Inhibition of Steroid 5α-Reductase Activity by Aliphatic Fatty Acids

Candidates for Chemoprevention of Prostate Cancer[a]

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INTRODUCTION

Prostate cancer is the most frequent cancer in men and the second most important cause of male cancer death in the United States.[1] Numerous studies have demonstrated a link of prostate cancer to high dietary fat intake.[2] However, up to now, there is no established mechanism to explain how fat might contribute to prostate cancer etiology. On the other hand, there are compelling reasons to believe that androgens play a central role in prostate carcinogenesis. Cell division in the prostate gland is controlled by dihydrotestosterone, the reduced metabolite of testosterone. Steroid reduction is carried out by a single enzyme, 5α-reductase (5αR, EC 1.3.99.5).[2] This suggests that prostate carcinogenesis could be prevented by modulation/inhibition of 5αR. Studies investigating the influence of the steroidal 5αR inhibitor, finasteride, on prostate cancer pathogenesis support this idea and are still in progress.

Results showing that prostate cancer development is influenced by androgens or by dietary fat do not exclude each other. Due to a high-fat and low-fiber diet, inhabitants of high prostate cancer risk areas (e.g., the United States) show high circulating testosterone levels and this may lead to enhanced 5αR expression.[2] The molecular conformation and the enzyme activity of membrane-bound enzymes, like 5αR,[3] are stabilized and highly regulated by the lipid environment. This basic biochemical finding for membrane-bound enzymes gives a rationale to argue that 5αR activity is modulated by the fatty acid composition in the prostate gland. Further evidence for this working hypothesis arises from the nonsteroidal compound, retinoic acid, a lipophilic molecule, which has also been found to inhibit 5αR.[4] We could show that human 5αR derived from

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freshly obtained human fibroblasts and prostate tissues[5] was inhibited by certain aliphatic free fatty acids. In order to determine structure-enzyme inhibition relationships for free fatty acids, we studied the influence of chain length, unsaturation, oxidation, and esterification on the potency to inhibit 5αR activity.

MATERIALS AND METHODS

Fatty Acids and Derivatives Investigated for 5α-Reductase Inhibition Activity

Caproic, caprylic, nonanoic, capric, undecanoic, lauric, tridecanoic, myristic, pentadecanoic, palmitic, margaric, and stearic acids ($C_{2:0}$ to $C_{18:0}$); oleic, linoleic, and linolenic acids ($C_{18:1}$ to $C_{18:3}$); *cis*-9(10)-epoxystearic acid (EPSA); 9- and 10-monohydroxystearic and 9,10-dihydroxystearic acids; 1- and 2-monolaurin (1-ML and 2-ML); 1,2- and 1,3-dilaurin; trilaurin; dilauroylphosphatidyl choline and ethanolamine; and lauric acid ethyl ester at high purification grade were used for the inhibition experiments.

Standard 5α-Reductase Assay (Type II Enzyme)

Fibroblasts were cultivated from a human genital tissue specimen. The cells were harvested and homogenized by sonication as previously described.[6] The 1600-g supernatant of sonication was used as the protein source. The 100-μL standard incubation assay contained 20 μg of protein, 0.1 M sodium citrate, 1 mM NADPH, and 0.05 μM [1,2(*n*)-^{3}H]-testosterone (spec. act. = 40–60 Ci/mmol) in 0.1 M Tris-HCl (pH 5.5). At appropriate concentrations, fatty acids were added in 5 μL ethanol. Samples were incubated for 30 min at 37 °C. The assays were carried out in quadruplicate. In addition, blanks were run, containing buffer instead of protein solution. The reaction was stopped by adding 500 μL $CHCl_3$-CH_3OH (2:1) (v:v). Steroid analysis was carried out using a 100-μL aliquot from the chloroform layer as described elsewhere.[7] The protein content was measured by the method of Lowry *et al.*[8]

Inhibition of Human Prostate Tissue Enzyme (Type I Enzyme) by Lauric Acid

Prostate tissue specimens were obtained from five patients who underwent transurethral resection. In one case, histological examination revealed the presence of malignant cells; in a second specimen, a myofibroma was found; and three patients presented a benign prostate hyperplasia. In each experiment, approximately 0.5 g (wet weight) of prostate tissue was cut into small pieces (<1 mm^3) and transferred into 5 mL of 10 mM Tris-HCl (pH 7.4) containing 2 mM EDTA, 5 mM NaN_3, and 10 mM $MgCl_2$. The material was disrupted using an Ultra Turrax S8N5G laboratory dispersion system (Jahnke & Kunkel, Staufen, Germany) and was filtered through a 120 μM stainless steel wire screen. The filter residue was washed with an additional 3 mL of Tris buffer. The combined filtrates were homogenized in a Potter-Elvechjem apparatus (Braun, Melsungen, Germany) using a tight-fitting glass pestle and were used as a source for 5αR

activity determination. The material retained by the stainless steel mesh did not show significant 5αR activity. The prostate tissue assay contained 98 pmoles of testosterone, 2 pmoles of tritium-labeled testosterone (spec. act. = 54 Ci/mmol), 0.72 mM NADPH, 5 mM glucose-6-phosphate, 0.6 U glucose-6-phosphate dehydrogenase, and up to 500 μg protein in a total volume of 400 μL incubation medium. For the inhibition experiments, 36 μg (0.45 mM) of lauric acid was added. 5αR incubation was carried out in triplicate at 37 °C for 1 h. The reaction was stopped by transferring the mixture into 2 mL $CHCl_3$-CH_3OH (2:1) (v:v). Steroid analysis was performed with a 500-μL aliquot of the chloroform layer as described for the standard assay.

