Effect of Estrogen Administration on Activities of Testosterone 5α-Reductase, Alkaline Phosphatase and Arginase in the Ventral and the Dorsolateral Prostates of Rats

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Synopsis

Daily treatment with 5 or 500 μg of estradiol benzoate of male adult rats for 7 days did not change the activity of testosterone 5α-reductase in the ventral prostate, while the activity decreased slightly in the dorsolateral prostate. The activity of alkaline phosphatase was increased by 60% over the respective control in the ventral prostate from rats treated with the larger dose of estrogen, but the estrogen treatment did not affect the alkaline phosphatase activity in the dorsolateral prostate. On the contrary, the estrogen treatment evoked three-fold elevation in the arginase activity of the dorsolateral prostate in contrast to the decreased arginase activity in the ventral prostate following estrogen administration. From these results, it was concluded that the alterations in some enzyme activity of the ventral and the dorsolateral prostates evoked by estrogen treatment were different from those observed in the respective lobes from castrated animals, although both estrogen administration and castration induced atrophy of the tissue. Furthermore, it might be also worthwhile to mention that the ventral and the dorsolateral prostates of rats responded in a different manner to estrogen administered.

It was known that estrogen administration to male adult rats caused an involution of male accessory sex organs (Price and Williams-Ashman, 1961). Shrinkage of these organs was also evoked after castration of male rats. Brandes (1966) reported that fine structures of the ventral prostate of rats observed after castration were similar to those observed after administration of estrogen.

A marked difference in contents of citrate, fructose and zinc (Mann, 1964) and in activities of some enzymes (Müntzing, 1972) has been observed between the ventral and the dorsolateral prostates of rats. Contents of these substances in the prostates were decreased after castration and were restored after treatment of animals with androgen (Mann, 1964). Activities of testosterone 5α-reductase (Shimazaki et al., 1969), alkaline phosphatase (Bialy and Pincus, 1967) and arginase (Pegg et al., 1970; Yamanaka et al., 1971) in ventral prostate of rats were also reported to be decreased after castration and to be increased after administration of androgen to castrated animals. Therefore, it seems to be interesting to compare the contents of these substances and activities of these enzymes in the ventral and the dorsolateral prostates from estrogen-treated animals with those in tissues from castrated rats. The present report deals with the effect of estrogen administration on the ventral as well as the dorsolateral prostates of rats, with special attention to the alkaline phosphatase and the arginase activities.

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Materials and Methods

Animals
Male Wistar rats weighing 450−550 g were used throughout the experiments. Animals were maintained on a standard laboratory chow in a room of constant temperature. They were sacrificed by cervical dislocation and the ventral and the dorsolateral lobes of the prostatic gland were excised separately and were pooled after freed of surrounding connective tissues. For estrogen treatment, estradiol benzoate was dissolved in sesame oil and was injected with a daily dose of 5 or 500 µg per head subcutaneously. When a castrated animal was used, the operation was performed via scrotal route under ether anesthesia.

Tissue preparation
The pooled tissue was homogenized in 5 volumes of 0.05 M Tris-HCl buffer (pH 7.4) using a teflon-glass homogenizer, and filtered through nylon cloth. For assay of arginase, Mn++ was added immediately after homogenization to a final concentration of 0.03 M.

Determination of the activities of testosterone 5α-reductase, alkaline phosphatase and arginase
The activity of testosterone 5α-reductase was determined by the method described previously (Shimazaki et al., 1971). Incubation of tissue homogenate was performed in the presence of 0.05 M Tris-HCl buffer (pH 7.2), 5.5×10⁻⁴ M NADPH and 3×10⁻⁶ M testosterone-4-¹⁴C (0.2 µc) for 30 min. After incubations, dihydrotestosterone (5α-androstane-17β-ol-3-one) and 5α-androstane-3α, 17β-diol were extracted and purified. Testosterone 5α-reductase activity was calculated from the amounts of these two metabolites, and expressed as µmoles of the sum of these two steroids formed/hr/g tissue (wet weight) or mg DNA.

Alkaline phosphatase activity was determined as outlined in Sigma Technical Bulletin No. 104 (1963), in which p-nitrophenylphosphate was used as substrate. Arginase activity was determined by the method described previously (Yamanaka et al., 1971) with a modification, 10% perchloric acid instead of trichloracetic acid was used for termination of the reaction. Enzyme activities of the latter two enzymes were expressed as µmoles of p-nitrophenol or urea formed/min/g tissue (wet weight) or mg DNA, respectively.

Other analytical methods
Nucleic acids were extracted by the method of Hutchinson et al., (1952). DNA was determined by the diphenylamine reaction and RNA by the orcinol reaction (Schneider 1960). Citrate was assayed by the method of Speck et al., (1946). Fructose determination was performed by the method described by Lindner and Mann (1960). Zinc was assayed according to Kahnke (1966) with atomic absorption spectrophotometer (Hitachi Model 518, Japan) after treatment of tissue with hot perchloric acid.

