Alterations in the Intraprostatic Hormonal Metabolism by the Pollen Extract Cernilton®N

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Introduction

A number of hypotheses have been implicated in the etiology of benign prostatic hyperplasia (BPH). The most important theories are: (1) an alteration of the androgen metabolism in BPH if compared to the normal prostate (NPR) leading to an accumulation of the biologically highly active androgen 5α-dihydrotestosterone (DHT) predominantly in the stroma; (2) a change in the androgen-estrogen ratio in favor of estrogens; (3) and an alteration in the intraprostatic interaction between stroma and epithelium [for an overview see (3)]. Such variable hypotheses do not allow a unified therapeutic concept for BPH.

For the medical treatment of BPH a variety of substances are utilized such as GnRH analogues, which reduce peripheral androgen and estrogen concentration (5,8), 5α-reductase inhibitors, which lower the intraprostatic DHT concentration (14), or aromatase inhibitors, which lower the peripheral estrogen concentration (12).

Besides these substances influencing the hormonal milieu, phytopharmaca are also utilized to treat patients with BPH who do not have indications for surgery. These drugs, such as the pollen extract Cernilton®N, lead to a subjective improvement in the patient's symptoms. The effect is supposedly based on an improvement in the inflammation or congestion of the prostate. To what extent these drugs influence the intraprostatic hormonal milieu is not known. We were interested in the question whether and to what degree phytopharmaca influence the intraprostatic androgen metabolism and may exert their effects by a change in the intraprostatic DHT content. To this end we characterized the main enzymes of the androgen metabolism (5α-reductase, 3α- and 3β-hydroxysteroid oxidoreductase) in the epithelium and stroma of the human prostate, and tested the in vitro influence of the phytopharmacon Cernilton®N on these enzymes.

Materials and Methods

The activity of DHT-metabolizing enzymes (5α-reductase, $3\alpha\text{-HSO}_{red}$, $3\beta\text{-HSOR}_{red}$) was determined in mechanically separated epithelial and stromal fractions from 10 normal and 20 hyperplastic prostate glands. To this end aliquots of the tissue homogenates were incubated with at least 4 different concentrations of the individual substrates (either exclusively in ^{3}H-labelled or ^{3}H-labelled and unlabelled form: testosterone to measure the 5α-reductase in concentrations from 14 to 600 nM, DHT to measure 3α- and $3\beta\text{-HSOR}_{red}$ in concentrations from 100 to 4860 nM). After addition of a co-factor NADPH-regenerating system (5 mM glucose-6-phosphate, 0.6 U glucose-6-phosphate dehydrogenase) the reaction was started with the co-factor NADPH (5α-reductase: 0.5 mM; 3α- and $3\beta\text{-HSOR}_{red}$: 1.5 mM) and the mixture incubated for 15 min at 37°C. To determine the effect of the pollen extract, epithelial and stromal fractions of three of the hyperplastic prostates were incubated with various concentrations (49;246;493µg/ml) of the water-soluble (wPE) or fat-soluble (fPE) fractions of the extract, mixed well and then submitted to the same procedure as described above. After the reaction was stopped by the addition of either, and following extraction, the steroids were separated by HPLC (reversed phase, stationary phase: Lichrosorb RP 18, mobile phase: acetonitrile: H_2O = 50:50). Quantification was performed by measuring the radioactivity in the individual chromatographic fractions (substrate and various metabolites).
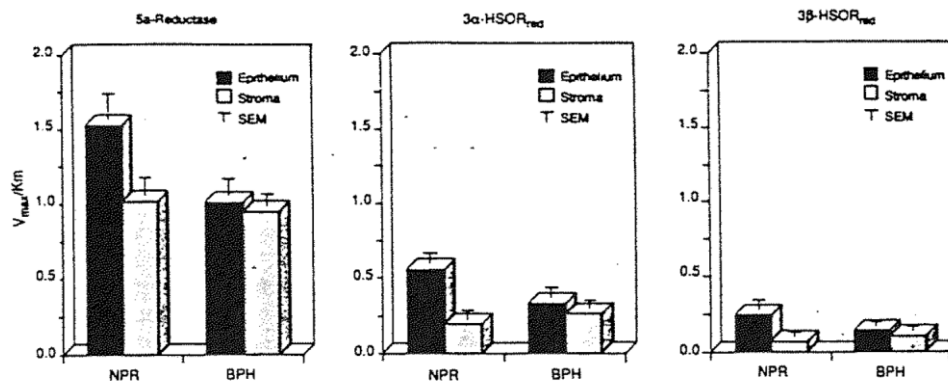
Fig. 1 Mean potential capacities (Vmax/Km) of 5α-reductase, 3α- and 3β-hydroxysteroid oxidoreductase (3α- and 3β-HSOR$_{red}$) in epithelium and stroma of 10 normal (NPR) and 20 hyperplastic (BPH) prostate glands.

