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Papers

Kinetic analysis of androstenedione 5α-reductase in epithelium and stroma of human prostate

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In the human prostate, various androgen-metabolizing enzymes are present. Among these enzymes, testosterone 5α-reductase seems to be dominant. However, androstenedione is also a potential substrate of the prostatic 5α-reductase. To address the question of to what extent the reduction of androstenedione to androstanedione occurs, the present study describes in detail the kinetic characteristics (K_m and V_{max}) and possible age-dependent alterations of this enzymatic step in epithelium and stroma of the human prostate. In normal prostate (NPR), the mean K_m (nM) and V_{max} (pmol/mg protein · h) were about twofold higher in stroma (K_m, 211; V_{max}, 130) than in epithelium (K_m, 120; V_{max}, 56), whereas in the benign prostatic hyperplasia (BPH), the mean K_m (nM; mean ± SEM) and V_{max} (pmol/mg protein · h; mean ± SEM) were about sixfold higher in stroma (K_m, 688 ± 121; V_{max}, 415 ± 73) than in epithelium (K_m, 120 ± 10; V_{max}, 73 ± 8). In BPH, those differences between epithelium and stroma were highly significant ($p < 0.001$). However, the efficiency ratios (V_{max}/K_m) of neither BPH nor NPR showed any significant differences between epithelium (NPR, 0.47; BPH, 0.62 ± 0.06) and stroma (NPR, 0.70; BPH, 0.63 ± 0.05). With respect to age-related changes, only stroma showed a significant increase of K_m ($p < 0.01$) and V_{max} ($p < 0.05$) with age. In summary, in both epithelium and stroma of the human prostate, a 5α-reductase converts in measurable amounts androstenedione to androstanedione. The kinetic data were, in part, different between epithelium and stroma; the reason for this difference remains unclear. In comparison to other metabolic conversions, such as testosterone to dihydrotestosterone and androstenedione to testosterone, it is unlikely that, in the human prostate, the adrenal androgen androstenedione contributes significantly to the formation of testosterone and, further, of dihydrotestosterone. (*Steroids* **62**:589–594, 1997)

Keywords: 5α-reductase; human prostate; androstenedione; steroid

Introduction

A balanced androgen metabolism is thought to be a pivotal prerequisite for the normal androgen responsiveness of the human prostate.[1,2] Moreover, it is widely accepted that the level of the various androgens in the human prostate is regulated by a complex pattern of different androgen-metabolizing enzymes.[3] Regarding these enzymes, in previous in vitro studies,[4–8] we have compared the reductive [5α-reductase, 3α(β)-hydroxysteroid oxidoreductase, and hydroxylases] and oxidative [17β-hydroxysteroid oxidoreductase (17β-$HSOR_{ox}$)] metabolism of testosterone. The enzyme activity of the 17β-$HSOR_{red}$, giving rise to the conversion of androst-4-ene-3,17-dione (androstenedione) to testosterone, has been studied thoroughly as well.[8] It turned out that among these androgen-metabolizing enzymes, the dihydrotestosterone (DHT)-forming testosterone 5α-reductase is the most powerful one. So, it seems that the DHT level in the normal and benign hyperplastic human prostate (BPH) is primarily under the regulatory force of that testosterone 5α-reductase. It is known that the adrenal androgen androstenedione is another potential substrate for the 5α-reductase. Therefore, in this study, we describe the enzyme kinetics (K_m and V_{max}) of the conversion of androstenedione to 5α-androstane-3,17-dione (androstanedione). This conversion is an alternative to the above-mentioned 17β-$HSOR_{red}$-regulated conversion of androstenedione to

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testosterone. The extent of those pathways in the human prostate may allow us to evaluate whether androstenedione itself is able to contribute substantially to the testosterone formation and, subsequently, to the formation of DHT.

