J. steroid Biochem. Vol. 22, No. 4, pp. 513–519, 1985
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0022-4731/85 $3.00 + 0.00
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METABOLISM OF ANDROGENS IN THE SEMINAL VESICLES AND THE DIFFERENT LOBES OF THE PROSTATE IN YOUNG MATURE RATS

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(*Received* 16 *April* 1984)

Summary—Oxidation and reduction of 4-androstene-3,17-dione (androstenedione), 17β-hydroxy-4-androsten-3-one (testosterone), 17β-hydroxy-5α-androstan-3-one (DHT), 5α-androstan-3α,17β-diol (3α-A′diol) and 5α-androstane-3β,17β-diol (3β-A′diol) were measured in homogenates from ventral (VP), dorsal (DP) and lateral prostate (LP), the coagulating gland (CG) and seminal vesicle (SV) of the intact sexually mature rat using NAD(H) or NADP(H) as cofactors. The specific activity of the various enzymes varied significantly between the different organs. 5α-Reductase activity was highest in the DP and the CG, and undetectable in the LP. 17β-Hydroxysteroid oxidoreductase (17β-HSOR) activity was mainly confined to the LP. 3α-Hydroxysteroid oxidoreductase (3α-HSOR) activity was also highest in the LP. In the VP the highest 3α-HSOR activity was recorded using NAD(H) as cofactor. In the other organs, similar or higher enzymatic activities were measured using NADP(H) as added cofactor. 3β-Hydroxysteroid oxidoreductase (3β-HSOR) activity was high in the LP and low or undetectable in the other tissues. Our results indicate that isoenzymes of 3α-HSOR, 3β-HSOR and 17β-HSOR are present in the accessory sex organs of the rat.

INTRODUCTION

The rat accessory sex organs are composed of paired ventral, lateral and dorsal prostatic lobes, the coagulating glands and the seminal vesicles. These organs surround the urethra and the base of the bladder. The coagulating gland, also named anterior prostate, is considered to be a lobe of the rat prostate and homologous with the median lobe in the human prostate [1]. Characteristic differences in the histological structure of the various lobes of the prostate and seminal vesicles have been reported [2, 3, 4]. The different prostatic lobes have been studied with regard to differences in post-castration involution [5, 6], differences in estrogen and androgen responsiveness [1], Zn uptake [7] and functional properties [1, 8]. Based on histologic similarities and obtainable biochemical data, the different organs may be divided into three subgroups: The lateral lobe and seminal vesicle; the dorsal lobe and coagulating gland; and the ventral lobe forming a third group [3]. In many studies the lateral and dorsal lobes have been combined and studied as the "dorso-lateral prostate" [7, 9–12] despite the great difference in histologic appearance.

In the prostate and in most androgen-responsive organs, 17β-hydroxy-5α-androstan-3-one (dihydrotestosterone) is considered to be the active androgen at the cellular level. In these organs there are enzymes which may further metabolize dihydrotestosterone. It is not clear whether such metabolic transformation of dihydrotestosterone generally represents biological inactivation or in fact biological activation in specific target cells [13, 14]. Different androgens display different androgenic potencies *in vivo* [15] and some responses are better or faster evoked by steroids other than dihydrotestosterone [16]. Since many of the metabolic transformations of dihydrotestosterone are reversible, a discussion of androgen actions of other steroids must always include the possible metabolic transformation of the actual steroid to dihydrotestosterone. Information about the relative activities of the various enzymes that metabolize and form dihydrotestosterone has resulted in an increased understanding of the biochemical basis for benign hyperplasia in human prostate [17], and has also led to a method to determine the sensitivity of malignant tumours to endocrine treatment [12].

