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A COMPARATIVE STUDY OF THE ANDROGEN RECEPTOR APPARATUS IN ADULT RODENTS

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SUMMARY

Diversification of the androgen response in rat prostate, rat uterus and mouse kidney cannot be explained by differences in the cytosolic androgen receptor proteins extracted from these organs.

On the other hand, metabolism of the steroid hormone in the target cell and interaction in the target cell with non specific binding proteins could intervene in the diversification of the androgen response. As far as the steroid 5α-reductase is concerned, the absence in a given target cell of enzymes which compete for its substrate (testosterone) or which inactivate its reaction product (dihydrotestosterone) are probably of more importance than the apparent "nuclear" localisation of the enzyme. In particular, steroid 5α-reductase may play a rate limiting role in the production of dihydrotestosterone-receptor complex in the prostate. Transformation of 3α-androstanediol into dihydrotestosterone at the target organ level is catalyzed predominantly by the NAD(H)-dependent microsomal 3α-HSD. The high activity of this enzyme in several tissues such as the rat prostate, the rat kidney and the rat exorbital lacrimal gland may explain the particular responsiveness of these organs to exogenously administered 3α-androstanediol.

The question of whether testosterone-receptor complexes and dihydrotestosterone-receptor complexes have different physiological activity remains to be investigated. Preliminary experiments indicate that both receptor complexes can associate with nuclear chromatin *in vitro*.

The chain of events which leads from an androgen signal to an androgen effect passes through two more or less distinct divisions of the target cell machinery: the receptor apparatus and the effector apparatus. Looking at the response of various organs to androgens, one is faced with an enormous variety of androgen effects. Androgen effects involve gross morphological changes such as hyperplasia (e.g. prostate), hypertrophy (e.g. muscles), atrophy (e.g. thymus), as well as subtle changes in enzyme activities. Although differentiation of the *effector apparatus* most probably will explain the largest part of this diversification, it is possible that some diversification occurs at the level of the *receptor apparatus* also. Indeed, some target organs respond preferentially to a particular androgen or androgen derivative. The exorbital lacrimal gland and the kidney, for instance, are particularly responsive to 3α-androstanediol [1–5]. The search of pharmacologists for compounds with a predominant anabolic activity is also based on this hypothesis. In addition, even within a particular organ, different androgens may provoke different effects [6–8]. It is not known, however, whether these different effects occur in the same or in different cells.

In order to explore the possibility that diversification of the androgen receptor apparatus intervenes in the diversification of the androgen effect, we compared the androgen receptor apparatus in several target organs of adult rodents. Two important parts of this apparatus have been identified at present: the transformation of circulating androgens in "active metabolites" [9] and the binding of androgens to "receptor proteins" [10–12]. Both aspects will be discussed in this study.

I. A COMPARATIVE STUDY OF THE ANDROGEN RECEPTOR PROTEIN IN RAT PROSTATE, RAT UTERUS AND MOUSE KIDNEY

Recently some arguments have been advanced which support the contention that some diversification exists at the level of the androgen receptor protein. Male accessory sex tissues such as the rat prostate and the seminal vesicles contain dihydrotestosterone–receptor proteins and concentrate dihydrotestosterone in their cell nuclei. In several other androgen target organs, however, testosterone-receptors or nuclear uptake of testosterone has been reported. Testosterone itself, and not dihydrotestosterone, seems to be the active androgen in the mouse kidney [4], the mouse submaxillary gland [13], the rat musculus levator ani [14], the immature rat uterus [15], the Shionogii carcinoma [16] and a tumor cell line derived from hamster ductus deferens [17]. Similar results were obtained by continuous infusion of tritiated testosterone in rats (Heyns, unpublished results): in the prostate, dihydrotestosterone is the major androgen, while the mature rat uterus concentrates mainly testosterone (Table 1). The mouse kidney, which has been reported to contain testosterone-receptor proteins, also concentrates testosterone in comparable experiments [4, 18].

In the present study, we compared the *in vitro* properties of androgen receptor proteins in rat prostate, rat uterus and mouse kidney. Specific binding

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Table 1. Composition of unconjugated radioactivity in rat prostate or uterus after 6 hours of intravenous infusion of ^{3}H-testosterone (n = 6)

	% of radioactivity (% ± S.D.)		
	As polar metabolites	As testosterone	As dihydrotestosterone
Prostate	3·3 ± 0·3	4·4 ± 1·4	79·8 ± 4·8
Uterus	37·9 ± 7·0	48·2 ± 6·7	0·7 ± 0·2

Table 2. Comparison of apparent Kd values and number of receptor sites of androgen receptor proteins in rat prostate, mouse kidney and rat uterus

Ligand	Origin of cytosol	Apparent Kd (nM)	Sites (mol/mg protein) × 10^{-14}
Dihydrotestosterone	Rat prostate*	1·3	5·5
Testosterone	Rat prostate*	1·5	6·0
Testosterone	Mouse kidney*	1·2	2·2
Testosterone	Rat uterus	2·0	10

* Mean of 4 determinations.

was measured either by Sephadex G-50 chromatography (in uterus; Heyns, in preparation), or by ammonium sulfate precipitation (in prostate and kidney; Verhoeven, in preparation).