The enzymatic activity was determined from the distribution of the radioactivity in these fractions. The specific activity of the labelled substrate, the ratio between labelled and unlabelled substrate, the incubation time, the protein concentration, and the blanks were utilized for the calculation. All assays were performed in duplicate.

Proteins were measured according to Lowry (6). The kinetic parameters Km and Vmax were calculated from the Lineweaver-Burk plot using regression analysis (least square method). The Student's t-test was utilized to determine significant differences between the means. $P < .05$ was considered significant.

Results and Discussion

In the human prostate many androgen-metabolizing enzymes are present (see Fig. 1 in the chapter, ,,Hormone Metabolism in the Human Prostate"). The potential capacities of these enzymes vary greatly as our own published (10,11) and unpublished results show. The DHT-forming 5α-reductase and the DHT-removing 3α- and 3β-hydroxysteroid oxidoreductases (3α- and 3β-HSOR$_{red}$) have the highest potential capacity and therefore the greatest biological significance. It can therefore be assumed that these three enzymes are mainly responsible in the regulation of the intraprostatic DHT level.

Androgen Metabolism in the Normal and Hyperplastic Human Prostate

The potential capacity of an enzyme is expressed by the ratio Vmax / Km (10). In Fig. 1 the mean potential capacities for 5α-reductase, 3α-HSOR$_{red}$ and 3β-HSO$_{red}$ in epithelium and stroma of normal and hyperplastic prostates are shown. The 5α-reductase in the epithelium of normal prostate tissue has the highest potential capacity, where it is significantly higher than in the stroma, and also higher than in stroma or epithelium in hyperplastic prostate tissue. In the stroma there are no significant differences between NPR and BPH. The potential capacity of the 3α-HSOR$_{red}$ is significantly lower than that of the 5a-reductase, and the capacity of the 3β-HSOR$_{red}$ is again significantly lower than that of the 3α-HSOR$_{red}$. Both DHT- removing enzymes have significantly higher capacities in the epithelium of normal prostate tissue than in the stroma, and than in the epithelium and stroma of BPH tissue. The potential capacity of the 3α-HSOR$_{red}$ in NPR stroma is minimally lower, and that of the 3β-HSOR$_{red}$ even significantly lower than in BPH stroma.

