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AGE DEPENDENT CHANGES IN ANDROGEN METABOLISM IN THE RAT PROSTATE

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Summary—Oxidation and reduction of androstenedione, testosterone, dihydrotestosterone (DHT), 5α-androstan-3α,17β-diol and 5α-androstane-3β,17β-diol (3α- and 3β-A'diol) were measured in homogenates from the ventral prostate (VP), dorsal prostate (DP), lateral prostate (LP), the coagulating gland (CG) and seminal vesicles (SV) in intact rats of different ages from young mature (3–6 months) to senescent rats (20–30 months). Some very old intact rats (30–32 months) were treated with testosterone in order to rule out the effect of this hormone on androgen metabolism. The enzymatic activities for young mature rats were significantly altered by increasing age, both with regard to differences between the various organs as well as differences in cofactor requirement. With increasing age, the specific activity of most enzymes gradually decreased. With testosterone as substrate, 5α-reductase activity was significantly reduced in the old rats in all tissues studied and was undetectable in the oldest animals in the VP and the SV. On the other hand, 5α-reductase could not be recorded in any tissue in old rats when androstenedione was the substrate. 3α-Hydroxysteroid oxidoreductase (3α-HSOR) in the VP was the only enzyme which did not decrease in activity by increasing age. In the other lobes this enzyme activity decreased similar to 3β-hydroxysteroid oxidoreductase (3β-HSOR) and the 17β-hydroxysteroid oxidoreductase (17β-HSOR) activity. Administration of testosterone to old rats increased the specific activity of most of the enzymes studied.

INTRODUCTION

The rat accessory sex organs are composed of different prostatic lobes, the coagulating glands and the seminal vesicles. Prostatic cancer in rats can be induced by carcinogenic agents [1] or sex-hormone treatment [2], or may occur spontaneously in old rats [2, 3, 4]. These cancers occur most frequently in the ventral prostatic lobe [2, 4], and occasionally in the dorsal lobe [1, 3]. There is only one report on cancer of the coagulating gland [5]. As in man, benign prostatic hyperplasia also occurs in the rat prostate where it is confined to the ventral lobe [4, 6]. There are reports on the influence of ageing on the receptor content of the rat ventral prostatic lobe [7, 8], androgen metabolism *in vitro* in this lobe [9] and *in vivo* uptake of androgens in different tissues in the rat [10]. There are, however, no reports on the changes in androgen metabolism *in vitro* in the different lobes of the rat prostate and seminal vesicles from adolescens to senescens. The present study is a systematic and detailed investigation on numerous androgen metabolizing enzymes in the accessory sex organs of old rats.

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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 [16]. VP: Ventral prostate. DP: dorsal prostate. LP: lateral prostate. SV: seminal vesicles. CG: coagulating gland (anterior prostate).

EXPERIMENTAL

Animals

Wistar rats of different ages were used. The young animals (3–6 months old) 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. Fifty male retired breeders, 12 months of age, were delivered by Møllegårds Avlslaboratorium, Denmark, and kept in the same animal quarter until sacrified at different ages as indicated in the results. Seven rats, three 30-months old and four 32-months old, respectively, were treated for 10 days with testosterone proprionate (1 mg/bw/day) and sacrified the 11th day. These rats are indicated in the results as 30–32 months, T-treated. All animals were exposed to controlled light (14 h light and 10 h darkness), and temperature (19–21°C) conditions. Rat chow and tap water were provided *ad libitum*.

Old animals were used in experiments only if they were in good health condition and the accessory sex organs appeared normal. During the 20 months the rats were kept in our animal quarters, five were

sacrified because of different malignant tumours [5], and 9 were sacrified or died of other or unknown diseases.