Experimental

Chemicals

[1,2,6,7-^{3}H]Androst-4-ene-3,17-dione (specific activity, 2.69–3.40 TBq/mmol) was purchased from Amersham Buchler (Braunschweig, Germany), and the unlabeled steroids were purchased from Sigma Chemical Company (St. Louis, Missouri, USA). The eluant for high-performance liquid chromatography and the scintillation solution Rialuma were obtained from Mallinckrodt Baker (Griesheim, Germany). All other chemicals were from Merck AG (Darmstadt, Germany), Serva (Heidelberg, Germany), and Boehringer (Mannheim, Germany).

Tissue preparation

Normal prostatic (NPR) tissue was obtained from four brain-dead kidney donors, aged 35–56 years. BPH tissue was obtained from 15 men, aged 55–87 years, by suprapubic prostatectomy. In each case, a written consent for this study was given. After surgical extirpation, the tissue was immediately chilled in ice-cold 150 mM NaCl. Afterward, the specimens were divided into small pieces and stored in plastic tubes at −196°C. For each tissue specimen, histology was examined by an experienced pathologist.

The prostatic epithelium and stroma were separated exactly as described previously.[7] Using this separation procedure, the relative purities of the epithelial and the stromal fractions were more than 83% and were estimated by measuring acid phophatase as a marker for epithelial cells and hydroxyproline as a marker for stromal elements in both tissue fractions.[4,6–8] For enzyme measurement, aliquots of the frozen homogenates were pulverized in a porcelain mortar chilled with liquid nitrogen. The powder was allowed to thaw in small tubes, which were kept in an ice bath for about 1 h.

Measurement of 5α-reductase activity

After optimization of the assay conditions using BPH tissue, 5α-reductase activity was measured under optimized incubation conditions. Briefly, the incubation mixtures (final volume, 202 μl), each prepared in duplicate, were composed of buffer-diluted tissue homogenate (300–700 μg of protein, pH 7.5), a NADPH-generating system (5 mM glucose-6-phophate and 0.6 units of glucose-6-phophate dehydrogenase), and varying concentrations (35–5250 nM) of androstenedione (either [^{3}H]androstenedione alone or [^{3}H]androstenedione plus unlabeled androstenedione). The reaction was started by adding 0.5 mM NADPH, and the mixtures were incubated at 37°C for 30 min. The reactions were stopped by adding 3 ml of ether, and the steroids were extracted twice with ether (2 × 60 s). The ether phases were evaporated to dryness (Vortex Evaporator, Haake Buchler, Saddle Brook, New Jersey, USA), redissolved in 500 μl ether, and again evaporated to dryness. The dried steroids were redissolved in 50 μl of acetonitrile containing 100 μg of the following steroids as tracer: testosterone, androstenedione, DHT, androstanedione, androsterone, and epiandrosterone. The steroids were separated by reversed-phase high-performance liquid chromatography as described in detail previously.[8] The eluant was composed of a filtered and helium-degassed mixture of acetonitrile/methanol/H_2O (47:12:41, v/v/v). The recovery was, on average, 80% of the starting material. The androstenedione 5α-reductase activity was calculated from the percentage of radioactively labeled androstanedione, androsterone, and epiandrosterone, taking into consideration recovery, blank values, the specific activity of [^{3}H]androstenedione, and the ratio of added [^{3}H]androstenedione to unlabeled androstenedione. Furthermore, due to the inevitable presence of 17β-HSOR activity (metabolizing androstenedione to testosterone), the androstenedione concentration actually available for 5α-reductase was determined in each experiment by subtraction of the concentration of formed testosterone and DHT from the originally added androstenedione concentration. The percentage of formed testosterone and DHT decreased with increasing androstenedione concentration. In epithelium and stroma of NPR, the mean reduction (%) of the originally added androstendione concentration ranged from 0.42 to 1.79 and from 0.34 to 0.93, respectively. In epithelium and stroma of BPH, the mean reduction (%; mean ± SEM) of the originally added androstenedione concentration ranged from 0.42 ± 0.07 to 1.42 ± 0.25 and from 0.78 ± 0.13 to 3.56 ± 0.41, respectively.