Metabolism of androgens in the ventral prostate of the rat has been extensively studied *in vivo* and *in vitro* [18–29]. A few studies on the uptake and metabolism of androgens *in vivo* in the different lobes and SV

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Trivial names and abbreviations used: Androstenedione: 4-androstene-3,17-dione; Testosterone: 17β-hydroxy-4-androsten-3-one; Androstanedione: 5α-androstane-3,17-dione; Dihydrotestosterone (DHT): 17β-hydroxy-5α-androstan-3-one; 3α-A′diol: 5α-androstan-3α,17β-diol; 3β-A′diol: 5α-androstane-3β,17β-diol; Androsterone: 3α-hydroxy-5α-androstan-17-one; Epiandrosterone: 3β-hydroxy-5α-androstan-17-one; 17β-Hydroxysteroid oxidoreductase: 17β-HSOR; 3α(3β)-Hydroxysteroid oxidoreductase: 3α(3β)-HSOR; NADPH: Refers in this study to a NADPH-generating system (see Experimental). VP: Ventral prostate. DP: Dorsal prostate. LP: Lateral prostate. SV: Seminal vesicle. CG: Coagulating gland (anterior prostate).

have revealed marked differences between these tissues [30, 31, 32].

We have done an extensive comparative and systematic *in vitro* study of the androgen metabolism in the different lobes of the rat prostate and seminal vesicle.

EXPERIMENTAL

Chemicals

[1α,2α(*n*)-^{3}H]Testosterone (S.A. 53 Ci/mmol), [4-^{14}C]4-androstene, 3,17-dione (S.A. 58 mCI/mmol), [4-^{14}C]5α-dihydrotestosterone (S.A. 59 mCi/mmol), [1α,2α(*n*)-^{3}H]5α-androstan-3α,17β-diol (S.A. 40 Ci/mmol) and [1α,2α(*n*)-^{3}H]5α-androstane-3β,17β-diol (S.A. 40 Ci/mmol) were purchased from the Radiochemical Centre, Amersham, England. Unlabelled steroids were delivered by Steraloids Inc., U.S.A.

Labelled steroids were purified by thin layer chromatography (TLC). TLC was performed on 20 × 20 cm silica gel 60 7254 plates (33), purchased from E. Merck, Darmstadt, or on 20 × 20 cm silica gel F 1500 LS 254 W plates (34), purchased by Schleicher Schüll, Dassel, W. Germany.

From Sigma Chemicals Corp. NADP, NAD, glucose-6-phosphate, glucose-6-phosphate dehydrogenase were delivered. Scintillation fluid (Scint Hei-l) was obtained from F. Heidenreich, Oslo, Norway, and was diluted with absolute ethanol (2:100, v/v).

Solvents used for high-pressure liquid chromatography (HPLC) analysis were of HPLC grade (Rathburn Chemicals, Walkerburn, Great Britain). All other chemicals were obtained from E. Merck, Darmstadt, and were of *pro analysis* quality. Protein content of the various tissues was measured by the use of Bio-Rad Protein assay with bovine gamma globulin as a reference.

Animals

Intact Wistar male rats (age 60–90 days, weight 250–350 g) were used. The animals were purchased from Institutt for Folkehelse, Oslo, and kept in the animal quarters at Regionsykehuset in Trondheim for a minimum of 3 days before experiments were started. They were kept under controlled light (14 h light and 10 h darkness) and temperature (19–21°C) conditions. Rat chow and tap water were provided *ad libitum*.

Preparation of cell homogenates

The rats were killed by decapitation and immediately the different lobes of the prostate, the coagulating glands and the seminal vesicles were dissected free from surrounding tissue and taken out. Of the lateral and dorsal lobes only the lateral and the dorsal tip, respectively, were used to avoid the intermediate zone which contains both cellular elements [2] and also to avoid the ampullary gland which lies in close proximity to the lateral lobe [1]. A magnifying glass is helpful for this dissection. The ventral lobe, the coagulating glands and the seminal vesicles were taken out *in toto*. The intraluminar content of the seminal vesicles was pressed out. The different tissues were kept on ice, minced with fine scissors and suspended in a volume ice-cold Tris–buffer (0.05 M Tris adjusted to pH 7.4 at 37°C), depending on tissue weight. The tissue was homogenized using a glass/Teflon homogenizer with a tight-fitting ball-type piston at 300 rpm for 2–4 min. The homogenates were centrifuged at 800 *g* at 4°C for 20 min. The supernatant fraction was decanted and used in the incubation studies. The protein content of this cell extract varied between 2 and 12 mg/ml.

