



Rat Steroid 5α -Reductase Kinetic Characteristics: Extreme pH-dependency of the Type II Isozyme in Prostate and Epididymis Homogenates

P. N. Span,^{1*} A. G. H. Smals,¹ C. G. J. Sweep² and Th. J. Benraad²

¹Department of Medicine, Division of Endocrinology and ²Department of Experimental and Chemical Endocrinology, University Hospital, Nijmegen, The Netherlands

Reevaluating the assay for rat steroid 5α -reductase isozymes in prostate and epididymis homogenates we encountered an extreme pH-dependency of the type II isozyme. The time-course of the metabolism of testosterone (T) to 17β -hydroxy- 5α -androstan-3-one (DHT) at acidic pH shows an initial burst when the homogenate is not brought to pH before the start of the incubation. Therefore, the rat type II 5α -reductase isozyme does not follow Michaelian law under these conditions making a single time point measurement invalid. Assessing the pH-optimum of 5α -reduction in both rat prostate and epididymis homogenates we found a strong substrate dependency: at high substrate concentrations a pH-optimum for the type II isozyme of pH 5.0 was found, whereas at lower concentrations pH 5.5 is optimal. Establishing V_{\max} (maximum velocities) and K_m (affinity constants) for the 5α -reduction of T at pH 4.5–8.0, the efficiency optimum V_{\max}/K_m appeared to be pH 5.5 in both prostate and epididymis homogenates. Specifically at acidic pH these kinetic characteristics of the type II isozyme vary many-fold. Discrepancies in literature concerning 5α -reductase characteristics can, at least in part, be attributed to the choice of optimal pH, or to pH shifts during the assay.

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INTRODUCTION

Steroid 5α -reductase (5α -reductase E.C. 1.3.99.5) is a NADPH-dependent enzyme capable of 5α -reducing a number of steroids with a 4,5 double bond and a 3-oxo group, including glucocorticoids, progestogens, mineralocorticoids, androgens [1] and non-androgens such as the pheromone precursor 4,16-androstadien-3-one [2, 3].

5α -Reductase can serve an anabolic purpose, catalyzing the conversion of testosterone (T) into the more potent androgen dihydrotestosterone (DHT) [4]. DHT binds with higher affinity to the androgen receptor than does T [5, 6]. DHT has been implicated in the pathogenesis of benign prostatic hyperplasia (BPH), prostate cancer, acne vulgaris, androgenic alopecia and hirsutism [7–11]. 5α -Reductase can also play a catabolic role, as 5α -reduced metabolites are susceptible to $3\alpha/3\beta$ -reduction. These 3-hydroxylated

metabolites can be excreted after subsequent hydroxylation or conjugation.

In 1970, Voigt *et al.* described a 5α -reductase with a pH-optimum of 5.5 in homogenates of human foreskin [12]. It was not until 1976 that Moore and Wilson postulated the existence of two 5α -reductase subtypes with distinct pH-optima [13], later designated type I and II according to the chronological order in which their cDNA's were isolated [14–17]. Research on patients with a type II 5α -reductase deficiency (pseudohermaphroditism) has underlined the importance of the type II isozyme in the development of several androgen-dependent organs [18–20]. This signifies an anabolic role of this type II isozyme. The role of the type I 5α -reductase isozyme is not known as patients with a deficiency have not yet been described, but is assumed to be catabolic [14].

The 5α -reductase isozymes are highly conserved as rat and human tissues both exhibit two subtypes [14–17, 21, 22]. Amino acid sequence homology between rat and human subtypes is higher than between type I and type II of either species [1]. Both rat and human

*Correspondence to P. N. Span.

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type II 5α -reductase show a narrow acidic pH-optimum, while both type I isozymes have a broad pH-optimum of 6.0–8.0 [14, 22].

In literature the pH-optimum of surmised type II activity in rat or human tissue homogenates differs from pH 5.0 [14, 22], to 5.5 [12, 20, 23–26], to 6.2 [27] or even 7.0 [28, 29]. Recent work of Thigpen *et al.* has shed new light upon the concept of pH-optimum [30]. They postulated the efficiency optimum V_{\max}/K_m . Unlike the classical pH-optimum which only determines velocities at a single substrate concentration, the efficiency optimum has the advantage of taking into account the substrate dependency of enzyme reaction velocity. However, apparent affinity constants (K_m 's) for 5α -reductase isozymes differ widely in literature [cf. 23].

