NOTE

Inhibition of Nuclear Testosterone 5α-Reductase in Rat Ventral Prostate by Estrogens and Anti-Androgens

JUN SHIMAZAKI, YUMIKO OHKI, ATSUSHI KOYA AND KEIZO SHIDA

Department of Urology, Gunma University School of Medicine, Maebashi

Synopsis

The activity of testosterone 5α-reductase in purified nuclei of rat ventral prostate was inhibited by addition of estradiol-17β, estrone or estriol in vitro. Rate of the inhibition of the enzyme activity by these estrogens was of similar degree. Therefore, this inhibitory effect does not seem to be correlated to their estrogenic potency.

Effect of three anti-androgens (Ro 2-7239, SK & F 7690 and TSAA 291) on the activity of testosterone 5α-reductase was also examined and it was revealed that SK & F 7690 and TSAA 291 showed the inhibitory effect on the enzyme activity.

Ventral prostate of rats metabolizes testosterone through oxidative and also reductive pathway (Ofner, 1968; Wilson and Gloyna 1970). Among many reactions involved in testosterone metabolism, formation of 5α-dihydrotestosterone (5α-androstane-17β-ol-3-one) seems to be biologically important because of its potent biological activity (Williams-Ashman and Reddi, 1971; Robel et al., 1971), and of its uptake and retention in nuclei of target tissue cells (Liao and Fang, 1969; Mainwaring, 1970). Furthermore, changes in the rate of this transformation were noted during the maturation and aging process of animals (Shimazaki et al., 1969; Harper et al., 1971).

Testosterone 5α-reductase in ventral prostate was localized in nuclear and cytoplasmic membraneous fractions (Frederiksen and Wilson, 1971; Shimazaki et al., 1971; Roy, 1971). Recently Moore and Wilson (1972) reported that this enzyme was specifically localized in the nuclear membrane fraction of rat ventral prostate. The activity of nuclear 5α-reductase in rat ventral prostate was influenced by direct addition of estradiol-17β to the incubation mixture for the assay of the enzyme activity (Shimazaki et al., 1971). The present report deals with the inhibitory effect of various steroidal compounds on the activity of nuclear 5α-reductase in rat ventral prostate.

Materials and Methods

Animals and tissue preparation

Male Wistar rats weighing 300–400 g were used throughout the experiment. The ventral prostate of rats was removed immediately after sacrifice and pooled on ice. The methods for homogenization of ventral prostates and for isolation of purified nuclei was described previously (Shimazaki et al., 1971). The recovery of DNA in purified nuclear fraction was approximately 50% and RNA/DNA ratio in this fraction was below 0.28.

Determination of testosterone 5α-reductase

The activity of testosterone 5α-reductase was determined according to our previous report (Shima-
Incubation was carried out with purified nuclear fraction equivalent to 300 mg of original tissue in the presence of 100 μmoles of Tris-HCl (pH 7.2), 1 mg of NADPH and 0.2 μc of testosterone-4-14C (specific activity 58.2 mc/mM, Radiochemical Centre, Amersham) in final volume of 2 ml. The substrate concentration was adjusted to $6 \times 10^{-5}$ M by adding unlabeled testosterone. Testosterone and other steroidal compounds were dissolved in 0.3 ml of dimethyl sulfoxide, and added to incubation flasks. Incubation was performed at 37°C for 60 min. Activity was expressed as mmoles of 5α-dihydrotestosterone formed /g of tissue equivalent/hr.

### Results and Discussion

It was reported previously (Shimazaki et al., 1971) that supplement of estradiol-17β, dihydrophosphate of estradiol-17β and hexestrol diphosphate to the incubation mixture was inhibitory on the activity of testosterone 5α-reductase in prostatic nuclei. In the present experiment the inhibitory effect of estrone and estriol on the enzyme activity was also observed (Table 1). Estrogenic potency of estradiol-17β, estrone and estriol was very different when tested by in vivo method (Emmens, 1962), however, extent of the inhibition by these estrogens was within a similar range. Metabolism of testosterone by minced prostatic tissue was influenced by direct addition of estrogenic compounds to the incubation mixture (Shimazaki et al., 1965; Farnsworth 1969; Belham and Neal, 1971), and this might be at least partly due to inhibition of testosterone 5α-reductase by estrogens. However estrogen was reported to have various effects on male target tissues, for example, effect on the rate of substrate entry to prostatic cells (Giorgi et al., 1972) and inhibition on the attachment of 5α-dihydrotestosterone to cytosol receptor from prostate (Jungblut et al., 1971). It has been also well documented that estrogens interact with glutamic dehydrogenase (Yielding and Tomkins, 1962), respiratory and electron transport systems (Guidry et al., 1952; Cochran and DuBois, 1954; Kuss and Jütting, 1967), glucose-6-phosphate dehydrogenase (McKerns, 1963), and ATPase (Robinson, 1970). Recently Harper et al. (1970) and Fahmy and Griffiths (1968) reported the inhibition of DNA polymerase from thymus and prostate by estrogens.

**Table 1. Inhibition on nuclear testosterone 5α-reductase by various steroidal compounds**

<table>
<thead>
<tr>
<th>Additions</th>
<th>No. of exp.</th>
<th>Testosterone 5α-reductase (μmole of 5α-dihydrotestosterone formed /g/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>no</td>
<td>4</td>
<td>12.53 ± 0.84</td>
</tr>
<tr>
<td>Estradiol-17β</td>
<td>4</td>
<td>8.49 ± 1.03**</td>
</tr>
<tr>
<td>Estrone</td>
<td>3</td>
<td>7.93 ± 1.35*</td>
</tr>
<tr>
<td>Estriol</td>
<td>3</td>
<td>7.18 ± 0.71***</td>
</tr>
<tr>
<td>Ro 2–7239</td>
<td>3</td>
<td>9.73 ± 0.73</td>
</tr>
<tr>
<td>SK &amp; F 7690</td>
<td>4</td>
<td>7.61 ± 1.40*</td>
</tr>
<tr>
<td>TSAA 291</td>
<td>3</td>
<td>7.77 ± 0.18**</td>
</tr>
</tbody>
</table>

a) Concentration of inhibitors added was the same as that of the substrate ($6 \times 10^{-5}$ M).

b) M ± S.E.

Statistical analysis was performed between the values of no inhibitor and of inhibitor experiment by t-test; $p < 0.05$ (*), $p < 0.02$ (**), $p < 0.01$ (***)
hibitory effect of cyproterone acetate and chlormadinone acetate was detected in testosterone 5α-reductase of rat ventral prostate. Cyproterone acetate was known to suppress the uptake and retention of testosterone and 5α-dihydrotestosterone in prostatic tissue (Fang and Liao, 1969; Belham and Neal, 1971). Therefore, it might be interesting that anti-androgenic compounds exert their effects on target tissues in several ways; inhibitory effect on the binding site for steroids, or inhibition of the activity of testosterone 5α-reductase, etc.

Acknowledgement

The authors are indebted to Mr. Fukumoto, Japan Roche Co. (Ro 2–7239), Dr. K. de Acosta, Smith Kline & French Laboratory (SK & F 7690) and Dr. R. Nakayama, Takeda Chemical Industries (TSAA 291) for supplying anti-androgens. These experiments were partly supported by a Grant of the Ministry of Education, Japan.

References


