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In Vitro Inhibition of Androstenedione 5α-Reduction by Finasteride in Epithelium and Stroma of Human Benign Prostatic Hyperplasia

H. Weisser* and M. Krieg

Institute of Clinical Chemistry, Transfusion and Laboratory Medicine, University Clinic Bergmannsheil, Bürkle-de-la-Camp-Platz 1, 44789 Bochum, Germany

Finasteride is a well known steroid 5α-reductase inhibitor. In this context, recently we have shown that in human benign prostatic hyperplasia (BPH) finasteride inhibits the 5α-reduction of testosterone to dihydrostestosterone (DHT) more effectively in the epithelium as compared to the stroma. The aim of the present study was to describe in epithelium and stroma of human BPH the effect of finasteride on the 5α-reduction of androstenedione, that is the second main circulating androgen in men, to androstanedione. Using a finasteride concentration of 75 nM and an androstenedione concentration of 220 nM, the mean inhibition [% ± SEM] of 5α-reductase activity was significantly higher in epithelium (69 ± 2) than in stroma (52 ± 4). Both in epithelium and stroma, this inhibition of 5α-reductase activity was dose-dependent and competitive. Dixon plots as well as slope replots of Lineweaver–Burk plots showed that the mean inhibition constant K_i (nM ± SEM) was significantly lower in epithelium (10 ± 1 and 11 ± 2, respectively) than in stroma (33 ± 7 and 28 ± 4, respectively) indicating a significantly stronger inhibitory effect of finasteride in epithelium. From those mean K_i values, it follows that in human BPH finasteride inhibits equally well both the 5α-reduction of androstenedione to androstanedione and testosterone to DHT. Based on these inhibition studies, there is no evidence for the coexistence of substrate-specific 5α-reductases converting either testosterone or androstenedione. However, the striking difference in finasteride sensitivity of the 5α-reduction between epithelium and stroma could be due to a cell-type specific expression of structurally different 5α-reductases as well as to a different access of finasteride to 5α-reductase in epithelium and stroma where, compared to each other, the lipid environment is significantly different. © **1998 Elsevier Science Ltd. All rights reserved.**

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INTRODUCTION

The androgen metabolism of the human prostate is regulated by a variety of androgen metabolizing enzymes [1]. Among those the testosterone 5α-reductase possesses the highest potential activity [2]. It irreversibly converts testosterone into the most potent androgen dihydrostestosterone (DHT) [3]. Thus, 5α-reductase dictates the cellular availability of DHT and consequently the androgen responsiveness of the human prostate. Besides testosterone, androst-4-ene-3,17-dione (androstenedione), i.e. the second main circulating androgen in men, is also a potential substrate of 5α-reductase (Fig. 1). In previous own studies, in the epithelium and stroma of human prostate its 5α-reduction into androstanedione has been described in detail [4]. Similar to the 5α-reduction of testosterone, in those studies with androstenedione as substrate significant differences with regard to the amount (V_{max}) and substrate affinity (K_m) were found between the epithelial and stromal 5α-reductase activity. Moreover, the V_{max} and K_m values of the androstenedione 5α-reductase were two- to six-fold higher as compared to those values found for the testosterone 5α-reductase. In an earlier study, we have also shown that finasteride inhibits the conversion of testosterone into DHT in epithelium and