Enzymatic assays

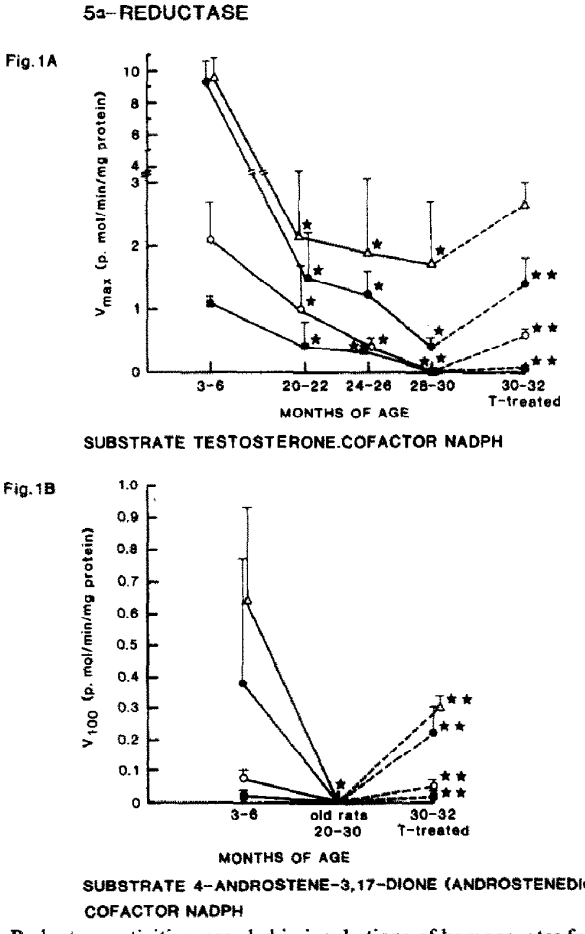
Details related to chemicals, preparation of cell homogenates, conditions of incubations, separation of steroids and assay of enzymatic activities have been described previously [11–15]. The V_{max} of the different enzymatic activities were calculated from a minimum of 4 different incubations and given as mean ± SD. Statistical values were obtained by Student's t-test.

The activity of 5α-reductase using androstenedione as substrate and 17β-hydroxysteroid oxidoreductase when testosterone or androstenedione were substrates was so low that a V_{max} could not be calculated. The activities of these enzymes were recorded in incubations containing 1.4 μM (100 ng) of the substrate and named V_{100}.

RESULTS

Changes in enzymatic activities with increasing age

5α-Reductase (Figs 1A–B). With increasing age, the activity of this enzyme decreased in all the organs studied, except in the LP where no 5α-reductase could be detected neither in young and old rats nor in old rats after testosterone treatment. When androstenedione was the substrate, no 5α-reductase activity could be recorded in untreated old animals in any tissue, whereas low activity could be measured in the DP and the CG in the oldest rats with testosterone as substrate.



Figs 1A–B. 5α-Reductase activities recorded in incubations of homogenates from different prostatic lobes and seminal vesicle in the intact young (3–6 months old) rats, intact old rats and intact old rats who have been treated with testosterone propionate (1 mg/kg b.wt) in 10 days before experiments were started (T-treated). 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 (NADPH) and radiolabelled testosterone (Fig. 1A) or androstenedione (Fig. 1B). The metabolites were separated by HPLC or TLC. In Fig. 1A. V_{max} was calculated from incubations with different amounts of testosterone added (0.7–8.6 μM). In Fig. 2B 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, $n \geq 4$. *Significant different ($P \leq 0.05$) from young rats (3–6 months old). **Significant different ($P \leq 0.05$) from untreated old rats (28–30 months old). ○——○: Ventral prostate (VP). ●——●: Dorsal prostate (DP). □——□: Lateral prostate (LP). ■——■: Seminal vesicle (SV). △——△: Coagulating gland (CG). Using this assay, no 5α-reductase was detectable in the LP.

After testosterone treatment to 30 and 32 months old animals (see Experimental), the activity of 5α-reductase increased compared to the values obtained in the untreated old animals in all tissues and substrates studied to a specific activity of approximately half of that in the young animals.

