Journal of Steroid Biochemistry, 1976, Vol. 7, pp. 1005–1012. Pergamon Press. Printed in Great Britain

IN VITRO BINDING AND METABOLISM OF ANDROGENS IN VARIOUS ORGANS: A COMPARATIVE STUDY

M. KRIEG and K. D. VOIGT

Second Medical Clinic, Department of Clinical Chemistry, University of Hamburg, Martinistraße 52, 2000 Hamburg 20, F.R.G.

SUMMARY

A comparative study of *in vitro* binding and metabolism of 5α-dihydrotestosterone (5α-DHT), testosterone (T) and 19-nortestosterone (19-Nor-T) in the prostate (PR), bulbocavernosus/levator ani (BCLA) and skeletal muscle (SM) of the rat was performed. After the homogenate of the organs was incubated at 0°C for 2 h, the 100,000 *g* cytosol was processed. Binding was analyzed by agargel electrophoresis at low temperature and androgen metabolism by t.l.c.

The overall physico-chemical characteristics of the androgen receptor are similar if not identical in the PR, BCLA, and SM. The dissociation constants being within the organs different, in the prostate K_D(19-Nor-T) < K_D(5α-DHT) < K_D(T) and in the BCLA K_D(5α-DHT) < K_D(19-Nor-T) < K_D(T). The range being 7×10^{-10} M–4×10^{-9} M. Significant differences were found between the available androgen binding sites expressed as fmol/mg cytosol protein: being 170 in PR, 24 in BCLA and 2 in SM. In the PR after 5α-DHT incubation at 0°C, 26% of the extracted radioactivity was found as 5α-androstanediols, while after T incubation 10% was converted to 5α-DHT and 6% to 5α-androstanediols. In the BCLA and SM only small amounts of 5α-DHT were metabolized to 5α-androstanediols (5–8%), and no conversion of T to 5α-DHT was measurable.

In conclusion, no different physico-chemical characteristics of the androgen receptor of the PR, BCLA and SM, but rather differences in the affinity of the receptor to various steroids, differences in metabolism of the steroids and especially differences in the amount of available androgen binding sites might be responsible for the different androgen dependency of PR, BCLA and SM.

INTRODUCTION

Our knowledge of the interaction of androgens with specific intracellular binding proteins has been derived mostly from the prostate [1, 2]. On the other hand the knowledge of the interaction of androgens with organs like the bulbocavernosus/levator ani (BCLA) or skeletal muscle, which show also a significant degree of androgen responsiveness [3–8], is relatively poor.

This prompted us to study in more detail and always in comparison with the prostate the binding behaviour of 5α-dihydrotestosterone (5α-DHT),* testosterone and the "anabolic" steroid 19-nortestosterone in the BCLA and skeletal muscle, i.e. the musculus quadriceps femoris. Because of the extensive metabolism of androgens in target organs, some metabolic studies are included in this investigation.

The findings indicate that the three organs differ in their androgen binding more quantitatively in terms of available binding sites than qualitatively in terms of physico-chemical characteristics of the binding protein. Discussed is a positive correlation between the extent of androgen action *in vivo* and the amount of androgen receptor protein available for a steroid, the cellular 5α-reductase activity and the degree of affinity of the receptor to the various androgens.

* The following trivial names and abbreviations are used: 5α-Androstanediols = 5α-androstane-3α,17β-diol + 5α-androstane-3β,17β-diol; BSA = bovine serum albumin; Cortisol = 11β,17,21-trihydroxy-4-pregnene-3,20-dione; Cyproterone acetate = 6-chloro-17-hydroxy-1α,2α-methylene-4,6-pregnadiene-3,20-dione acetate; 5α-Dihydrotestosterone (5α-DHT) = 17β-hydroxy-5α-androstan-3-one; 3α-diol = 5α-androstane-3α,17β-diol; Estradiol-17β = 1,3,5(10)-estratriene-3,17β-diol; 19-Nortestosterone = 17β-hydroxy-4-estren-3-one; 5α-Reductase = 3-oxo-5α-steroidΔ^4-dehydrogenase; Testosterone = 17β-hydroxy-4-androsten-3-one.

MATERIAL AND METHODS

Chemicals. [1,2,4,5,6,7-^{3}H]-5αDihydrotestosterone (S.A. 130 Ci/mmol) was obtained from Amersham Buchler (Braunschweig). [1,2-^{3}H]-5α-Dihydrotestosterone (S.A. 40 Ci/mmol) and [1,2,6,7-^{3}H]-testosterone (S.A. 85 Ci/mmol) were obtained from NEN Chemicals (Dreieichenhain). [6,7-^{3}H]-19-Nortestosterone (S.A. 29 Ci/mmol) was kindly provided by Organon International (Oss), as well as, antiserum raised in rabbits against 11α-OH-testosterone-11-succinyl-BSA. The radioactive solution was evaporated to dryness and the hormone redissolved in ethanol. Radiochemical purity was monitored by t.l.c. and was greater than 96%. Non-radioactive steroids were obtained from Merck AG (Darmstadt). Cyproterone acetate was generously supported by Schering AG (Berlin). Ribonuclease A (RNase), desoxyribonuclease (DNase), pronase and lipase were purchased from Serva (Heidelberg).

Animals. Male Wistar rats, weighing 340–400 g, were castrated 24 h before the experiments were started.