*Correspondence to H. Weisser. Fax: 49-234-3026614; E-mail: heike.weisser@ruhr.uni.bochum.de
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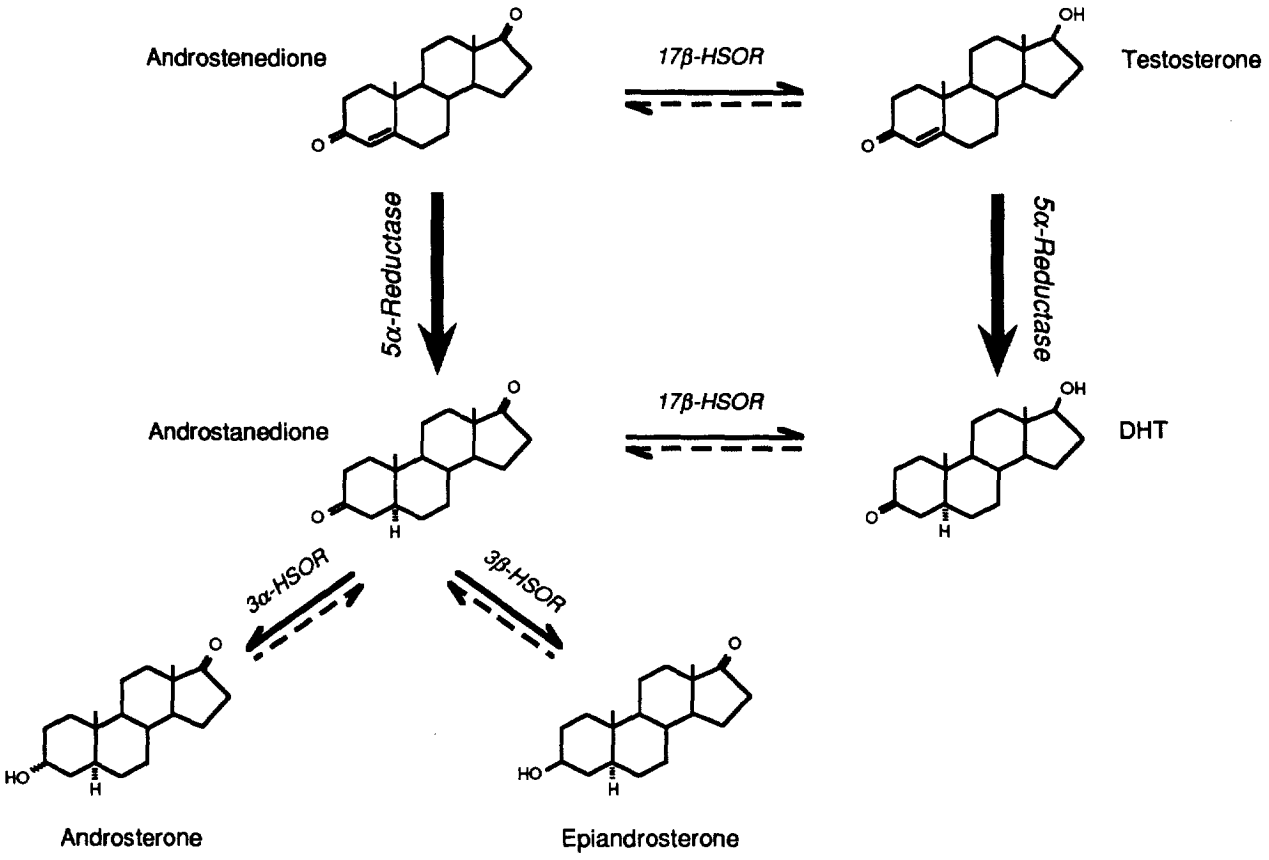

Fig. 1. Scheme of the androstenedione metabolism in human BPH. List of abbreviations: 17β-HSOR, 17β-hydroxysteroid oxidoreductase; 3α-HSOR, 3α-hydroxysteroid oxidoreductase; 3β-HSOR, 3β-hydroxysteroid oxidoreductase.

stroma of human BPH, the inhibition being significantly stronger in the epithelium [5]. Therefore, in the present study the inhibition of androstenedione 5α-reduction to androstanedione within the BPH by finasteride was investigated and compared with those previously described inhibitory effects of finasteride on the testosterone 5α-reduction. Using finasteride as an inhibitor and two substrates for the 5α-reductase, such experiments could be of importance regarding the question whether or not substrate-specific 5α-reductases are actually operating in epithelium and stroma of human BPH.

EXPERIMENTAL

Chemicals

[1,2,6,7-^{3}H]-androst-4-ene-3,17-dione (S.A. 2.69–3.40 TBq/mmol) was purchased from Amersham Buchler (Braunschweig, Germany) and the unlabeled steroids were from Sigma (St. Louis, MO). 17β-(*N*-t-butyl)carbamoyl-4-aza-5α-androst-1-en-3-one (finasteride) was a gift from Merck, Sharp and Dohme (Munich, Germany). The eluant for HPLC 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

BPH tissue was obtained from 5 men, aged 59–85 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. Afterwards, the specimens were divided into small pieces and stored in plastic tubes at −196°C. For each tissue specimen, the respective histology was proven by an experienced pathologist.

