DIFFERING PATTERNS OF ACID PHOSPHATASE AND CATHEPSIN D ACTIVITIES IN THE RAT VENTRAL PROSTATE GLAND DURING CASTRATION-INDUCED PROSTATIC INVOLUTION

By
Heikki J. Helminen, Jan L. E. Ericsson and Bengt Arborgh

ABSTRACT
The activities of acid phosphatase and cathepsin D in the rat’s ventral prostate lobe were investigated under normal conditions and during castration-induced involution. The specific activities of the acid hydrolases were higher in the prostatic »tissue« fraction than in the »secretion« fraction of the gland, and the total activities of acid phosphatase and cathepsin D in the »secretion« were increased up to about 24% and 8%, respectively, of the activities in the whole lobe.
During involution, the specific activities of both hydrolases in the tissue showed a rapid increase, while total activity and activity »per cell« (on a DNA basis) of acid phosphatase declined, while the total activity of cathepsin D remained essentially unaltered at first and ultimately decreased; the activity of cathepsin D »per cell« was first increased and later showed a slight decrease.
Cycloheximide administration retarded the gross atrophy and decrease in protein and DNA content, and was associated with a lowered total, specific and »per cell« activity of cathepsin D, and an »increased« total activity of acid phosphatase. The observations are compatible with an increased de novo synthesis of cathepsin D and a decreased synthesis of acid phosphatase during prostatic involution.
The findings were interpreted as indicating that in the ventral prostate lobe of the rat, acid phosphatase is in part a secretory (and hormone-sensitive) enzyme, and in part a »conventional« lysosomal enzyme, while cathepsin D is predominantly an enzyme which is bound to conventional lysosomes.
Lysosomes are known to be actively involved in the atrophy of many tissues (Weber 1969; Weinstock & Iodice 1969; Woessner 1969). During mammary gland involution, the lysosomes undergo profound structural changes (Helminen & Ericsson 1968a,b,c) which appear to be reflected in changes in biochemically measurable activities of the lysosomal enzymes (Helminen & Ericsson 1970a).

Castration-induced prostatic involution is a somewhat neglected «model» for the study on the mechanisms of tissue involution (Brandes 1966; Harkin 1963). In comparison with mammary gland involution, the prostate model offers two points of special interest. Firstly, the secretory fluid of the prostate is known to contain at least one lysosomal enzyme, i.e. acid phosphatase, which has been shown to be derived from the epithelial cells of the prostate (Mann 1963; Price & Williams-Ashman 1961; Rosenkrantz 1969; Helminen & Ericsson 1970b); secondly, castration gives rise to a relatively delayed involution of the prostate gland, without drastic morphological transformation of the cells on account of stagnation of the secretory fluid. Thus, the involution can be considered as being solely due to deprivation of the steroid hormone action on the prostate, so that the subsequent cellular and lysosomal alterations are unrelated to secondary effects such as distension of the alveoli – as appears to be the case in the mammary gland during involution (Helminen & Ericsson, in press).

The fine structural alterations in the rat prostatic epithelium during involution have recently been studied in some detail (Helminen & Ericsson, in press). It was shown that the fine structural changes in the epithelium begin to occur on days 2–3 of involution and are characterized by the formation of extensive whorls of endoplasmic reticulum, rapidly followed by the appearance of large autophagic vacuoles in the supranuclear area of the epithelial cells (Helminen & Ericsson, in press). Some epithelial cells become necrotic, and probably on account of this, increased numbers of macrophages are observed in the epithelium (Helminen & Ericsson, to be published). Thus, the combined action of cellular autophagy, necrosis of the epithelial cells, and heterophagy activity in the macrophages appears to be responsible for the prostatic atrophy. However, up to date, there is little information about the quantitative changes in the lysosomal enzymes which occur in the involuting prostate gland (Stafford et al. 1949; Lasnitzki et al. 1966).