Tissue processing and labeling. BCLAs and ventral prostates of five rats and skeletal muscle, i.e. musculus quadriceps femoris, of three rats were pooled in each experiment. The tissue was pulverized in a mortar chilled in liquid nitrogen. The fine tissue powder was transferred to the centrifuge tubes and 1 vol. buffer (0.01 M Tris–HCl, 0.002 M EDTA, 0.005 M $NaNO_3$, 0.01 M $MgCl_2 \cdot 6\,H_2O$, 0.002 M 2-mercapto-ethanol, pH 7.4 at +2°C) was added to the BCLA and skeletal muscle and 2 vol. to the prostate. All procedures were carried out near 0°C. The thawed homogenate was labelled for 2 h at 0°C with various concentrations of tritiated steroids (2×10^{-8}–2×10^{-10} M). After the incubation, the homogenate was centrifuged at 35.000 rev/min for 1 h and the clear supernatant was defined as 100,000 *g* cytosol, in which the final concentration range of the tritiated steroids was 2×10^{-8} M–3×10^{-10} M.

Agargel electrophoresis. Agargel electrophoresis at low temperature was performed according to Wagner [9] and details have been published previously [10].

Estimation of the available binding sites and apparent dissociation constants (K_D). By convention, the difference between the tritiated steroid bound in the absence and presence of the excess unlabeled steroid is referred to as "specific" binding. Therefore, we determined the peak decrease in the anodic part of the gel (left from the start) obtained by adding in a parallel tube a 100-fold excess of the respective unlabeled steroid together with the tritiated one to the homogenate. From this binding difference we calculated the specifically bound hormone. The means of at least three experiments were plotted according to Scatchard [11], whereby total cytosolic tritiated steroid concentration was determined in an aliquot of the respective cytosol and the free fraction by subtracting the specifically bound fraction from total one. The dissociation constant (K_D) and the maximal binding sites were found by the intercepts of the straight line with the "bound" and "bound/free" axis.

Metabolic studies. Under identical conditions, as mentioned above for the binding studies, after 2 h of incubation at 0°C the homogenate of the organs was processed into the 100,000 *g* cytosol. The steroids were then extracted with ether and chloroform and separated by t.l.c. on silica gel in chloroform:acetone (9:1, v/v). To separate 5α-DHT from androsterone and epiandrosterone the respective fraction from the first chromatography was acetylated and then chromatographed on Al_2O_3G (type E) in cyclohexane:ethylacetate (9:1, v/v). Details have been reported previously [12].

Treatment of the cytosol with anti-testosterone antibody. The principle of the method was first described by Castañeda and Liao [13]. We used this method for the qualitative characterization of the steroid receptor complex: To 100 μl of the cytosol, which has been processed from homogenate incubated with tritiated steroids, 10 μl of a 1:50 diluted anti-testosterone antibody solution was added and incubated for 2 h at 0°C. The antibody, having a 30% cross reaction with 5α-DHT, was able to bind more than 90% of unbound or unspecifically bound testosterone or 5α-DHT. After incubation, antibody bound tritiated steroid was separated from receptor bound one by agargel electrophoresis, as the antibody migrated to the cathode while the receptor protein migrated to the anode.

Other methods. (1) Enzyme digestion: Pronase, lipase, DNase and RNase in a final concentration of 1 mg/ml cytosol were added to aliquots of the cytosol and incubated at 0°C for 90 min. (2) Protein concentration of the cytosol was measured by the biuret reaction. (3) Before measurement of radioactivity the gel slices were frozen in the counting vials in order to destroy the gel structure and to facilitate the elution of the radioactivity into the scintillation fluid. The elution was carried out for at least 2 h under gentle shaking. Analysis was performed in a Packard scintillation counter 3380 (efficiency 40%) without external standardization because of a constant AES ratio.

RESULTS

Common characteristics of the [^3H]-androgen binding in target organs

The well defined androgen binding peak shown in Fig. 1 can be demonstrated by agargel electrophoresis in the 100,000 *g* cytosol of the rat prostate and bulbocavernosus/levator ani (BCLA) with tritiated 5α-DHT, testosterone and 19-nortestosterone, while in the skeletal muscle this binding peak can be achieved only with highly labeled 5α-DHT and testosterone. The common characteristics of this binding are summarized in Table 1. The principles of point 6 are described in more detail under the Material and Methods section "Treatment of the cytosol with anti-testosterone antibody".

Androgen binding in the rat skeletal muscle cytosol

Analyzing the skeletal muscle cytosol (Fig. 2) of male and female rats, besides a specific 5α-DHT binding peak (II) which fulfills all points of Table 1, a further peak (I) is demonstrated constantly which is not displaceable by a 100-fold excess of unlabeled 5α-DHT and which does not display most of the common characteristics: only points 1, 5 and 9 of Table 1 hold true for this binding protein. Using highly labeled testosterone, a specific binding (peak II) can be demonstrated which is much smaller than the respective peak obtained with [^{3}H]-5α-DHT and which has therefore not been characterized quantitatively.