Each BPH was separated in epithelium and stroma exactly as described earlier [6, 7]. Briefly, aliquots of minced tissue of one BPH were thawed in an ice bath and homogenized after addition of four volumes (w/v) of 10 mM Tris buffer (2 mM EDTA, 5 mM NaN_3, 10 mM $MgCl_2 \cdot 6\,H_2O$, pH 7.5, at 4°C) with a Bühler homogenizer HO 4 (Bühler, Tübingen, Germany) in three periods of 30 s at 8,000 rpm with cooling intervals of 30 s. The homogenate was filtered through nylon gauze (pore size 150 μm) under slight suction. The filtrate was centrifugated at 1,800*g* for 10 min, the pellet resuspended in four volumes of Tris buffer (w/v) and defined as epithelium. The tissue retained

after filtration was homogenized in four volumes of Tris buffer (w/v) with an Ultra Turrax (Jahnke und Kunkel, Staufen, Germany) in three periods of 30 s, with 30 s cooling intervals. The homogenate was passed through nylon gauze under strong suction. The retained tissue was resuspended in four volumes of Tris buffer (w/v) and defined as stroma. Using this separation procedure, the relative purity of the epithelial as well as the stromal fraction was more than 83%, estimated by measuring acid phosphatase as a marker for epithelial cells and hydroxyproline as a marker for stromal elements in both tissue fractions [3, 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

The 5α-reductase activity was determined after optimization of the assay conditions regarding incubation time, protein and NADPH concentration using BPH tissue (4). Briefly, the incubation mixtures (final volume 202 μl), each prepared in duplicate, were composed of 40 μl Tris buffer-diluted tissue homogenate of one BPH (300–700 μg protein, pH 7.5), 20 μl NADPH-generating system (glucose-6-phosphate, 5 mM final concentration; glucose-6-phosphate dehydrogenase, 0.6 U final concentration) and varying concentrations of androstenedione from stock solutions in ethanol (60–5300 nM final concentration) alone (either as [^{3}H]androstenedione or [^{3}H]androstenedione plus unlabeled androstenedione) or in the presence of varying concentrations of finasteride (10–125 nM final concentration). Tris buffer was supplemented to 182 μl. The reaction was started by adding 20 μl NADPH (0.5 mM final concentration) and the mixtures were incubated at 37°C for 30 min. The reactions were stopped by adding 3 ml 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, NJ), redissolved in 500 μl ether and again evaporated to dryness. The dried steroids were redissolved in 50 μl acetonitrile containing 100 μg of the following steroids as tracer: testosterone, androstenedione, 5α-dihydrotestosterone, androstanedione, androsterone and epiandrosterone. The steroids were separated by reversed-phase high performance liquid chromatography as described in detail earlier [7, 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-hydroxysteroid oxidoreductase activity (metabolizing androstenedione to testosterone), the androstenedione concentration actually available for 5α-reductase was determined in each experiment by subtracting the concentration of formed testosterone and DHT from the originally added androstenedione concentration.

Other methods

Protein content was determined according to the method of Lowry *et al.* [9], using BSA as standard. Acid phosphatase activity (EC 3.1.3.2) 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 0.983 ± 0.006 (mean ± SEM) and 0.993 ± 0.003, respectively. The inhibition pattern of finasteride was determined by Lineweaver–Burk and Dixon plots [12]. K_i values were derived from Dixon plots and from replots of Lineweaver–Burk plots (slopes versus finasteride concentration). The statistical significance of the means was determined by Student's *t*-test.

RESULTS

Single point assays and dose-dependence

In epithelium and stroma of human BPH the effect of finasteride on the 5α-reduction of androstenedione was investigated using a finasteride and androstenedione concentration of 75 and 220 nM, respectively. The mean inhibition [% ± SEM] of 5α-reductase activity was significantly ($p = 0.005$) higher in epithelium (69 ± 2) than in stroma (52 ± 4) (Fig. 2).

