Evidence that human prostatic 5α-reductase is located exclusively in the nucleus

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Received 19 February 1985, revised version received 22 April 1985

The 5α-reductase activities in human prostatic nuclei and microsomes were compared The activities in both subcellular fractions were identical with respect to pH dependence, heat inactivation and Michaelis constants for NADPH and testosterone Subcellular distribution studies using DNA and nicotinamide mononucleotide adenyl transferase as nuclear markers showed that the amount of 5α-reductase present in the microsomes was directly proportional to the amount of nuclear contamination These results indicate that human prostatic tissue contains only one form of 5α-reductase, which is located exclusively in the nucleus

This finding has important implications for the mechanism of steroid action in the prostate.

Human prostate *5α-Reductase* *Nuclear localization*

1. INTRODUCTION

The widely accepted model of androgen action in the prostate is that testosterone is converted in the cytoplasm into the more potent androgen, dihydrotestosterone (DHT). The DHT then binds to the cytoplasmic androgen receptor and the receptor-DHT complex is translocated into the nucleus [1–4]. Subcellular distribution studies indicate that 5α-reductase, the enzyme which catalyses the reduction of testosterone to DHT, is found in the microsomes and in the nuclear membrane [5–8]. The question of why the prostate has 5α-reductase activity in both the nucleus and microsomes has not been addressed by other workers. One possibility is that the enzyme activity in each fraction has a distinct function. While the function of the microsomal 5α-reductase would obviously be the generation of cytoplasmic DHT, it is difficult to envisage what role the nuclear 5α-reductase could have as in the model described in [1,2], there are no unoccupied androgen receptors in nucleus.

In an attempt to gain some insight into the role of the nuclear enzyme we have compared some of the properties of the 5α-reductase activity in the nuclear and microsomal fractions of human prostatic tissue to determine whether they are distinct isoenzymes. We have also re-examined the subcellular distribution of human 5α-reductase using markers for nuclear material.

Abbreviation. DHT, 5α-dihydrotestosterone, 17β-hydroxy-5α-androstan-3-one

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2. EXPERIMENTAL

2.1. *Materials*

Alcohol dehydrogenase, androstenedione, ATP and β-nicotinamide mononucleotide were obtained from Sigma, Poole, England. Hyperplastic prostatic tissue was obtained from patients following open surgery, and stored at −70°C before use. The sources of all other materials were described in [9].

2.2. *Homogenisation and subcellular fractionation*

Prostatic tissue was homogenised in 5 vols of buffer containing 100 mM Tris–HCl, pH 7.5, 1

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00145793/85/$3.30

mM EDTA, 20% (v/v) glycerol, 15 mM β-mercaptoethanol according to one of the following methods·

Method 1:

Prostatic tissue was chopped finely with scissors and homogenised using two 30-s bursts at full power of a Ystral homogeniser (Scottish Scientific Instrument Centre, Edinburgh).

Method 2:

Prostatic tissue was briefly immersed in liquid nitrogen and then ground to a fine paste using a metal file. The paste was then homogenised in a hand-held glass-Teflon homogeniser (3–10 strokes)

Method 3:

As in method 2 except that the paste was homogenised in a Dounce homogeniser using 1–10 strokes of the loose-fitting pestle.

Unhomogenised tissue was removed by centrifugation at $20 \times g$ for 15 min and the resulting supernatant was used as the homogenate in further experiments. The nuclear and microsomal fractions of the homogenate were prepared by the method of De Duve et al. [10].

2.3. *Subcellular distribution of 5α-reductase*

Homogenates prepared by the methods described above were centrifuged at $800 \times g$ for 20 min to produce nuclear pellets and post-nuclear supernatants (PNS). Initial experiments indicated that at least 90% of the 5α-reductase activity in the PNS was located in the microsomes (not shown). The extent of contamination of PNS by nuclear material was estimated using nuclear markers. These were either DNA, or nicotinamide mononucleotide adenyl transferase (NMNAT), an enzyme which is located solely in the nucleus [11].

5α-Reductase was assayed by the method of Houston et al. [9], using [^{3}H]testosterone as substrate. NMNAT was assayed as in [12]. DNA was estimated by the method of Giles and Myers [13]. The significance of differences between the properties of the nuclear and microsomal 5α-reductase was assessed using Students *t*-test.