The high binding affinity and low binding capacity of peak II is shown in Figs. 3 and 4. Figure 3 shows that the maximal binding capacity is very low. The values have been obtained from four different experiments, whereby each point represents the binding difference of peak II before (= total binding) and after

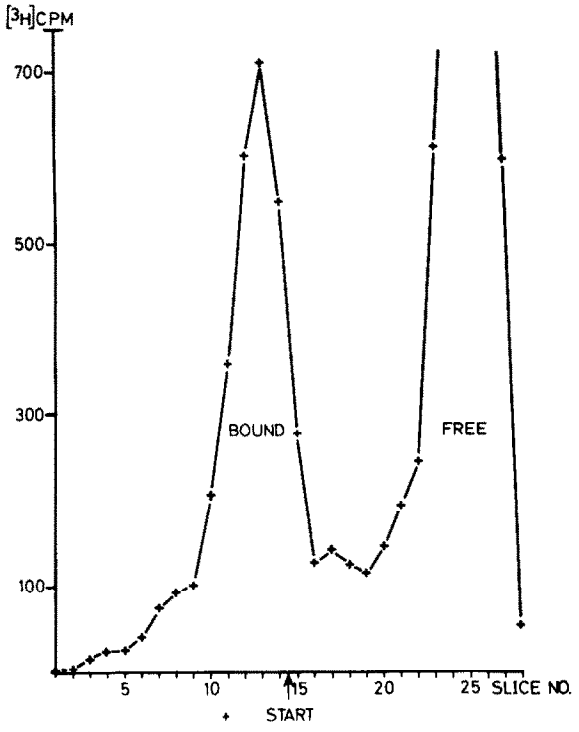

Fig. 1. Typical cytosolic binding pattern of rat androgen target organs, analyzed by agargel electrophoresis. Organ homogenate was incubated with the tritiated androgen for 2 h at 0°C. After processing the 100,000 *g* cytosol, 40 µl were applied between slice nos. 14 and 15 (arrow). Bound hormone migrates to the anode (left), unbound to the cathode (right). Radioactivity was measured in c.p.m./slice, each slice being 3 mm wide.

(= non-specific binding) adding a 100-fold excess of unlabeled 5α-DHT. Each experimental point is plotted in Fig. 4 according to Scatchard [11], and a regression line can be calculated ("bound/free" = 2.41–1.05 × "bound"; $r = 0.66$; $P < 0.01$) with a resulting K_D of 2.4×10^{-9} M and an intercept with the abscissa corresponding to 2.3×10^{-15} mol 5α-DHT-binding sites per mg cytosol protein.

Androgen binding in the bulbocavernosus/levator ani (BCLA) muscle cytosol

As shown in Fig. 5, a saturation of the cytosolic androgen receptor is obtained with tritiated 5α-DHT, 19-nortestosterone and testosterone. Each point is the mean of at least three different experiments. The corresponding Scatchard plots revealed straight lines (Fig. 6). The lowest apparent K_D (7.1×10^{-10} M) is found with 5α-DHT and the highest (1.7×10^{-9} M) with testosterone. The binding sites, derived by the intercepts of the straight lines with the abscissa, are in the same range of order for each steroid, amounting to 27 fmol/mg protein when calculated from the experiments with 5α-DHT.

Androgen binding in the prostate

Also in the prostate cytosol, the androgen receptor can be saturated with each of the three tritiated steroids used (Fig. 7). Starting the experiments, we added

Table 1. Common characteristics of the specific [^{3}H]-androgen binding peak found by agargel electrophoresis. The binding of Fig. 1 fulfilled each of the nine points

1. Migration to the anode
2. Migration distance of the peak maximum: ~4.5 mm
3. Destroying by temperature (45°C, 1 h)
4. Destroying by pronase (0°C, 1.5 h)
5. No destroying by lipase, DNase and RNase
6. No significant "stripping" of the binding by an antibody against testosterone and 5α-DHT
7. Displacement by low excess (50–100-fold) of various androgens
8. Displacement by high excess (1000-fold) of cyproterone acetate or estradiol-17β
9. No displacement by high excess of cortisol

increasing amounts of tritiated 5α-DHT in a relative low concentration range which resulted in a Scatchard plot as shown in the middle panel of Fig. 8. A straight line can be found only in the higher concentration range. Therefore, in the following experiments with 19-nortestosterone and testosterone, we used only higher steroid concentrations. Also in the prostate, the highest K_D is found with testosterone, while the lowest is obtained with 19-nortestosterone. The binding sites, derived by the intercepts of the straight lines with the abscissa, are in the same range of order for each steroid, amounting to 170 fmol/mg protein when calculated from the experiments with 5α-DHT.

Androgen metabolism in vitro *at* 0°C

Table 2 summarizes our study on androgen metabolism *in vitro* at 0°C. After a 2 h incubation of the

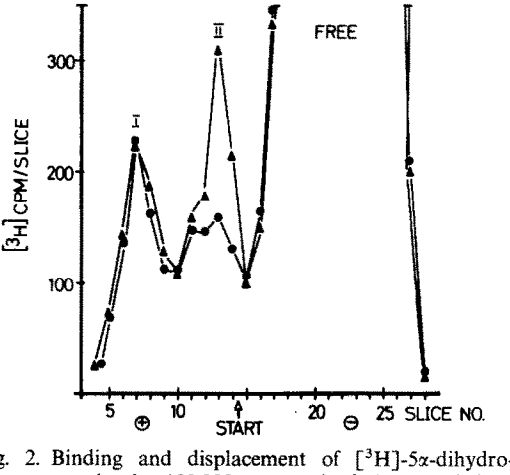

Fig. 2. Binding and displacement of [^{3}H]-5α-dihydrotestosterone in the 100,000 *g* cytosol of the rat skeletal muscle, analyzed by agargel electrophoresis. Pooled skeletal muscle homogenate of three rats was incubated for 2 h at 0°C with 2.2×10^{-8} M (final concentration) tritiated 5α-dihydrotestosterone alone (▲) or in the presence of a 100-fold excess of unlabeled 5α-dihydrotestosterone (●). After processing the cytosol, 40 µl were applied between slice nos. 14 and 15 (arrow). Bound hormone migrates to the anode (left), unbound to the cathode (right). Radioactivity was measured in c.p.m./slice, each slice being 3 mm wide.