Other methods

Protein content was determined according to the method of Lowry et al.[9] using BSA as the standard. Acid phosphatase (EC 3.1.3.2) activity was measured by the method of Walter and Schütt.[10] K_m and V_{max} values were derived from Lineweaver–Burk plots,[11] from which regression lines were computed by the method of the least squares. The mean correlation coefficients of the regression lines in BPH for epithelium and stroma were 0.982 ± 0.008 (mean ± SEM) and 0.995 ± 0.001, respectively. The respective data for NPR tissue were 0.987 and 0.995. As described previously, the efficiency of androstenedione 5α-reductase (V_{max}/K_m) was estimated by the equation $v = V_{max} \cdot S/(K_m + S)$, where S is the endogenous androstenedione concentration.[4,6,8] Because under in vivo conditions the K_m value of the androstenedione 5α-reductase are much higher than S,[12] it follows that $K_m + S \approx K_m$ and $v = V_{max}/K_m \cdot S$. Assuming identical substrate concentrations, the potential enzymatic efficiency, previously termed by us as potential activity, is given by the V_{max}/K_m ratio. The statistical significance of the means was determined by Student's t test. The significance of age-related changes was determined by the Spearman rank correlation coefficient (R). $p < 0.05$ was considered significant.

Results

Effect of NADPH, protein, and incubation time

As shown in Figure 1, androstenedione 5α-reductase activity was measured in the epithelium and stroma of BPH, varying the NADPH concentration (0.01–10 mM), protein concentration (0.2–0.7 mg/incubation mixture), or incubation time (10–120 min). The optimal NADPH concentration was found to be 0.5 mM. Concerning protein, linearity of 5α-reductase activity was found over the whole tested range. Routinely, the protein concentration used was between 0.3 and 0.7 mg/incubation mixture. Concerning incubation time, linearity was proven up to 60 min. Routinely, an incubation time of 30 min was used.

Kinetic parameters (K_m, V_{max}, V_{max}/K_m) of the 5α-reduction of androstenedione in the epithelium and stroma of NPR and BPH

The activity, i.e., the velocity of formation of metabolites of androstenedione 5α-reductase, was measured under optimized incubation conditions in epithelium and stroma of