In the three organs studied, high affinity, low capacity androgen binding proteins were found (Table 2). Their affinity was of the same order of magnitude as that of receptor proteins for other steroid hormones. The number of binding sites, however, was some 10 times lower than the number of sites for estrogens [19] or glucocorticoids [20] in their respective target organs.

The *testosterone and dihydrotestosterone-binding proteins of rat prostate* have very similar steroid-binding properties. Indeed, the number of binding sites for both steroids is almost identical while the apparent affinities for both compounds are only slightly different (Table 2). Moreover, competition studies with various non-labelled steroids (Table 3) show a marked similarity for [^{3}H]-testosterone and [^{3}H]-dihydrotestosterone binding. Since other properties of these cytosol binders, such as sedimentation behavior and salt precipitation, are also much alike, it might well be that in rat prostate cytosol, dihydrotestosterone and testosterone bind to the same protein.

A striking similarity was found also, when the *ligand specificity was compared in rat uterus, rat prostate and mouse kidney.* Very strong competition is observed with two anabolica (19-nor-testosterone and dianabol). The anti-androgens cyproterone acetate and chlormadinone acetate are also very active. The competition observed with 17β-estradiol is in accordance with several *in vivo* findings in the literature [21]. 3β-Androstanediol is a much better competitor than its 3α-epimer, and epitestosterone is nearly inactive (Table 3).

Despite these similarities, it remains to be explained why in our experiments, as in the literature [22], *dihydrotestosterone is bound more tightly in the prostate while testosterone binding prevails in kidney and uterus.* It is our impression that the latter differences—which in our hands are much less important than in the literature—can be explained by methodological factors rather than by differences in receptor proteins.

The *first* and probably the most important factor involved in the very extensive metabolism even at

Table 3. Ligand specificity of the androgen receptor protein in rat prostate, mouse kidney and rat uterus cytosol

Origin of cytosol	Prostate		Kidney	Uterus
labelled ligand	Testosterone	Dihydrotestosterone	Testosterone	Testosterone
	K_i Value			
Competitor	nM	nM	nM	nM
Testosterone	4·0	3·3	1·3	2·0
Dihydrotestosterone	2·0	2·0	4·1	4·3
Epitestosterone	74	33	20	33
5α-Androstan-3α,17β-diol	—	35	26	39
5α-Androstan-3β,17β-diol	—	9·9	10	7·2
17β-Estradiol	—	12	5·3	6·1
17α-Methyl-19-nor-testosterone	1·5	0·9	0·2	0·4
Dianabol	2·2	2·0	4·8	3·3
Cyproterone acetate	—	4·1	5·4	2·6

Table 4. Metabolism of androgens in cytosol preparations at 4°C

Origin of cytosol	Androgen	Per cent untransformed androgen	
		In total cytosol	In receptor bound fraction
Rat prostate	Testosterone	76	87
	Dihydrotestosterone	56	92
Mouse kidney	Testosterone	81	88
	Dihydrotestosterone	20	77
Rat uterus	Testosterone	90	96
	Dihydrotestosterone	10	89

4°C of dihydrotestosterone in cytosol; testosterone, on the contrary, remains virtually unaltered under the same experimental conditions. After 4 hours of incubation at 4°C, only 56, 20 and 10% of the incubated dihydrotestosterone could be recovered unchanged from prostate, kidney and uterus, respectively (Table 4). In all three tissues the main metabolite was 3α-androstanediol which shows weak binding to the cytosol receptor.

A *second* factor is the considerably higher non-specific binding of dihydrotestosterone in the cytosol from all organs studied. In equilibrium dialysis experiments in the presence of 10^{-6} M of testosterone or dihydrotestosterone, the bound/unbound ratios were from 2 to 5 times as high for dihydrotestosterone as for testosterone after 24 h of incubation.