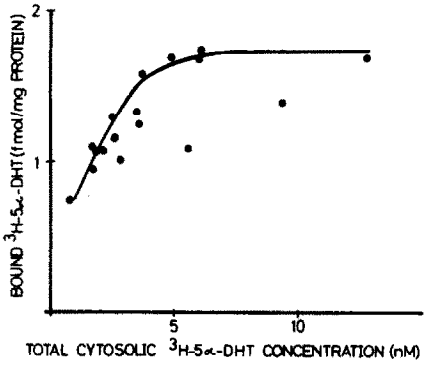

Fig. 3. Saturation of the rat skeletal muscle cytosol receptor, analyzed by agargel electrophoresis. Increasing amounts of [^{3}H]-5α-dihydrotestosterone (5α-DHT) were added to the homogenate. The concentration of the cytosolic [^{3}H]-5α-DHT was measured and plotted versus specifically bound [^{3}H]-5α-DHT, determined in the respective cytosol by subtracting from the totally bound the non-specifically bound 5α-DHT. Each experimental point from four different experiments is plotted.

organ homogenates with tritiated steroids, varying amounts of 5α-DHT are converted to 5α-androstanediols in the processed cytosol, being highest in the prostate while BCLA and skeletal muscle metabolize only small amounts of 5α-DHT to the 5α-androstanediols. When incubating with testosterone, a substantial amount is converted to 5α-DHT and 5α-androstanediols only in the prostate. Furthermore, when adding 5α-androstane-3α,17β-diol to the prostate, the metabolism to 5α-DHT is not as high as the conversion of 5α-DHT to the diols after adding 5α-DHT.

Comparison of androgenic endpoints

Table 3 and Fig. 9 compare the binding sites, K_Ds, 5α-reductase activity and qualitative binding characteristics found in the prostate, BCLA, and skeletal muscle. Most striking is the difference in the amount

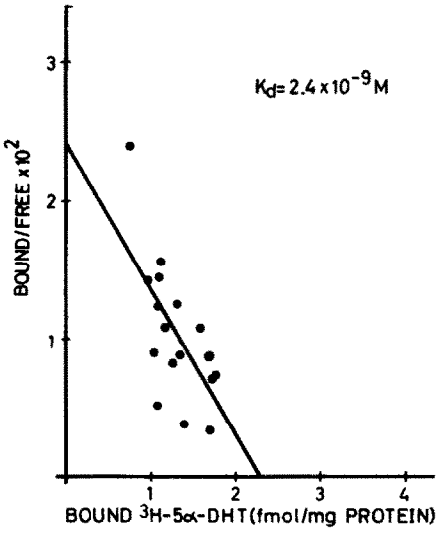

Fig. 4. Scatchard plot of the specific [^{3}H]-5α-dihydrotestosterone (5α-DHT)-binding in the rat skeletal muscle cytosol. The plot is derived directly from the saturation study of Fig. 3.

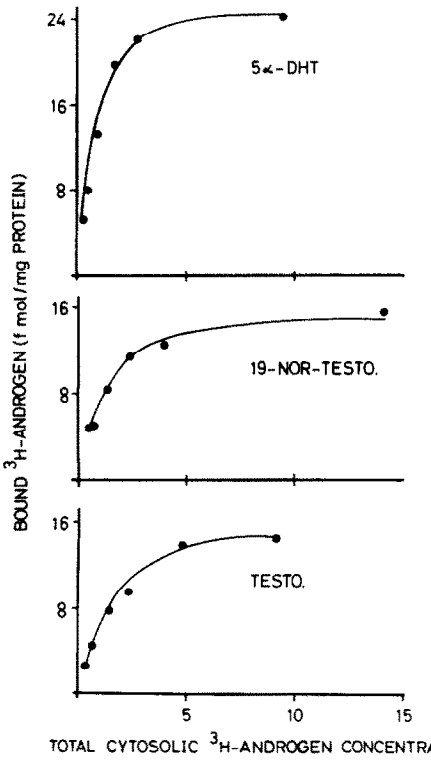

Fig. 5. Saturation of the bulbocavernosus/levator ani muscle cytosol receptor with tritiated 5α-dihydrotestosterone (5α-DHT), 19-nortestosterone (19-nor-testo) and testosterone (testo.), analyzed by agargel electrophoresis. Increasing amounts of the tritiated androgens were added to the homogenate. The concentration of the total cytosolic tritiated androgens was measured and plotted versus specifically bound tritiated androgens, determined in the respective cytosol by subtracting from the totally bound the non-specifically bound androgen. The points are means of at least three experiments.

of available binding sites and the fact that the K_D within one organ is, as far as investigated, always twice as low for 5α-DHT as for testosterone. Striking differences were found also in the 5α-reductase activity, being *in vitro* at 0°C practically absent in the BCLA and skeletal muscle while in the prostate even at 0°C conversion of testosterone to 5α-DHT occurred.

