Influence of Sex Hormones on Prostatic Secretion Protein, a Major Protein in Rat Prostate

Åke Pousette, Per Björk, Kjell Carlström, Björn Forsgren, Bertil Högborg, and Jan-Åke Gustafsson

ABSTRACT

Prostatic secretion protein (PSP) or estramustine-binding protein is a major protein in rat ventral prostate. The amount of PSP was measured per mg of cytosolic protein at different ages and after castration or administration of sex hormones. The amount of PSP is relatively low before puberty (25 µg/mg of protein) but increases at about 28 days of age to about 670 µg/mg of protein and then decreases to a constant level of about 300 to 400 µg/mg of protein, which is stable until at least 9 months of age. Following castration, the amount of PSP decreased relatively slowly, but 6 days after castration less than 20% of the original amount of PSP was detected. Treatment with testosterone propionate (1 mg/day) for 2 weeks (starting 2 weeks after castration) restored precastration levels of PSP.

It is concluded that PSP is an androgen-sensitive protein, and it is suggested that PSP should be considered as a probe for estimation of androgenic action on the prostate. PSP is similar to the so-called prostatic binding protein as well as to prostatein, and it is quite possible that the three proteins represent one and the same entity.

INTRODUCTION

PSP or estramustine [1,3,5(10)-estratriene-3,17β-diol,3-N-bis(2-chloroethyl)carbamate (LEO 275)] binding protein was originally discovered when searching for the mechanism of action of estramustine phosphate, a drug used in the treatment of advanced prostatic carcinoma (6). Distribution studies on male rats using radioactive estramustine have shown that this compound is taken up and retained by the ventral prostate lobe (1, 4). Estramustine was found to bind to a specific protein in rat ventral prostatic cytosol with a K_d of 2 x 10^-8 M (3, 4). This protein was purified to homogeneity, and antibodies were raised against it (2). A radioimmunoassay was developed for quantitation of the protein, and using this technique it was found that PSP was present in very high amounts in the ventral prostate as well as in the prostatic fluid. The protein was also present in other male accessory sexual glands, although in lower amounts (2). Recent studies have shown that an estramustine-binding protein similar to PSP is also present in prostate from other species, including humans (10). The biological action of PSP is not yet elucidated, but the high prostatic content of this secretory protein may indicate that it has an important function in the regulation of male fertility.

RESULTS

Amount of PSP in Rat Ventral Prostatic Cytosol at Different Ages and after Castration

In an effort to help to understand the biological role of PSP, the present study was carried out to investigate the age dependence of PSP as well as its gonadal regulation.

MATERIALS AND METHODS

Male Sprague-Dawley rats were used in all experiments. Testesctomy was carried out under ether anesthesia using the scrotal route. Hormone administration was performed by daily i.m. injections of 1 mg of testosterone (17β-hydroxy-4-androst-ene-3-one) propionate or progesterone (Sigma Chemical Co., St. Louis, Mo.) dissolved in 0.25 ml of propanediol or by s.c. injections of 2 mg of estradiol valerate (Schering AG, Berlin, Germany) once a week (7).

Preparation of Cytosol. After decapitation of the rat, the ventral prostate was immediately removed and homogenized in 3 volumes of ice-cold Buffer A [Tris-HCl, 0.05 M; EDTA, 1 mM; dithiothreitol, 1 mM; NaCl, 0.005 M (pH 7.4)] using an Ultra-Turrax homogenizer. The homogenate was centrifuged for 60 min at 105,000 x g. After removal of the floating lipid layer, the high-speed supernatant was decanted and stored at -70°C until analyzed.

Determination of protein was made according to the method of Lowry et al. (9) using bovine serum albumin as a standard.

Radioimmunoassay. The radioimmunoassay was performed as described earlier (2). The frozen cytosol was thawed on ice. DASP's (Organon N.V., Oss, Holland) were used at a concentration of 1 ampul/100 ml of buffer for the separation of free and bound antigen. The antisera was diluted 1:10,000. The standard curve ranged from 5 to 1000 ng of pure estramustine-binding protein per ml of buffer. 125I-labeled protein was used at a concentration of approximately 100 ng/ml.