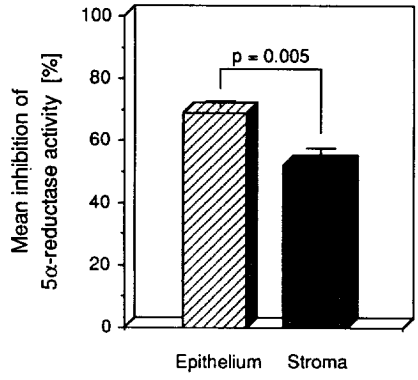

Fig. 2. Inhibition of androstenedione 5α-reductase in epithelium and stroma of human BPH ($n = 5$) using a finasteride and androstenedione concentration of 75 nM and of 220 nM, respectively. Each bar indicates the mean ± SEM of five experiments performed in duplicate.

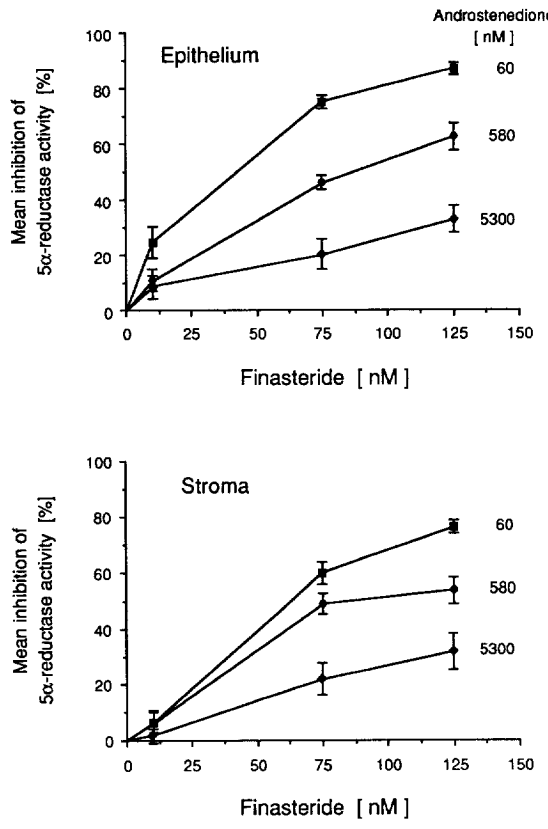

Fig. 3. Dose-dependent inhibition of androstenedione 5α-reductase in epithelium and stroma of BPH ($n = 5$) by finasteride. Each point is the mean ± SEM of five experiments performed in duplicate.

As shown in Fig. 3, in epithelium and stroma this inhibition was clearly dose-dependent. Moreover, the mean inhibition decreases significantly with increasing androstenedione concentration (Fig. 3).

Determination of the inhibition pattern of androstenedione 5α-reductase by finasteride in epithelium and stroma of human BPH

In epithelium and stroma, the inhibition pattern of androstenedione 5α-reductase activity by finasteride was determined by Lineweaver–Burk plots using varying concentrations of androstenedione (60–5300 nM) and finasteride (10–125 nM). An example of such inhibition plots is shown in Fig. 4. Both in epithelium and stroma finasteride acts as a competitive inhibitor indicated by a dose-dependent increase of the K_m value, i.e. a dose-dependent loss of substrate affinity. As shown in Fig. 5, the inhibition pattern was confirmed in each case by Dixon plots.

Determination of the inhibitor constant K_i

In epithelium and stroma, the inhibition constant K_i was determined from Dixon plots and from slope replots of Lineweaver–Burk plots. The mean inhibition constant K_i (nM ± SEM) for that competitive inhibition was significantly ($p < 0.005$ and $p = 0.01$, respectively) lower in epithelium (11 ± 2 and 10 ± 1, respectively) than in stroma (28 ± 4 and 33 ± 7, respectively). Thus, the affinity of finasteride is higher for the epithelial than for the stromal androstenedione 5α-reductase.

DISCUSSION

In this study, for the first time the inhibitory effect of finasteride on the 5α-reduction of androstenedione has been investigated in human BPH, separated in epithelium and stroma. Compared with earlier studies on the 5α-reduction of testosterone in BPH [3, 5], the present data may contribute to the question whether the 5α-reduction of androstenedione is equally well inhibited as the 5α-reduction of testosterone.