Results

Effect of estrogen administration
Animals were divided into three groups. To the first group 0.2 ml of sesame oil was injected daily. To the other two groups daily dose of 5 µg and 500 µg of estradiol benzoate were injected respectively for 7 days. Rats were sacrificed 24 hrs after the final injection.

Changes in the body weight during the experimental period were compared in three experimental groups (Table 1). No significant changes were observed in sesame oil injected animals, and a slight loss of the weight during the experiment for 7 days was noticed in animals given 5 µg of estradiol benzoate. However, a significant loss of the weight during the experimental period was observed in animals given 500 µg of the steroid.

The wet weight of both the ventral prostate and the dorsolateral prostate in the estrogen treated groups decreased statistically significantly during the treatment period. Testicular weight was also reduced in the estrogen treated groups, and on the contrary, slightly enlarged adrenals were noticed in these groups. Moreover, these changes in organ weight were slightly greater in rats given 500 µg of estradiol benzoate than in those treated with 5 µg of the same steroid.

DNA content in the ventral prostate was slightly reduced after estrogen treatment, while DNA in the dorsolateral prostate in these groups was not significantly altered (Table 2). Contents of RNA in the ventral prostate were significantly reduced after estrogen treatment. Reduction in RNA content was also observed in the dorsolateral
Table 1. Body weight, and weights of ventral and dorsolateral prostates, adrenal and testis after treatment with estradiol benzoate (EB).

<table>
<thead>
<tr>
<th>Daily dose of EB</th>
<th>Body weight</th>
<th>Organ weight(a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>µg</td>
<td>start of treatment</td>
<td>ventral prostate</td>
</tr>
<tr>
<td></td>
<td>g</td>
<td>g</td>
</tr>
<tr>
<td>0(b) (15)</td>
<td>482.0±6.7</td>
<td>481.0±6.7</td>
</tr>
<tr>
<td>5 (25)</td>
<td>478.2±7.0</td>
<td>465.6±6.9</td>
</tr>
<tr>
<td>500 (25)</td>
<td>484.6±6.8</td>
<td>425.9±5.9(c)</td>
</tr>
</tbody>
</table>

Data are shown as M±S.E.

Injections were performed daily for 7 days. 24 hours after the last injection, animals were sacrificed.

a) Injection with sesame oil.

b) Numbers in parentheses show the number of rats used.

c) Changes in the body weight during the experimental period were statistically different from those of rats received sesame oil (p<0.001).

d) Organ weight is expressed as mg/100g body weight.

Differences between sesame oil injected and estrogen injected animals were statistically significant (*; p<0.05, **; p<0.01, ***; p<0.001).

The degree of the RNA reduction during the treatment period appeared to be greater than that of the reduction of the organ weight.

The content of citrate in the ventral prostate was significantly reduced after estrogen treatment. The similar tendency in the reduction of citrate content was also observed in the dorsolateral prostate. Contents of fructose and zinc in the dorsolateral prostate were also reduced after injection with either dose of estradiol benzoate.

The activity of the testosterone 5α-reductase in the ventral prostate from estrogen treated groups was not markedly changed when compared with that from sesame oil injected animals (Table 3). In dorsolateral prostates, the activity of the testosterone 5α-reductase seemed to be reduced by estrogen administration.

The activity of the alkaline phosphatase in the ventral prostate from animals given 500 µg of estradiol benzoate was significantly increased when compared with the activity on the wet weight basis. However, when the enzyme activity was compared with that on per mg DNA basis, no significant difference in those experimental groups was observed. Therefore, the increase in the
Table 3. The activity of testosterone 5α-reductase, alkaline phosphatase and arginase in the ventral and dorsolateral prostates of estradiol benzoate (ES) treated rats.

<table>
<thead>
<tr>
<th>Daily dose of EB</th>
<th>Testosterone 5α-reductase</th>
<th>Alkaline phosphatase</th>
<th>Arginase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mgmol. /hr. /g.</td>
<td>mgmol./hr. /mg DNA</td>
<td>µmol./min /mg g</td>
</tr>
<tr>
<td>Ventral prostate</td>
<td>5.92±1.01</td>
<td>4.43±0.87</td>
<td>11.26±0.79</td>
</tr>
<tr>
<td>5 (25)</td>
<td>4.84±1.77</td>
<td>2.49±0.86</td>
<td>12.21±1.01</td>
</tr>
<tr>
<td>500 (25)</td>
<td>4.45±1.02</td>
<td>2.41±0.77</td>
<td>18.13±3.06*</td>
</tr>
<tr>
<td>Dorso-lateral prostate</td>
<td>8.54±1.97</td>
<td>7.22±0.61</td>
<td>3.63±0.36</td>
</tr>
<tr>
<td>5 (25)</td>
<td>6.08±2.10</td>
<td>3.31±1.36</td>
<td>3.45±0.68</td>
</tr>
<tr>
<td>500 (25)</td>
<td>6.08±1.92</td>
<td>3.83±1.61</td>
<td>3.49±0.83</td>
</tr>
</tbody>
</table>

Data are shown as M±S.E.