A *third* factor which should be considered is the duration of the experimental procedure. Indeed, the dissociation of the dihydrotestosterone-receptor complex is considerably slower than that of the testosterone-receptor complex (Fig. 1). Consequently dihydrotestosterone-binding will be favored in slow methods

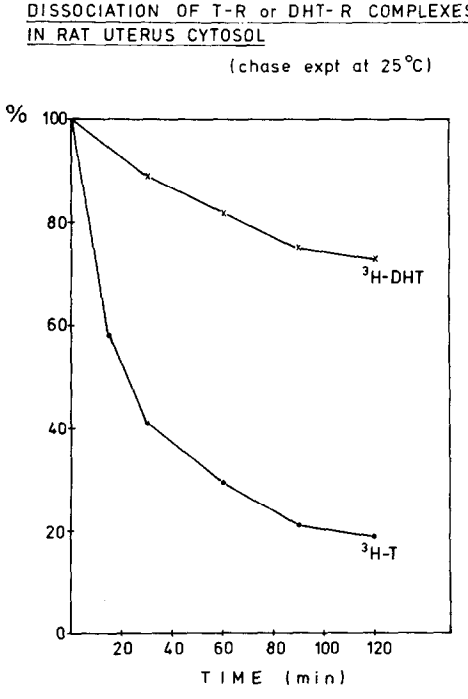

Fig. 1. Dissociation of the dihydrotestosterone–receptor complex as compared with the dissociation of the testosterone–receptor complex.

such as sucrose density gradient centrifugation, in which the androgen receptor complex is allowed to dissociate during approximately 18 h. In fact, using the latter method, we found predominantly dihydrotestosterone in all three organs studied. On the contrary, much less dissociation intervenes during the short time it takes to perform gel filtration or ammonium sulfate precipitation.

In *conclusion*, it is our opinion that a single androgen receptor is responsible both for the binding of testosterone and dihydrotestosterone, in rat prostate, rat uterus and mouse kidney. The finding of preferential binding of either dihydrotestosterone or testosterone is not due to differences in ligand specificity of this receptor but to other factors such as metabolism, aspecific binding or dissociation of the androgen-receptor complex during the experimental procedure.

II. ANDROGEN METABOLISM IN TARGET CELLS

Because of the marked similarity of the steroid-binding properties of the receptor proteins, we turned our attention to another mechanism which may finally determine whether testosterone itself or dihydrotestosterone will be bound to the receptor protein: i.e. metabolism at the level of the target organ. Two reactions have been investigated in some detail:

1. The transformation of testosterone into 5α-dihydrotestosterone, which is an irreversible reaction catalyzed by a steroid 5α-reductase.
2. The interconversion of 5α-dihydrotestosterone and 3α-androstanediol, a reaction which is reversible and which is catalyzed by several 3α-hydroxysteroid dehydrogenases (3α-HSD).

1. *The "nucleus-associated" steroid 5α-reductase*

The recent interest in the steroid 5α-reductase has been stimulated by three findings. Firstly, the reaction product, dihydrotestosterone, is a very potent androgen. Secondly, dihydrotestosterone is the major androgen found in several accessory sex tissues [23]. Thirdly, it has been reported that the steroid 5α-reductase is the only androgen metabolizing enzyme associated with nuclei from accessory sex tissues [9].

We studied three aspects of the 5α-reductase: the occurrence of nucleus-associated 5α-reductase activity in organs with varying degrees of androgen responsiveness, the properties of this enzyme in the various