DISCUSSION

The data presented in this investigation indicate that the binding protein of three androgen dependent organs, i.e. the prostate, BCLA and skeletal muscle, shows similar if not identical qualitative binding characteristics when analyzed by agargel electrophoresis. Together with the facts that the three tritiated androgens 5α-DHT, testosterone and 19-nortestosterone exhibit similar high binding affinities and that each of them can be displaced by an excess of the other two, a common receptor protein for their actions in androgen dependent organs is assumed. Verhoeven *et al.* [14] came to the same conclusion when comparing the androgen binding behaviour of

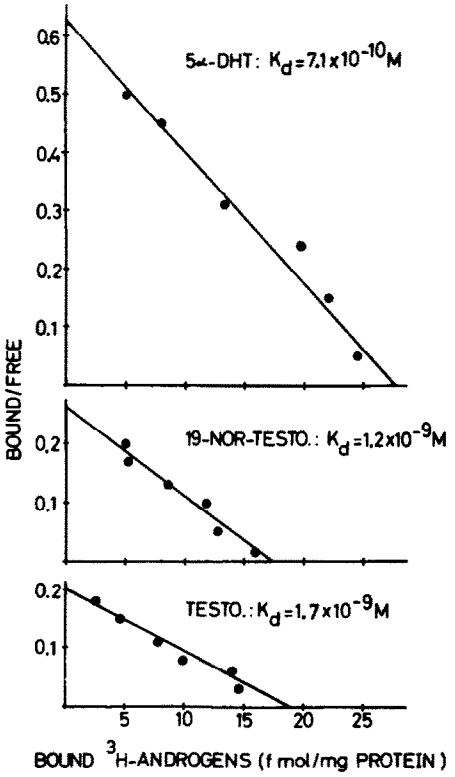

Fig. 6. Scatchard plots of the specific binding of tritiated 5α-dihydrotestosterone (5α-DHT), 19-nortestosterone (19-nor-testo.) and testosterone (testo.) in the rat bulbocavernosus/levator ani muscle cytosol. The plots were derived directly from the saturation study of Fig. 5.

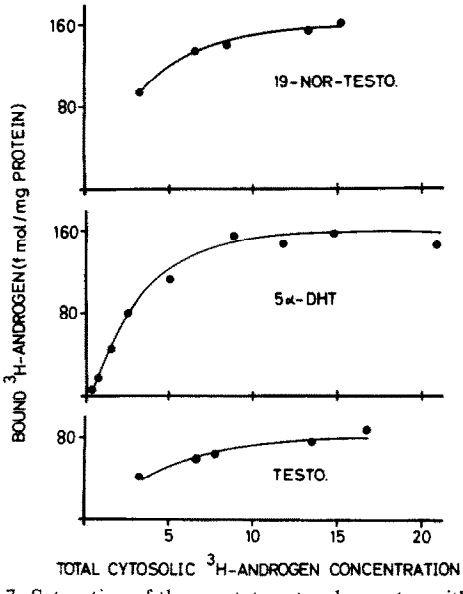

Fig. 7. Saturation of the prostate cytosol receptor with tritiated 19-nortestosterone (19-nor-testo.), 5α-dihydrotestosterone (5α-DHT) and testosterone (testo.), analyzed by agargel electrophoresis. Increasing amounts of the tritiated androgens were added to the homogenate. The concentration of the total cytosolic tritiated androgens was measured and plotted versus specifically bound tritiated androgens, determined in the respective cytosol by subtracting from the totally bound the non-specifically bound androgen. The points are means of at least three experiments.

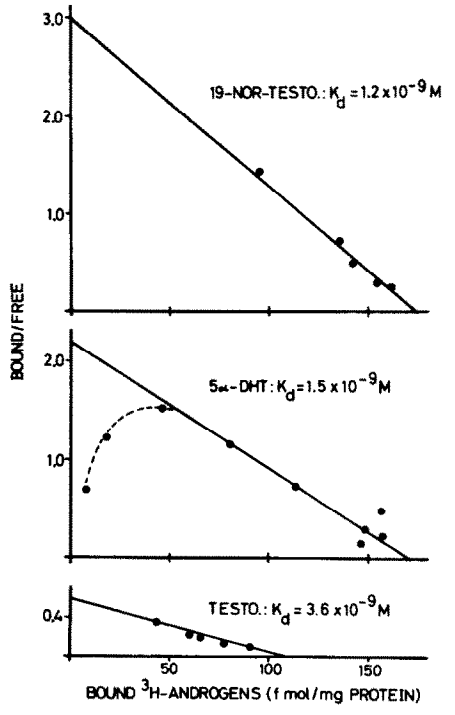

Fig. 8. Scatchard plots of the specific binding of tritiated 19-nortestosterone (19-nor-testo.), 5α-dihydrotestosterone (5α-DHT) and testosterone (testo.) in the rat prostate cytosol. The plots are derived directly from the saturation study of Fig. 7.

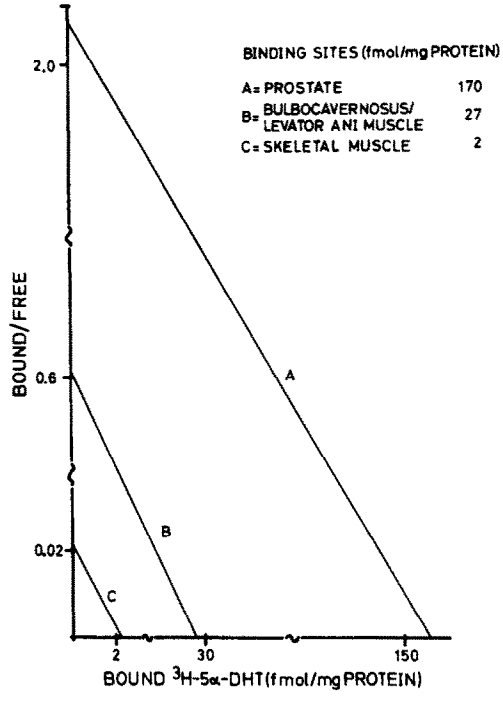

Fig. 9. Comparison of the Scatchard plots and the respective maximal binding sites, found for [^{3}H]-5α-dihydrotestosterone (5α-DHT) in the prostate (A), bulbocavernosus/levator ani (B) and skeletal muscle (C) cytosol. The straight lines have been depicted from Figs. 4, 6, and 8.