Conditions of incubation

Varying amounts of steroids (50, 75, 100, 150, 300 and 600 ng which correspond to a final concentration of 0.7–8.6 μM were used unless otherwise stated) were transferred to conical glass centrifuge tubes. The C_{14} labelled steroids were used as substrate without addition of unlabelled steroids while aliquots of H_3 labelled steroids (approx 2.5 10^5 cpm, 1–2.5 ng) were added together with unlabelled steroids. The solvents were evaporated to dryness under a stream of nitrogen. To the dry material was added 100 μl of Tris–HCl buffer (pH 7.4 at 37°C) and one of the following cofactors: βNAD (Grade III), βNADH (Grade III), and NADP (Sigma grade) at a final concentration of 0.1 mM, or a NADPH-generating system consisting of 10 μl NADP (2.5 mM in Tris–HCl), 20 μl glucose-6-phosphate (12.5 mM in Tris–HCl) and 10 μl glucose-6-phosphate dehydrogenase (250 μg/ml in Tris–HCl). After addition of buffer and cofactor the tubes were vortexed and preincubated at 37°C for 5 min in a water bath with shaking. One-hundred μl of homogenate was then added to each tube and incubated at 37°C for 15 or 45 min, depending on the expected enzymatic activity. Per cent conversion of the substrate varied normally from 5 to 20%.

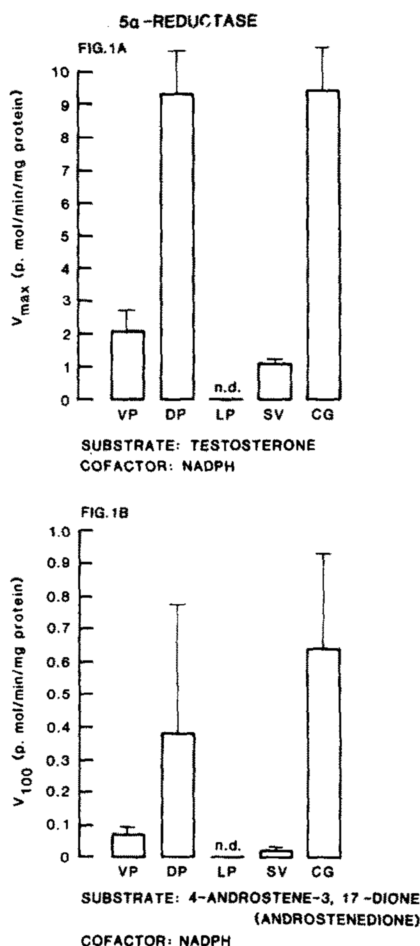
The incubations were terminated by the addition of 2 ml ice-cold ethyl acetate followed by vigorous stirring. The ethyl-acetate contained 15 μg/ml of each of the following non-radioactive steroids: Testosterone, androstenedione, dihydrotestosterone, androstanedione, 3α-A′diol, 3β-A′diol, androsterone and epiandrosterone. The tubes were extracted with this solution, followed by two extractions with 2 ml ethyl acetate containing no added steroids. Blank samples from each of the different steroids used in the experiment were prepared by adding ethyl acetate containing the above listed steroids before the addition of homogenates at zero time.

Separation of steroids

For the measurement of 17β-HSOR activity using testosterone or androstenedione as substrates, the metabolites were separated by HPLC and in-line monitoring of radioactivity [35, 36]. All other sep-

arations of steroids were performed by TLC as previously published [33, 34].

In the TLC systems the standards were visualized by spraying with a 0.2% etanolic solution of 2,7-dichlorofluorescein and viewed under ultraviolet light (366 nm) [34]. Each fraction was scraped off the TLC-plate, transferred to liquid scintillation vials containing 15 ml Scint Hei 1 scintillation cocktail diluted with 2% absolute ethanol. The radioactivity was determined in a Rack Beta liquid scintillation counter 1215 (LKB Wallac, Turku, Finland).



