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COMPARISON OF THE RECEPTOR BINDING PROPERTIES OF NANDROLONE AND TESTOSTERONE UNDER *IN VITRO* AND *IN VIVO* CONDITIONS

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Summary—Previous *in vitro* binding studies with androgen receptors in rat seminal vesicles (Toth M. and Zakar T., *J. steroid Biochem.* **17** (1982) 653–660) have shown that the difference in the effects of nandrolone (N) and testosterone (T) is caused by the fact that 5α-reduction increases the affinity of T and decreases the affinity of N. We confirmed this result using androgen receptors in rat prostate and intact human MCF-7 cells. We also analysed the receptor binding properties of N, T, dihydronandrolone (DHN) and dihydrotestosterone (DHT) *in vivo* following a combined 2-h infusion of a physiological dose of [^{3}H]T and the same dose of [^{3}H]N in castrated male rats, which permitted a direct comparison of the accumulation of [^{3}H]N, [^{3}H]T, [^{3}H]DHN and [^{3}H]DHT in different sub-cellular fractions of various tissues. There was a considerable accumulation of radioactivity in the liver, but no retention of active compounds. In the prostate there was a preferential retention of [^{3}H]DHT over [^{3}H]DHN in the receptor fractions whereas in thymus, spleen and muscular tissues [^{3}H]N and [^{3}H]T were retained in equal amounts. The kidney showed a preferential retention of [^{3}H]N over [^{3}H]T. The present results explain the relatively strong effect of nandrolone compared to that of testosterone on target tissues devoid of 5α-reductase activity (e.g. muscular tissue) compared to its relatively weak effect on tissues with a relatively high 5α-reductase content (e.g. prostate).

INTRODUCTION

Androgen-sensitive tissues respond with different sensitivities to the administration of testosterone and its derivatives or nandrolone and its derivatives [1, 2].

For the male secondary sex organs a high responsiveness to testosterone was found; characteristics of this high responsiveness are a high androgen receptor concentration, high activity of the enzyme 5α-reductase and a low 3α-reductase activity [3]. It has also been reported that nandrolone and testosterone have different effects on male secondary sex organs [1, 4]. Nandrolone, when injected in oily solution, is much less androgenic than testosterone with regard to its effects on the prostate, but is just as potent regarding its effect on the levator ani muscle [1]. This difference in anabolic and androgenic effects has been questioned; for instance, it has been stated by Wilson and Griffin [5] that "All modified androgens have equal enhancement or impairment of all known physiological functions of androgens" and "This is not surprising in view of the fact that all known actions of androgens are mediated by a single high-affinity receptor in the cytoplasm of target cells". The second statement is probably correct but the first statement is not, since the importance of the function of the enzyme 5α-reductase with regard to distinguishing the different effects of anabolic and androgenic compounds has not been taken into consideration. Recently, an explanation has been offered for the difference in "myotropic" and "androgenic" activity between nandrolone and testosterone [6]; 5α-reduction of testosterone increases its affinity for the androgen receptor in rat tissue, whereas 5α-reduction of nandrolone decreases the binding affinity. In the present study we have investigated whether this explanation is also valid for human androgen receptors. Results obtained *in vitro*, however, do not always reflect the *in vivo* situation. We have therefore also examined the binding properties of testosterone, nandrolone and their 5α-reduced metabolites under *in vivo* conditions following a combined infusion of a physiological dose of [^{3}H]testosterone and the same dose of [^{3}H]nandrolone in castrated male rats. The specific activity of the radioactive steroids was sufficiently high to detect binding to receptor sites and the radioactive compounds were separated by reversed phase high performance liquid chromatography. Non-specific binding of radioactive compounds was estimated in a rat pretreated with 0.25 mg testosterone propionate and 0.25 mg nandrolone propionate shortly before the start of the infusion of the tritiated steroids.