In the present investigation the activities of two typical «marker enzymes» for lysosomes – acid phosphatase and cathepsin D – in the rat ventral prostate before and during involution have been measured. It was anticipated that such quantitative studies would yield new information on the role of lysosomal enzymes in hormone-governed atrophy of the epithelial organ – especially when considered in relation to the previously known fine structural and histochemical data. Acid phosphatase was chosen since its role as a «secretory enzyme» in the prostate has been well documented (Helminen & Ericsson
1970b; Rosenkrantz 1969), and cathepsin D because it is generally known that this enzyme is involved in tissue degradation (Weber 1969; Weinstock & Iodice 1969). The effect of cycloheximide, an inhibitor of protein synthesis at the cytoplasmic (ribosomal) level, on the regression of the gland and the activities of the two lysosomal enzymes has also been studied.

MATERIALS AND METHODS

Animals

100 adult male rats (age 3–6 months) of the Sprague-Dawley strain were used in the experiments. The weights of the animals ranged from 324 g to 445 g. The rats received standard »Purina« pellets and water *ad libitum*. Prior to sacrifice the rats were anaesthetized with ether, the abdomen was opened with a pair of scissors, and the blood was drained off by opening the heart. The connective/fat tissue pads surrounding the prostates were peeled off and the ventral prostate lobes were exposed. The prostates were carefully dissected free, taking care not to sever the lateral lobes.

Preparation of »whole lobe«, »tissue« and »secretion« fractions

The lobes were weighed on a precision balance and subsequently thoroughly homogenized in 6.0 ml of ice-cold 0.15 M KCl in a Potter-Elvehjem type of teflon homogenizer operated at 1000 r.p.m. The homogenates were designated as »whole lobe« fractions. Ten glands were treated in this way. In order to obtain representative samples of the secretory fluid of the prostate (Niemi 1966; Niemi 1967, personal communication), intact lobes were placed on tiny grids of stainless steel (bended to fit in test tubes). The lobes were then cut into thin slices with a pair of sharp scissors, and the grids were pushed down into the tubes. The bottom of the test tube was conical in shape; accordingly, there was an empty space between the net and the bottom of the tube. The tubes containing the tissues were centrifuged at 400 X g for 10 min, after which the nets were picked up and the yellowish fluid at the bottom of the tubes was re-centrifuged at 800 X g for 10 min. The fluid was collected and the volume of the cell-free supernatant, designated as »secretion«, was measured with micropipettes. The »tissue« fraction that was collected on the grids was homogenized in ice-cold 0.15 M KCl as described above. All the procedures mentioned above were performed in a cold room (about 4°C). The »whole lobe«, »tissue«, and »secretion« fractions (ten specimens of each) were frozen to −20°C and stored in the refrigerator until biochemical determinations for protein, DNA, acid phosphatase, and cathepsin D were carried out. Freezing and thawing did not diminish the enzyme activities; neither did the addition of Triton X-100 (final concentration 0.5%v) increase the activities of the acid hydrolases.

Castrated animals

Castrations was performed under light ether anaesthesia. The prostates were collected 2, 3, 5, 7 and 10 days after the operation. The number of experimental animals was adjusted to 10 at each interval, except for the 2 and 10 day intervals, where the number of rats used was 5. The untreated animals served as a control group. In order to remove the secretory fluid of the prostates, all the lobes were centrifuged and homogenized before biochemical measurements were performed according to the procedure outlined above.

749
Administration of cycloheximide

The effect of cycloheximide, an inhibitor of protein synthesis, on the prostatic involution was investigated. Cycloheximide (obtained from Sigma Chemical Co., St. Louis, Mo., USA) was dissolved in saline and administered intramuscularly once a day. The dose given was 0.10 mg per 100 g of body weight. In general the rats tolerated this relatively high dose of cycloheximide quite well, though it caused some loss of body weight (Fig. 1). Acute toxicity did occur, however, and some of the animals died. The administration of cycloheximide was initiated simultaneously with the castration. The control groups (see above) received a daily injection of saline. Tissues to be used for biochemical assays were collected on days 3, 5 and 7 after castration (10 animals in each group). The collection of prostates was carried out as for the corresponding control groups.