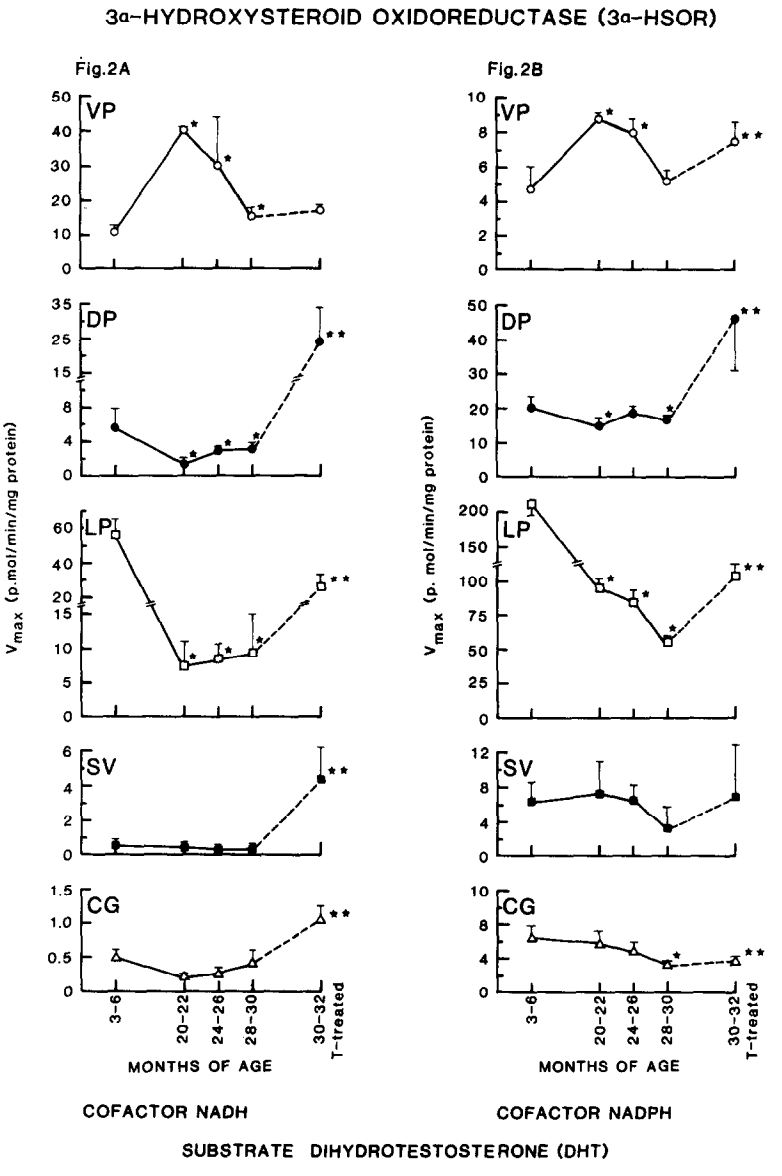
With NADH as cofactor, no activity was demonstrated in any of the tissues studied (data not shown in the figures).