RESULTS AND DISCUSSION

The IC_{50} of fatty acids that inhibit 5αR (type II) activity are given in TABLE 1. The 5αR inhibition activity of saturated fatty acids depends on the hydrocarbon-chain length. When C_{18} fatty acids were investigated, only the unsaturated compounds showed 5αR inhibition activity. When EPSA is converted to a hydroxy compound by oxirane ring cleavage, the enzyme inhibition activity is lost. This result, together with the inhibition activity of nonsubstituted medium-chain ($C_{10:0}$ to $C_{14:0}$) fatty acids, suggests that hydrophobicity is a prerequisite for 5αR activity inhibition. The enzyme inhibition activity of lauric acid ($C_{12:0}$) is lowered by esterification to monoglycerides and is totally lost by esterification to diglycerides and triglycerides and to the ethyl ester. The 5αR inhibition by fatty acids also requires strongly polar end-groups. 5αR activity inhibition by fatty acids has already been reported by Liang and Liao.[9] We found that the relative inhibitory potencies for the type II enzyme of saturated and unsaturated fatty acids are, in decreasing order, $C_{18:2}$, $C_{18:3}$, $C_{13:0}$, $C_{12:0}$, $C_{11:0}$, $C_{18:1}$, and $C_{10:0}$ (TABLE 1). We could show that lauric acid, which was inactive in the assay system of Liang and Liao, was active in reducing the activity of type II enzyme (TABLE 1) and of type I enzyme[5] (FIGURE 1), which is present in human prostate tissue homogenate. Taken together, the results suggest that certain free fatty acids have the potency to inhibit *in vitro* the activity of both 5αR isozymes.[10]

TABLE 1. IC_{50} for 5α-Reductase (Type II) Inhibition by Free Fatty Acids and Derivatives

Fatty Acid	IC_{50} [10^{-4} M]	IC_{50} [mg/L]	Fatty Acid	IC_{50} [10^{-4} M]	IC_{50} [mg/L]
$C_{10:0}$	9.9	169.8	$C_{18:1}$	4.3	120.2
$C_{11:0}$	3.5	64.6	$C_{18:2}$	0.8	23.6
$C_{12:0}$	1.6	31.1	$C_{18:3}$	1.0	27.3
$C_{13:0}$	1.3	28.5	EPSA	2.0	59.7
$C_{14:0}$	6.4	146.5	1-ML	6.3	171.5
$(IC_{40})^a$			2-ML	4.7	127.6

[a] $C_{14:0}$ did not show 50% enzyme inhibition at concentrations up to 1.5 mM.

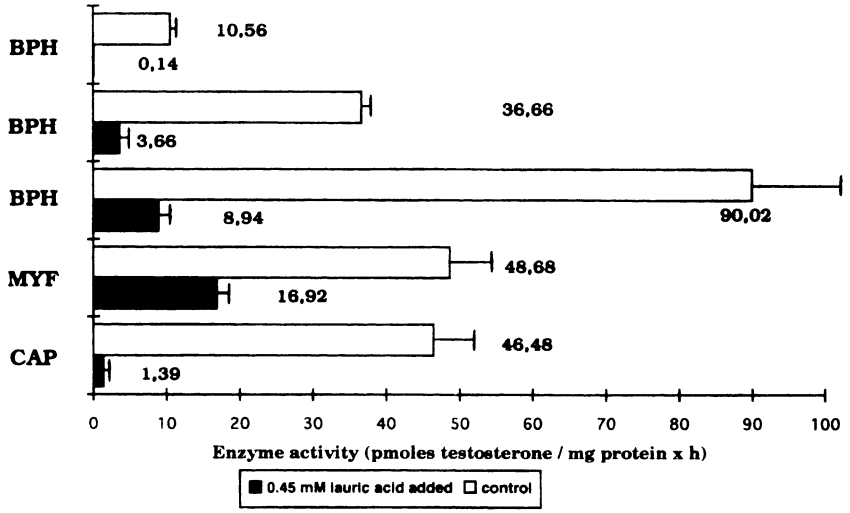

FIGURE 1. Inhibition of 5α-reductase (type I) by lauric acid in different human prostatic specimens. The mean ± SE of three experiments is shown. Terms: BPH = benign prostatic hyperplasia, MYF = prostatic myofibroma, CAP = prostatic cancer.

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