Biochemical assays

Protein was measured according to the method of Lowry et al. (1951) and DNA according to the method of Burton (1956). Acid phosphatase and cathepsin D were assayed by the methods of Berthet & de Duve (1951) and Anson (1938), respectively, as modified by Bowers et al. (1967). A detailed description of the assay conditions has been published previously (Helminen & Ericsson 1970a). The activity of acid phosphatase in the tissue was also determined using p-nitrophenyl phosphate (obtained from Sigma Chem. Co., USA) as the substrate (Vanha-Perttula 1971). The final concentration of the substrate in the incubation medium was 3 mM. The results are expressed as follows: Specific activities in nmoles (eqs.) P(i) (»tyrosine«)/min/mg protein; total activities in nmoles (eqs.) P(i) (»tyrosine«)/min/whole gland and per 100 g of body weight; activities calculated »per cell« in nmoles P(i) (»tyrosine«)/min/µg DNA. The body weights used for the calculations were those recorded immediately before castration (and not those recorded after cycloheximide/saline administration). The significance of the differences measured between the separate experimental groups was tested by Student’s t-test.

Table 1.

Data on the rat ventral prostate gland and the protein content of various fractions.

<table>
<thead>
<tr>
<th>Weight of the lobe</th>
<th>»Whole lobe« protein content</th>
<th>»Tissue« protein content</th>
<th>Volume of the »secretion«</th>
<th>Protein conc. of »secretion«</th>
</tr>
</thead>
<tbody>
<tr>
<td>193 ± 8 mg per 100 g</td>
<td>29.5 ± 1.9 mg per 100 g</td>
<td>20.6 ± 1.3 mg per 100 g</td>
<td>72 ± 6 µl per 100 g</td>
<td>136 ± 5 mg/ml per 100 g</td>
</tr>
<tr>
<td>(b. w.) (10)</td>
<td>(b. w.) (10)</td>
<td>(b. w.) (10)</td>
<td>(b. w.) (10)</td>
<td></td>
</tr>
</tbody>
</table>

Values are given as means ± sem. Figures in brackets refer to the number of experiments.

750
Table 2.
Activities of acid hydrolases in the rat ventral prostate gland.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Acid phosphatase</th>
<th>Cathepsin D</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Specific activity</td>
<td>Total</td>
</tr>
<tr>
<td>A. »Whole lobe«</td>
<td>6.7 ± 0.5 (10)</td>
<td>197.0 ± 15.0 (10)</td>
</tr>
<tr>
<td>B. »Tissue«</td>
<td>7.3 ± 0.4 (10)</td>
<td>150.0 ± 12.0 (10)</td>
</tr>
<tr>
<td>C. »Secretion«</td>
<td>1.5 ± 0.5 (10)</td>
<td>15.0 ± 2.0 (10)</td>
</tr>
</tbody>
</table>

Results are expressed as described in the text; means ± sem. Figures in brackets refer to the number of experiments.

RESULTS

Normal ventral prostate

The results of the assays performed on the normal prostate glands and the fractions are shown in Tables 1 and 2. As can be calculated from the figures presented, the centrifugation procedure – which was undertaken with the intention of acquiring representative samples of prostatic secretion – diminished the protein content of the prostate gland by 30–33 %. The volume of the fluid gathered from the »control tissues«, liver, spleen and thymus was negligible as compared with the volume of the fluid collected from the prostates. The »whole lobe« and »tissue« fractions exhibited considerably more enzyme activity than the »secretion« fraction (Table 2). Specific activities of the enzymes were highest in the »tissue« fraction, though they were almost equally high in »whole lobe« fractions, and low in the »secretion« fraction, especially the activity of cathepsin D. The »secretion« fraction contained between 8 and 24 % of the activity of acid phosphatase in the »whole lobe« fraction; corresponding values for cathepsin D activity were 5 and 8 %.

* The higher values for each enzyme (24 % and 8 %) refer to calculations based on the subtraction of the activity in the »whole gland« from that in the »tissue« fraction, the lower values (8 % and 5 %) refer to those obtained directly from the measurements on the »secretion« fraction.
Effect of castration and cycloheximide administration on the body weights of the rats. The weights are correlated to the initial weights before castration. • — •, «normal» castrated rats; × — × — ×, cycloheximide-treated castrated rats. Note: Each dot in the illustrations represents the mean of 10 independent determinations (except for 2 and 10 day intervals where only 5 estimations were performed). The graphic illustrations were obtained by drawing horizontal lines through the means. The vertical bars illustrate standard errors of the mean. The weights of the prostates, contents of protein, DNA, total activities of acid phosphatase and cathepsin D, are expressed per 100 g of body weight. The body weights measured immediately before castration were used in the calculations (and not the weights recorded during the experiments). Figures in the graphic illustrations refer to the level of statistical significance of the differences observed between the experimental groups at 3, 5 and 7 day intervals (P < 0.05, almost significant; P < 0.02; significant; P < 0.001, highly significant).