In fact, this is the case, because our K_i values are nearly identical regardless whether testosterone or androstenedione has been taken as substrate [5]. So, finasteride treatment in men obviously will not only lead to the well documented inhibition of the 5α-reduction of testosterone but also of androstenedione, which is secreted daily in considerable amounts by the adrenal gland. Hence, it can be ruled out that the finasteride inhibition of testosterone 5α-reductase will concomitantly be counter-balanced by an regulatory increase of the 5α-reduction of androstenedione to androstanedione, subsequently followed by an increased conversion of androstanedione to DHT. Rather, finasteride treatment seems to block intraprostatic DHT formation from testosterone and from androstenedione (via androstanedione) as well. In accordance with such an effective blockade of the androstenedione metabolism by finasteride is the observation in vivo of an increased androstenedione level both in the prostate [13] and in blood under finasteride treatment [14].

Moreover, the lower K_i-values found in the epithelium as compared to the stroma suggest that finasteride inhibits the androgen induced cellular processes in epithelium much stronger. In fact, more recently histomorphological investigations showed in BPH of men under finasteride treatment both marked atrophy and apoptosis in the epithelial cells, whereas in stroma cells such processes were of minor intensity [15].

The significant difference of the K_i-values between epithelium and stroma could be in favour of the coexistence of two 5α-reductase isoenzymes, one being primarily located in the epithelium, the other in the stroma. We came to the same conclusion already in an earlier own publication, in which the inhibition of the testosterone 5α-reductase by finasteride has been investigated [5]. Thus, it is attractive to speculate that the differences in K_i values found in this study between epithelium and stroma of BPH might have at least something to do with two 5α-reductase isoenzymes found in BPH tissue by molecular biological techniques [16–19]. In one of those studies it has