EXPERIMENTAL

Materials

[^{3}H]Testosterone (1.61×10^{12} Bq/mmol), [^{3}H]nandrolone (1.07×10^{12} Bq/mmol), and [^{3}H]5α-dihydrotestosterone (5.41×10^{12} Bq/mmol) were manufactured by the Radiochemical Centre, Great Britain. [^{3}H]R1881 (5.41×10^{12} Bq/mmol) was manufactured by New England Nuclear. The non-radioactive hormones were prepared by Organon International B.V.

Code names and abbreviations

The following trivial names and abbreviations were used: Testosterone (T) = 17β-hydroxy-4-androsten-3-one; Nandrolone (N; 19-nortestosterone) = 17β-hydroxy-4-estren-3-one; 5α-Dihydrotestosterone (DHT) = 17β-hydroxy-5α-androstan-3-one; 5α-Dihydronandrolone (DHN) = 17β-hydroxy-5α-estran-3-one; Metribolone (R1881) = 17-hydroxy-19,21dinor-17β-pregna-4,9,11-trien-3-one; 5α-reductase = 3-oxo-5α-steroid 4-ene-dehydrogenase; EDTA = ethylenediaminetetracetic acid; HPLC = high performance liquid chromatography; Adion = androstenedione.

Animals and infusion technique

Experiments were conducted using male Wistar rats (300 g) 24 h after castration. The conscious animals were infused via the vena jugularis with a mixture of 5.2 nmol of [^{3}H]N and 5.2 nmol of [^{3}H]T in 1.2 ml of rat serum in saline (1:1) at a a constant rate of 0.010 ml/min for 2 h with a micro-infusion pump and a polypropylene cannula. Previous studies [7] had shown that administration of T up to 1.7 nmol/h for 14 days resulted in pronounced nuclear accumulation whereas higher doses resulted in only slight additional increases. Moreover, a substitution dose of 8 nmol/h of T for 14 days (by implant) in castrated rats resulted in prostate weights and DNA content comparable to those of intact animals [7]. An infusion time of 2 h was chosen for the present study since previous studies had shown that the content of [^{3}H]T in prostatic nuclei reached a plateau between 30 and 60 min of infusion [8]. In order to study non-specific accumulation of [^{3}H]T and [^{3}H]N a similar experiment was performed in a rat pretreated with 0.25 mg (0.73 μmol) testosterone propionate and 0.25 mg (0.73 μmol) nandrolone propionate subcutaneously 16 and 1 h before the start of the infusion experiment.

Tissue preparation and isolation of subcellular fractions

The animals were killed under ether anaesthesia immediately after termination of the infusion and the tissues were removed and placed in buffer A (10 mmol/l Tris–HCl, 3 mmol/l EDTA, 3 mmol/l $MgCl_2$, 2.5 mmol/l dithioerythritol, 20 mmol/l sodium molybdate and 0.25 mol/l sucrose, pH 7.4) in ice.

The following procedures, were used to obtain (A) the homogenates, (B) the protamine-sulphate pellets from the cytosol fractions, which contain the receptor-bound radioactivity and (C) the 8000 N/kg nuclear pellets which were used for the preparation of the KCl-extractable supernatants and the non-KCl extractable residues.

(A) Tissues were homogenized in buffer A at 0°C (5 ml of buffer per g of tissue). Heart, skeletal (gastrocnemius) and levator ani muscle were disrupted using 2 × 3-s full bursts of the polytron homogenizer and prostate, liver spleen, kidney and thymus were homogenized in a glass-to-glass potter homogenizer. The homogenates were centrifuged at 8000 N/kg for 5 min.

(B) The supernatant fractions were further centrifuged at 1,100,000 N/kg for 20 min at 4°C and the cytosol fractions were collected and mixed (1:1, v/v) with a protamine-sulphate solution in Tris-buffer (2.2 mg/ml). After 10 min at 0°C the solution was centrifuged (10,000 N/kg) and the pellets were collected for subsequent extraction with ethyl acetate.