Ventral prostate after castration

The castration procedure itself did not have any noticeable effect on the weights of the rats, except that the operation caused a transient delay in the increase of the weights of the animals whereas the cycloheximide-treated animals suffered a considerable weight loss (Fig. 1). The weights of the ventral prostates, and the contents of protein and DNA in the tissues, decreased in a progressive fashion after castration (Fig. 2); however, in the cycloheximide-treated animals, the reduction was markedly delayed.

Fig. 2.
The effect of castration and cycloheximide treatment on the weight of the ventral prostate (Fig. 2 a), protein content (Fig. 2 b) and content of DNA in the prostatic «tissue» (Fig. 2 c). For explanations see legend for Fig. 1.

752
The total activity of acid phosphatase (»tissue activity«) is illustrated in Fig. 3 a. The activity of the enzyme declined rapidly after castration; thus, on day 5, only about 47 % of the activity was retained. In the cycloheximide-treated animals the decline appeared to be slower, at least at the early intervals (Fig. 3 a). This pattern was the same, irrespective of whether β-glycerophosphatase or p-nitrophenyl phosphatase served as the substrate for the enzyme. The specific activity of acid phosphatase increased in the prostate tissue during involution (indicating an augmented concentration) and a maximal concentration was reached on days 7–10 (Fig. 3 b). It appeared that this increase could be partially inhibited by the administration of cycloheximide (Fig. 3 b). When calculated on a »DNA basis« (»per cell basis«) the acid phosphatase activity showed a steady decreasing tendency throughout the experimental period; this trend was apparent both with and without cycloheximide (Fig. 3 b).

The activities of cathepsin D during involution are graphically illustrated in Fig. 4. The total activity ultimately showed a descending tendency (Fig. 4 a). However, the curve differed significantly from that for acid phosphatase (Fig. 3 a) in that instead of decreasing rapidly, cathepsin D remained high up to day 7 of involution. At this interval, there was still about 74 % of the initial activity present. The activity of cathepsin D in the cycloheximine-treated animals was significantly lower than the level of the corresponding activities in the castrated »control« animals. The specific activity of cathepsin D increased rapidly during involution; an increase was also found in the cycloheximide group. However, the slope of the curves was not so steep (Fig. 4 b). When the activity of cathepsin D was determined on a »per cell« basis, there was a significant rise up to day 5. This enhancement of the enzyme activity was largely inhibited by cycloheximide (Fig. 4 c). On day 10, the activity in the group not treated with cycloheximide returned to values close to those found before the induction of involution.

**DISCUSSION**

Present knowledge regarding the biology of the prostate, substantiates the view that the function of this accessory sex organ is under direct hormonal control (Mann 1963; Price & Williams-Ashman 1961), and that the testes are the principal source of the controlling hormone — testosterone (Grayhack 1963;
Tullner 1963; Li 1963). Although much work has been devoted to the task of unravelling the role of testosterone in the metabolism and physiology of the prostate, the primary site and mode of action of the hormone still remains to be defined (Baulieu 1970; Korner 1969; O'Malley 1971; Williams-Ashman & Reddi 1971).

It has been well documented that the production of the secretory fluid from the prostate ceases gradually after castration and that it can be restored by testosterone administration (Huggins et al. 1939; Rosenkrantz 1969). Simultaneously with the alterations in the levels of the hormone in the blood, the whole prostatic lobe and its epithelial cells undergo involution, while injections of testosterone restore the normal structure and function of the gland (Huggins et al. 1939; Rosenkrantz 1969).