The total incubation mixture consisted of 100 µl 125I-labeled protein, 100 µl of standard or appropriately diluted sample, and 100 µl of antisera. After incubation at room temperature for 2 hr, 1 ml of DASP suspension was added to each tube, and the stopped tubes were rotated slowly at room temperature for 16 to 20 hr. After centrifugation at 1000 x g for 3 min, the supernatant was aspirated, and the DASP containing the bound radioactivity were washed 3 times with 0.9% NaCl solution. The bound radioactivity was measured in a well-type γ counter.

Assay of serial dilutions of rat ventral prostatic cytosol gave a dose-response curve identical to the standard curve for the prostatic secretion protein.

Statistics. One-way analysis of variance was used. Means were compared by studentized range test of means. Confidence interval was set to 0.95.

RESULTS

Amount of PSP in Rat Ventral Prostatic Cytosol at Different Ages and after Castration

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Ages. The amount of PSP was first quantitated at different ages. Five pools of cytosol were prepared for each age group. In younger rats, several prostates were used to give sufficient material for one pool, but in adult rats only one prostate was used for each pool.

The amount of PSP is strongly age dependent (Table 1). In 2- and 3-week-old rats, a relatively low amount of PSP is present, both when calculated as absolute amount and when calculated as amount per mg of cytosolic protein. In 28-day-old rats, the specific content of PSP has increased about 20-fold, and at this age PSP constitutes about 60 to 70% of the cytosolic protein. With increasing age, the total amount of PSP increases, but the specific content of the protein is relatively constant (300 to 400 μg/mg protein, corresponding to about 30 to 40% of the total amount of cytosolic protein) at least up to 9 months of age.

During this study, it was found that the amount of PSP in 8- to 10-week-old rats varied between 18 to 38% of total cytosolic protein when using rats of different strains and from different breeders. The weight of the ventral prostate varied from 0.13 to 0.5 g. However, the variation of these parameters, when comparing identically treated animals in the same experiment, was acceptable.

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>wt of ventral prostate (g)</th>
<th>Absolute amount of PSP (mg)/ventral prostate</th>
<th>μg PSP/mg of cytosolic protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>0.013 ± 0.004</td>
<td>0.0019 ± 0.0011</td>
<td>21.7 ± 4.5</td>
</tr>
<tr>
<td>20</td>
<td>0.027 ± 0.004</td>
<td>0.01 ± 0.003</td>
<td>30.8 ± 4.8</td>
</tr>
<tr>
<td>28</td>
<td>0.07 ± 0.008</td>
<td>0.21 ± 0.04</td>
<td>668 ± 156</td>
</tr>
<tr>
<td>35</td>
<td>0.18 ± 0.02</td>
<td>0.96 ± 0.11</td>
<td>387 ± 57</td>
</tr>
<tr>
<td>56</td>
<td>0.49 ± 0.06</td>
<td>7.99 ± 2.20</td>
<td>390 ± 53</td>
</tr>
<tr>
<td>90</td>
<td>0.82 ± 0.07</td>
<td>16.5 ± 2.34</td>
<td>413 ± 70</td>
</tr>
<tr>
<td>180</td>
<td>1.19 ± 0.29</td>
<td>30.8 ± 11.5</td>
<td>319 ± 56</td>
</tr>
<tr>
<td>270</td>
<td>1.30 ± 0.18</td>
<td>24.1 ± 6.03</td>
<td>269 ± 66</td>
</tr>
</tbody>
</table>

* Mean ± S.D.