Table 2. Percentage distribution of the main metabolites obtained by thin-layer chromatography in the 100,000 *g* cytosol of the rat prostate, bulbocavernosus/levator ani and skeletal muscle after 2 h incubation of the organ homogenate at 0°C with tritiated 5α-dihydrotestosterone (5α-DHT), testosterone, 5α-androstane-3α,17β-diol (3α-diol), and 19-nortestosterone. The concentration of added steroids to the homogenate was $1–2 \times 10^{-8}$ M

	Metabolites found (%)	Tritiated steroids added: 5α-DHT	Testosterone	3α-Diol	19-Nor-testosterone
Prostate	5α-Androstanediols	26	6	81	—
	Testosterone	≪5	74	≪5	—
	5α-DHT	67	10	12	—
	19-Nor-testosterone	—	—	—	81
Bulbocavernosus/ levator ani muscle	5α-Androstanediols	5	<5	96	—
	Testosterone	≪5	83	≪5	—
	5α-DHT	84	<5	≪5	—
	19-Nor-testosterone	—	—	—	87
Skeletal muscle	5α-Androstanediols	8	<5	94	—
	Testosterone	≪5	87	≪5	—
	5α-DHT	78	≪5	≪5	—
	19-Nor-testosterone	—	—	—	89

Table 3. Comparison of available binding sites, dissociation constants (K_D), 5α-reductase activity and qualitative binding characteristics in various androgenic endpoints

	5α-DHT-binding sites (fmol/mg protein)	K_D's ($\times 10^{-10}$ M) 5α-DHT	Testosterone	5α-Reductase activity	Binding characteristics (qualitative)
Prostate	170	15	36	present	↑
Bulbocavernosus/ levator ani muscle	27	7	17	not measurable	nearly identical
Skeletal muscle	2	24	not determined	not measurable	↓

the rat prostate and kidney. Also Liao and Liang [15] discussed a common receptor protein in androgen sensitive tissues. On the other side, Steinetz *et al.*, in a short communication [16] suggested that at least the BCLA may have two types of receptor, one sensitive to anabolic and the other to androgenic stimulation. It should be emphasized, however, that all these assumptions are based on indirect evidence and that only the purification of the receptor protein and protein structure analysis will give a definite answer as to whether or not a single androgen receptor population exists in androgen dependent organs.

The detailed characterization of the androgen binding pattern in the rat skeletal muscle and the comparison of our findings with the data from others [17, 18] will be discussed elsewhere [19]. Despite some discrepancies, one may state that the androgen responsiveness of the rat skeletal muscle [6–8] could be mediated by this receptor protein. Furthermore, we may conclude from our *in vitro* studies, supported by the finding [20] of always a greater weight increase of various muscles of the guinea-pig after 5α-DHT than testosterone administration, that the affinity of the receptor is higher to 5α-DHT than testosterone, though due to the extreme small testosterone peak a quantitative binding study has not been performed.

Concerning the androgen binding in the BCLA, earlier results from the laboratory have been confirmed [5, 21]. The highest affinity to the receptor displays 5α-DHT followed by 19-nortestosterone and testosterone. The finding of Jung and Baulieu [22], that in the perineal muscle testosterone is bound with higher affinity than 5α-DHT, is not supported by our data. The question arises whether the affinity differences of the three steroids to the BCLA receptor will reflect their differences in biological activities in terms of growth stimulation. Compiling the literature [23–34], the bioassay data are not quite consistent. Most investigators found that 19-nortestosterone and its esters acted more upon the BCLA than testosterone and its respective esters. Furthermore, 5α-DHT showed always greater biological activity than testosterone. We also found a tremendous weight increase of the BCLA of castrated rats when 5α-DHT was administered s.c. for 12 days [5].

The apparent K_Ds, as determined in the prostate, must be discussed in the light of two facts:

(1) Even *in vitro* at 0°C metabolism occurs. When 5α-DHT is added to the homogenate, 26% of the extracted radioactivity, which is nearly the same as Verhoeven *et al.*, found [14], is recovered as 5α-androstanediols, which are little if at all bound to the receptor [35]. This might not lead to a significant underestimation of the total available binding sites for 5α-DHT under saturation conditions, i.e. when at least twice the amount of 5α-DHT needed for saturation has been added to the medium. In experiments, however, in which the concentration of 5α-DHT is smaller than the available binding sites, the aforementioned metabolism of 5α-DHT to the

5α-androstanediols will further reduce the amount of 5α-DHT interacting with the receptor.