Figs 1A–B. 5α-Reductase activities recorded in incubations of homogenates from the intact young mature rat ventral prostate (VP), dorsal prostate (DP), lateral prostate (LP), seminal vesicle (SV) and coagulating gland (CG). The tissues were homogenated in Tris–HCl (pH 7.4 at 37°C) and the 800 *g* supernatant fraction was incubated in 45 min at 37°C in the presence of a NADPH-generating system and radiolabelled testosterone (Fig. 1A) or androstenedione (Fig. 1B). The metabolites were separated by thin layer chromatography. In Fig. 1A V_{max} was calculated from incubations with different amounts of testosterone added (0.7–8.6 μM). in Fig. 1B the enzymatic activities were recorded as V_{100} which correspond to the activity in incubations containing 1.4 μM (100 ng) of androstenedione. Values are given as mean ± SD. $n \geq 4$. n.d.: not detectable.

Assay of enzymatic activities

The sum of metabolites from each enzymatic reaction was calculated and the amount of enzyme activity was recorded in pmol/min/mg protein at each concentration of steroid added. The V_{max} for each of these enzymatic reactions was calculated from double reciprocal plots (Lineweaver–Burk). Each enzyme activity is calculated for 4–10 different incubation experiments and is given as mean ± standard deviation.

The activity of 17β-hydroxysteroid oxidoreductase with both testosterone and androstenedione as substrates, was so low in all the various organs, that a reliable V_{max} could not be calculated. Likewise the V_{max} of 5α-reductase using androstenedione as substrate could not be estimated due to a very low enzyme activity. The activity of these enzymes was recorded in pmol/min/mg protein in incubations containing 1.4 μmol (100 ng) of the substrate (V_{100}).

RESULTS

5α-Reductase

The enzymatic activity of 5α-reductase in the 800 *g* supernatant fraction from the different prostatic lobes and the SV is shown in Figs 1A and 1B. The V_{max} of this enzyme using testosterone as substrate varied significantly from one organ to the other. The highest activity was recorded in the DP and the CG. In the LP, no activity of 5α-reductase was recorded using our assay, while the VP and SV showed intermediate values. No 5α-reductase could be detected in the lateral prostatic lobe using different buffers or incubation conditions.

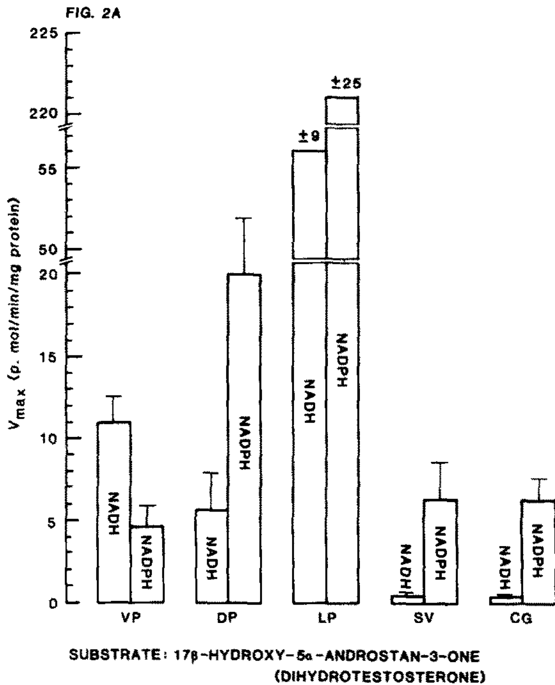
5α-Reductase activities measured in incubations containing a fixed concentration of androstenedione (see Experimental) were very low (Fig. 1B). The relative differences in the activities between the various tissues were similar to those observed with testosterone as substrate.