3. RESULTS

3.1. *Comparison of the properties of nuclear and microsomal 5α-reductase*

The pH dependence of the nuclear and microsomal 5α-reductase was compared, as shown in fig 1a). The 5α-reductase activity in both fractions behaved very similarly with a broad pH optimum between 6.0 and 7.5 The susceptibility of the nuclear and microsomal 5α-reductases to heat inactivation at 50°C was studied (fig 1b). The 5α-reductase activity in both fractions was very heat-labile with the activity being completely destroyed within 5 min

Both the nuclear and microsomal 5α-reductases respond hyperbolically to their substrates Fig.2a shows that the apparent K_m for testosterone (K_m^T) of the 5α-reductase in each fraction was very similar. The mean K_m^T of the nuclear 5α-reductase was 29.4 ± 8.4 nM whereas that of the microsomal enzyme was 31.0 ± 8.2 nM (mean ± SD of 3 separate experiments). The 5α-reductase activity in each subcellular fraction was completely dependent on the presence of NADPH No activity was detected in the absence of co-factor or when NADPH was replaced by NADH (not shown). The K_m for NADPH (K_m^{NADPH}) of the nuclear and microsomal enzymes were very close (fig.2b). The K_m^{NADPH} of the nuclear 5α-reductase was $2.1 \pm 0\,5$ μM compared with a value of 2.62 ± 0.9 μM for the microsomal enzyme (mean ± SD of 3 separate experiments). The differences in the kinetic parameters of the nuclear and microsomal 5α-reductases were not significant ($P > 0\,1$).

3.2. *Subcellular distribution studies*

During the studies reported above it was observed that the proportion of 5α-reductase activity in the microsomes was increased if the tissue was homogenised using the Ystral, which generates high shear forces. This suggested that some, or all, of the microsomal 5α-reductase was of nuclear origin and was present in the microsomes as a result of nuclear degradation during homogenisation. We therefore systematically studied the effect of homogenisation on the subcellular distribution of 5α-reductase.

Prostatic tissue was homogenised with varying amounts of shear force using the methods described in section 2 The amounts of 5α-reductase, NMNAT and DNA in the homogenate, nuclear pellet and PNS were determined. As shown in fig 3, the proportion of the nuclear markers, DNA and NMNAT present in the PNS increased with increasing shear force. This was accompanied by a