(C) The 8000 N/kg pellets were washed three times with 1 ml buffer B (10 mmol/l Tris–HCl, 1.5 mmol/l EDTA, 2.5 mmol/l dithioerythritol, pH 7.4). The nuclear fractions were extracted three times with 0.5 ml buffer B containing 0.6 mol/l KCl pH 8.6. The solution was centrifuged (10 min, 300,000 N/kg, 4°C) to separate the nuclear extract from the residue.

Extraction of radioactivity

The radioactivity in the total homogenates, protamine sulphate pellets, nuclear extracts and residues were extracted three times with 3 ml of ethyl acetate containing 1 μg each of unlabelled testosterone, 5α-dihydrotestosterone, nandrolone and 5α-dihydronandrolone. Following evaporation to dryness under nitrogen, the pellets were dissolved in 0.200 ml ethyl acetate for further separation by reversed phase HPLC. The extraction procedure resulted in complete (>95%) extraction of [^{3}H]testosterone, [^{3}H]nandrolone and [^{3}H]5α-dihydrotestosterone.

Chromatographic procedures

The separation of extracted radioactive steroids was performed by reversed phase HPLC on an octadecyl-silica support. The chromatograph consisted of a HPLC pump (Waters, model 510) in combination with a sample injector (Rheodyne, model 7125) fitted with a 100 μl sample loop, a u.v. monitor (LKB, 2158 Uvicord), a dual channel recorder (Kipp en Zonen, model BD41), an integrator (Spectra Physics, SP 4200) and a fraction collector (LKB, 2211 Superrac). The reversed-phase support was Supelcosil LC-18-DB 5 μm (Supelco) prepacked in a 25 cm × 4.6 mm i.d. stainless steel column. The separation column was preceded by a 2 cm long cartridge guard column filled with the same support. Ultrapure Milli-Q water (Millipore) and Uvasol-quality acetonitrile (Merck) were used for the preparation of the mobile phase. Mobile phase composition water–acetonitrile = 53:47 (v/v). Type FH filters (Waters, 0.5 μm) were used for filtration of the mobile phase. Prior to use, the mobile phase was filtered and degassed in an ultrasonic bath. Before each series of sample runs the HPLC system was calibrated by chromatography of a reference mixture of non-radioactive T (10 μg), N (10 μg), DHT (15 μg) and DHN (15 μg). All chromatographic runs were performed at a flow ate of 1.0 ml/min at ambient temperature. The column effluent was monitored at

206 nm. The sample solution (100 μl) was then chromatographed. To allow an easy matching of the radioactivity tracing with the peak position of the u.v. pattern, each sample was spiked with 5–10 μg quantities of non-radiolabelled N, T, DHT and DHN. From each sample run 80 effluent fractions were collected at 15 s intervals with 0.25 ml/fraction and a total run time of 20 min. Radioactivity of all fractions was measured off-line in a liquid scintillation counter.

Estimation of binding affinities for the androgen receptors in rat prostate and human MCF-7 cells

The details of the procedures for receptor binding studies using rat prostate (A) and human MCF-7 cells (B) have been described previously [9, 10].

(A) Frozen prostates from Wistar rats, which were castrated 18 h earlier, were homogenized in Tris–glycerol buffer (10 mmol/l Tris–HCl, 1 mmol/l EDTA, 200 g/l glycerol, 0.5 mmol/l dithioerythritol, 20 mg/l NaN_3, pH 7.4). Cytosol was collected following centrifugation 1,100,000 N/kg for 20 min at 4°C) and aliquots (0.100 ml) of the cytosol fraction were incubated with 0.100 ml of the protamine sulphate solution (2.2 mg/ml) in Tris–glycerol buffer for 10 min at 4°C. The protamine sulphate pellets were incubated with [^{3}H]R1881, either alone or in the presence of increasing concentrations of competitor as described previously [9]. Non-specific binding of tritiated ligand was assessed in the presence of 50-fold excess unlabelled R1881. The radioactivity bound to the protamine sulphate pellet was collected in 10 g/l sodium dodecylsulphate solution (0.200 ml) and counted in a liquid scintillation counter.

