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Potential Activities of Androgen Metabolizing Enzymes in Human Prostate

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The entire androgen metabolism of the human prostate is an integral part of the DHT mediated cellular processes, which eventually give rise to the androgen responsiveness of the prostate. Therefore, the potential activities of various androgen metabolizing enzymes were studied. Moreover, the impact of aging on the androgen metabolism and the inhibition of 5α-reductase by finasteride were studied. In epithelium (E) and stroma (S) of normal (NPR) and hyperplastic human prostate (BPH), for each enzyme being involved in the conversion either of testosterone via DHT, 3α- and 3β-diol to the $C_{19}O_3$-triols or from testosterone to androstenedione and vice versa, the amount (V_{max}) and Michaelis constant (K_m) were determined by Lineweaver–Burk plots. Furthermore, V_{max}/K_m quotients were calculated, which served as an index for the potential enzyme activity. 17 enzymes showed a mean $V_{max}/K_m \geq 0.10$. The top four were the 5α-reductases in E and S of NPR and BPH. Among those, the highest activity was found in E of NPR (1.6 ± 0.2). Moreover, in E a significant age-dependent decrease of 5α-reductase activity occurred, whereas in stroma rather constant activities were found over the whole age range. Similar age-dependent alterations were found for the cellular DHT levels. Finally, the finasteride inhibition of 5α-reductase (IC_{50};nM) was stronger in E (35 ± 17) than in S (126 ± 15). In conclusion, 5α-reductase is: (a) the outstanding androgen metabolizing enzyme in NPR and BPH; (b) dictating the DHT enrichment in the prostate; (c) under the impact of aging; and (d) preferentially inhibited by finasteride in E.

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INTRODUCTION

The cellular availability of sufficient amounts of 5α-dihydrotestosterone (DHT) is thought to be a prerequisite for normal growth and function of the human prostate [reviewed in 1]. Moreover, the development of human benign prostatic hyperplasia (BPH) seems to be a pathobiological process that is at least in part dependent on DHT [reviewed in 2]. The effect of DHT at the cellular level is mediated by its binding to receptor proteins with high affinity and specificity. Subsequently, the DHT receptor complex initiates the activation of androgen responsive elements at the genome level that ultimately leads to androgen specific cellular responses [reviewed in 3]. Due to the fact that DHT is one of numerous androgen metabolites at the cellular level, the entire androgen metabolism is inherently connected with the aforementioned DHT mediated cellular events. Thus, a balanced androgen metabolism seems to be an indispensable condition for the normal androgen responsiveness of the prostate. On the other hand, it follows that an imbalance of the cellular androgen metabolism could lead to an enhanced or diminished responsiveness.

Therefore, *in vitro* studies on the numerous metabolic steps of androgen metabolism in the human prostate have been performed by us [4–7]. In those studies, for each enzyme, both its amount (V_{max}) and Michaelis constant (K_m) have been determined under optimized incubation conditions. Furthermore, V_{max}/K_m quotients for each enzyme have been calculated. According to the Michaelis–Menten equation, such quotients may serve as an index for the potential activity of an enzyme [5].

Our present paper will deal in more detail with

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Abbreviations: 5α-reductase, 3-oxo-5α-steroid: $NADP^+$-4en-oxidoreductase (EC 1.3.1.22); 3α(β)-HSOR, 3α- or 3β-hydroxysteroid: NAD(P)$^+$oxidoreductase (EC 1.1.1.50/51); 17β-HSOR, 17β-hydroxysteroid: NAD(P)$^+$17-oxidoreductase (EC 1.1.1.63/64); 3β-diol-6(7)α(β)-hydroxylase, 5α-androstane-3β,17β-diol hydroxylating enzyme at 6α, 6β, 7α or 7β; red and ox, reductive and oxidative direction of an enzymatic reaction, respectively; 3α-diol, 5α-androstane-3α,17β-diol; 3β-diol, 5α-androstane-3β,17β-diol; $C_{19}O_3$-triols, sum of 5α-androstane-3β,6(7)α(β), 17β-triols; finasteride, 17β-(*N*-*t*-butyl)carbamoyl-4-aza-5α-androst-1-en-3-one.

these potential activities of the androgen metabolizing enzymes in the epithelium (E) and stroma (S) of both the normal prostate (NPR) and BPH. Moreover, the strong impact of aging on 5α-reductase in the human prostate, the close correlation between 5α-reductase activities and DHT concentrations at the cellular level of the human prostate, and the different inhibition of 5α-reductase in E and S of BPH by finasteride are further subjects of this paper.