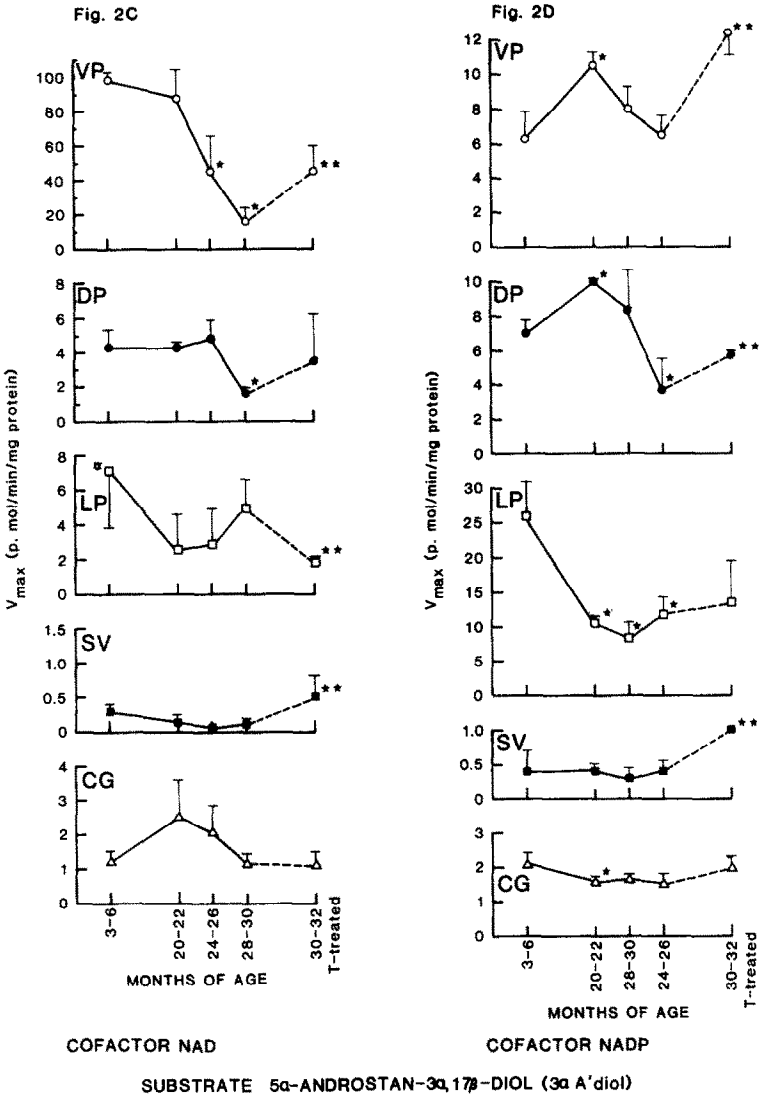
3α-Hydroxysteroid oxidoreductase (3α-HSOR) (Fig. 2A–D). The 3α-HSOR activity is calculated from incubations with dihydrotestosterone (DHT) as substrate (reductive enzymatic activity 3α-HSOR RED—Figs 2A–B) or 3α-A'diol as substrate (oxidative enzymatic activity—3α-HSOR OX—Figs 2C–D). Cofactors were added to the incubations as indicated in the figures.

In the VP this enzyme preferred NAD(H) as cofactor, opposite to all the other tissues studied in which NADP(H) gave higher enzymatic activities. The 3α-HSOR RED in the VP was higher in the 20–25-month old animals than in the young rats, like the 3α-HSOR OX in the VP and the DP when NADP was cofactor. In the LP, the high activity of this enzyme was significantly reduced in the old animals, whereas in the DP, the SV and the CG small or insignificant changes were observed with increasing age.

In the VP, the 3α-HSOR RED activity was unchanged or slightly elevated following testosterone treatment to old rats, while the 3α-HSOR OX activity increased significantly. In the other tissues investigated, the 3α-HSOR RED activity increased and the 3α-HSOR OX activity remained unchanged or decreased in response to this treatment.

3β-Hydroxysteroid oxidoreductase (3β-HSOR)





Figs 2A–D. 3α-Hydroxysteroid oxidoreductase (3α-HSOR) activities recorded in incubations of homogenates from different lobes of the rat prostate and seminal vesicle in intact rats of different age and in old rats who have received testosterone propionate (1 mg/kg b.wt for 10 days) prior to experiment (T-treated). V_{max} was calculated from incubations with different amounts (0.7–8.6 μM) of dihydrotestosterone (Figs 2A–B) or 3α-A'diol (Figs 2C–D) and cofactors as indicated in the figures. Values are given as mean ± SD, $n \geq 4$. *Significant different ($P \leq 0.05$) from young rats (3–6 months old). **Significant different ($P \leq 0.05$) from untreated old rats (28–30 months old). Conditions and abbreviations, see legend to Fig. 1. *(Fig. 2C) This V_{max} of 3α-HSOR in the LP would not be calculated (> ∞). This value correspond to the enzymatic activities in incubations containing 4.3 μM of the substrate (3α-A'diol).