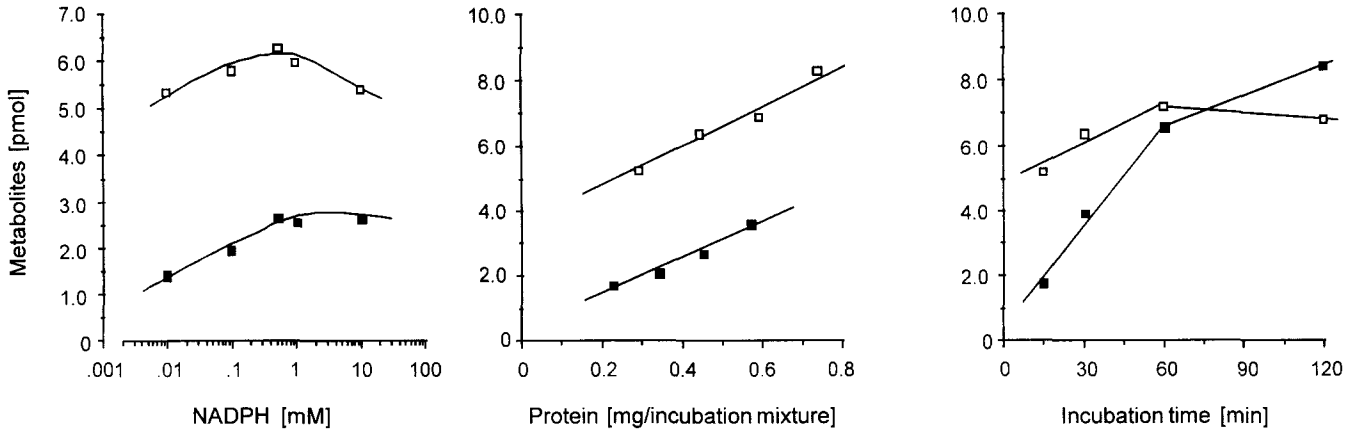

Figure 1 Activities of androstenedione 5α-reductase in the epithelium (■) and stroma (□) of one BPH as a function of NADPH concentration (Left), protein concentration (Center), and incubation time (Right). The protein concentration for variation of NADPH and incubation time ranged from 0.34 to 0.44 mg/incubation mixture. The incubation time for variation of NADPH and protein was 30 min. The androstenedione concentration was 50 nM. All experiments were performed in duplicate and were corrected for blank values.

NPR and BPH as a function of the androstenedione concentration. Each K_m and V_{max} value was determined by Lineweaver–Burk plots according to the Michaelis–Menten model. The mean K_m and V_{max} values are summarized in Figure 2. In NPR, the mean K_m (nM) and V_{max} (pmol/mg protein · h) were about twofold higher in stroma (K_m, 211; V_{max}, 130) than in epithelium (K_m, 120; V_{max}, 56). The mean V_{max}/K_m values were 0.47 and 0.70 in the epithelium and stroma, respectively. In BPH, the mean K_m (nM; mean ± SEM) and V_{max} (pmol/mg protein · h; mean ± SEM) were about sixfold higher in stroma (K_m, 688 ± 121; V_{max}, 415 ± 73) than in epithelium (K_m, 120 ± 10; V_{max}, 73 ± 8). In BPH, those differences between epithelium and stroma were highly significant ($p < 0.001$). However, as was the case for NPR, in BPH, the efficiency ratios (V_{max}/K_m; mean ± SEM) were not significantly different between epithelium and stroma (0.62 ± 0.06 vs. 0.63 ± 0.05).

Relationship between kinetic parameters (K_m, V_{max}, V_{max}/K_m) of androstenedione 5α-reductase and donor's age

In Figures 3 and 4, all K_m, V_{max}, and V_{max}/K_m values are plotted vs. the age of the donors. In the stroma (Figure 3), a significant age-dependent increase of K_m ($p < 0.01$) and V_{max} ($p < 0.05$) was found; i.e., with age the affinity of 5α-reductase to androstenedione decreased significantly, whereas the concentration of active androstenedione 5α-reductase increased significantly. In the epithelium, the 5α-reductase activity remained relatively constant over the whole age range (Figure 4). Concerning the efficiency ratio (V_{max}/K_m), no significant age-dependent alteration was observed in either epithelium or in stroma (Figures 3 and 4).

Discussion

Altered androgen metabolism has been postulated to be involved in the age-dependent development of BPH.[1,2] In previous studies, we investigated in detail the androgen-metabolizing enzymes that are involved in the reductive and oxidative pathways of testosterone.[3–8] Herein, we describe for the first time the 5α-reduction of androstenedione in detail. A comparison of kinetic data like K_m, V_{max}, and V_{max}/K_m of androstenedione 5α-reductase with those of other prostatic androgen-metabolizing enzymes, such as testosterone 5α-reductase and androstenedione 17β-$HSOR_{red}$ allows us to estimate whether, in epithelium and stroma of NPR and BPH, the adrenal androgen androstenedione could be an important source for growth-promoting metabolites like DHT.