MATERIALS AND METHODS

Chemicals

All chemicals used were purchased from companies mentioned in earlier publications [4–8].

Tissue

Overall, the NPR and BPH tissue was obtained from 10 men, aged 15–58 years, and 70 men, aged 50–95 years, respectively. Tissue origin and handling up to the separation of epithelium and stroma have been described in detail earlier [4–8].

Measurement of enzyme activity

The amount (V_{max}) and the substrate affinity in terms of the Michaelis constant (K_m) have been determined for each of the following enzymes by Lineweaver–Burk plots and also, in part, by Eadie–Hofstee plots: 5α-reductase, 3α-$HSOR_{ox}$, 3α-$HSOR_{red}$, 3β-$HSOR_{ox}$, 3β-$HSOR_{red}$, 3β-diol-6α-hydroxylase, 3β-diol-6β-hydroxylase, 3β-diol-7α-hydroxylase, 3β-diol-7β-hydroxylase, 17β-$HSOR_{ox}$ and 17β-$HSOR_{red}$. The optimization of the multiple point assay, separation of the metabolites by HPLC and determination of V_{max} and K_m have been published in detail elsewhere [4–8].

Inhibition experiments with finasteride

With regard to 5α-reductase, the 50% inhibitory concentration (IC_{50}), the inhibition pattern, as well as the inhibition constant (K_i) of finasteride in E and S of BPH have been published in detail elsewhere [9].

Measurement of DHT in the human prostate

After steroid extraction and defatting, followed by the separation of the extracted steroids by HPLC, we measured DHT in NPR and BPH tissue by RIA. Details have been published elsewhere [8].

Miscellaneous

The quotient V_{max}/K_m serves as an index of the potential enzyme activity, derived from the reduced Michaelis–Menten equation $V \approx V_{max}/K_m \cdot S$ [5]. The statistical significance of the differences between means was determined either by using Student's t-test or by applying the analysis of variance (ANOVA) with repeated measures. Regression lines were calculated by the method of least squares. The significance of age-dependent changes was determined using the Spearman rank correlation coefficient (R).

RESULTS

Mean potential enzyme activities in the human prostate

All prostatic enzymes being involved in the metabolism either of testosterone via DHT, 3α- and 3β-diol to the $C_{19}O_3$-triols or from testosterone to androstenedione and vice versa have been analyzed. According to the index V_{max}/K_m, 17 of those enzymes showed a mean potential activity ≥ 0.10 (Table 1). Among these 17 potential enzyme activities, the top four were those of the 5α-reductase in E and S of NPR and BPH. Among the 5α-reductase activities, the highest

Table 1. Mean (±SEM) potential activities (V_{max}/K_m) of various androgen metabolizing enzymes in epithelium (E) and stroma (S) of normal (NPR) and hyperplastic human prostate (BPH)

Enzyme	Substrate	Cofactor	Tissue	n	V_{max}/K_m
5α-Reductase	Testosterone	NADPH	NPR,E	5	1.6 ± 0.2
5α-Reductase	Testosterone	NADPH	BPH,E	20	1.1 ± 0.1
5α-Reductase	Testosterone	NADPH	BPH,S	20	1.0 ± 0.1
5α-Reductase	Testosterone	NADPH	NPR,S	5	0.9 ± 0.1
3α-$HSOR_{red}$	DHT	NADPH	NPR,E	10	0.56 ± 0.08
3α-$HSOR_{red}$	DHT	NADPH	BPH,E	20	0.33 ± 0.04
3α-$HSOR_{red}$	DHT	NADPH	BPH,S	20	0.26 ± 0.02
3α-$HSOR_{ox}$	3α-diol	NAD^+	NPR,E	3	0.26 ± 0.11
3β-$HSOR_{red}$	DHT	NADPH	NPR,E	10	0.24 ± 0.03
3α-$HSOR_{red}$	DHT	NADPH	NPR,S	10	0.19 ± 0.02
7α-Hydroxylase	3β-diol	NADPH	BPH,E	4	0.19 ± 0.04
3α-$HSOR_{ox}$	3α-diol	NAD^+	BPH,S	10	0.18 ± 0.03
3β-$HSOR_{red}$	DHT	NADPH	BPH,E	20	0.14 ± 0.02
7α-Hydroxylase	3β-diol	NADPH	BPH,S	4	0.12 ± 0.02
3β-$HSOR_{ox}$	3β-diol	NAD^+	BPH,E	10	0.11 ± 0.02
3α-$HSOR_{ox}$	3α-diol	$NADP^+$	NRP,E	7	0.10 ± 0.03
3β-$HSOR_{red}$	DHT	NADPH	BPH,S	20	0.10 ± 0.01