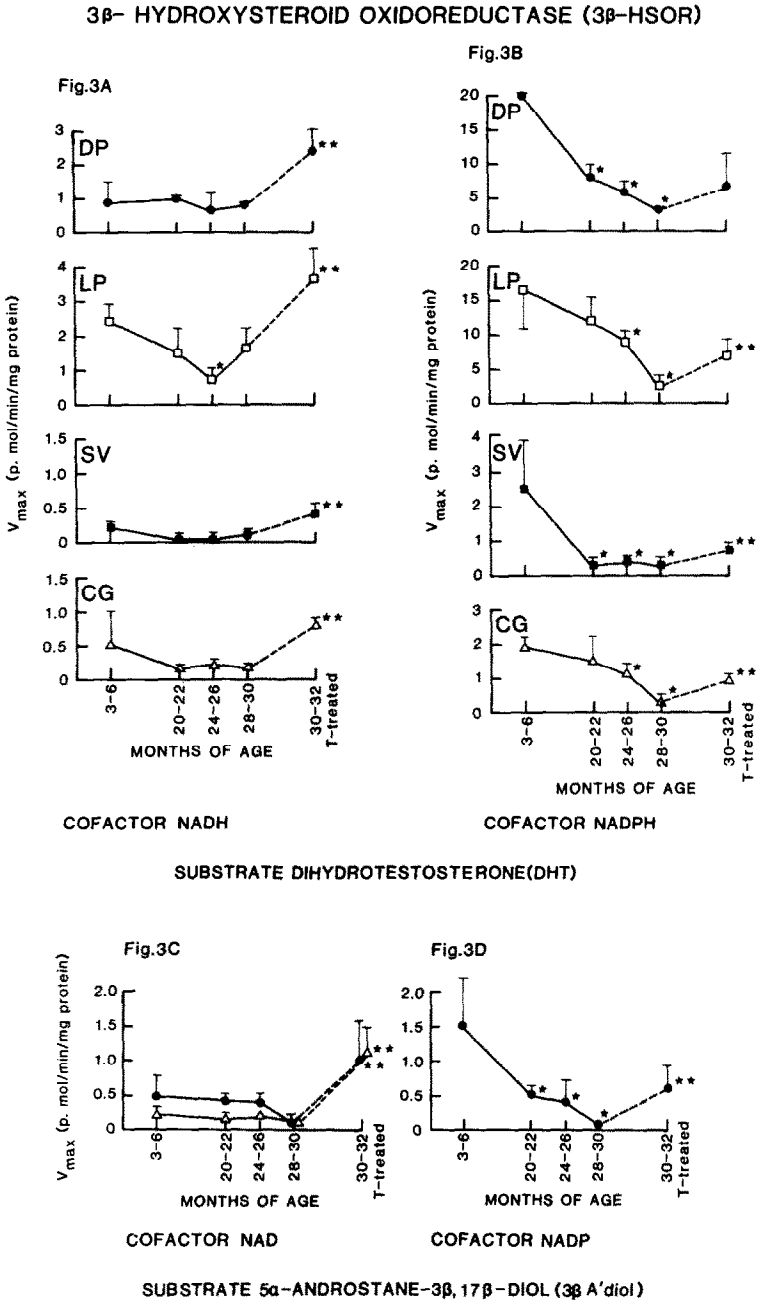
(*Figs 3A–D*). The 3β-HSOR activities are calculated from incubations with dihydrotestosterone (DHT) as substrate (reductive enzymatic activity—3β-HSOR RED—Figs 3A–B) or 3β-A'diol as substrate (oxidative activity—3β-HSOR OX—Figs 3C–D) Cofactors were added to the incubations as indicated in the figures.

The 3β-HSOR RED activity was detectable in all organs studied except the VP, while the 3β-HSOR OX could only be detected in the DP and CG.