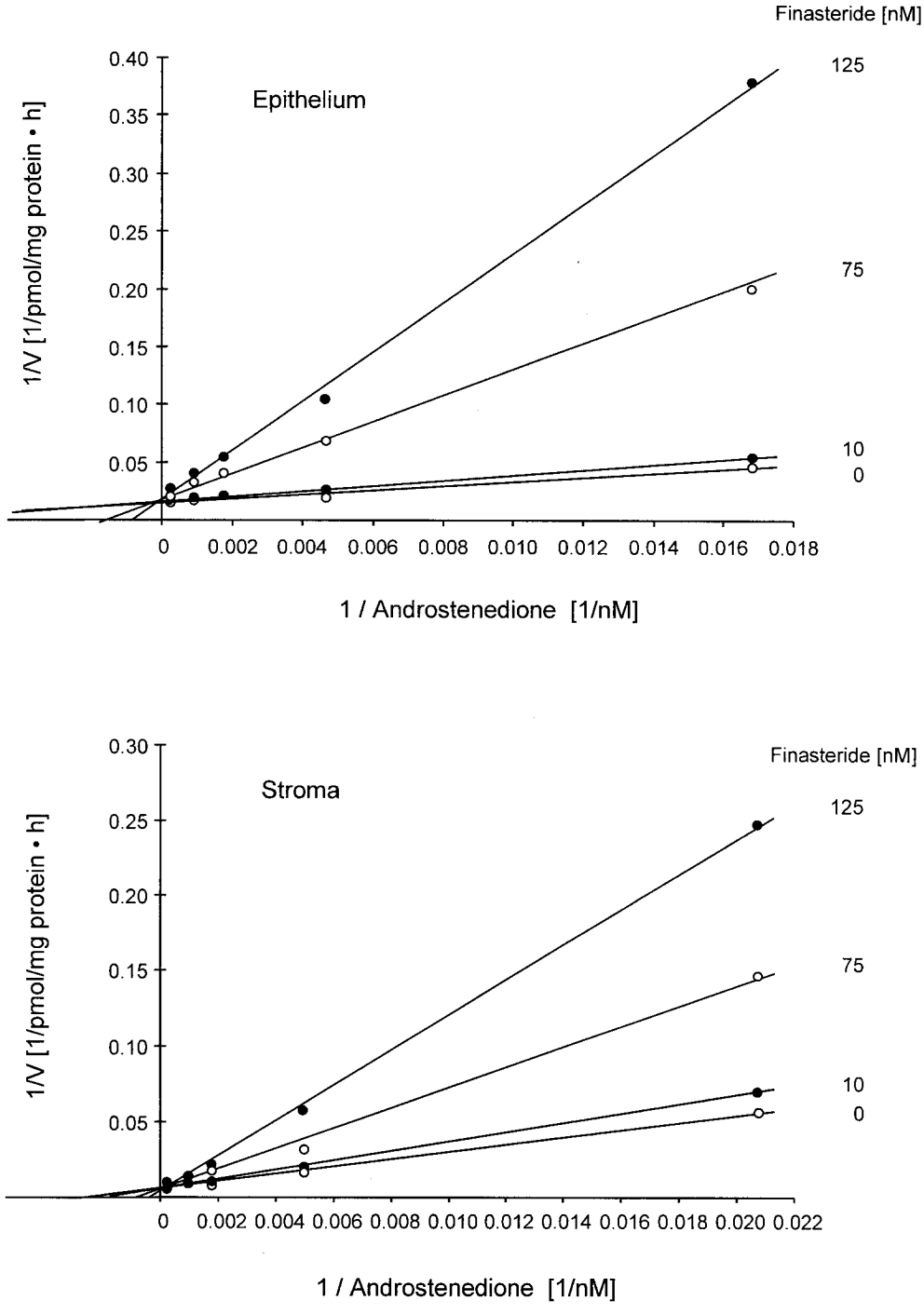

Fig. 4. Lineweaver–Burk plots of the inhibition of androstenedione 5α-reductase in epithelium and stroma of BPH by finasteride. The inhibitory effect was determined throughout in a contemporaneous assay, carried out at varying finasteride as well as androstenedione concentrations. The intercept of the straight lines with the *x*-axis indicates $1/K_m$ (K_m, affinity constant).

been reported that in epithelium of BPH only 5α-reductase type 1 mRNA was found, whereas in stroma type 1 and type 2 mRNA were present [19].

However, it must be kept in mind that such differences could also be due to the cellular environment in which the 5α-reductase is embedded. In this context, it is known that the 5α-reductase is nearly exclusively located in nuclear and microsomal membranes. Removed from its membrane environment, the 5α-reductase becomes more or less inactive. Thus, for an optimally active 5α-reductase the presence of membrane components are absolutely necessary. Moreover, inhibitors like finasteride basically are lipophilic compounds, which operate the better the more the lipid environment meets their structural criteria [20]. In this context, therefore, it cannot be ruled out that differences in the lipid composition between the epithelial and stromal compartment of