Because of these inconsistencies we set out to re-evaluate the assay for both isozymes in two rat androgen target tissues: the epididymis, containing predominantly type II 5α -reductase [1, 14], and the prostate, containing both subtypes [14]. We detected a pH-dependent non-linear time-course in the 5α -reduction of T in both rat tissue homogenates which could be resolved by bringing the enzyme to the appropriate pH before the start of the reaction. Furthermore, we demonstrated that the reported controversy about the pH-optimum of type II 5α -reductase could, at least in part, be explained by the substrate dependency of 5α -reduction. Also, we investigated the pH-dependency of enzyme kinetics of both 5α -reductase isozymes. Our results indicate an extreme pH-dependency of the type II isozyme with regard to enzyme characteristics V_{\max} , K_m and V_{\max}/K_m . This pH-dependency could explain—in conjunction with the intricacy of establishing a pH-optimum—many of the discrepancies in affinity constants and velocities found in literature.

MATERIALS AND METHODS

Materials

[1,2,6,7- ^3H]Testosterone (3.74 TBq/mmol) and [1 α ,2 α (n)- ^3H]17 β -hydroxy- 5α -androstane-3-one (dihydrotestosterone) (2.00 TBq/mmol) were purchased from Amersham (Amersham, U.K.). [9,11- ^3H]5 α -androstane-3 α ,17 β -diol (1.48 TBq/mmol) was obtained from Du Pont-New England Nuclear (Boston, MA). All radiolabelled steroids were purified by high performance liquid chromatography (HPLC, see below) before use. Testosterone was purchased from Steraloids (Wilton, NH). (L+)-Ascorbic acid, ATP (adenosine 5'-triphosphate disodium salt) and EDTA (ethylenediaminetetra-acetate disodium salt dihydrate) were obtained from Merck (Darmstadt, Germany). Glutathione and α -monothioglycerol (3-mercaptopropanediol) were obtained from Sigma Chemical (St Louis, MO). TLCK (tosyl-lysine chloro-

methyl ketone) and TPCK (tosyl-phenylalanine chloromethyl ketone) were from Calbiochem (La Jolla, CA) and Pefabloc was obtained from Boehringer (Mannheim, Germany). Diethylether (p.a.), n-hexane (Lichrosolv) and 2-propanol (Lichrosolv) were purchased from Merck. All other chemicals used were of analytical grade.

Protein levels were determined by a method modified from Lowry [31] against a standard of bovine serum albumin (OHRD 20/21, Hoechst-Behring, Marburg, Germany). The assay was modified for microtiter-plates and had a sensitivity of 25 μg per well. Endogenous steroid levels were determined by radioimmunoassay, as described previously [32]. Steroid concentrations were: testosterone 0.2 nM, androstenedione 0.06 nM and corticosterone 5.79 nM in the prostate homogenate, and testosterone 0.2 nM and androstenedione 0.05 nM in the epididymis homogenate. As these concentrations are low compared to the concentrations employed—and as the homogenates are diluted over 20-fold in the final assay—endogenous steroids could not interfere with the measurement of 5α -reductase enzyme activity.

Buffers

Homogenization buffer consisted of 20 mM phosphate (Merck), 1 mM monothioglycerol and 0.25 M sucrose (Merck), pH 7.0. The incubation buffer consisted of 200 mM Tris (2-amino-2-hydroxymethylpropane-1,3-diol, Merck), citric acid monohydrate (Merck) and 2 mM NADPH tetrasodium salt (Merck) in a final volume of 1 ml, pH 4.5–8.0. Final assay pH was checked in control tubes without tracer, before and after incubation.

Tissue preparation

Wistar rats 7–13 weeks old (200–250 g) were killed by decapitation and whole prostates and epididymides were removed, freed of adhering fat and placed in liquid nitrogen for transport. Tissues were kept at -80°C or processed immediately. All subsequent procedures were performed on ice. Tissues were thawed and minced with razor blades into small pieces. Minced tissue was homogenized in ice-cold homogenization buffer with a 7 ml Dounce tissue grinder (Kontes Glass Co., Vineland, NJ) with a loose and a tight fitting pestle. The homogenate was filtered twice through nylon netting of 50 and 140 mesh respectively to remove cell debris. By this procedure, without centrifugation, a full homogenate with nuclei and cytosol is obtained. For rat prostate the final preparation contained 29.1 mg protein/ml, while rat epididymis homogenate contained 1.1 mg protein/ml.