Our studies indicate that, in the NPR and BPH, the mean K_m and V_{max} values of androstenedione 5α-reductase were about twofold to sixfold higher in stroma than in epithelium. In BPH, such differences between epithelium and stroma were highly significant. In various studies of our own using identical experimental protocols,[5,13] the mean K_m and V_{max} values of testosterone 5α-reductase were found to be well in accordance with our previously published data.[4] Thus, a comparison of these data with the present K_m and V_{max} values of androstenedione 5α-reductase seems to be admissible. Based on such a comparison, it can be stated that, also for the testosterone 5α-reductase, significantly higher K_m and V_{max} values were found in stroma than in epithelium.[3–5] Moreover, the K_m and V_{max} values of the androstenedione 5α-reductase were always higher than those of the testosterone 5α-reductase. Taken as V_{max}/K_m, in comparison to the reduction of testosterone to DHT, an up to fourfold lower catalytic efficiency of the reduction of androstenedione to androstanedione was found. On the other hand, the catalytic efficiency of the 5α-reduction of androstenedione to androstanedione is about sevenfold higher than the 17β-reduction of androstenedione to testosterone. This suggests that it is unlikely that, in the human prostate, the adrenal androgen androstenedione contributes significantly to the formation of testosterone and, subsequently, of DHT.

Turning to the correlation of K_m and V_{max} with the age of the donor, in stroma, significant increases of K_m and V_{max} were found. Thus, in stroma, aging leads to a loss of substrate affinity (increase of K_m), counterbalanced by an

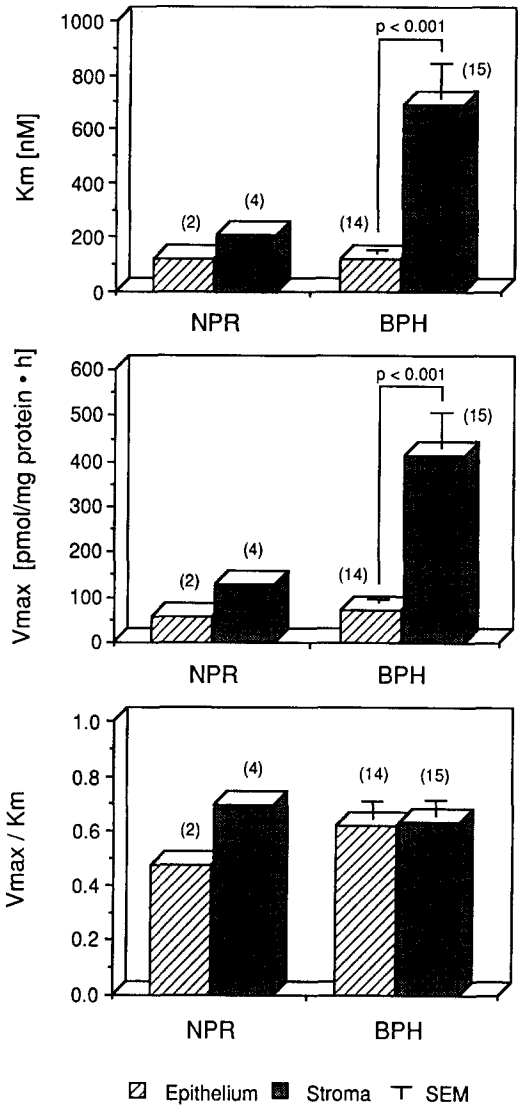

Figure 2 Mean apparent Michaelis constants (K_m), maximal velocities (V_{max}), and potential enzymatic efficiency (V_{max}/K_m) of androstenedione 5α-reductase in the epithelium and stroma of NPR and BPH tissue. The number of prostates studied is shown in parentheses.

increase of the concentration of active androstenedione 5α-reductase (V_{max}). Similar age-dependent alterations have been shown previously for testosterone 5α-reductase.[4] In epithelium, such an age-dependent alteration of K_m and V_{max} of the androstenedione 5α-reductase was not found, being partly in contrast to the epithelial testosterone 5α-reductase, the K_m of which decreased in an age-dependent manner.[3,4]

Differences in the affinity constant (K_m) between the androstenedione and testosterone 5α-reductase, as well as a partially different age dependency, could be due either to the existence of structurally different 5α-reductases or to posttranslational modulations of a single 5α-reductase. Martini et al.[14] have already postulated the existence of at least two substrate-specific 5α-reductases in rat prostatic tissue. One, being responsible for testosterone reduction,