The prostatic secretion is known to contain one lysosomal enzyme in relatively high concentration, viz. acid phosphatase (Rosenkrantz 1969) and probably several other »acid« hydrolases with low-activity (Price & Williams-Ashman 1961). The presence of acid phosphatase in the prostatic secretory fluid has been shown to be under the control of androgenic hormones (Gutman & Gutman 1939; Kent et al. 1969). The occurrence of small amounts of cathepsin D in the fluid from the ventral prostate believed to represent secretion has been reported (Helminen 1969); this observation was confirmed in the present study. Much higher activity of cathepsin D is obtained in the secretory fluid from the coagulating gland (Helminen 1969). As compared with cathepsin D, the activity of acid phosphatase in the »secretion« was considerably higher in the ventral lobe. It was recently demonstrated that the cellular mechanism of acid phosphatase release into the alveolar lumen in the ventral lobe was mediated through a merocrine type of secretion (Helminen & Ericsson 1970b).

The centrifugation procedure adopted in this study for the collection of secretory fluid is a comparatively crude method. However, it has two important advantages and proved to be useful for obtaining estimates of the enzyme activities in the actual tissue. First, the bulk of the fluid collected and designated as »secretion« must be derived from the true secretory fluid, since the centrifugation of liver, spleen or thymus slices did not yield any fluid at the bottom of the test tubes. Second, the DNA content of the fluid from the prostates was negligible, indicating that the collected fluid did not contain any whole epithelial cells, blood cells or nuclei.

Fig. 4.
The effect of castration and of cycloheximide administration on the content of cathepsin D activity (Fig. 4a) (per 100 g of body weight); specific activity of cathepsin D (Fig. 4c) and activity of cathepsin D calculated per »cell basis« (Fig. 4c). For explanation see legend for Fig. 1.

756
The centrifugation studies of the normal ventral prostate glands showed that the specific activities of the enzymes were lower in the prostatic secretion than in the prostatic tissue. A reasonable explanation for this may be that other proteins of the secretory fluid are expelled in greater proportion from the epithelial cells into the alveolar lumen than acid phosphatase and cathepsin D.

In principle, the results shown in Figs. 1 and 2 are in good agreement with the results published previously concerning the effects of castration on body weight, weight of the prostate and protein and DNA content of the prostate (Arvola 1961; Kochakian 1963). New information was obtained in the investigation on the in vivo effect of cycloheximide. Thus, the reduction in the absolute weights of the prostate lobes and protein and DNA contents of the prostates was not as marked in the »cycloheximide animals« as in the »normal«, castrated rats. The differences between the groups were found to be statistically significant. Cycloheximide therefore — whatever its mode of action (e.g. inhibition of protein (including enzyme) synthesis or reduction of the mobility of the macrophages) — was capable of inhibiting the progress of the atrophy of the prostate. With regard to the higher values for DNA in the cycloheximide group (Fig. 2 c), the findings can be interpreted as indicating that cycloheximide in part prevented the destruction of the epithelial cells of the prostate. The slow decline in DNA in the cycloheximide groups may well be explained by an inhibition of synthesis of the lytic enzymes both in epithelial cells and macrophages, or by the prevention of macrophages from reaching and digesting the necrotic epithelial cells.

Before discussing the enzyme changes it must be emphasized that the available information about tissue involution supports the view that lysosomal changes that take place in the cells during tissue atrophy are secondary in character. This view is based on the fact that fine structural and lysosomal changes begin to occur on day 2 of prostatic involution and later, while remarkable biochemical changes occur as early as 1–2 days after castration (Santti & Villee, in press; Williams-Ashman & Liao 1963). Thus, the deprivation of hormonal stimulus can be regarded as the primary, and changes in lysosomal function as the secondary, cause for the involution of the prostate.

Earlier studies, for instance of the mammary gland and the uterus (Helminen & Ericsson 1970a; Woessner 1969), have shown that the specific activities of the lysosomal enzymes increase during involution. This was also found to be the case in the prostate, as illustrated by the graphs in Figs. 3 b and 4 b. Such an increase does not necessarily signify augmented synthesis, but rather »conservation« and concentration of the lysosomal enzymes in the tissues resulting from degradation of other constituents of the cells.