5α -Reductase assay

Radiolabelled testosterone in ethanol was brought to final concentration by isotopic dilution with non-labelled steroid in Pyrex culture tubes (borosilicate

glass, 12 × 75 mm, Corning Inc., Corning, NY). Ethanol was evaporated under a mild nitrogen stream at room temperature. Incubation buffer was added and the tubes were put in a shaking water bath at 37°C for at least 10 min before the start of the incubation to ensure dissolving of substrate (97%). A tube with cofactor (NADPH) in incubation buffer and a tube with the homogenate were placed in the water bath for 10 min to obtain the appropriate temperature. In a number of experiments, performed to optimize the assay, several anti-oxidants or protease inhibitors were added before pre-heating the homogenate, i.e. 10 min before starting the incubation. Immediately after adding the cofactor (200 μ l) to the substrate, the incubation was started by adding the pre-heated homogenate (10–50 μ l). After 10–30 min the incubation was terminated by adding 100 μ l of 3 M NaOH. To extract metabolites, 4 ml of ice-cold ether was added, the tubes were capped and shaken. The water phase was frozen in an alcohol bath with dry-ice, the organic phase was decanted and evaporated under nitrogen. Metabolites were dissolved in 100 μ l hexane for HPLC.

HPLC

Metabolized steroids were separated on a Hibar LiChrosorb Diol-column (length 250 mm, 5 μ m, Merck), equipped with a guard column (Resolve Silica, Waters Corp., Milford, MA). The HPLC-system included a Waters 610 Fluid Unit, a Waters 600E System Controller and a Waters U6K injector. The isocratic flow of the mobile phase (hexane-propa-nol 96:4, v/v) was 1.5 ml/min at a pressure of 680 psi. Radioactivity was monitored with a FloOne Beta Radiomatic A500 radio-chromatography detector (Packard-Canberra Benelux, Tilburg, The Netherlands) with a 500 μ l cell and a liquid scintillation flow of 1.5 ml/min (Aqua-Luma, Lumac-LSC, Olen, Belgium). The counting efficiency for tritium was 47%. The percentage formation of DHT and 5 α -androstane-3 α /17 β -diol were used to estimate 5 α -reductase activity. Overall experimental recoveries for testosterone, dihydrotestosterone and 5 α -androstane-3 α ,17 β -diol were 90–93%.

Enzyme characteristics

5 α -reductase enzyme characteristics were obtained by the Lineweaver–Burke method with a double reciprocal plot of velocity against substrate concentration at pH 4.5–8.0. Affinity constants (K_m 's) and maximum velocities (V_{max}) were determined and the efficiency ratio V_{max}/K_m was calculated.

RESULTS

Enzyme stability

Initially, cofactor was added to the incubation mixture containing substrate, and the reaction was started by adding the pre-heated homogenate. Measured velocities, however, differed with time used to pre-heat the homogenate. The enzyme appeared to be unstable at 37°C. Within 10 min only 8% of the total enzyme activity could yet be recovered. This applied to 5 α -reductase isozymes type I and II in both rat tissues. 1 mM monothioglycerol was added to the homogenization buffer beforehand. Neither anti-oxidants (ascorbic acid (50 mg/l) or glutathione (10 mg/l), or both), nor EDTA (1 mM), ATP (2 mM), nor protease-inhibitors [Pefabloc (100 mM), TPCK (200 mM) or TLCK (100 mM), alone or simultaneously], prevented the deterioration of enzymatic activity at 37°C. After the enzyme had deteriorated, adding NADPH before starting the incubation did not restore enzyme activity. However, adding NADPH before the 10 min pre-heating of the homogenate greatly preserved enzymatic activity. Keeping the homogenate on ice with NADPH until adding it to the incubation mixture gave best results, preserving 80% of enzyme activity. Although the homogenate then would not attain the appropriate temperature at the start of the incubation, we used this protocol in the subsequent study.

Non-linear time-course

The time-course in metabolism can be considered linear, allowing for a single time point measurement to estimate initial velocities, until substrate depletion leads to a substantial decrease in velocity. Percentual conversion of T to DHT was not allowed to exceed 15%, by varying incubation times with different substrate concentrations, to prevent this substrate depletion. Analysis of the time-course of testosterone metabolism, however, showed an "initial burst": in the first 30 s the measured velocity was higher than during the subsequent 10 min. This phenomenon was observed only at acidic pH in both rat prostate and epididymis homogenates [Fig. 1(A and B)]. For the type I isozyme, in rat prostate homogenate at pH 7.0, the time-course of testosterone metabolism was linear [Fig. 1(C)]. The initial burst precluded the correct estimation of initial velocities of 5 α -reductase type II at acidic pH in a one-time point measurement.