Animals used in this experiment were the same as those in the experiment of Table 1.

Effect of castration

On the 5th day after castration, weight of the ventral prostate was 59.6±9.5 mg/100 g body weight (p<0.001 from intact rats) and weight of the dorsolateral prostate was 42.8±5.1 mg/100 g body weight (p<0.001 from intact rats). These shrinkages were accompanied by a significant decrease in the contents of RNA, citrate and fructose. The activity of alkaline phosphatase and arginase in these prostates were compared with those from intact animals (Table 4). The activity of alkaline phosphatase in the ventral prostate from castrated animals was slightly reduced than that from intact rats, and the activity of this enzyme in the dorsolateral prostate was not changed in all the three groups.

Table 4. The activity of alkaline phosphatase and arginase in the ventral and dorsolateral prostates of intact and castrated rats.

<table>
<thead>
<tr>
<th></th>
<th>Alkaline phosphatase</th>
<th>Arginase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µmol./min /g</td>
<td>µmol./min /g</td>
</tr>
<tr>
<td>Ventrall prostate</td>
<td>14.16±0.09</td>
<td>2.80±0.27</td>
</tr>
<tr>
<td>intact (5)b)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>prostate castrated</td>
<td>11.16±0.71</td>
<td>1.48±0.12*</td>
</tr>
<tr>
<td></td>
<td>4.48±0.04</td>
<td>1.96±0.15</td>
</tr>
<tr>
<td>Dorsolateral prostate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>intact (5)</td>
<td>5.04±1.34</td>
<td>2.29±0.08</td>
</tr>
<tr>
<td>prostate castrated</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are shown as M±S.E.

a) Rats were sacrificed on 5th day after castration.

Weights of the ventral and dorsolateral prostates were 59.6±9.5 mg/100 g body weight and 42.8±5.1, respectively.

b) Numbers in parentheses show the number of rats used.

*; p<0.05 from intact animals.
lateral prostate was not changed by castration. The arginase activity in the ventral prostate was significantly decreased after castration, while the arginase activity in the dorsolateral prostate was not changed after castration.

Discussion

In the present experiment, it was observed that estrogen treatment caused weight decrease in the ventral and dorsolateral prostates, accompanying the loss of fructose, citrate and zinc in the organs. These changes are almost similar to those observed after castration of animals (Mann, 1964).

According to Fencl and Villee (1973), the amount of testosterone metabolized in vitro by the ventral prostate of rats decreased after castration, while estrogen treatment did not affect the rate of testosterone metabolism. The similar result also appeared in the report by Moore and Wilson (1973), and this was further reconfirmed by the present experiment. In the dorsolateral prostate, the activity of the testosterone 5α-reductase was found to be decreased slightly after injection of estradiol benzoate to animals. The similar response of testosterone 5α-reductase in the dorsolateral prostate of rats after injection of estracyte was also reported by Kirdani et al. (1974).

It was reported by Anderson and Müntzing (1971) that activity of alkaline phosphatase in the ventral prostate of rats showed an elevation after estrogen treatment and a decrease after castration when compared with that of intact animals. The similar observations were also made in the present experiment, however, no changes in the activity of alkaline phosphatase after estrogen treatment were detected in the dorsolateral lobes of prostate.

From the present experiment, it could be concluded that castration and estrogen treatment induced the similar organ weight loss in the ventral and the dorsolateral prostates, but the effect of castration and estrogen treatment on activities of some enzymes in the tissues seems to be remarkably different. Furthermore, estrogen administration seems to induce different alterations in enzyme activities of different lobes of the prostates of rats. Different responses of the ventral and the dorsolateral prostates of rats to sex hormones (Tisell, 1971), HCG (Rosoff and Martin, 1968) and prolactin (Moger and Geshwind, 1972) have been reported.

Estrogen administration to male rats resulted in a lowering of the plasma androgen concentration (Danuta et al., 1973). This effect has been considered to mainly due to inhibition of the pituitary gonadotropic secretion (Perklev and Gröning, 1969), to the change in the steroid metabolic pattern of the liver (Schriefers, 1967) and to the inhibitory effect on the testicular androgen biosynthesis (Samuels et al., 1964; Oshima et al., 1967). However, decrease in the level of circulating androgen evoked by estrogen administration cannot explain the different effect of estrogen and castration on the prostates. Therefore it might be plausible to consider that the involution of the prostates after estrogen treatment is not only a sequence of reducing the circulating androgen but might be also results of some direct effect of estrogen on the prostatic tissue itself. Increased release of prolactin also may possibly be a part of the effects of estrogen administration (Salloch et al., 1971).

Acknowledgement

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References

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