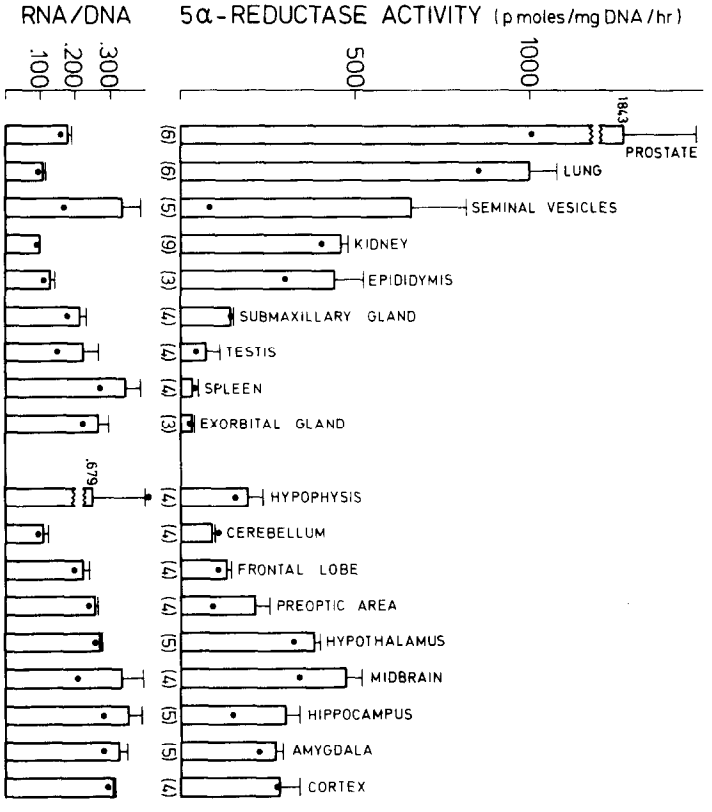

Fig. 2. Measurements of 5α-reductase activity in nuclear preparations of a wide variety of organs and brain regions of male rats.

nuclear preparations, and the subcellular distribution of the enzyme in androgen responsive and androgen insensitive organs.

Figure 2 summarizes the results of a series of measurements of 5α-reductase activity in nuclear preparations of a wide variety of organs and brain regions of male rats. The purity of the nuclear fractions was checked by measurements of RNA/DNA ratios. The following conclusions may be drawn from these data [24].

One. Several androgen-dependent organs figure between the organs with the highest nucleus-associated 5α-reductase activity.

Two. Several other androgen target organs, like the exorbital lacrimal gland, the submaxillary gland and the testis, have low nuclear 5α-reductase activity.

Three. At least one organ which is rather androgen-insensitive figures between the tissues with very high nucleus-associated 5α-reductase activity, namely the lung.

In a second set of experiments, we compared the properties of the 5α-reductase in several nuclear preparations. A comparison of our data in kidney nuclei and lung nuclei with similar data reported for other tissues leads to the conclusion that essentially the same enzyme properties are found in all the nuclear preparations: a very high affinity for testosterone, requirement of NADPH as cofactor and a pH-optimum around neutrality. Competition is observed with a lot of non-androgenic 4-ene-3-keto steroids such as progesterone, 17α-hydroxyprogesterone and epitestosterone. None of the steroidal or non-steroidal antiandrogens interfere with the 5α-reductase. Steroids with a 11-hydroxy function are not attacked by this enzyme [24–28]. Both the low K_m [29], the optimal pH [30], the specificity for NADPH [31] and the inability to reduce glucocorticoids [32] distinguish this enzyme from the 5α-reductase (system) in rat liver.

Finally, we investigated the subcellular distribution of 5α-reductase activity in 5 organs with different degrees of androgen responsiveness, namely the prostate, the rat kidney, the submaxillary gland, the lung and the midbrain of the rat. In all these organs, the subcellular distribution was roughly identical. The particulate fractions—mitochondria and microsomes—accounted for the major part of the cellular activity. The amount of nucleus-associated 5α-reductase activity exceeded the contamination with microsomal marker enzymes only in the prostate and the kidney. In the prostate, nucleus-associated 5α-reductase activity accounted for 25% of the total cellular activity, in the kidney for 20%. These figures are considerably lower than those which have previously been reported [30, 33, 34]. Moreover, although measurements were made under strictly linear conditions, it is our impression that 5α-reductase activity in microsomal preparations may have been underestimated. In fact, 5α-reductase activity has been calculated as the sum of the formation of dihydrotestosterone and 3α-

Table 5. A comparison of several properties of the 3α-hydroxysteroid dehydrogenases in rat kidney

	Enzyme		
	NADPH-soluble	NADPH-particulate	NADH-particulate
Sex difference	♀ > ♂	♀ ⋘ ♂	♀ < ♂
$(NH_4)_2SO_4$ Precipitation	60–80%	—	—
Sephadex G-100	MW ≃ 30,000	Vo*	Vo*
Optimum activity at pH	5·5–7	8	4·5
Influence of ionic strength	—	↑↑	—
Influence of phosphate	—	↓↓	—
50% Denautration after 10 minutes incubation at:	52°C	41°C	58°C
~ Km for dihydrotestosterone	0·3 nM	> 100 nM	2·4 nM
~ Km for 3α-androstanediol	> 100 nM	3·14 nM	0·6 nM

* Triton-solubilized enzyme or trace of this enzyme found in the cytosol fraction. Vo = void volume.

androstanediol. In view of the complexity of the metabolism particularly in microsomal preparations, it cannot be excluded that other unidentified but 5α-reduced metabolites were not included in those calculations.

In conclusion, although the presence of 5α-reductase activity in target tissues may be of paramount importance, the so-called "nuclear localization" of this enzyme may be only a very secondary feature due, for instance, to the presence of outer nuclear membranes (contiguous with the endoplasmic reticulum) in the investigated nuclear preparation.