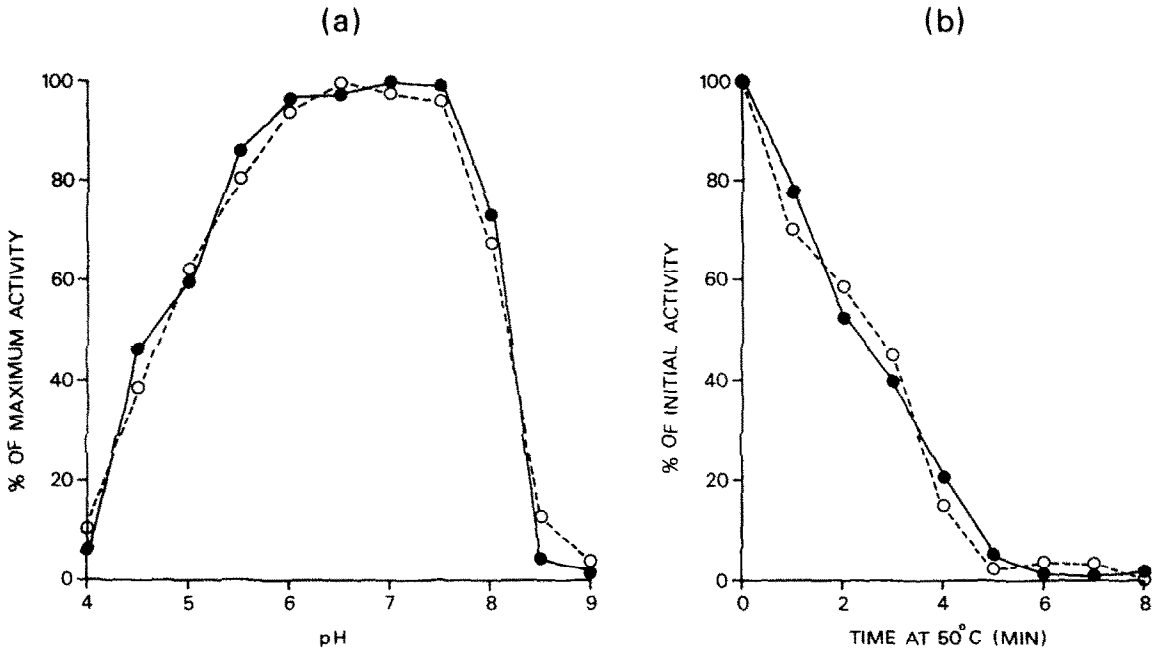

Fig 1 (a) pH dependence of nuclear and microsomal 5α-reductase. Prostatic homogenates were produced using method 2 5α-Reductase was assayed as in [9] in a Tris/citrate buffer adjusted with NaOH to the pH indicated (●) Nuclear 5α-reductase, (○) microsomal 5α-reductase (b) Heat inactivation of nuclear and microsomal 5α-reductase Prostatic homogenates were produced using method 2 Nuclei (●) and microsomes (○) were incubated at 50°C Samples were removed at the times indicated and assayed for 5α-reductase activity

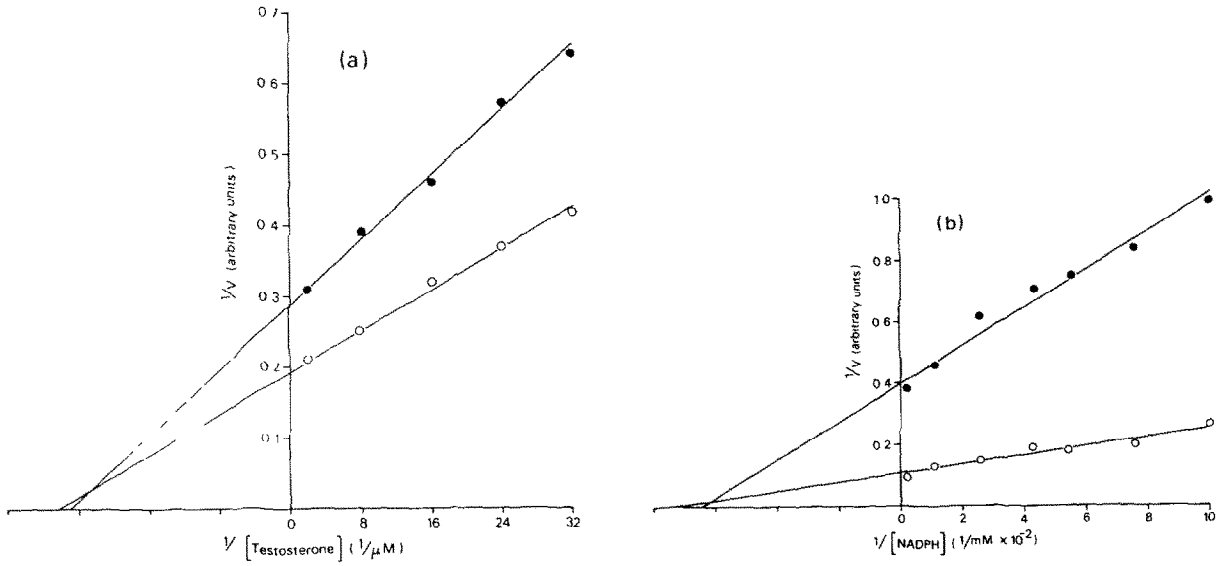

Fig 2 Activities of nuclear and microsomal 5α-reductase at different substrate concentrations Homogenates were produced by method 2 and nuclear (●) and microsomal (○) fractions were prepared 5α-reductase was assayed as in [9] except that in (a) the concentration of testosterone was varied as indicated and that in (b) the concentration of NADPH was varied as indicated The data shown are the means of duplicate assays

corresponding decrease in the proportion of these markers in the nuclear fraction (not shown). We therefore concluded that the degree of contamination of the PNS by nuclear material increased with the amount of shear force employed in the homogenisation method. In fig.3, the proportion of 5α-reductase in the PNS is plotted as a function of the amount of nuclear contamination of the PNS as assessed using DNA or NMNAT. The proportion of 5α-reductase in the PNS varied from 26 to 95% depending on the homogenisation method. In addition, the 5α-reductase activity in the PNS was directly proportional to the amount of contamination of the PNS by nuclear material In these experiments it was not possible to produce post-nuclear supernatants with less than 29% nuclear contamination owing to the fibromuscular nature of prostatic tissue. However, by extrapolation it can be seen that at 0% nuclear contamination there is essentially no 5α-reductase activity in the PNS.