With increasing age, the 3β-HSOR showed a moderate decline or remained unchanged, depending on organ and cofactor. Testosterone treatment elevated the activity of this enzyme in all tissues studied, compared to the activity in the oldest untreated animals.

17β-Hydroxysteroid oxidoreductase (17β-HSOR) (*Figs 4A–B and Table* 1). The 17β-HSOR reductive activity was calculated from incubations with androstenedione (17β-HSOR RED—Fig. 4A), whereas the oxidative enzymatic activity (17β-HSOR OX) was calculated using the following substrates: testosterone (Fig. 4B) and dihydrotestosterone, 3α-A'diol and 3β-A'diol (Table 1). The values presented in Figs 4A–B are calculated from incubations with a fixed concentration of the substrate, while the activities in Table 1 are recorded as V_{max} (see Experimental).

The activity of 17β-HSOR OX/RED in Figs 4A–B

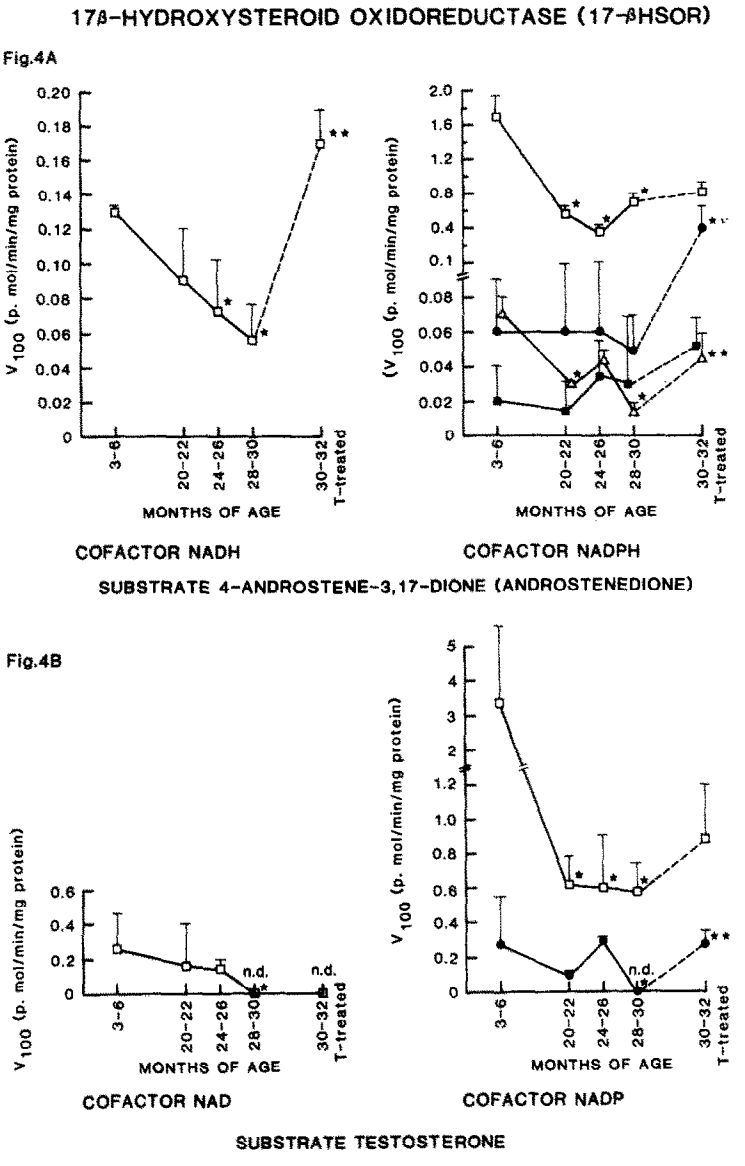


Figs 3A–D. 3β-Hydroxysteroid oxidoreductase (3β-HSOR) activities recorded in incubations of homogenates from different lobes of the rat prostate and seminal vesicle in intact rats of different age and in old rats who have received testosterone propionate (1 mg/kg b.wt for 10 days) prior to experiment (T-treated). V_{max} was calculated from incubations with different amounts (0.7–8.6 μM) of dihydrotestosterone (Figs 3A–B) or 3β-A'diol (Figs 3C–D) and cofactors as indicated in the figures. Values are given as mean ± SD, $n \geq 4$. *Significant different ($P \leq 0.05$) from young rats (3–6 months old). **Significant different ($P \leq 0.05$) from untreated old rats (28–30 months old). Conditions and abbreviations, see legend to Fig. 1.