(B) Aliquots (0.100 ml) of the suspension of MCF-7 cells (10^7 cells/ml) were added to the wells of the microtitration plates and incubated for 45 min at 37°C with [^{3}H]5α-dihydrotestosterone either alone or with increasing concentrations of unlabelled competitor. The subsequent freeze-thaw/high salt extraction procedure followed by a dextran-coated charcoal method in low salt has been described previously [10].

RESULTS

In vivo uptake and subcellular distribution of [^{3}H]N, [^{3}H]T and metabolites following combined infusion in castrated rats

A combined dose of tritiated N and T was administered in a continuous infusion for 2 h to castrated male rats. The total radioactivity in blood and homogenates from various tissues and the percentages of total radioactivity associated with N, T, DHN and DHT, found after termination of the infuse, are shown in Table 1. The highest concentration of radioactivity was found in the liver (24 × the concentration in blood). However, no radioactive N, T, DHN and DHT was detectable; following separation by reversed phase HPLC practically all radioactivity was found in a polar fraction and in a fraction with a retention time similar to that of Adion or Androsterone (Fig. 1A). Higher concentrations of radioactivity relative to that found in blood were also detected in prostate (6 ×), kidney (3 ×) and thymus (1.7 ×). The levels in the muscular tissues and in the spleen were more or less comparable to those found in blood (Table 1).

The concentration of radioactive N, T, DHN and DHT in the protamine-sulphate pellet from the cytosol fraction, nuclear extract and nuclear residue of various tissues are shown in Table 2. The most distinct finding is the high concentration of DHT and DHN in the various subcellular fractions of the prostate compared to that found in the subcellular fraction from other tissues. Kidney, thymus, levator ani, heart muscle, spleen and skeletal muscle (gastrocnemius) have an enzyme pattern (low 5α-reductase and high 3α-reductase activity, ref. 2, 3, 12) which explains the accumulation of N and T instead of DHN and DHT. The HPLC elution patterns of radioactive compounds present in the protamine-sulphate pellets are shown in Fig. 1. The only peaks of radioactive material present in the protamine sulphate pellets from the prostate and the kidney were those corresponding to N, T, DHN and DHT (Figs 1C and D) whereas [^{3}H]N and [^{3}H]T were the only compounds present in the protamine-sulphate

Table 1. Total radioactivity in homogenates from various tissues, percentage of total radioactivity associated with N, T, DHT and DHT and concentrations, after continuous infusion for 2 h of 5.2 nmol of [^{3}H]N and 5.2 nmol of [^{3}H]T in 1.2 ml diluted rat serum

Tissue	Radioactivity in homogenate (nCi/g tissue)	Percentages of total radioactivity				Concentration (fmol/g tissue)			
		N	T	DHN	DHT	N	T	DHN	DHT
Prostate	1108	5.8	2.1	19.9	51.2	2216	534	7603	13019
Liver	4441	—	—	—	—	—	—	—	—
Kidney	551	12.7	9.2	0.9	1.1	2413	1163	120	151
Spleen	238	16.8	17.5	—	1.1	1379	955	—	59
Thymus	302	24.7	30.6	—	1.1	2572	2120	—	76
Heart muscle	248	20.5	33.2	—	—	1753	1888	—	—
Skeletal muscle[a]	171	25.5	39	—	—	624	636	—	—
Levator ani	158	21.4	21.5	—	—	1166	780	—	—
Blood	180 (nCi/ml)	21.5	31.5	—	—	1097	974	—	—

— Below detection limit.
[a]Gastrocnemius.