In our initial protocol pH was presumably obtained almost immediately, as the homogenization buffer was a 20 mM phosphate buffer, the incubation buffer used was a 200 mM Tris–citrate buffer and only 10–50 μ l of the homogenate were added to a final volume of 1 ml incubation buffer of the appropriate pH. However, bringing the enzyme to pH before starting the incubation by adding 0.2 ml of incubation buffer

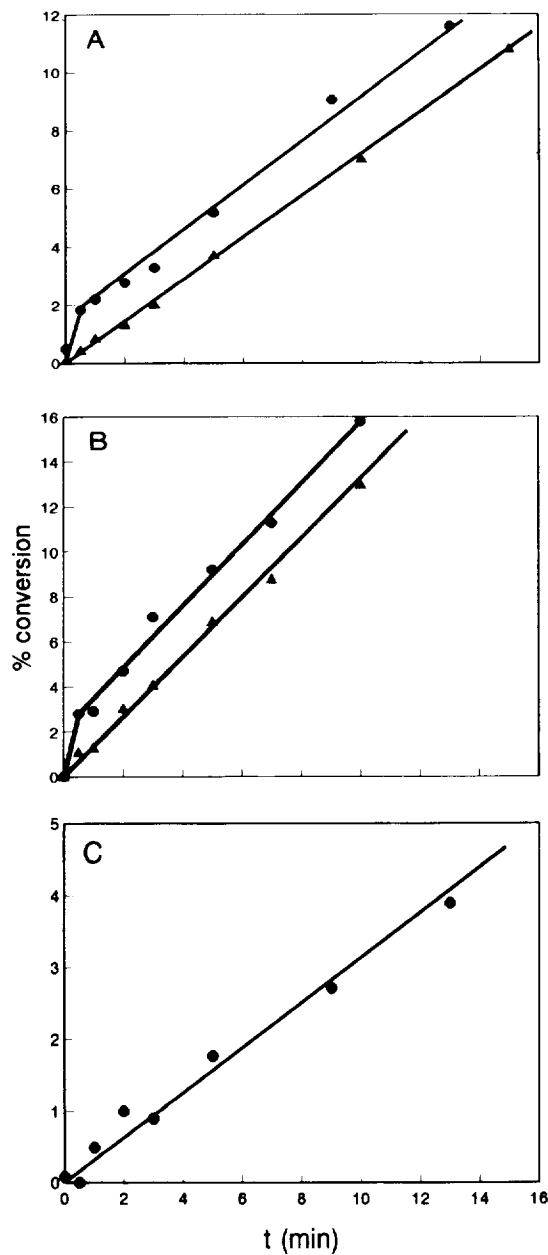


Fig. 1. Time-course in the conversion of T to DHT. (A) Rat prostate homogenate. (B) Rat epididymis homogenate. 5α -reductase was assessed at pH 5.5 using 30 nM T as substrate. When the homogenate is kept in homogenization buffer pH 7.0 an initial burst is found (●). When the enzyme is brought to pH 5.5 by the addition of incubation buffer, the time-course is linear (▲). (C) In rat prostate homogenate the time-course at pH 7.0 and using 300 nM T (type I 5α -reductase) is linear (●). The protocol and control of buffer pH are as described in Materials and Methods.

of the appropriate pH to the homogenate, the “initial burst” could be prevented [Fig. 1(A and B)].

pH-dependency of rat 5α -reductase isozymes

pH-profiles showed that rat prostate contains both 5α -reductase isozymes [Fig. 2(A)]: enzyme activity can be detected at pH 5.0–5.5, indicative of type II