All other androgen metabolizing enzymes measured in this study showed V_{max}/K_m quotients <0.10. Abbreviations of enzymes and substrates are explained elsewhere in this paper.

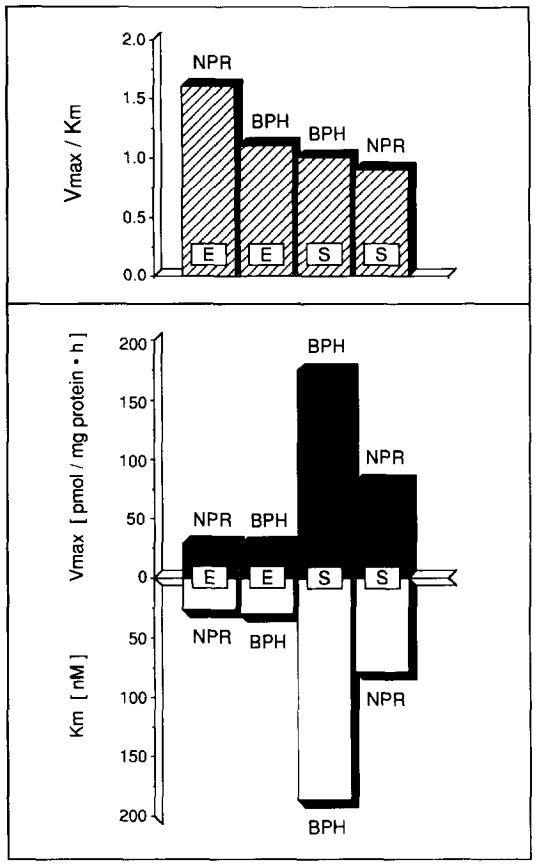

Fig. 1. Mean V_{max}/K_m (upper panel) and V_{max} and K_m (lower panel) of 5α-reductase in epithelium (E) and stroma (S) of normal (NPR; $n = 5$) and hyperplastic human prostate (BPH; $n = 20$). V_{max}, maximal activity of 5α-reductase in terms of enzyme concentration; K_m, Michaelis constant of 5α-reductase.

potential activity was found in E of NPR. Furthermore, the lowest 5α-reductase activity was still significantly higher than the highest enzyme activity of the 3α-$HSOR_{red}$ group, the latter being responsible for the conversion of DHT to 3α-diol. Moreover, the 3α-$HSOR_{red}$ in NPR epithelium and BPH stroma was in part counterbalanced by the respective 3α-$HSOR_{ox}$, which is catalyzing the conversion of 3α-diol to DHT. Finally, only two 3β-diol-hydroxylases, which are catalyzing the irreversible conversion of 3β-diol to $C_{19}O_3$-triols, were listed. Comparatively, their potential activities were rather low.

Mean V_{max} and K_m values of 5α-reductase

As shown in Fig. 1 (lower panel), relatively low mean V_{max} values were associated with relatively low mean K_m values and vice versa. In other words, a relatively low amount of 5α-reductase was balanced by a relatively high substrate affinity, whereas a relatively high amount of 5α-reductase was counterbalanced by a relatively low substrate affinity. This inverse relationship between the amount of 5α-reductase and its substrate affinity led to mean V_{max}/K_m quotients, which were, despite significant differences between NPR epithelium and the other three prostate fractions, of the same order of magnitude (Fig. 1, upper panel).

