very little metabolism of 5α-androstane-3β,17β-diol to DHT. Therefore, I agree with you that the metabolism cannot be the reason for this competition.

Heyns. It could also be a question of affinity that you don't have an affinity high enough to show up as binding activity but enough to show up as competition.