(2) Using low 5α-DHT concentrations, in the prostate one gets no straight line with a negative slope of the Scatchard plot but rather an increase of the "bound/free" ratio. Very similar data have been obtained in the uterus [36]; as explanation, a cooperative binding is discussed. Such phenomenon can alternatively be explained by degradation of the receptor protein. It is known that the receptor protein is more stable with bound ligand [37, 38]. From this, it follows that the lower the androgen concentration in the medium, the more receptor protein is accessible to degradation. Both possibilities, cooperativity and/or degradation, lead to the same result, which can be avoided only by using either higher steroid or lower protein concentrations [36]. As far as 5α-DHT is concerned, its conversion to 5α-androstanediols will potentiate the phenomenon. Therefore, the determination of K_D for 5α-DHT in the prostate seems problematical and might result in slightly too high values. This is reflected by the wide range of K_Ds reported from 10^{-9} M [14, 39, 40, 41] to 10^{-13} M [42]. The grades of the affinity of the steroids to the receptor in prostate are similar to those found in the BCLA. The only exception being the slightly higher affinity of 19-nortestosterone when compared to 5α-DHT. This is also in contrast to a previous report [21] in which we have found for the prostate, under high receptor saturating conditions, statistically proved higher binding for 5α-DHT than for 19-nortestosterone to the receptor. In the literature three groups [14, 39, 41] also observed by competition studies that 19-nortestosterone was slightly more effective than 5α-DHT, while Liao *et al.* [42] reported just the opposite. In conclusion and based on previous results [21], we tend to believe that in the prostate-5α-DHT is bound more than 19-nortestosterone, which, by the way, will better explain the bioassay data showing that 5α-DHT is more active upon the prostate than 19-nortestosterone [29, 33].

From our comparative study *in vitro*, three points might be important for a better understanding of the graded androgen responsiveness of the organs *in vivo* (prostate > BCLA > skeletal muscle), as well as, the graded "myotrophic/androgenic" ratios of the steroids (19-nortestosterone > testosterone): (1) The amount of available androgen binding sites is quite different in the organs (prostate > BCLA > skeletal muscle). (2) The 5α-reductase activity is present in the prostate even *in vitro* at 0°C, while not measurable in the BCLA and skeletal muscle, thus reflecting well the situation *in vivo* [5, 12, 44–47]. (3) A single class of androgen binding sites, which is similar if not identical in each investigated organ, has a higher affinity for 5α-DHT and 19-nortestosterone than for testosterone. From these points, it seems attractive for us to speculate that there exists a positive correlation between the extent of androgen action at the cellular level of target organs and the amount of androgen receptor protein available for a steroid, the cellular 5α-reductase activity and the degree of affinity of the receptor to the various androgens or their metabolites. When applying this reasoning to the prostate, it must show a high androgenic responsiveness due to the high amounts of available binding sites and high 5α-reductase activity present, the latter converting testosterone to 5α-DHT which is bound with higher affinity to the receptor than testosterone. Concerning the skeletal muscle, on the other hand, this organ must be a poor androgenic endpoint as only low amounts of receptor protein are available which will bind with comparatively low affinity testosterone itself due to the absence of significant 5α-reductase activity. Discussing the BCLA along this line, it must be more responsive to androgens than the skeletal muscle due to higher amounts of androgen binding sites, but less than the prostate as it does not show measurable amounts of 5α-reductase activity and has less receptor protein. Furthermore, the higher "myotrophic/androgenic" ratio of 19-nortestosterone compared to testosterone is in accordance with this hypothesis, as stated in a previous paper [21].

Acknowledgements—This work was supported by the DFG, Sonderforschungsbereich 34 "Endokrinologie".

REFERENCES

1. Liao S. and Fang S.: *Vit. Horm.* **27** (1969) 19–90.
2. King R. J. B. and Mainwaring W. I. P.: Steroid–Cell Interactions. Butterworth & Co., London, 1974.
3. Wainman P. and Shipounoff G. C.: *Endocrinology* **29** (1941) 975–978.
4. Eisenberg E. and Gordan G. S.: *J. Pharmacol. exp. Ther.* **99** (1950) 38–44.
5. Krieg M., Szalay R. and Voigt K. D.: *J. steroid Biochem.* **5** (1974) 453–459.
6. Kochakian C. D.: *Proc. Soc. exp. Biol. Med.* **32** (1935) 1064–1065.
7. Breuer C. B. and Florini J. R.: *Biochemistry* **4** (1965) 1544–1549.
8. Breuer C. B. and Florini J. R.: *Biochemistry* **5** (1966) 3857–3865.
9. Wagner R. K.: *Hoppe Seyler's Z. physiol. Chem.* **353** (1972) 1235–1245.
10. Krieg M., Steins P., Szalay R. and Voigt K. D.: *J. steroid Biochem.* **5** (1974) 87–92.
11. Scatchard G.: *Ann. N.Y. Acad Sci.* **51** (1949) 660–672.
12. Buric L., Becker H., Petersen C. and Voigt K. D.: *Acta endocr., Copenh.* **69** (1972) 153–164.
13. Castañeda E. and Liao S.: *J. biol. Chem.* **250** (1975) 883–888.
14. Verhoeven G., Heyns W. and DeMoor P.: *Steroids* **26** (1975) 149–167.
15. Liao S. and Liang T.: In *Hormones and Cancer* (Edited by K. W. McKerns). Academic Press, New York 1974, pp. 229–260.
16. Steinetz B. G., Giannina T., Butler M. and Popick F.: *Endocrinology* **89** (1971) 894–896.
17. Michel G. and Baulieu E.-E.: *J. Endocr.* **65** (1975) 31–32.
18. Gustafsson J.-A. and Pousette A.: *Biochemistry* **14** (1975) 3094–3101.
19. Krieg M.: *Steroids* **28** (1976) 261–274.
20. Kochakian C. D.: *Klin. Wschr.* **39** (1961) 881–884.