5α-reductase activity, DHT content and aging

The correlation of the individual V_{max} and K_m values of 5α-reductase with donors' age showed in E and S a significant increase of K_m. On the other hand, an increase of V_{max} with age was found only in the S (data not shown). Thus, the resultant V_{max}/K_m quotients of 5α-reductase decreased significantly with age in the prostate E, whereas in the prostate S rather constant V_{max}/K_m quotients were found over the whole age range (Fig. 2, left panel). Moreover, the highest V_{max}/K_m quotients were found in E of relatively young prostates. Like the V_{max}/K_m quotients of 5α-reductase, the DHT levels were highest in E of relatively young prostates (Fig. 2, right panel) and they decreased with age in the E. In contrast, in the prostate S rather constant DHT levels were found over the whole age range (Fig. 2, right panel). All in all, the 5α-reductase activity and the DHT level were closely interconnected.

5α-reductase inhibition by finasteride

In E and S of five BPH, the concentration of finasteride required to inhibit 5α-reductase by 50% (IC_{50}) was determined from inhibition curves (Fig. 3). At a testosterone concentration of 298 nM, the mean IC_{50} value in BPH epithelium (35 ± 17) was about three times lower than in BPH stroma (126 ± 15). At other testosterone concentrations used (14, 27, 70 and 582 nM, respectively) similar differences in the IC_{50} values between E and S were found. The K_i values (nM ± SEM), derived from Dixon plots, were 8 ± 1 and 27 ± 3 in E and S, respectively. At a concentration of $\geqslant 10$ nM, finasteride inhibited 5α-reductase in E and S of human BPH competitively.

DISCUSSION

This paper presents data on V_{max}/K_m quotients of numerous androgen metabolizing enzymes, which we have analyzed in E and S of NPR and BPH [4–7]. In our earlier studies on 5α-reductase, it has been established that the V_{max}/K_m quotient is suitable to serve as an index for the potential enzyme activity [5]. According to the reduced Michaelis–Menten equation ($V \approx V_{max}/K_m \cdot S$), for the purpose of estimating the actual rate (V) of a certain metabolic step the respective substrate concentration (S) has additionally to be considered. At present, such substrate concentrations at the cellular level of the prostate are not exactly known. Likewise, the actual cellular redox potential is as yet unknown. Thus, it remains uncertain as to whether the supplementation of our enzyme assays with cofactors like NADPH, $NADP^+$, NADH, NAD^+ matches the *in vivo* situation. On the other hand, there is evidence from the literature that the "free"

testosterone level in blood [10], which is probably the natural substrate level for 5α-reductase, is approximately twice as high as the cellular DHT level [11], which is probably the natural substrate level for those enzymatic steps which immediately succeed the conversion of testosterone to DHT. Moreover, it has been shown that the content of DHT and of some other androgen metabolites are rather similar in E and S of the human prostate [8, 11–16]. Finally, it has been reported that in the cytosol of mammalian cells the ratios of NADPH/$NADP^+$ and NAD^+/NADH are high in favor of NADPH and NAD^+, respectively [17, 18]. Taken together, it appears that *in vivo* the differences in activity between 5α-reductase and all other androgen metabolizing enzymes could be even higher than those indicated by the V_{max}/K_m quotients. On the other hand, referring to the comparison between E and S, it is more likely that the reported differences in the V_{max}/K_m quotients between both organ compartments match the *in vivo* situation. In summary, despite some uncertainties, the dominance of 5α-reductase among all androgen metabolizing enzymes in the human prostate is impressively underlined by the V_{max}/K_m quotients of this study.

According to the outstanding role which 5α-reductase plays at the cellular level of the human prostate the global DHT enrichment in the human prostate [19] is best explained by such a dominance of 5α-reductase, which catalyzes the irreversible conversion of testosterone to DHT. Moreover, the mean V_{max}/K_m quotients of 5α-reductase suggest that in E of NPR the comparatively highest DHT enrichment occurs. In fact, very recently we were able to confirm such preferential DHT enrichment in E [8]. Moreover, the hypothesis of elevated DHT content in BPH, as compared to NPR, has recently been challenged [14, 20–22].