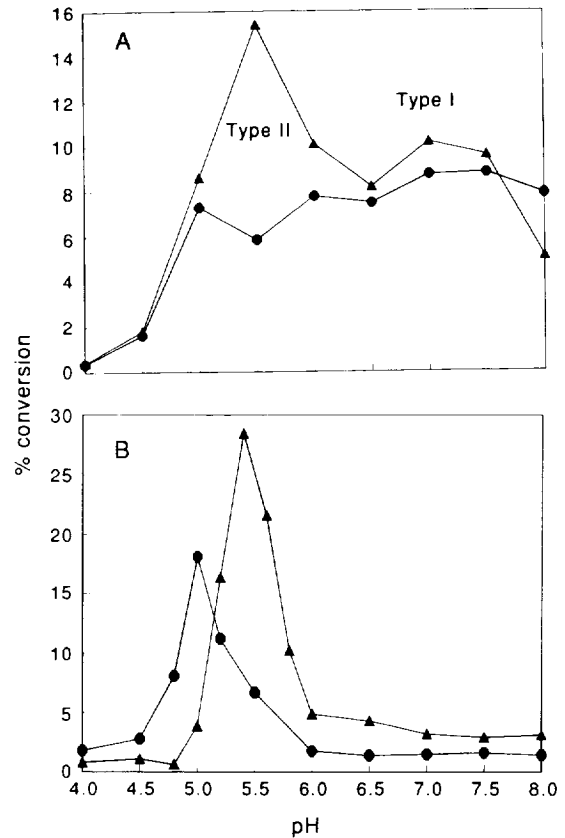


Fig. 2. pH dependency of T conversion in: (A) rat prostate homogenate; and (B) rat epididymis homogenate. 5α -reduction was assessed with 1 μ M T (●) or with 10 nM T (▲) at pH 4.0–8.0 as described in Materials and Methods. The optimum pH for the type II isozyme at acidic pH is dependent on the substrate concentration used.

5α -reductase, and at pH 6.0–8.0, the optimum described earlier of the type I isozyme. Rat epididymis showed high type II activity at acid pH, but only minimal activity at pH 6.0–8.0 [Fig. 2(B)]. The type II 5α -reductase isozyme has been reported to have a narrow pH-optimum of either pH 5.0 or 5.5. In our hands both pHs were optimal, albeit under different conditions [Fig. 2(A and B)]. A careful analysis of substrate dependency at both pHs showed that at testosterone concentrations above 80 nM an optimum of pH 5.0 would be found, whereas smaller amounts of testosterone are metabolized more efficiently at pH 5.5 [Fig. 3(A and B)]. The type II isozyme in the rat prostate shows the same substrate-dependency at acidic pH as in the epididymis. In the epididymis only a low maximum velocity, with a high affinity, is attained at pH 7.0 [Fig. 3(B)]. In rat prostate homogenate the type I isozyme (at pH 7.0) is more efficient in metabolizing high concentrations of T [Fig. 3(A)].

The type I isozyme in rat prostate homogenate has a broad classical optimal pH of 6.0–8.0 [Fig. 2(A)]. Enzyme characteristics V_{max} , K_m and V_{max}/K_m for this isozyme did not differ substantially in this pH-range [Fig. 4(A, B and C)]. The enzyme characteristics for the type II isozyme, however, differed widely. At

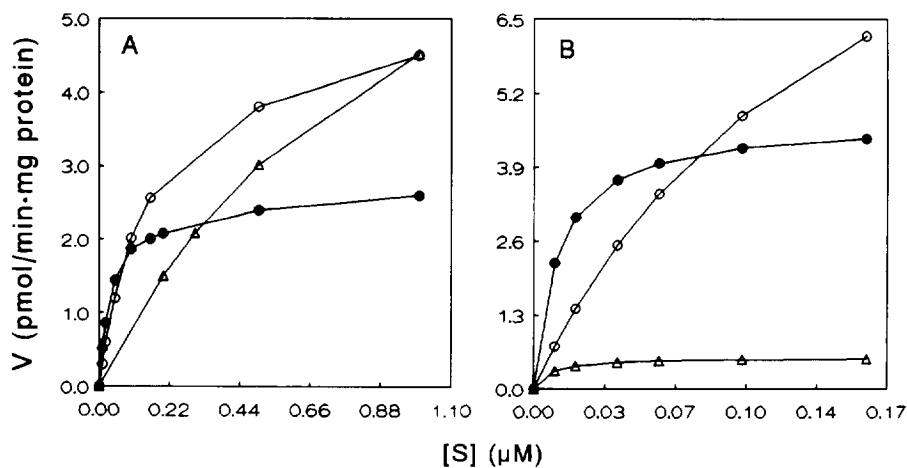


Fig. 3. Substrate dependency of pH-optimum. (A) Rat prostate homogenate. (B) Rat epididymis homogenate. 5 α -reductase enzyme activity was assessed at pH 5.0 (○), at pH 5.5 (●) and at pH 7.0 (△). At low substrate concentrations in both tissues the highest initial velocity is found at pH 5.5. Higher T concentrations are more efficiently metabolized at pH 5.0. At T concentrations higher than 1 μ M, pH 7.0 would become optimal in rat prostate, but not in rat epididymis homogenates.

acidic pH, V_{max} and K_m showed a strong pH-dependency in both tissues (Fig. 4). At pH 4.5–6.0 V_{max} and K_m varied by a factor of 20. The efficiency ratio

V_{max}/K_m was also extremely pH-dependent in the acidic range and had an optimum at pH 5.5 in rat prostate and epididymis [Fig. 4(C and F)].