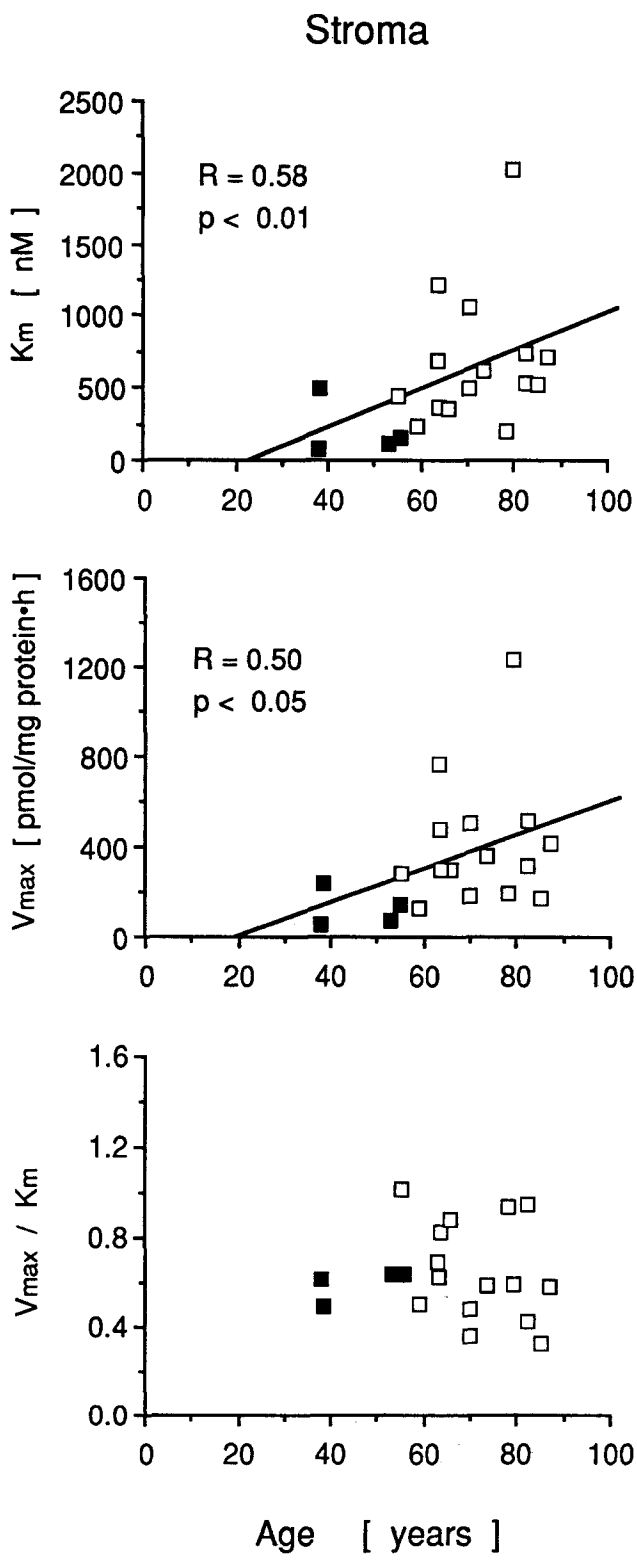

Figure 3 Correlation between K_m, V_{max}, and V_{max}/K_m of androstenedione 5α-reductase in the stroma of NPR (■) and BPH (□) tissue and the age of the donors. Each K_m and V_{max} was determined by Lineweaver–Burk plot. The significance of the age-related changes was determined by the Spearman rank correlation coefficient (R).