A comparison of the potential capacities of the DHT-forming 5α-reductase and the DHT removing 3α-HSOR$_{red}$ and 3β-HSOR$_{red}$ allows the conclusion that there is no higher accumulation of DHT in BPH as compared to NPR. This conclusion is, however, only valid under the assumption of similar mean testosterone concentrations in men with normal and hyperplastic prostates. These results of the potential capacities therefore do not support the DHT accumulation hypothesis for BPH, but rather support the recently published data on DHT concentrations in normal prostate tissue (13,15) which demonstrated a higher concentration of DHT in normal prostate tissue removed immediately after death than in BPH tissue.
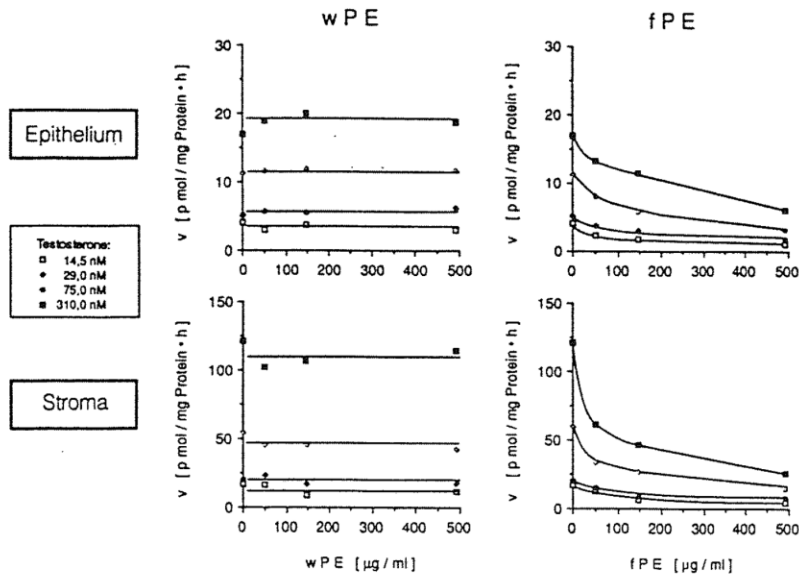
Fig. 2 Influence of the water-soluble fraction (wPE) of the pollen extract (left column) and the fat-soluble fraction (fPE) of the pollen extract (right column) on the enzyme activity (v). The activity of 5α-reductase in the epithelium (upper row) and stroma (lower row) is shown as an example. The enzyme activities were measured at different concentrations of the substrate testosterone (14.5–310 nM) and varying concentrations of wPE and fPE (49–493 µg/ml incubation mixture). All measurements were done in duplicate.

Alteration of the Intraprostatic Androgen Metabolism by the Pollen Extract Cernilton®N

To determine the effect of the pollen extract Cernilton®N on the enzymes of the intraprostatic androgen metabolism, the activities of the DHT-forming 5α-reductase and the DHT-metabolizing 3α-HSOR$_{red}$ and 3β-HSOR$_{red}$ were measured in epithelium and stroma of three hyperplastic prostates with varying concentrations of substrates as well as different concentrations of the water-soluble (wPE) and fat-soluble (fPE) fraction of the pollen extract. The activity of the 5α-reductase was not affected by wPE in a concentration range from 49 to 493 µg / ml incubation mixture in epithelium or stroma (Fig. 2). The activities of 3α-HSOR$_{red}$ and 3β-HSOR$_{red}$ were similarly not affected by this substance within the same concentration range (data not shown). However, fPE demonstrated in epithelium and stroma an inhibitory effect on the 5α-reductase (Fig. 2). The formation of DHT from testosterone is therefore significantly inhibited by the addition of fPE to the incubation mixture. Additionally, fPE also inhibited the activity of 3α-HSOR$_{red}$ and 3β-HSOR$_{red}$ in epithelium and stroma (data not shown). Therefore the metabolism of DHT to 5α-androstanediol is also diminished. The fat-soluble extract of another phytopharmacon

(Serenoa repens B, Permixon®) was also found to inhibit the activity of 5α-reductase and 3α-HSOR$_{red}$ in human foreskin fibroblasts (9). This would indicate that nonspecific acting ingredients of such fat-soluble extracts are responsible for the inhibition of the enzymes.

To determine the kinetic mechanisms of the inhibitory effect of fPE, the enzyme activities were plotted for the different substrate and inhibitor concentrations in a double-logarithmic plot according to Lineweaver-Burk as shown exemplarily for the 3α-HSOR$_{red}$ in epithelium and stroma in Fig. 3. For all enzymes, 5α-reductase, 3α-HSOR$_{red}$ and 3β-HSOR$_{red}$, it was found in epithelium and stroma that the presence of fPE in the incubation mixture of the tissue homogenate did not change the Km, but that the Vmax changed corresponding to the concentration. Therefore the fPE acts as a non-competitive inhibitor of these enzymes, or in other words, the ingredients of the fat-soluble fraction do not bind at the active center for testosterone or DHT, but at another location, thereby altering the turnover number.