Table 2

<table>
<thead>
<tr>
<th>Time after castration/</th>
<th>n</th>
<th>wt of ventral prostate (g)</th>
<th>Absolute amount of PSP (mg)/ventral prostate</th>
<th>μg PSP/mg of cytosolic protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 day</td>
<td>5</td>
<td>0.34 ± 0.05</td>
<td>3.61 ± 1.57</td>
<td>191 ± 22</td>
</tr>
<tr>
<td>2 days</td>
<td>5</td>
<td>0.39 ± 0.08</td>
<td>5.13 ± 1.54</td>
<td>278 ± 61</td>
</tr>
<tr>
<td>6 days</td>
<td>5</td>
<td>0.07 ± 0.01</td>
<td>0.021 ± 0.006</td>
<td>36 ± 19</td>
</tr>
<tr>
<td>15 days</td>
<td>5</td>
<td>0.03 ± 0.01</td>
<td>0.003 ± 0.001</td>
<td>34 ± 8</td>
</tr>
<tr>
<td>20 days</td>
<td>5</td>
<td>0.04 ± 0.02</td>
<td>0.003 ± 0.001</td>
<td>27 ± 16</td>
</tr>
</tbody>
</table>

* Mean ± S.D.

Effects of Castration and Androgen Treatment on the Amount of PSP. Rats were killed at 56 days of age following varying periods of castration (0, 2, 6, 15, and 20 days) and were analyzed for amount of PSP in the ventral prostate (Table 2). The absolute amount as well as the specific content of PSP had reached a low base-line level 6 days following castration, and this level was stable at least up to 20 days after castration. When 56-day-old rats (castrated 14 days earlier) were given androgen (see "Materials and Methods"), normal levels of PSP were restored after 2 weeks of treatment (Table 2). The effects of castration and androgen substitution on the specific content of PSP in ventral prostatic cytosol indicate that PSP is more sensitive to androgenic stimulation than the average ventral prostatic cytosolic protein.

Effects of Estrogen and Progesterone on the Amount of PSP. Treatment was started on 42-day-old rats, and the animals were killed at 56 days of age (Table 3). The effect of...
castration was mimicked by treatment of normal rats with estrogen for 2 weeks, which decreased the weight of the prostate as well as the total and specific contents of PSP in the ventral prostate. It is not known if the effect seen represented a direct effect of estrogen on the prostate or if it was mediated via the testes or the central nervous system. Treatment with progesterone tended to decrease the weight of the prostate as well as the total amount of PSP somewhat but had no effect on the specific content of PSP. When castrated rats were given both androgen and estrogen, the effects on the prostate and PSP were the same as when only androgen was given, showing that the effect of estrogen was completely counteracted by androgen.

DISCUSSION

The results presented in this investigation strongly support the contention that PSP is an androgen-sensitive protein. PSP decreases following castration and increases after substitution with androgen, both in terms of total content in the ventral prostate and in terms of specific content expressed as µg of PSP per mg of total cytosolic protein in the gland. The pronounced increase in prostatic concentration of PSP seen in 28-day-old rats as compared to 20-day-old animals is probably a result of the increased testicular activity occurring at about 4 weeks of age.

PSP was originally discovered due to its capacity to bind and concentrate estramustine, which is the dephosphorylated metabolite of Estracyt, a drug used in the treatment of prostatic carcinoma. Recent studies in our laboratory have shown that a protein similar to PSP is present in the prostate gland from other species including humans (10). This opens up the possibility that a patient’s response to Estracyt treatment might be predicted by analyzing the tumor content of PSP. Furthermore, the androgen dependence of PSP suggests that Estracyt uptake in the tumor tissue could be facilitated by pretreatment with androgen. It should be borne in mind, however, that translational proteins in cancer tissue are not necessarily stimulated by transcriptional events that regulate tissue proteins in well-differentiated benign tissues. Another clinical implication of the androgen dependence of PSP is that treatment of castrated patients with Estracyt may represent a suboptimal use of the drug. Studies on the hormonal regulation of human PSP may be of guidance in the attempts to optimize Estracyt therapy.

In previous publications, we have pointed out the similarities among PSP, PBP (5), and prostatein (8). The 2 latter proteins also represent major prostatic proteins secreted from the cells into the lumina of the tubuli of the gland. The age and androgen dependence of PBP (5) is similar to what has been found in this study for PSP, lending further support to the contention that PBP and PSP represent one and the same protein.

REFERENCES

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