Table 2. Retention of [^{3}H]N, [^{3}H]T and 5α-reduced metabolites [^{3}H]DHN and [^{3}H] DHT in subcellular fractions of various tissues after a continuous infuse for 2 h of 5.2 nmol of [^{3}H]N and 5.2 nmol of [^{3}H]T in 1.2 ml diluted rat serum

	Protamine-sulphate pellet (fmol/g tissue)				Nuclear extract (fmol/g tissue)				Nuclear residue (fmol/g tissue)			
Tissue	N	T	DHN	DHT	N	T	DHN	DHT	N	T	DHN	DHT
Prostate	152	40	338	638	397	322	1087	2307	—	2	26	93
Kidney	83	26	6	5	143	17	5	4	22	—	5	5
Spleen	13	10	—	—	81	47	41	—	27	23	—	—
Thymus	35	26	—	—	477	404	—	—	59	62	—	—
Heart muscle	12	12	—	—	97	85	—	4	54	40	4	4
Skeletal muscle[a]	6	—	—	—	62	33	—	—	73	52	—	—
Levator ani	19	20	—	—	287	186	—	10	32	19	—	—

— Below detection limit.
[a]Gastrocnemius.

pellet from the thymus and heart (Figs 1E and F) and spleen, skeletal muscle and levator ani (not shown). These patterns, with only those peaks which correspond to compounds with a relatively strong affinity for the androgen receptor, are different from the elution patterns of extracts from the total homogenates (not shown) and blood (Fig. 1B) which also show several other identifiable peaks (e.g. androstenedione, estrenedione, androsterone, androstanediols and estranediols) and non-identifiable peaks (mostly polar metabolites). The radioactivity in the peaks from the eluates of the protamine-sulphate pellets is bound to low-capacity binding sites since the non-specific binding found in the pellets from a rat pretreated with unlabelled nandrolone propionate and testosterone propionate (see Experimental) was less than 10% of the values found in the experiment with tracer alone (Figs 1C–F, dotted lines). Analysis of the protamine-sulphate pellet from the prostate shows substantial binding of DHT, a lower amount of N and DNH and a much lower amount of T (Fig. 1C and Table 2). In the protamine-sulphate pellet from the kidney N was present in the highest concentration (Fig. 1D), whereas T and N were bound in equal amounts in thymus (Fig. 1E), heart muscle (Fig. 1F), levator ani, spleen and skeletal muscle (Table 2). The patterns found after separation of the nuclear extracts and nuclear residues by HPLC were similar to those found with the protamine-sulphate pellet; there was preferential nuclear accumulation of DHT in the prostate, nuclear levels of N were relatively high in the kidney, and the levels of N and T were similar in thymus, levator ani, heart muscle, spleen and skeletal muscle (Table 2).

Specificity of N, T, DHN and DHT for androgen receptors in rat prostate and intact MCF-7 cells

The relative affinities of N, T, DHT and DHN for the androgen receptor in rat prostate and the androgen receptor in MCF-7 cells are listed in Table 3. The data show that 5α-reduction of T strongly potentiates androgen receptor binding; the ratio of affinities of DHT/T was 2.0 and 2.7 for the androgen receptor in rat prostate and human MCF-7 cells respectively. On the other hand, 5α-reduction of N strongly reduced binding; the ratio of affinities of DHN/N was 0.5 and 0.6 for rat and human androgen receptors respectively.

DISCUSSION

Applying a constant infusion of a combination of [^{3}H]N and [^{3}H]T, we found that only N, T, DHN and DHT but no other metabolite of N and T are retained specifically in the protamine sulphate fractions of prostate and kidney, whereas N and T are retained in the protamine-sulphate fractions from spleen, thymus and muscular tissues. The results explain why these tissues are sensitive to the effects of N and T; the same androgen receptor system is present as was shown before for muscular and prostate tissues [2, 13]. However, a difference in the effects of T and N was found. DHT was more dominant than DHN in the prostate, whereas N was at least equally dominant as T in tissue without or with a low 5α-reductase activity (kidney, spleen, thymus and various muscular tissues, ref. 2, 3, 12). The important role of 5α-reduction for the effects of N and T on the prostate is clearly demonstrated in this study. In the prostate both T (as was found in many other studies; for reviews see references 2 and 11) and N are converted to 5α-reduced metabolites. The fact that the ratio DHN/N is lower than DHT/T and the absolute amount of N is higher than T in the protamine-sulphate fraction of the prostate suggest that N, although it is converted to DHN in the prostate, is not as good a substrate for 5α-reductase as T. Previous studies have shown that the specificity of N for the enzyme 5α-reductase is similar to that of T [14] or lower than that of T [15, 16]. The importance of metabolizing enzymes for the biological effects of nandrolone and testosterone is also shown by the almost complete absence of N, T, DHN and DHT in the homogenate of the liver and in the protamine-sulphate fraction of the liver. It is known from the literature that T is readily inactivated by the liver and that even large amounts of T when taken orally do not produce abnormalities in liver function [5]. It has also been reported [5] that 17α-alkylated derivatives of T may cause abnormalities in liver function because of slower hepatic inactivation. In the present study N was found to be more dominant than T in