The 5α-reductase activities in Fig. 1 are restricted to incubations in the presence of a NADPH-generating system. With NADH as a cofactor, no activity of this enzyme was demonstrated in any of the tissues studied (data not shown).

3α-Hydroxysteroid oxidoreductase (3α-HSOR)

As shown in Figs 2A–B the enzymatic activity of 3α-HSOR calculated from incubations with dihydrotestosterone (reductive activity—3α-HSOR RED) or 3α-A′diol (oxidative activity—3α-HSOR OX) as substrates varied significantly between the tissues studied and was dependent on the cofactors added.

The 3α-HSOR RED activity (Fig. 2A) was very high in the LP, and significantly lower in the other organs. In the VP the ratio of NADH to NADPH-dependent activity was approximately 2.5, while in the other tissues this ratio was 0.10–0.25.



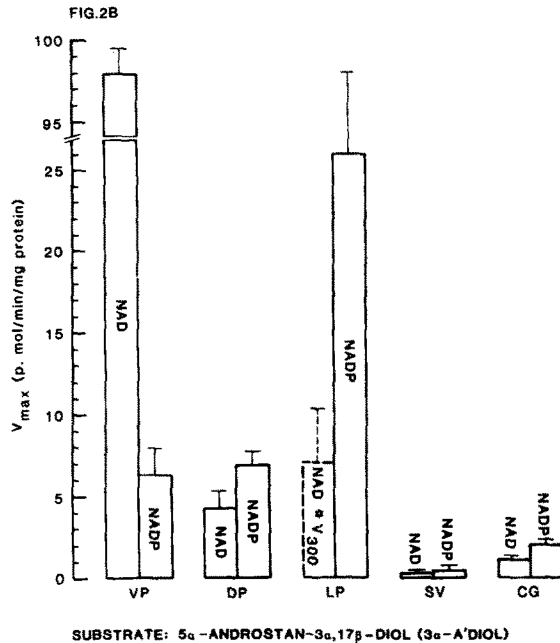


Fig. 2A–B. 3α-Hydroxysteroid oxidoreductase (3α-HSOR) activities recorded in incubations of homogenates from different lobes of the rat prostate and seminal vesicle (conditions, see legend Fig. 1). V_{max} was calculated from incubations with different amounts (0.7–8.6 μM) of dihydrotestosterone (Fig. 2A) or 3α-A′diol (Fig. 2B), and cofactors as indicated in the figures. Values are given as mean ± SD. $n = 4$. *(Fig. 2B). This V_{max} of 3α-HSOR in the LP could not be calculated (> ∞). V_{300} correspond to the enzyme activity recorded in incubations containing 4.3 μM (300ng) of the substrate (3α-A′diol).

A comparable diversity between the tissues is shown in Fig. 2B where the NAD-dependent 3α-HSOR OX activity in the VP was very high and increased 15-fold by using NAD compared to NADP as cofactor. In the other lobes and the SV this ratio was less than 1. The 3α-HSOR OX activity was intermediate in the LP and low in the DP, and CG and the SV.

3β-Hydroxysteroid oxidoreductase (3β-HSOR)

The 3β-HSOR activities shown in Figs 3A–B are calculated from incubations with dihydrotestosterone (reductive activity—3β-HSOR RED) or 3β-A′diol (oxidative activity—3β-HSOR OX) as substrates.

The 3β-HSOR RED activity (Fig. 3A) could not be detected in the VP. With NADPH as cofactor, the highest activities were recorded in the LP and the DP. The NADH-dependent activity was intermediate in the LP and the DP, low but detectable in the SV and CG.

In the VP, the LP and the SV, there were no signs of 3β-HSOR OX activity (Fig. 3B). In the DP a low activity of this enzyme was found, with a ratio of NADP to NAD dependent activity of approx 3. A very low activity was detected in the CG, but only when NAD was added as cofactor.