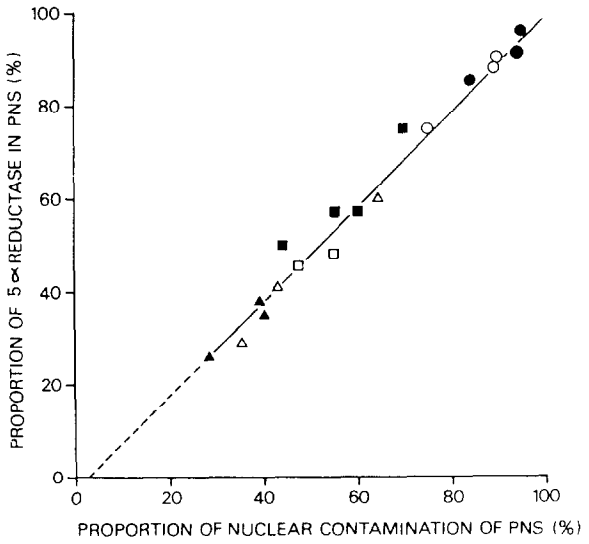

Fig.3 Correlation between nuclear contamination and 5α-reductase activity in post nuclear supernatants Nuclear pellets and post-nuclear supernatants were prepared from homogenates produced by either method 1 (●,○), method 2 (■,□) or method 3 (▲,△) as described in section 2 Samples were taken from each fraction and assayed for 5α-reductase and for DNA (closed symbols) and NMNAT (open symbols) The proportion of nuclear contamination in the PNS is defined as the amount of marker in the PNS/amount of marker in homogenate The proportion of 5α-reductase in PNS is defined as amount of 5α-reductase in PNS/amount of 5α-reductase in homogenate The line is the best fit to the data and was calculated by a least-squares method

4. DISCUSSION

These results show that the nuclear and microsomal 5α-reductases are indistinguishable with respect to pH dependence and susceptibility to heat inactivation. Moreover, the enzymes in both fractions have identical affinities for NADPH and testosterone; this suggests that there is no preferential utilisation of testosterone by either the microsomal or nuclear enzymes There is therefore no evidence in the present study that the 5α-reductase in human prostatic nuclei and microsomes are distinct isoenzymes This would also appear to be true for the rat prostate and epididymis as several properties of the nuclear and microsomal 5α-reductase from these tissues are also identical [14,15].

In view of the finding that the prostate contains only one form of 5α-reductase, we re-examined the subcellular distribution of the enzyme using DNA and NMNAT as markers of nuclear contamination. Our interpretation of the results shown in fig 3 is that human prostatic 5α-reductase is located exclusively in the nucleus and that the 5α-reductase activity found in the microsomes is the result of contamination by nuclear material. We believe that a similar situation occurs in other tissues, given that there appears to be only one isoenzyme in rat prostate and epididymis, and that most workers find that the bulk of 5α-reductase activity is located in the nucleus [7,8]

The finding that DHT production is located in the nucleus rather than in the cytoplasm is clearly at variance with the model of androgen action in the prostate in which DHT production and the formation of the DHT-receptor complex occur in cytoplasm prior to translocation into the nucleus. Recently, however, several workers [16–18] have provided evidence that in oestrogen responsive tissues the classical two-step model may require modification An alternative hypothesis was proposed in which both occupied and unoccupied receptors are located exclusively in the nucleus and that the binding of oestrogen to the receptor results in an increase in the affinity of the receptor for nuclear acceptor sites [18]. While there is as yet no direct evidence that this is the case with other

steroid hormones, our finding that DHT is produced solely in the nucleus is compatible with such a mechanism and suggests that the androgen receptor may also be located exclusively in the nucleus.

ACKNOWLEDGEMENT

This work was supported by a grant from the Medical Research Council.

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