Undoubtedly, V_{max}/K_m quotients are helpful to compare enzyme activities with each other. However, details about the corresponding V_{max} and K_m values are of interest as well. Therefore, in addition to V_{max}/K_m quotients, the mean V_{max} and K_m values of 5α-reductase have been presented. It turns out that with regard to V_{max} and K_m there are significant differences particularly between E and S. Moreover, an apparently close correlation between V_{max} and K_m of 5α-reductase is given. In other words, in E of the prostate a relatively low amount (low V_{max}) of 5α-reductase is found, which apparently has a relatively high substrate affinity (low K_m). On the other hand, in S the relatively high amount (high V_{max}) corresponds with a 5α-reductase of relatively low substrate affinity (high K_m). It is conceivable that the existence of isoenzymes, which has been reported previously [23], gives rise to such a close correlation between V_{max} and K_m as far as their mean values are concerned.

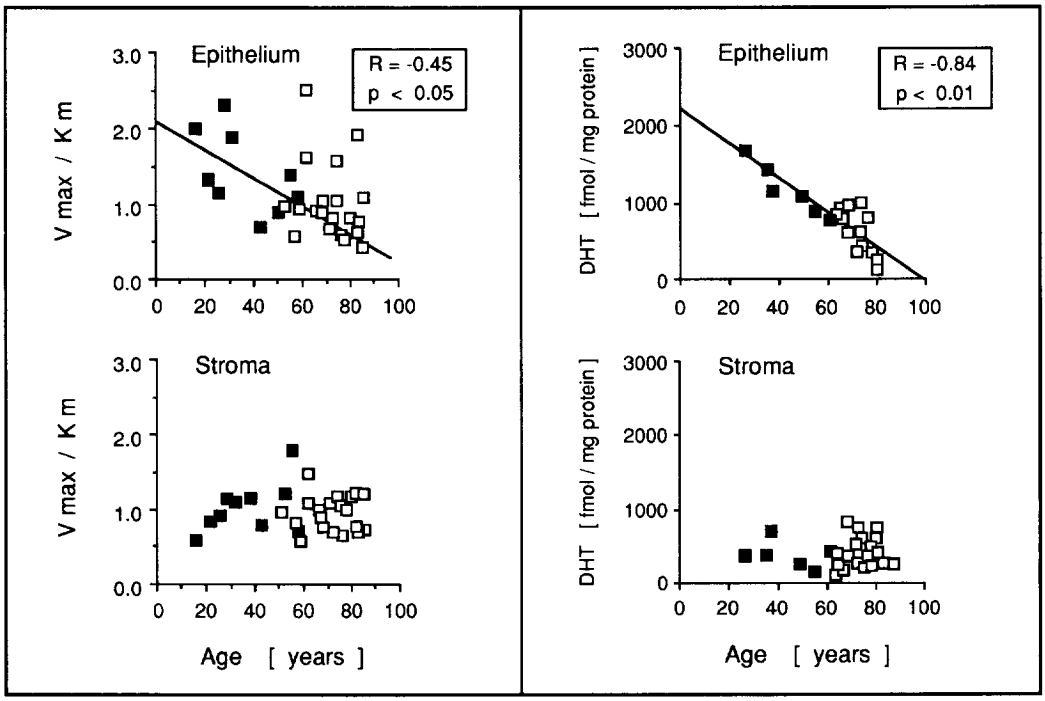

Fig. 2. Correlation between either the potential activity (V_{max}/K_m) of 5α-reductase (left panel) or the endogenous DHT content (right panel) in E and S of NPR (■) and BPH (□) and the age of donors. The significance of the age-related changes was determined by the Spearman rank correlation coefficient (R). V_{max}, maximal activity of 5α-reductase in terms of enzyme concentration; K_m, Michaelis constant of 5α-reductase.