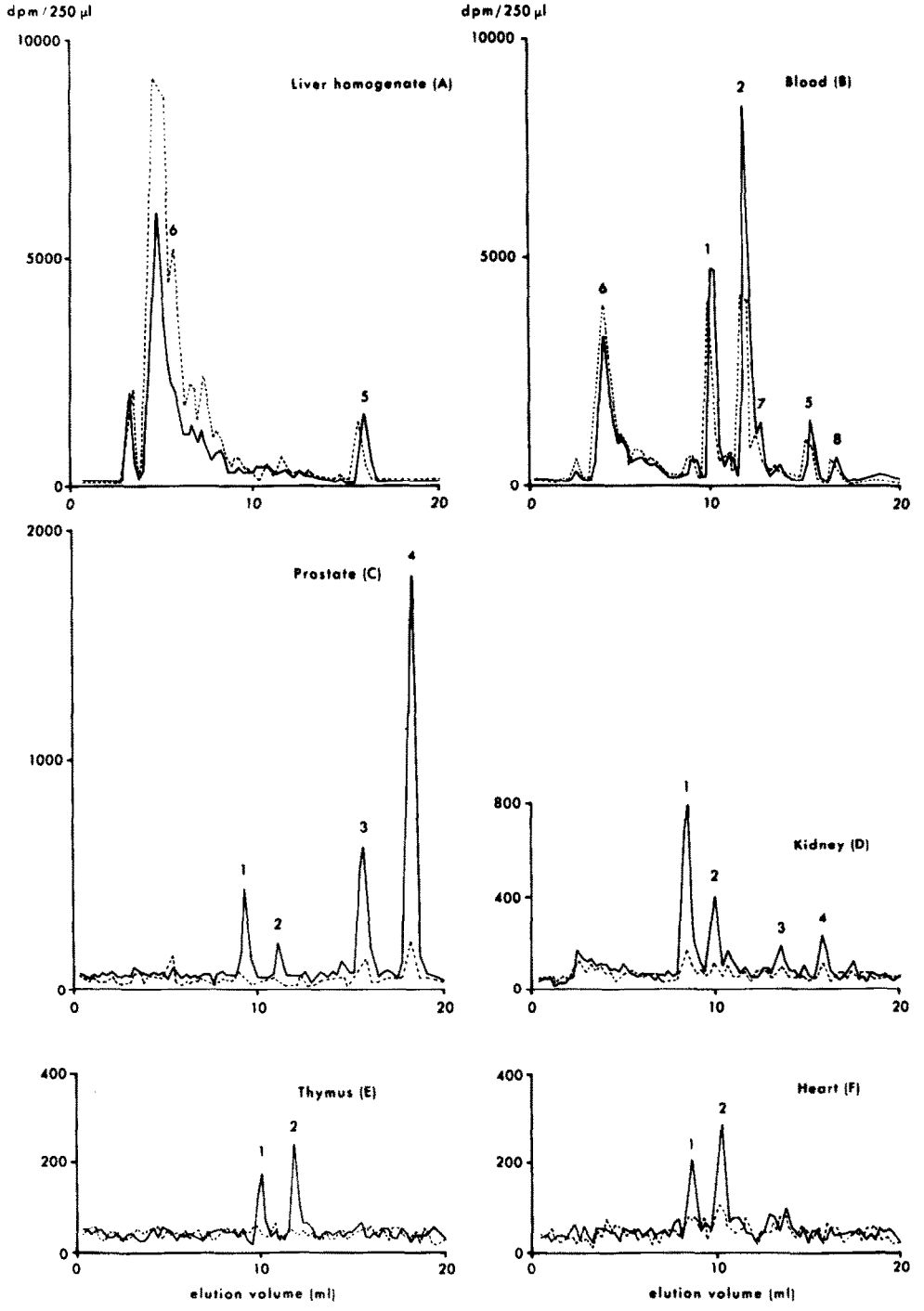

Fig. 1. Separation by reversed phase HPLC of radioactive compounds present in 0.100 ml ethyl acetate extract. Following a 2 h infusion of a combined dose of [^{3}H]N and [^{3}H]T, blood samples, homogenates and subcellular fractions from various tissues were taken and extracted with ethyl acetate. After separation fractions of 0.250 ml were collected and the radioactivity of each fraction was estimated. Straight lines: results obtained from a castrated rat infused with[^{3}H]N and [^{3}H]T. Dotted line: result from a rat injected with testosterone propionate and nandrolone propionate (see Experimental) before the start of the infusion with [^{3}H]N and [^{3}H]T. (A) Extract of the total liver homogenate (B) Extract from blood at the end of the infusion experiment. (C) Extract from the protamine sulphate pellet from prostate. (D) Extract from the protamine sulphate pellet from kidney. (E) Extract from the protamine sulphate pellet from thymus and (F) Extract from the protamine sulphate pellet from heart muscle. Peak identification was done by addition of unlabelled reference steroids: 1 = N, 2 = T, 3 = DHN, 4 = DHT, 5 = Adion/androsterone, 6 = polar metabolites, 7 = estrenedione, 8 = 3α-estranediol.

Table 3. Relative affinities of T and N and their 5α-reduced metabolites for the androgen receptors in rat prostate and human MCF-7 cells

Compound	Rat prostate (4°C) (R1881 = 100%)	Intact MCF-7 cells (37°C) (5α-dihydrotestosterone = 100%)
Testosterone (T)	38 ± 10 (13)*	38 ± 6 (6)[a]
5α-Dihydrotestosterone (DHT)	77 ± 13 (6)	100