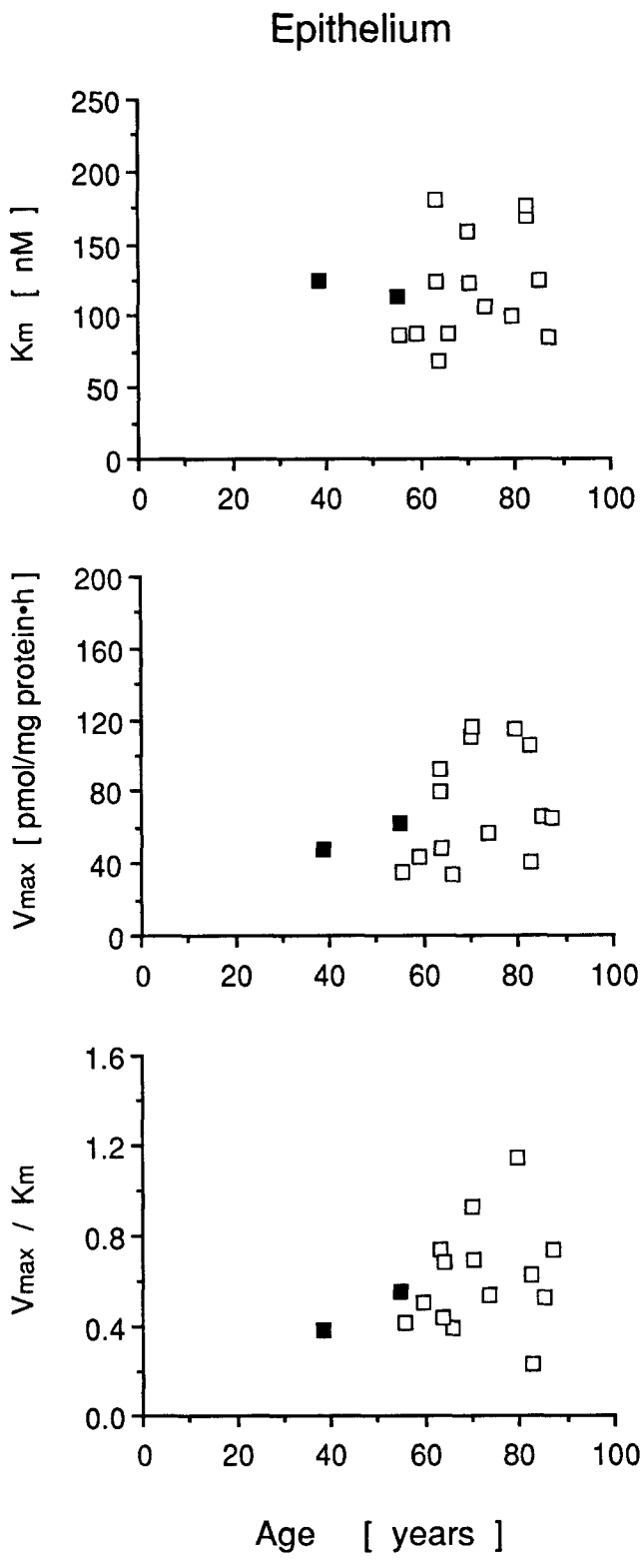

Figure 4 Correlation between K_m, V_{max}, and V_{max}/K_m of androstenedione 5α-reductase in the epithelium of NPR (■) and BPH (□) tissue and the age of the donors. Each K_m and V_{max} was determined by Lineweaver–Burk plot. The significance of the age-related changes was determined by the Spearman rank correlation coefficient (R).

seems to be sensitive to age and to the inhibition by 4-hydroxy-4-androstene-3,17-dione. The other, being responsible for androstenedione reduction, did not show such sensitivity. In addition, Hudson and Wherrett[15] compared the nuclear 5α-reduction of testosterone and androstenedione in human BPH. Their K_m values for the 5α-reduction of testosterone in the epithelium and stroma of human BPH are in accordance with our data. However, in contrast to our data, their study on the BPH stroma showed an apparent K_m for androstenedione 5α-reductase, which was about 10 times lower than that for testosterone. Moreover, their V_{max} values were lower using androstenedione as substrate instead of testosterone. In this context, it has to be noted that Hudson and Wherrett[15] described the 5α-reduction of androstenedione by the nuclear fraction, whereas in the present study, whole-cell homogenates were used. Further investigations are necessary to clarify whether the abovementioned differences are due to an asymmetrical distribution of androstenedione 5α-reductase between the different subcellular fractions.