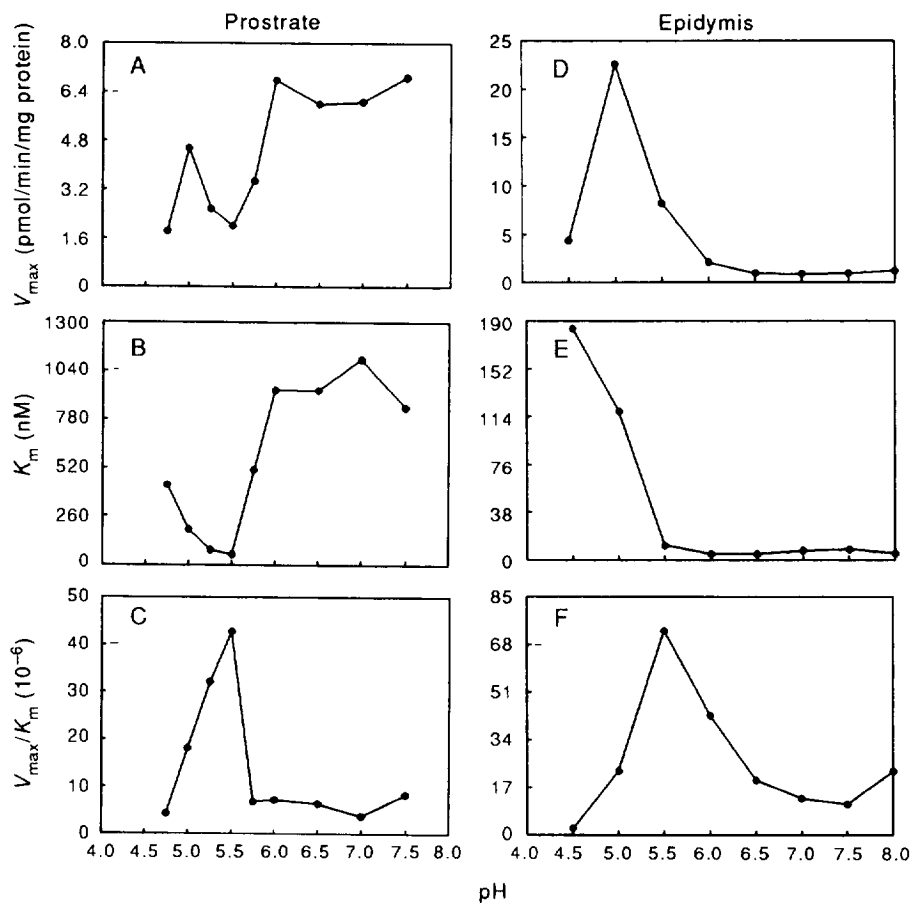


Fig. 4. Enzyme characteristics V_{max} (A, D), K_m (B, E) and V_{max}/K_m (C, F) at pH 4.75–7.5 (rat prostate A, B, C) and pH 4.5–8.0 (rat epididymis D, E, F). V_{max} and K_m were determined by a double reciprocal plot of estimated initial velocity against substrate concentration according to Lineweaver-Burke. The V_{max} vs pH shows the pH-optimum at high (saturating) substrate concentrations. V_{max}/K_m plotted vs pH shows its pH-optimum at pH 5.5 in both tissue homogenates. In both tissues enzyme characteristics vary many-fold, especially at acidic pH.

DISCUSSION

Several papers have shown the existence of at least two isozymes of 5 α -reductase in both rat [14, 16, 21] and human [16, 17, 22] tissues. Most recent research has focused on the measurement of 5 α -reductase mRNA and 5 α -reductase expressed in transfected cell systems [15–17, 21, 30, 33, 34]. However, correlation between mRNA level and assayable 5 α -reductase activity (protein) is sometimes poor, especially in human tissues [1, 22, 35]. Therefore research at the protein level is still warranted. As kinetic data concerning 5 α -reductase differ widely in literature, reported affinity constants of testosterone for 5 α -reductase range from 10 nM to 15 μ M [23, 30], we reevaluated the assay of this enzyme in two androgen-dependent rat tissues, the prostate and the epididymis. We used a full homogenate to keep conditions as close to the *in vivo* environment as is possible in this kind of *in vitro* measurements. In rat prostate both isozymes type I (with a physiological pH-optimum) and type II (acidic pH-optimum) are found [14]. In rat epididymis the pH-optimum suggests mainly type II 5 α -reductase activity [14], as does the apparent affinity constant at pH 7.0 (see below).