2. *The interconversion between 5α-dihydrotestosterone and 3α-androstanediol*

A comparative study has been made of the presence, the properties and the subcellular distribution of various enzymes with 3α-hydroxysteroid dehydrogenase activity in the rat kidney, prostate, lung, submaxillary gland and several other organs. It has been attempted to correlate these *in vitro* findings with the function of various 3α-HSD *in vivo*.

Rat kidney was studied most intensively. Three dehydrogenases with completely different characteristics could be identified in this tissue (Table 5):

1. an NADPH-linked enzyme, located in the high speed supernatant, which is about two times more active in female animals than in male ones;

2. an NADPH-linked enzyme in the microsomal fraction which occurs almost exclusively in male animals;

3. an HADH-dependent microsomal dehydrogenase which is 2 up to 3 times as active in male than in female rats;

Traces of the two enzymes associated with the particulate fractions were also found in the cytosol.

In accordance with previous investigations in the literature, the NADPH-dependent soluble enzyme was found in all organs investigated. The NADH-linked microsomal dehydrogenase was detected in several other tissues including the prostate. The NADPH-dependent enzyme was found in male rat kidneys only.

Table 5 summarizes some differences in the properties and kinetic characteristics of the three 3α-HSD in rat kidney. Essentially the same results were obtained for the two enzymes which are found in the prostate. As far as the *transformation of dihydrotestosterone into 3α-androstanediol* is concerned, it can easily be observed that the NADPH-3α-HSD of the cytosol has an exceedingly high affinity for dihydrotestosterone. Taking into account that NADPH/NADP ratios are considerably higher than 1 in mammalian tissues, it may be anticipated that this enzyme plays an important role in the formation of 3α-androstanediol. The exceedingly high *in vitro* transformation of dihydrotestosterone into 3α-androstanediol

Table 6. Correlation between the transformation of 3α-androstanediol into dihydrotestosterone in tissue slices and the presence of the microsomal NADH-dependent 3α-hydroxysteroid dehydrogenase

	Dihydrotestosterone production in tissue slices from 3α-androstanediol (%)	NADH-3α-HSD activity (μmol/g protein/h)
Male kidney	25	135
Female kidney	16	53
Lung	4	37
Submaxillary gland	16	18
Exorbital lacrimal gland	48	179
Prostate	69	154
Seminal vesicles	17	4

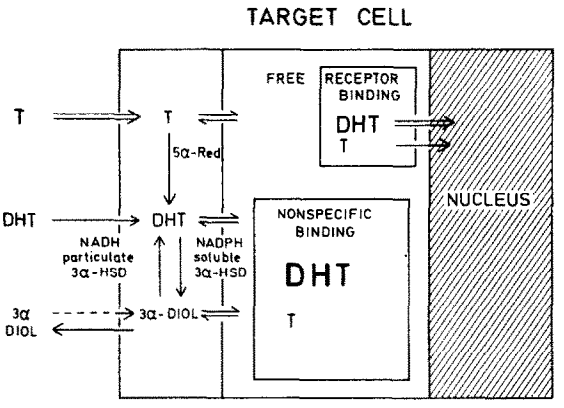

Fig. 3. Receptor apparatus in rodents: proposed chain of events.

by cytosol preparations even at 4°C and in the absence of exogenous cofactors may be caused by this enzyme.

Looking at *the inverse reaction*, it should be noted that the NAD(H)-linked microsomal enzyme has a high affinity for 3α-androstanediol. Only this enzyme effectively transformed 3α-androstanediol into dihydrotestosterone at low substrate concentrations. Taking into account the excess of oxydized NAD in all tissues investigated it may be anticipated that the presence of the NAD-dependent microsomal enzyme in a particular tissue will favor conversion of 3α-androstanediol into dihydrotestosterone in this tissue.

As already shown, 3α-androstanediol is a poor ligand for the androgen receptor protein. In view of the particular responsiveness of tissues such as the kidney and the exorbital lacrimal gland to 3α-androstanediol, we investigated: whether transformation of the diol into dihydrotestosterone occurred in tissue slices and whether a correlation existed between the transformation of 3α-androstanediol into dihydrotestosterone in particular tissues and the presence of the above mentioned NAD-linked dehydrogenase in the same tissues (Table 6). Such a relationship was indeed found for the rat prostate, the rat kidney and the exorbital lacrimal gland. It may be concluded that transformation of 5α-androstanediol into dihydrotestosterone at the target organ level may be an important factor in the responsiveness of several tissues to 3α-androstanediol.

DISCUSSION

Figure 3 gives a schematic view of the hypothetical model derived from the presented data.

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