Nandrolone (N)	75 ± 8 (4)	92 ± 16 (7)
5α-Dihydronandrolone (DHN)	35 ± 7 (4)	50 ± 16 (5)
Metribolone (R1881)	100	110 ± 6 (3)

[a]Mean ± SEM (number of experiments).

the kidney whereas in the blood both compounds are present in about equal amounts. The results of *in vitro* incubation studies with homogenates from kidney (results not shown) suggest that T is readily converted to its 17-keto metabolite whereas nandrolone is more stable. The retention of DHT in prostate nuclei was 2.3 pmol/g tisue or 1.5 pmol/mg DNA in the *in vivo* infusion experiment. This concentration is comparable to the saturation value of 1.8 pmol/mg DNA found in perfused rat prostate [8]. It can be concluded that a constant infusion of 5.2 nmol T/2 h is a physiologic dose as was found previously in a study with testosterone implants [7]. Prostatic 5α-reductase activity resulted in a considerable formation of DHT which is shown here to have a 2 to 3-fold higher binding affinity than T for the androgen receptor (both in human and rat tissues) and consequently is a more androgenic compound than T. The accumulation of DHN in prostate nuclei and other fractions of the prostate was only half that of DHT. Our binding studies and those of other investigators [6, 17] show that DHN has a lower affinity for the androgen receptor than N. It has also been reported [18] in *in vivo* studies that DHN has relatively weak androgenic activity. These results show that the conversion of N to DHN result in a weakening of its biological activity and that 5α-reduced androgens are not always more potent than their corresponding 4-ene-compounds.