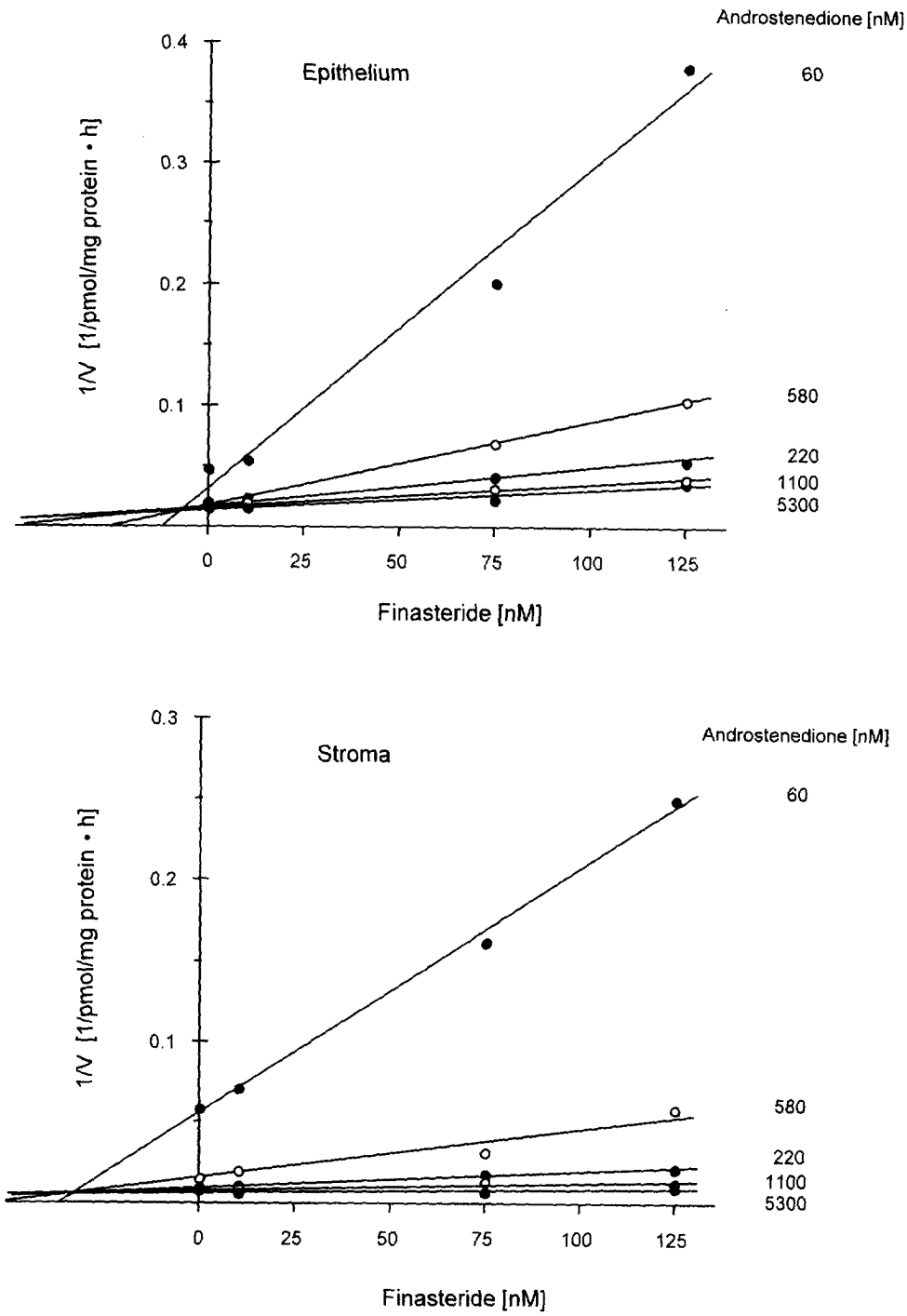

Fig. 5. Dixon plots of the inhibition of androstenedione 5α-reductase in epithelium and stroma of BPH by finasteride. The inhibitory effect was determined throughout in a contemporaneous assay, carried out at varying finasteride and androstenedione concentrations. K_i (inhibitor constant) was determined from the intersection of the straight lines.

BPH may at least in part be responsible for the different activities and sensitivities of the membrane-bound 5α-reductase in such epithelium and stroma [21]. Regarding the influence of the cellular environment on the 5α-reductase activity, it should also be pointed out that kinetic data like V_{max}, K_m and K_i of the 5α-reductase are hardly comparable if using BPH tissue on the one side and 5α-reductases expressed in transfected cells on the other.

Finally, it is remarkable that in the present study the K_i values obtained with finasteride are identical regardless whether testosterone or androstenedione has been taken as substrate. Hence, our data are not in favour of the existence of substrate-specific isoen-

zymes as postulated by Martini *et al.* [22]. Based on their data, they discussed for the rat prostate the existence of at least two substrate-specific 5α-reductases. One being responsible for the conversion of testosterone and sensitive to age as well as to the inhibitory effect of 4-hydroxy-4-androstene-3,17-dione (4-OH-A). The other, being responsible for the conversion of androstenedione and insensitive to age and to the effects of 4-OH-A. Moreover, molecular biological studies showed that the 5α-reductase type 2, expressed in transfected CHO cells, exhibited a similar K_m value (0.2 μM) for testosterone and androstenedione. On the other side, the 5α-reductase type 1 was characterized by an about sixfold lower K_m value for androstenedione (0.3 μM) than for testosterone (1.7 μM) [23]. However, earlier own studies on BPH tissue showed an about two- to sixfold higher V_{max} and K_m value for the 5α-reductase if androstenedione instead of testosterone was used as substrate [4]. Further studies, for example, using a 5α-reductase type 1 specific inhibitor such as LY306089 are certainly needed to clarify these striking discrepancies.

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