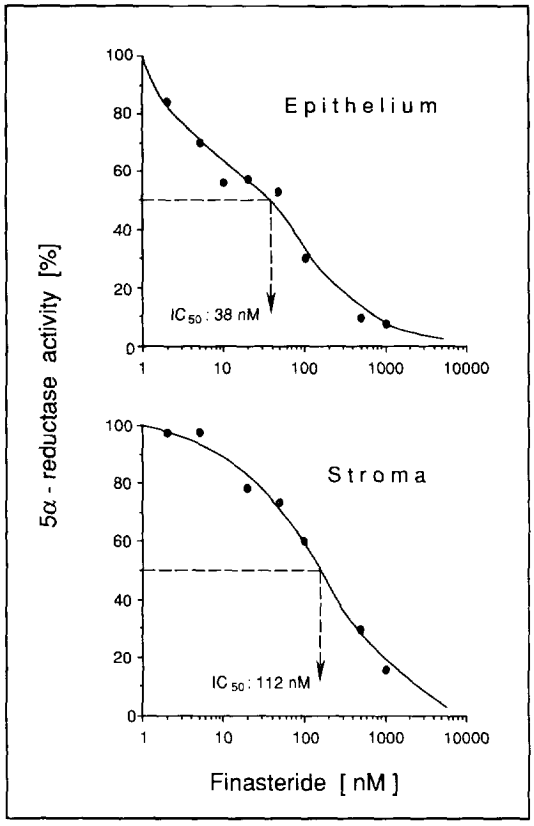

Fig. 3. Inhibition of 5α-reductase in E and S of human BPH by finasteride. The inhibition curves were obtained at a testosterone concentration of 298 nM. The mixtures were incubated at 37°C for 15 min. 5α-reductase activity in the absence of finasteride was taken as 100%. All experiments were performed in duplicate. IC_{50}, concentration of finasteride required to inhibit 5α-reductase by 50%.

However, mean values of parameters like V_{max} and K_m are masking the apparent impact of aging on those parameters [4–7]. For example, referring to the 5α-reductase, a significant linear increase of the individual K_m values in E and S occurs with age, whereas regarding V_{max}, such a linear increase has been restricted to the S [5]. These linear alterations of the individual K_m and V_{max} values with age clearly demonstrate that the aforementioned differences in the mean values of V_{max} and K_m, found for instance between E and S, are not necessarily due to tissue specific differences. Alternatively, as shown in this study and elsewhere [5, 6, 8], such differences of mean values either between E and S or between NPR and BPH can be the result of linear changes of the individual values with age.

Due to such alterations of V_{max} and K_m with age, in S the calculated individual V_{max}/K_m quotients remained rather constant over the whole age range, whereas in E a linear decrease of the V_{max}/K_m quotients was found. In other words, in S the potential enzyme activity of 5α-reductase remains constant over the whole age range, whereas in E a linear decrease of the 5α-reductase activity occurs. Supposing that the potential 5α-reductase activity, expressed as V_{max}/K_m, is indeed the most powerful activity among all enzyme activities of the androgen metabolism in the prostate, then the age-dependent alterations of the V_{max}/K_m quotients in E should correspond with respective alterations of the DHT level. In fact, this holds true. In E of the prostate both the V_{max}/K_m quotient and DHT level decrease with age, whereas in S both parameters are rather constant over the whole age range. Moreover, the relatively high V_{max}/K_m quotients in E of relatively young prostates correspond with equally high DHT levels. Therefore, our data suggest that the DHT level of the human prostate is primarily regulated by 5α-reductase activity.

Such regulation of DHT levels by 5α-reductase has been confirmed in clinical trials with the 5α-reductase inhibitor finasteride. In those trials, a dramatic suppression of the intraprostatic DHT formation has been found [24]. In this context, our *in vitro* studies with finasteride are the first to demonstrate that the IC_{50} and K_i values are significantly lower in the E as compared to the S of human BPH. Therefore, it has been postulated that *in vivo* finasteride will preferentially inhibit the epithelial 5α-reductase [9]. Moreover, it is interesting to note that the sensitivity of epithelial 5α-reductase to finasteride, found in our study, is in accordance with data on the cloned 5α-reductase 2 of the human prostate. However, the sensitivity of stromal 5α-reductase to finasteride is neither compatible with the sensitivity of the cloned 5α-reductase type 2 nor of type 1 [25, 26].

At present, we are unable to thoroughly explain the regulatory forces which give rise to the age-dependent alterations of V_{max} and K_m of 5α-reductase in the human prostate. Moreover, the significance of these phenomena for the pathogenesis of BPH is far from being understood. In addition, the reasons have to be explored why our enzyme data, which are based on conventional biochemical techniques, are only partially in line with those data based exclusively on gene technology. In this respect, post-translational events could give rise to the various phenomena reported in this study. Referring to such still hypothetical post-translational events, based on our own preliminary data [27], it is conceivable that alterations of lipid membranes, in which 5α-reductase is intimately embedded, could evoke those phenomena.

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