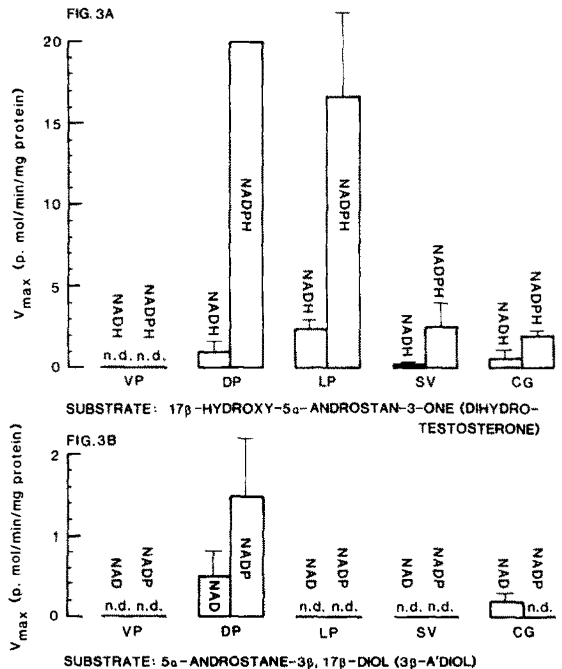


Fig. 3A–B. 3β-Hydroxysteroid oxidoreductase (3β-HSOR) activities recorded in incubations of homogenates from different lobes of the rat prostate and seminal vesicle (conditions, see legend Fig. 1). V_{max} was calculated from incubations with different amounts (0.7–8.6 μM) of dihydrotestosterone (Fig. 3A) or 3β-A′diol (Fig. 3B) and cofactors as indicated in the figures. Values are given as mean ± SEM. $n \geq 4$. n.d.: not detectable.

17β-Hydroxysteroid oxidoreductase (*17β*-HSOR)

The 17β-HSOR activities calculated from incubations with 5 different substrates are shown in Figs 4A–E. The reductive activity (17β-HSOR RED) was calculated using androstenedione as substrate (Fig. 4A), while oxidative activities (17β-HSOR OX) were calculated from incubations with the following substrates: Testosterone (Fig. 4B), dihydrotestosterone (Fig. 4C), 3α-A'diol (Fig. 4D) and 3β-A'diol (Fig. 4E). The enzymatic activities in Figs 4A-B were calculated from incubations with a fixed concentration of the substrate, while in Figs 4C–E a V_{max} was calculated (see Experimental).

The lateral prostate contained the highest oxidative as well as reductive enzymatic 17β-HSOR activity. The 17β-HSOR RED activity in the LP was highest with NADPH as cofactor. In the DP, the SV and CG very low activity of this enzyme was recorded, and only when NADPH was used as cofactor. The 17β-HSOR OX in the LP showed no significant difference in activity between NAD and NADP as added cofactor when DHT or 3β-A'diol were substrates, while the activity was highest using NADP as cofactor when testosterone and 3α-A'diol were substrates. In the SV, 17β-HSOR OX could not be demonstrated, whereas in the VP, the DP and the CG this enzymatic activity was low or undetectable, depending on substrate and cofactor.

DISCUSSION

In our study we have chosen the 800 *g* supernatant fraction as a source of enzyme, and the nucleus-associated enzymes are therefore not included. In the VP, 25–50% of 5α-reductase is localized to the nuclear fraction [18, 19, 20], whereas the corresponding figure for the SV is less [37]. The other enzymes which we have studied are mainly located outside the nucleus [21–30]. The exact subcellular distribution of the different androgen metabolizing enzymes in the various prostatic lobes and SV are still unknown.