In Fig. 4 the mean potential capacities (ratio Vmax / Km) for the three enzymes in epithelium and stroma of the three hyperplastic prostates are depicted without (Fig. 4 A) and with (Fig. 4
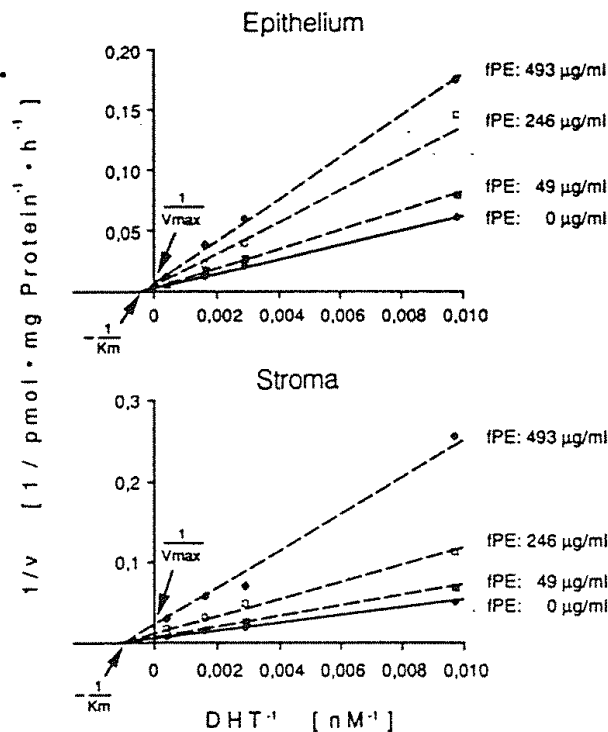
Epithelium

Stroma

Fig. 3 Inhibition of the enzyme activity (v). The inhibition of the 3α-HSOR$_{red}$ by different concentrations (49–493 µg/ml incubation mixture) of the fat-soluble fraction (fPE) of the pollen extract as a function of the concentration of the substrate DHT in epithelium and stroma of a hyperplastic prostate is shown as an example (double logarithmic plot according to Lineweaver-Burk).

B) additional fPE (493 µg / ml incubation mixture). It is easily seen that the potential capacities of the 5α-reductase as well as the 3α- and 3β- HSOR$_{red}$ in epithelium and stroma are drastically reduced, but that the inhibitory effect of the fPE on the three enzymes is different. The mean potential capacity of the 3α-HSOR$_{red}$ is more inhibited in both compartments than that of both 5α-reductase and 3β-HSOR$_{red}$.

To estimate the expected changes in DHT content after in vitro incubation with fPE, the mean potential capacities of the three enzymes without additional fPE were assumed to be 1.0, and the percent activity after addition of the highest concentration of fPE (493 µg / ml incubation mixture) was calculated. The mean percentage activity of 5α-reductase after addition of fPE is shown next to the mean percentage activity of the DHT metabolizing enzymes 3α- and 3β-HSOR$_{red}$ (Fig. 5). It can be seen that the activity of the 3α-HSOR$_{red}$ in particular in the stroma, but also in the epithelium is more inhibited than that of the 5α-reductase, while the inhibition of the 3β- HSOR^{red} is similar to that of the 5α-reductase. The different reaction of the enzymes may be explained by the different intracellular localization. The 3α-HSOR$_{red}$ is equally

distributed between cytosol and cytosolic membranes, while the 3β-HSOR$_{red}$ is mainly membrane-bound (1). The 5α-reductase is exclusively found in the perinuclear and microsomal membranes (2,4,7). Although our studies were conducted in a cell-free milieu, the membrane-bound enzymes are probably surrounded by membrane particles and should be only minimally influenced by fat-soluble extract.