Recent molecular and genetic studies demonstrate the existence of at least two 5α-reductases.[16,17] These two isoenzymes, chronologically termed 5α-reductases type 1 and type 2, have been sequenced and are characterized by different pH requirements for optimal in vitro enzyme activity, different substrate affinities, and different sensitivities to finasteride. 5α-Reductase type 1 is characterized by a neutral-basic pH optimum and a relative insensitivity to finasteride, whereas 5α-reductase type 2 has an acidic pH optimum and is sensitive to inhibition of finasteride. The human 5α-reductases type 1 and 2 revealed a similar K_m value (0.2 μM) for testosterone and androstenedione as far as type 2 is concerned.[16,17] In contrast, type 1 was characterized by an about sixfold higher K_m value for testosterone (1.7 μM) than the K_m for androstenedione (0.3 μM).

All in all, a comparison of our data with those from the literature is extremely limited by quite different experimental protocols (e.g., adjusted pH, whole-cell homogenates, crude nuclear fraction, and transfected cells). However, based on our identical experimental conditions, it seems that in epithelium and stroma of human prostate 5α-reductase converts testosterone to DHT with an up to fourfold higher efficiency (V_{max}/K_m) than that for androstenedione to androstanedione.[3] As yet, whether the differences in substrate affinity between epithelium and stroma are due to the existence of structurally different 5α-reductases in these two cellular compartments remains unclear. Studies on the intraprostatic expression of these two isoenzymes have demonstrated controversial results. Some authors have reported the presence of only 5α-reductase type 2 in whole human prostate,[18] whereas others have described both 5α-reductases type 1 and type 2 mRNA.[19] Recently, Bruchovsky et al.[20] reported on the cell type-specific localization of 5α-reductase isoenzyme mRNA in epithelium and stroma of human prostate. In stroma, 5α-reductases type 1 and type 2 mRNA was expressed, whereas in epithelium, only 5α-reductase type 1 mRNA was found. The authors concluded that these data explain the disparate activities of 5α-reductase in epithelium and stroma. However, studies by us and others demonstrated an apparent testosterone K_m value in the low nanomolar range for the epithelial 5α-

reductase,[4,5,21] whereas the biochemical characterization of human 5α-reductase type 1 expressed in transfected cells demonstrated a micromolar affinity for testosterone (apparent K_m, 1.7 μM).[16,17] Moreover, the sensitivity of the epithelial 5α-reductase to finasteride was found to be similar to the sensitivity of the 5α-reductase type 2.[5] Thus, it is conceivable that the activity of 5α-reductase in epithelium and stroma of human prostate is not only dictated by a cell type-specific expression of 5α-reductase isoenzymes, but it is also modified by other factors. In this context, it is worthwhile to mention that 5α-reductase of the prostate is nearly exclusively located in nuclear and microsomal membranes and that the removal of this enzyme from its membrane environment causes a tremendous loss of specific activity. These findings suggest that membrane components are necessary for optimal 5α-reductase activity. Thus, the question arises whether the differences between 5α-reductase activity in transfected cells and prostatic cells as well as the differences between the epithelial and stromal 5α-reductase activity are due to differences in the membrane environment, in which a functionally active 5α-reductase has to be embedded. In this context, more recently, we have found that the lipid composition in epithelium and stroma of human BPH is significantly different.[22] Studies in our laboratory are currently underway to clarify the interdependence between 5α-reductase activity and lipid environment.

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