REFERENCES

1. Overbeek G. A.: *Anabole Steroide. Chemie und Pharmakologie.* Springer, Berlin (1966) pp. 1–80.
2. Krieg M. and Voigt K. D.: Biochemical substrate of androgenic actions at a cellular level in prostate, bulbocavernosus/levator ani and in skeletal muscle. *Acta endocr., Copenh.* **85** Suppl. 214 (1977) 43–89.
3. Deslypere J. P., Sayed A., Verdonck L. and Vermeulen A.: Androgen concentrations in sexual and non-sexual skin as well as in striated muscle in man. *J. steroid Biochem.* **13** (1980) 1455–1458.
4. Toth M.: Relative androgenic and myotropic activity plots of 19-nortestosterone. *J. steroid Biochem.* **14** (1981) 1085–1090.
5. Wilson J. D. and Griffin J. E.: The use and misuse of androgens. *Metabolism* **29** (1980) 1278–1295.
6. Toth M. and Zakar T.: Relative binding affinities of testosterone, 19-nortestosterone and their 5α-reduced derivatives to the androgen receptor and to other androgen-binding proteins: a suggested role of 5α-reductive steroid metabolism in the dissociation of "myotropic" and "androgenic" activities of 19-nortestosterone. *J. steroid Biochem.* **17** (1982) 653–660.
7. Bartsch W., Knabbe C. and Voigt K. D.: Regulation and compartmentalization of androgens in rat prostate and muscle. *J. steroid Biochem.* **19** (1983) 929–937.
8. Baker H. W. G., Baily D. J., Feil P. D., Jefferson L. S., Santen R. J. and Bardin C. W.: Nuclear accumulation of androgens in perfused rat accessory sex organs and testes. *Endocrinology* **100** (1977) 709–721.
9. Bergink E. W., Hamburger A. D., de Jager E. and van der Vies J.: Binding of a contraceptive progestogen Org 2969 and its metabolites to receptor proteins and human sex hormone binding globulin. *J. steroid Biochem.* **14** (1981) 175–183.
10. Bergink E. W., van Meel F., Turpijn E. W. and van der Vies J.: Binding of progestagens to receptor proteins in MCF-7 cells. *J. steroid Biochem.* **19** (1983) 1563–1570.
11. Tymoczko J. L., Liang T. and Liao S.: Androgen receptor interaction in target cells: biochemical evaluation. In *Receptor and Hormone Action* (Edited by B. W. O'Malley and L. Birnbaumer). Academic Press, New York (1978) pp. 122–157.
12. Krieg M., Smith K. and Bartsch W.: Demonstration of a specific androgen receptor in rat muscle. Relationship between binding, metabolism and tissue levels of androgen. *Endocrinology* **103** (1978) 1686–1694.
13. Saartok T., Dahlberg E. and Gustafsson J. A.: Relative binding affinity of anabolic-androgenic steroids: Comparison of the binding to the androgen receptors in skeletal muscle and in prostate, as well as to sex hormone-binding globulin. *Endocrinology* **114** (1984) 2100–2106.
14. Shimazaki J., Horaguchi T., Ohki Y. and Shida K.: Properties of testosterone 5α-reductase of purified nuclear fraction from ventral prostate of rats. *Endocr. Jap.* **18** (1971) 179–187.
15. Voigt W., Fernandez E. P. and Hsia S. L.: Transformation of testosterone into 17β-hydroxy-5α-androstan-3-one by microsomal preparations of human skin. *J. biol. Chem.* **245** (1970) 5594–5599.
16. Lotz W.: Importance of 5α-reduction for the trophic effects of androgens on rat accessory sex organs and LH control. *J. steroid Biochem.* **13** (1980) 1261–1264.
17. Liao S., Liang T., Fang S., Castaneda E. and Shao T.: Steroid structure and androgenic activity. Specificities involved in the receptor binding and nuclear retention of various androgens. *J. biol. Chem.* **248** (1973) 6154–6162.
18. Drill V. A. and Riegel B.: Structural and hormonal activities of some new steroids. *Recent Prog. Horm. Res.* **14** (1958) 29–76.