21. Krieg M., Dennis M. and Voigt K. D.: *J. Endocr.* **70** (1976) 379–387.
22. Jung I. and Baulieu E.-E.: *Nature New Biol.* **237** (1972) 24–26.
23. Hershberger L. G., Shipley E. G. and Meyer R. K.: *Proc. Soc. exp. Biol. Med.* **83** (1953) 175–180.
24. Barnes L. E., Stafford R. O., Guild M. E. and Olson K. J.: *Proc. Soc. exp. Biol. Med.* **87** (1954) 35–38.
25. Barnes L. E., Stafford R. O., Guild M. E., Thole L. C. and Olson K. J.: *Endocrinology* **55** (1954) 77–84.
26. Holtkamp D. E., Heming E.-E. and Mansor L. F.: *J. clin. Endocr. Metab.* **15** (1955) 848.
27. Saunders F. J. and Drill V. A.: *Endocrinology* **58** (1956) 567–572.
28. Saunders F. J. and Drill V. A.: *Proc. Soc. exp. Biol. Med.* **94** (1956) 646–649.
29. Drill V. A. and Riegel B.: *Rec. Prog. Horm. Res.* **14** (1958) 29–67.
30. Overbeek G. A. and De Visser J.: *Acta endocr., Copenh.* **38** (1961) 285–292.
31. Dorfman R. I. and Kincl F. A.: *Endocrinology* **72** (1963) 259–266.
32. Dorfman R. I. and Dorfman A. S.: *Acta endocr., Copenh.* **42** (1963) 245–253.
33. Hilgar A. G. and Hummel D. J.: In *Androgenic and Myogenic Endocrine Bioassay Data* (Edited by A. G. Hilgar and D. J. Hummel). Cancer Chemotherapy National Service Center, Bethesda 1964, pp. 36–213.
34. Overbeek G. A.: *Anabole Steroide.* Springer-Verlag, Berlin 1966.
35. Krieg M., Horst H.-J. and Sterba M.-L.: *J. Endocr.* **64** (1975) 529–538.
36. Erdos T., Bessada R., Best-Belpomme M., Fries J., Gospodarowicz D., Menahem M., Reit E. and Veron A.: In *Advances in the Biosciences* (Edited by G. Raspe). Pergamon Press, Vieweg, vol. 7 (1970) pp. 119–131.
37. Bell P. A. and Munck A.: *Biochem. J.* **136** (1973) 97–107.
38. Liao S., Fang S., Tymoczko J. L. and Liang T.: In *Male Accessory Sex Organs. Structure and Function in Mammals.* (Edited by D. Brandes). Academic Press, New York 1974, pp. 238–265.
39. Shain S. A. and Boesel R. W.: *J. steroid Biochem.* **6** (1975) 43–50.
40. Töpert M., Zabel I. and Ziegler M.: *Analyt. Biochem.* **62** (1974) 514–522.
41. Blondeau J. P., Corprechot C., LeGoascogne C., Baulieu E.-E. and Robel P.: *Vit. Horm.* **33** (1975) 319–344.
42. Liao S.: *Int. Rev. Cytol.* **41** (1975) 87–172.
43. Liao S., Liang T., Fang S., Castañeda E. and Shao T.-C.: *J. biol. Chem.* **248** (1973) 6154–6162.
44. Bruchovsky N. and Wilson J. D.: *J. biol. Chem.* **243** (1968) 2012–2021.
45. Gloyna R. E. and Wilson J. D.: *J. clin. Endocr. Metab.* **29** (1969) 970–977.
46. Tveter K. J. and Aakvaag A.: *Acta endocr., Copenh.* **65** (1970) 723–730.
47. Bruchovsky N.: *Endocrinology* **89** (1971) 1212–1222.

DISCUSSION

Munck. I wonder what significance the results expressed as binding sites per milligram protein has in physiological terms. Is that per milligram cytosol protein? Or is it per milligram tissue weight?

Kreig. It is the estimation of available binding sites per mg soluble cytosol protein.

Munck. Have you compared the binding sites per cell between tissues?

Kreig. No.

Munck. Would that not be more significant when you are comparing different tissues in this way?

Kreig. Yes, this might be true. However, in contrast to glandular tissues like the prostate, the estimation of binding sites per muscle cell is very difficult and up to now not practicable for us.

O'Malley. You could make the calculation on the basis of DNA.

Kreig. Yes, the calculation of binding sites per DNA would be an acceptable approach. However, in the literature, we found no examples concerning the estimation of binding sites which reverse the results if relating the amount of binding sites per mg DNA instead of mg soluble protein.

Siiteri. I would like to raise a note of caution also. As you recall, Dr. Wilson and I about five years ago found a great deal of dihydrotestosterone in benign prostatic hypertrophy tissue and suggested that this may be a causative factor. As it turned out we were unsuccessful in inducing experimentally benign prostatic hypertrophy in dogs by treatment with dihydrotestosterone. However, recent work in Dr. Wilson's laboratory indicates that for the very first time prostatic hypertrophy can be induced in the dog by the administration of androstenediol. In fact the action of androstenediol is potentiated by estradiol administered simultaneously. So I would urge you to consider in your thinking about interaction of steroids with receptors the fact that we cannot dismiss compounds which do not apparently bind to the receptor as being physiologically unimportant.