A major problem we initially encountered was the instability of both isozymes at 37°C. Anti-oxidants and protease inhibitors could not prevent this deterioration of enzyme activity and neither ATP nor EDTA did influence 5 α -reductase activity. It has been established that NADPH binds to 5 α -reductase first, allowing T to bind subsequently [36, 37]. Liang *et al.* reported that inactivation of human and dog 5 α -reductase preparations could be prevented by addition of the cofactor NADPH while homogenizing the tissues [38]. Mutant human 5 α -reductase enzymes in both fibroblasts and transfected cells with a shorter half-life than normal enzyme preparations have been reported with a lower affinity for NADPH [1, 4, 18, 20]. A role for the cofactor to stabilize the enzyme in the cell and to regulate enzyme turnover has been suggested [4]. Therefore, we investigated the influence of adding NADPH on the encountered enzyme deterioration. In our hands, NADPH pre-binding had a pronounced stabilizing effect on both rat isozymes, but could not restore enzyme activity after pre-heating at 37°C. Several studies have shown the importance of specific membrane components and the effect of phospholipases, which would influence membrane fluidity, on 5 α -reductase enzyme activity [39, 40]. During cell homogenization perturbations in the membrane environment of the tightly membrane-bound enzyme might cause a conformational change in the protein. Binding of the cofactor might make the enzyme less susceptible to these membrane perturbations during homogenization, leaving the enzyme receptive for testosterone binding and metabolism.

Apart from the deterioration of 5 α -reductase activity which could be prevented by the addition of NADPH, we encountered a non-linear time-course in the metabolism of testosterone at acidic pH in both rat epididymis and prostate (Fig. 1). Earlier the group of Martin *et al.* reported that the 5 α -reductase in the particulate fraction of BPH homogenates also did not follow first-order Michaelis–Menten kinetics due to a similar non-linear time-course at pH 5.5 [23, 41, 42]. This would have a profound effect on the measurement of enzyme characteristics K_m and V_{max} when a single time point measurement is used. When we brought the tissue homogenate to the desired pH before starting the incubation, a linear time-course was obtained. In rat prostate high substrate concentrations are efficiently metabolized at neutral pH by the type I isozyme. A sudden pH-shift from neutral to acidic pH might explain the initial burst encountered in this tissue, when one assumes that at the start of the incubation a part of the substrate is metabolized by the type I isozyme. cDNA for type I 5 α -reductase has been derived from the human prostate, although an acidic pH-optimum of enzyme activity in prostate extracts is found [22], typical for the type II isozyme. Recent evidence, however, does suggest the expression of type I 5 α -reductase activity (P.M. Martin & F.K. Habib, pers. comm.). Also in rat epididymis no [1, 14] or only minimal [43] type I 5 α -reductase activity is found (see below). Because of the lack of measurable type I activity in rat epididymis, no higher velocity at any substrate concentration is found at neutral pH in our assay. We believe interference between the two isozymes does not explain the encountered initial burst. Rather this burst is an intrinsic aspect of the type II isozyme *per se*. The sudden pH-change in the micro-environment of the membrane-bound enzyme, when starting the reaction, might induce a short change in activity by influencing membrane fluidity and/or inducing a conformational change of the isozyme.

In the rat epididymis type I 5 α -reductase has been described by immunocytochemistry [43]. Our results, however, are at variance with this finding. At pH 7.0 in the rat epididymis only minimal 5 α -reductase activity is found [Fig. 3(B)]. Furthermore, the affinity constants we found at pH 6.0–8.0 in this homogenate are very low (approx. 10 nM) [Fig. 4(E)], while the type I isozyme has a characteristic K_m for T as high as 1 μ M [Fig. 4(B)]. For the human type II isozyme, expressed in Chinese hamster ovary cell lines, Thigpen *et al.* established a high affinity for T of about 10 nM at neutral pH [30]. Although one has to be aware of the species difference and the difference in experimental conditions (in prostate or epididymis homogenates, and expressed human type II enzyme in CHO cells), we believe that the enzyme activity at neutral pH we found in rat epididymis can be attributed to the type II isozyme. One might argue that the immunocyto-

chemical detectable 5 α -reductase type I enzyme is inhibited or is non-active, as also discrepancies between mRNA and enzyme activity have been reported in human BPH-tissue [22]. However, immunocytochemistry is much more sensitive than our biochemical assay and is capable of detecting extremely small amounts of enzyme. Furthermore, type I 5 α -reductase is reportedly highly concentrated in the initial segment of the epididymis [43]. We might therefore not have been able to detect this activity in a homogenate of the whole epididymis. Whether this type I activity in the rat epididymis has any physiological significance, considering its low affinity for T, needs to be addressed in studies using discrete segments of this tissue.