was mainly localized to the LP. When NAD(H) was cofactor, this enzyme could only be recorded in the LP. Using NADP(H), 17β-HSOR OX could be detected only in the LP and the DP, while the 17β-HSOR RED was detectable in all tissues studied, except the VP.

With increasing age, the activity of 17β-HSOR recorded with the two substrates shown in Figs 4A–B showed a moderate or insignificant decline. Treatment with testosterone enhanced this enzyme in all tissues.

17β-HSOR OX activities enlisted in Table 1 are restricted to the LP, as this enzyme could not be detected in the other tissues studied neither in un-



Figs 4A–B. 17α-Hydroxysteroid oxidoreductase (17α-HSOR) activities recorded in incubations of homogenates from different lobes of the rat prostate and seminal vesicle in intact rats of different age and in old rats who have received testosterone propionate (1 mg/kg b.wt for 10 days) prior to experiment (T-treated). 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 the substrate. In Table 1 V_{max} was calculated from incubations with different amounts of substrate added (0.7–8.6 μM). Values are given as mean ± SD, $n \geq 4$. *Significant different ($P \leq 0.05$) from young rats (3–6 months old) **Significant different ($P \leq 0.05$) from untreated old rats (28–30 months old). Conditions, see legend to Fig. 1. ○——○: Ventral prostate (VP). ●——●: Dorsal prostate (DP). □——□: Lateral prostate (LP). ■——■: Seminal vesicle (SV). △——△: Coagulating gland (CG).

Table 1. 17β-Hydroxysteroid oxidoreductase (17β-HSOR) in the LP

		Age of rats (Months)				
Substrate	Cofactor	3–6	20–22	24–26	28–30	30–32 T-treated
3α-A'diol	NAD	1.5 ± 0.6	0*	0*	0*	0.75 ± 0.4**
3α-A'diol	NADP	5.6 ± 1.3	1.55 ± 0.2*	2.2 ± 0.7*	2.2 ± 0.7*	1.4 ± 0.2**
3β-A'diol	NAD	7.6 ± 2.1	0*	0*	0*	0.9 ± 0**
3β-A'diol	NADP	9.8 ± 4.9	3.3 ± 1.2*	1.5 ± 0.7*	2.3 ± 0.4*	1.7 ± 0.2**
DHT	NAD	11.8 ± 2.2	0*	0*	0*	0.45 ± 0.5
DHT	NADP	11.0 ± 2.2	1.6 ± 0.2*	1.3 ± 0.3*	1.1 ± 0.25*	3.5 ± 0.3**

Values are given as V_{max} in pmol/min/mg protein.

treated old animals nor in testosterone-treated old rats.

In the LP, the activity of 17β-HSOR OX was highest when DHT or 3β-A'diol were substrates. When NAD was the cofactor, no activity could be detected in the untreated old animals, while a slight but detectable rise occurred after testosterone treatment. In incubations with NADP, 17β-HSOR OX declined with increasing age for all the three substrates used. After testosterone treatment, the 17β-HSOR OX showed a rise in activity when 3α-A'diol was substrate, opposite to the decline observed with 3β-A'diol or DHT as substrates.