In our assay, using testosterone as substrate, the V_{max} of 5α-reductase was approximately the same in the DP and CG with a specific activity of 9.3 pmol/min/mg protein, far higher than in the VP [2, 1] and SV [1, 1]. In the LP, in fact no activity of this enzyme could be recorded. A similar relationship was found using androstenedione as sub-

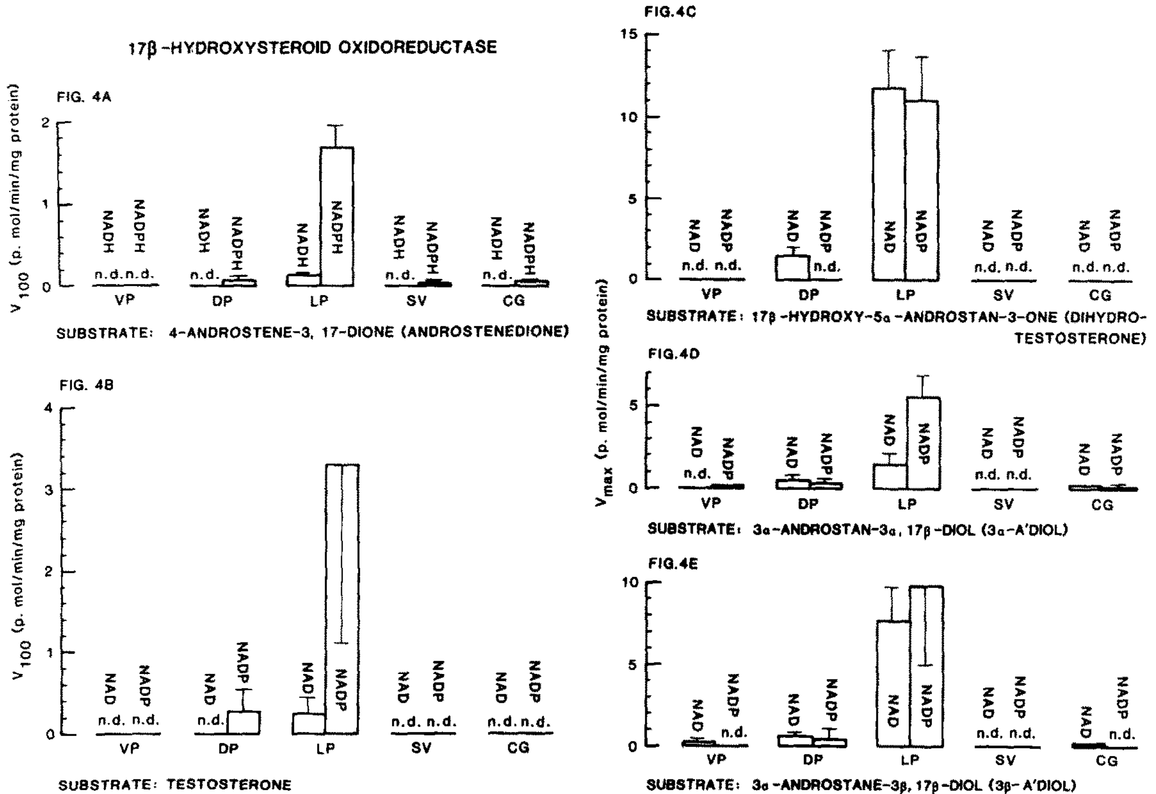


Fig. 4A–E. 17β-Hydroxysteroid oxidoreductase (17β-HSOR) activities recorded in incubations of homogenates from different lobes of the rat prostate and seminal vesicle (conditions, see legend Fig. 1). In Figs 4A-B the enzymatic activities were recorded as V_{100} which correspond to the activity in incubations containing 1.4 μM (100 ng) of androstenedione (Fig 4A) or testosterone (Fig. 4B) and cofactors as indicated in the figures. In Figs 4C–E V_{max} was calculated from incubations with different amounts (0.7–8.6 μM) of dihydrotestosterone (Fig. 4C), 3α-A'diol (Fig. 4D) or 3β-A'diol (Fig. 4E) added and cofactors as indicated in the figures. Values are given as mean ± SEM. $n \geq 4$. n.d.: not detectable.

strate. The ventral prostate has only one form of 5α-reductase in contrast to the liver, and it has different substrate specificity for different Δ4-3OXO-ketosteroids [19]. The constant ratios of enzymatic activities for the two substrates may indicate that it is the same 5α-reductase in the different prostatic lobes and the SV. In the LP, we were not able to detect any 5α-reductase activity. Studies on the nuclear fraction of this lobe are in progress.