Of more significance concerning the rate of lysosomal enzyme synthesis are the values obtained for the total activity and the activity »per cell« (calculated
on a DNA basis). Augmented de novo synthesis of enzyme proteins is either reflected as an increase in the total activity, or in the activity calculated on a »per cell« basis, or (as is usually the case) in both (depending on the extent and the time of occurrence of the necrosis). Cycloheximide can be expected to prevent such an increased synthesis, at least in part. Large scale invasion of the tissue by macrophages results in increased total and »per cell« activity and makes it difficult to decide whether changes in the enzyme production of pre-existing (epithelial) cells occur.

The results concerning acid phosphatase (Figs. 3a and c) indicate that there is a diminished synthesis of this enzyme in the epithelial cells during involution. Our morphological studies (Helminen & Ericsson, to be published) revealed that a moderate number of macrophages – known to be rich in acid hydrolases, including acid phosphatase – invade the prostatic tissue during involution. This suggests that if the enzyme activities in these enzyme-rich cells could be estimated and subtracted, the values calculated for the epithelial cells alone would show an even more significant decline than that presented in the diagrams. Apparently, because of the diminished synthesis of acid phosphatase, we were not able to record any effect with cycloheximide.

The situation is different concerning cathepsin D (Figs. 4a and c). Although the total activity does not increase (perhaps due to comparatively extensive cellular necrosis), there is a clear increase in enzyme activity as calculated on »per cell« basis. Cycloheximide treatment resulted in lower »total« and »per cell« activities than in the corresponding untreated animals. Since it appears unlikely that cycloheximide prevents the movement of macrophage-like cells into the tissue, the findings are compatible with an increased de novo synthesis of the enzyme during involution. The different patterns of enzyme activities obtained for acid phosphatase and cathepsin D are probably explained by the very low activity of the latter enzyme in the secretion. In accordance with the present findings, a clear increase in the activity of cathepsin D (signifying augmented synthesis) was observed in the mammary gland during involution (Helminen & Ericsson 1970a) and is probably triggered off by demands for high autophagic and heterophagic activity in involuting tissue.

Electron microscopic histochemical studies on the rat prostate indicate that a comparatively great proportion of newly synthesized acid phosphatase is channelled into secretory granules which release their contents into the alveolar lumens (Helminen & Ericsson 1970b). The remainder of the enzyme appears to be segregated into conventional lysosomes. Our present results seem to confirm this dual function of acid phosphatase as one »secretory« and one »regular« lysosomal enzyme. The secretory type of enzyme appears to be hormone (testosterone) sensitive. The immediate decline noted in total acid phosphatase is probably due mainly to cessation of the production of the secretory type of enzyme (morphological studies show diminished numbers of
acid phosphatase-containing granules during early involution) (Helminen & Ericsson, to be published). It is thus even possible that the production of acid phosphatase for conventional lysosomal purposes occurs at a pre-involutionary or even higher rate. Recent tissue fractionation and gel filtration studies by Vanha-Perttula et al. (to be published) on different fractions of the prostate substantiate the view mentioned above of one secretory, hormone-dependent acid phosphatase isoenzyme, and one »conventional« lysosomal acid phosphatase isoenzyme in the rat's ventral prostate. Cathepsin D, on the other hand, appears to be predominantly a »conventional« lysosomal enzyme. This interpretation would adequately explain the differences in patterns of total and »per cell« activities between acid phosphatase and cathepsin D. It could also explain the seemingly paradoxical effect of cycloheximide on total acid phosphatase activity (Fig. 3 a); cycloheximide appears to prevent or delay the collapse of the epithelial cells and prevents the progress of involution (cf. Fig. 2); hence there must be more acid phosphatase containing cells in this group as compared to the untreated group.

ACKNOWLEDGMENTS

Supported by grants from Nordisk Kulturfond and Duodecin Society. The skilled technical assistance of Mrs. Kerstin Wennberg and Mrs. Mervi Nurminen is gratefully acknowledged. The authors are indebted to Mr. Pertti Koskinen for statistical analysis of the results and to Professor Mikko Niemi and Associate Professor T. Vanha-Perttula for critically reading the manuscript.

REFERENCES

Received on May 26th, 1971.

761