Since these in vitro studies showed a stronger inhibition of the DHT catabolism compared to the DHT formation by the fat-soluble fraction of the phytopharmacon Cernilton®N, a lowering of the intraprostatic DHT level in tissue homogenates after fPE administration cannot be expected. On the contrary, an accumulation of DHT results, which should be similar to that in the normal prostate, however, at a generally lower activity level. This comparison is only valid under the condition that similar amounts of the fat-soluble extract are incorporated in the epithelial and stromal cells without being metabolized, and that these extracts reach the enzymes 5α-reductase, 3α- and 3β-HSOR$_{red}$ - which are located in different subcellular compartments - in similar concentrations. To make statements about the capacity of the
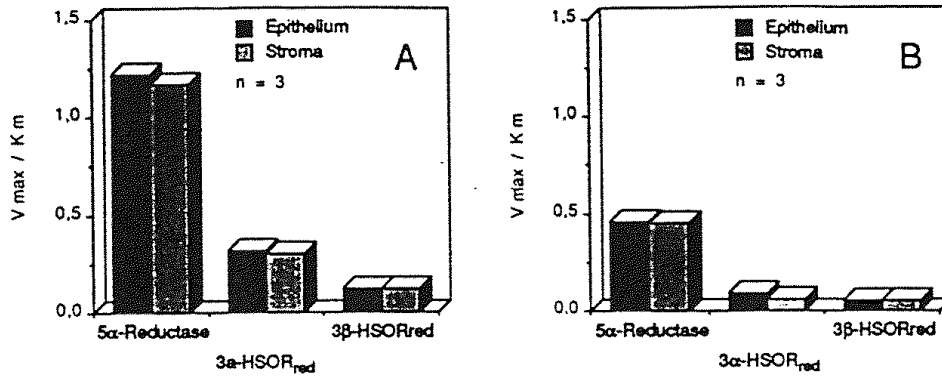
Fig. 4 Mean potential capacity (Vmax/Km) of 5α-reductase, 3α- and 3β-HSOR$_{red}$ in epithelium and stroma of three hyperplastic prostates without addition of fat-soluble fraction (fPE) of the pollen extract (A) and after addition of 493 µg fPE per ml of incubation mixture (B). All Vmax and Km values determined by Lineweaver-Burk plots.
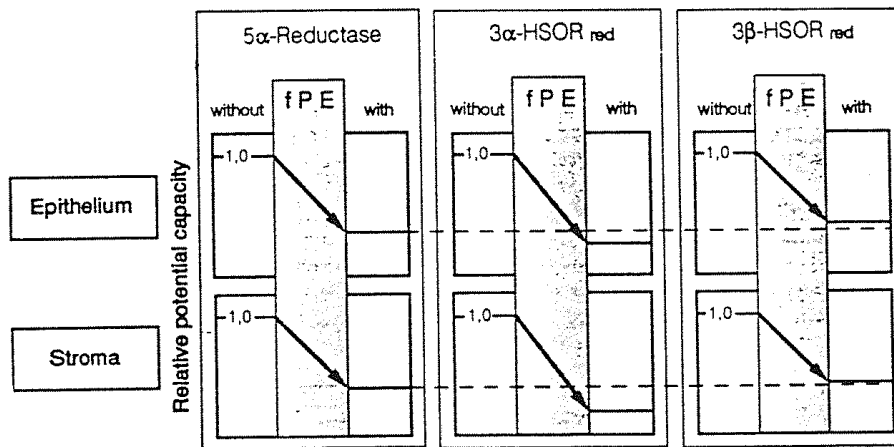

Fig. 5 Mean potential capacity (5α-reductase, 3α-HSOR$_{red}$ and 3β-HSOR$_{red}$) without (left columns) and after addition (right columns) of fat-soluble fraction (fPE) of the pollen extract in epithelium (upper row) and stroma (lower row) of three hyperplastic prostates. The potential capaci- ties without additional fPE were assumed as 1.0, and the percentage remaining potential capacity after addition of fPE was calculated. The dotted lines indicate the relative potential capacities of the 5α-reductase in epithelium and stroma after addition of fPE.

pollen extract to influence androgen metabolism in vivo, further studies of androgen metabolism have to be conducted in prostate glands of patients who have been treated for a defined period of time with the pollen extract.

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