Using NADH as cofactor no 5α-reduced products could be demonstrated, confirming that 5α-reductase has an absolute requirement for NADPH as the source of the reducing hydride ion [19].

The 3α-HSOR in the VP showed a NAD(H)-dependent activity unlike the other organs, where NADP(H) was the preferred cofactor. Conflicting results have been published with regard to cofactor and 3α-HSOR in the VP [24, 38], and may reflect that the NADH-dependent 3α-HSOR activity is mainly localized to the particulate fractions whereas the NADPH-dependent activity is mainly soluble [38]. Our ratio of NAD to NADP-dependent activity is comparable to the values published by Clark *et al.*[38]. In human normal and diseased prostatic tissue, NADH could not be substituted for NADPH to obtain maximum enzyme activity [25], indicating that the 3α-HSOR in the rat VP is different from human prostate 3α-HSOR. Another difference between the prostatic lobes is the 3α-HSOR OX/3α-HSOR RED ratio, which in the VP is higher than 1, whereas the reductive enzymatic activity dominated in the other lobes. Highest specific activity of 3α-HSOR was recorded in the LP.

In the VP, we were unable to detect any activity of 3β-HSOR (Figs 3A–B). While most *in vitro* studies of the VP in the rat have shown no activity of this enzyme [18, 24, 28], VP in organ culture has demonstrated a low and reversible activity [26], like many other androgen dependent organs of the rat [27]. In the LP and the SV no activity of 3β-HSOR OX was detected, whereas 3β-HSOR RED activity was present, preferring NADPH as cofactor.

In the DP and the CG the 3β-HSOR activity was reversible with a higher activity when NADP(H) was added as cofactor, except the 3β-HSOR OX in the CG which only could be demonstrated using NAD. In organ culture from the VP, NAD(H) was also the preferred cofactor [26], in contrast to other androgen dependent organs [27].

Our results indicate that LP is rich in 17β-HSOR activity (Figs 4A–E). A low concentration of this enzyme has been detected in the VP [29], whereas the concentrations were higher in other androgen dependent organs of the rat [39] and in rat prostatic cancer [40]. In our study, the cofactor added to obtain maximum activity of this enzyme is different in the various accessory sex organs, in agreement with previous reports [29]. In the LP, the highest activity of 17β-HSOR was recorded using NADP(H) as cofactor when testosterone, androstenedione or 3β-A'diol were substrates, while no significant difference in preferred cofactor was observed using 3α-A'diol or DHT as substrates. In the other prostatic lobes and the SV, the 17β-HSOR activity was very low or undetectable, depending on cofactor added.

In our study of enzymatic activities, the VP differ from the other lobes according to the activity of 5α-reductase, 3α-HSOR and 3β-HSOR. The very low 17β-HSOR is also a feature of SV and CG. The DP and the CG show similarities in the activities of 5α-reductase and 3α-HSOR, but not in 3β-HSOR and 17β-HSOR. The LP and the SV are strikingly different with regard to all the enzymes studied, and hence, anatomic similarities [3] are not reflected in the enzymatic activities recorded in this study.

5α-Reductase activity was only recorded when NADPH was added as cofactor, and the ratio of this enzymatic activity between testosterone and androstenedione as substrates was very similar in all the different tissues. This may indicate that no isoenzymes of 5α-reductase exist in these organs. On the other hand, the difference in preferred cofactor reqired for oxidative and reductive enzymatic activities and the difference in substrate specificity in the different tissues studied indicate that isoenzymes of 3α-HSOR, 3β-HSOR and 17β-HSOR exist in rat accessory sex organs. The biological significance of these isoenzymes remains to be investigated.

Acknowledgements—Dr. P. I. Lundmo, M. D. and Dr. Ing. A. Sunde are research fellows of the Norwegian Society for Fighting Cancer. This work was supported by a grant from this society We are indebted to Mr N. Nesjan for taking care of the animals and to Mrs D. Moholdt for typing the manuscript.

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