The pH-optimum of 5 α -reductase activity in rat or human tissue has been the subject of mild controversy. pH-optima in tissues considered to contain mainly type II 5 α -reductase differ in literature; an optimum of 5.0 has been described for the type II enzyme in rat epididymis and prostate and in human prostate [14, 22], and an optimum of 5.5 for the human epididymis and BPH tissue, fibroblasts and other human and rat tissues [12, 20, 23–26]. In addition, an optimum of 6.2 was found in the rat epididymis by Monsalve and Blaquier [27], while the group of Houston *et al.* reported a pH-optimum of 7.0 in the human prostate [28, 29]. The normal procedure to obtain a pH-optimum is by incubating an enzyme preparation with a single substrate concentration. Velocity, however, depends strongly on substrate concentration.

From the Michaelis–Menten equation: $v = (V_{\max}[S])/(K_m + [S])$ where $[S]$ is the T concentration follows: when $S \gg K_m$, a pH-profile would indicate V_{\max} vs pH.

And when $S \ll K_m$: $v = (V_{\max})/(K_m)[S]$. The velocities found are then proportional to V_{\max}/K_m .

In this paper we described the initial velocities at different testosterone concentrations and pHs. At testosterone concentrations below 80 nM a higher initial velocity is found at pH 5.5, whereas at higher testosterone concentrations the optimum in rat epididymis was 5.0. The pH-optimum shift from pH 5.0 to 5.5 reported for some mutant human 5 α -reductase enzymes might be explained by the reported change in substrate or cofactor affinity [4, 20]. As both testosterone and NADPH were kept at a constant concentration in these studies, a change in affinity by a mutation in the enzyme can cause the chosen concentration to induce a different pH-optimum, as a different affinity constant implies a different substrate dependency.

Unlike the classical pH-optimum, the efficiency ratio V_{\max}/K_m takes into account the substrate dependency of velocity. This ratio also reflects the potential *in vivo* velocity of DHT formation from T, as endogenous T concentrations are much lower than the K_m . In our hands, the optimum of the efficiency ratio for the type II isozyme was at pH 5.5 in rat prostate

and epididymis. This ratio has been reported to be optimal at pH 6.0 for a microsomal preparation of human prostatic tissue [44] or at pH 7.0 for the expressed human type II isozyme in a hamster ovary cell line [30]. Preliminary results from our laboratory also indicate that the efficiency optimum in human BPH tissue homogenate is at pH 6.5 (unpublished observations). In rat prostate both 5 α -reductase isozymes are present, so the V_{\max}/K_m ratio for the rat type II isozyme at neutral pH cannot be determined in this tissue, as at this pH the type I isozyme will interfere. Because of the high K_m of the type I isozyme, low efficiency ratios are found at neutral pH in rat prostate. The low efficiency ratio at this pH in rat epididymis, however, cannot be explained by the presence of type I 5 α -reductase. In contrast to the rat prostate, affinity constants are 100-fold lower at neutral pH in the epididymis. The decreased V_{\max}/K_m ratio at pH 6.5–8.0 in this tissue is caused by the 10-fold lower V_{\max} at this pH as compared to pH 5.5, with similar affinity constants.

Since we found the enzyme characteristics V_{\max} and K_m to be highly dependent on minor pH-changes in the acidic pH region, the choice of pH has considerable effects on reported kinetic data. Some effects of modifying substances might be explained by even minor shifts in the pH during the assay (as reported in 45 for EDTA).

The present study discloses that in rat prostate and epididymis homogenates the metabolism of T at acidic pH by the type II 5 α -reductase isozyme shows a non-linearity in the time-course when not brought to the desired pH before the start of the incubation. Therefore estimation of initial velocities at acidic pH in these tissues is incorrect in a single time point measurement. Bringing the enzyme to the appropriate pH before the start of the incubation leads to a linear time-course. When a pH-optimum is attained one has to consider the substrate dependency of enzyme reaction velocity. At high substrate concentrations a pH-optimum of pH 5.0 is found for the rat type II isozyme, while at low substrate concentrations an optimum of pH 5.5 is found, equalling the V_{\max}/K_m efficiency ratio pH-optimum in rat prostate and epididymis homogenates. The extreme pH-dependency of the rat type II 5 α -reductase isozyme strongly influences enzyme characteristics. The choice of pH for the assay of 5 α -reductase type II could explain discrepancies in reported affinity constants in literature.

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