DISCUSSION

Several studies on rat ventral prostate have shown reduced androgen receptor content [8, 10] as well as 5α-reductase activity [9, 16] and lowered DHT-levels with increasing age [17]. These data have been interpreted as a consequence of diminished plasma testosterone [9]. However, the ageassociated changes in the other accessory sex organs appear to be more complicated. According to Robinette *et al.*[10] the concentration of androgen receptors in old animals remains constant in the coagulating gland and the dorsal prostate, increases in the seminal vesicles, and declines in the ventral and the lateral prostatic lobes.

In our study, the activity of 5α-reductase declined with increasing age in all the various tissues, except in the LP where no 5α-reductase could be detected at any age. After testosterone treatment to the old animals, the activity of this enzyme increased to about half of the values obtained in the young animals (Figs 1A–B). Similar results have previously been reported with regard to VP [9, 16]. Our study also confirms the absolute requirement for NADPH as cofactor in the 5α-reduction of testosterone [18].

The activity of 3α-HSOR in the different tissues, using different substrates and cofactors (Figs 2A–D), showed a heterogenic picture as compared with 5α-reductase. Regarding to differences between the lobes, the 3α-HSOR in the VP preferred NAD(H) as cofactor, whereas the other tissues required NADP (H) to obtain maximum enzymatic activity. This preference of NAD(H) in the VP of the rat has been reported in another study [19], in contrast to the 3α-HSOR in normal and diseased human prostatic tissue which prefers NADP(H) [20].

Like the 3α-HSOR, the 3β-HSOR showed very different activity depending on tissue, age, substrate and cofactors (Fig. 3A–D). This enzyme was apparently not present in the VP, consistent with previous reports [21], and in contrast to other genital tissues and androgen dependent organs [22]. In the LP and the SV, only reductive 3β-HSOR activity was present whereas the DP and the CG contained both oxidative and reductive activity. With increasing age, the specific activity of 3β-HSOR was moderately reduced, with some modification related to tissue and cofactor. Generally, there was an increase in 3β-HSOR activity after testosterone treatment to the oldest rats.

There was no activity of 17β-HSOR (Figs 4A–B) in the VP and very low in the other tissues in agreement with *in vivo* studies [10, 23], and unlike the high activity recorded in other organs in the rat, like testis and the kidney [24]. In the old animals, this enzyme was essentially unchanged or moderately reduced, and raised after testosterone treatment in most tissues. The main localization of this enzyme was in the LP, and NADP(H) was the preferred cofactor.

When the androgens outlined in Table 1 were used as substrates, only minute amounts of 17β-HSOR activity could be detected in the VP, the DP, the SV and the CG in young animals [12]. In these tissues, this enzyme was apparently not present neither in old rats nor after stimulation with testosterone. In the LP, however, this enzymatic activity was high in young rats (Table 1), and could also be recorded in old rats, but only when NADP was the cofactor. The responses in activity to testosterone treatment differed, depending on substrate and cofactor.

With increasing age, the changes in enzymatic activities do not reflect the variations in receptor content in these different tissues [10]. The 5α-reductase activity showed a decline parallel to the fall in serum testosterone reported in old age [9], probably due to diminished testicular function.

In the prostate and in other androgen responsive organs, dihydrotestosterone is considered to be the androgen which bind to the receptor and initiate the events of androgen response. Since Baulieu *et al.* introduced their "multipurpose concept" [25] of specific effects of different 5α-reduced androgens, research have not gained conclusive answers to whether these metabolites represent metabolic steps in biological inactivation, storage function or in fact have biological functions in specific target cells.

Our study indicate that the 5α-reductase which irreversibly form dihydrotestosterone is the same enzyme in all the different prostatic lobes and accessory sex organs. On the other hand, the variation of enzymatic activities between the different tissues, the changes with increasing age as well as the responses to testosterone treatment, indicate a variety of isoenzymes of 3α,3β and 17β-HSOR, which reversibly form and remove dihydrotestosterone in the tissues. The physiological significance of the multiplicity of these enzymes is not understood although it might fit with the theory of specific effect of other 5α-reduced androgen than DHT at the cellular level. Much more work has to be done, however, before any conclusion can be drawn concerning possible physiological effects of the various androgen metabolites.

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