Increased activity of plasminogen activators during involution of the rat ventral prostate

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Plasminogen activator activity was measured in the ventral prostates of non-castrated, castrated, and androgen-treated rats to determine whether changes in this activity correlated with the process of glandular involution. While the activity was very low in cytosolic extracts from the prostates of non-castrated rats, 2 days following castration the plasminogen activator activity increased in a near-linear fashion such that by day 7 it was 10-fold higher in terms of specific activity (per mg of protein) and cellular concentration (per mg of DNA). During this interval there was a rapid decrease in the cell population of the prostates. Treatment of the 7-day castrated rats with the potent androgen, dihydrotestosterone, both reduced the plasminogen activator activity and restored the cell number in a dose-related manner. Gel electrophoretic analysis revealed two major bands of plasminogen activator activity in the cytosolic extracts from 4- and 7-day castrated rats, plus additional minor bands in samples from 10- and 14-day castrated rats. Approx. 10% of the cellular concentration of plasminogen activator activity was recovered in association with an 18000g pellet fraction from the prostates; this fraction showed less heterogeneity of the plasminogen activator forms as observed by gel electrophoresis. Inhibitor studies indicated that the 18000g pellet fraction from the prostates of non-castrated rats possessed some plasminogen activator inhibitor activity, but the relative concentration of the inhibitor activity was small. We conclude that the involution of the prostate is probably associated with increased synthesis of plasminogen activators through a de-repression process which may involve loss of androgen receptors.

One unique property of many hormone-sensitive tissues, such as the prostate, is the capacity of the component cells to undergo a process of self-destruction, termed autophagia, when the concentration of the appropriate hormone falls below normal (Bruchovsky *et al.*, 1978). The autophagic mechanism in prostatic cells, acquired during pubertal development, is suppressed by circulating androgens (Brandes, 1966). Activation of this mechanism, following castration, leads to a reduction in the size of the prostate (i.e. organ involution) which is due largely to a proportional loss in cell number (Lesser & Bruchovsky, 1973).

Involutionary changes in the rat prostate are confined mainly to the epithelial cells and include the early appearance of large number of lysosomes and the formation of autophagic vacuoles (MacKenzie *et al.*, 1963; Brandes, 1974). In addition, there are major changes in biochemical parameters, including declines in the production of

prostatic secretions (Kirchheim & Scott, 1965; Paris & Brandes, 1974), protein synthesis (Butler & Schade, 1958; Brandes & Groth, 1963), DNA synthesis (Coffey *et al.*, 1968; Lesser & Bruchovsky, 1973), RNA content and complexity (Butler & Schade, 1958; Parker & Mainwaring, 1977), and the concentration of androgen receptors (Bruchovsky & Craven, 1975; Van Doorn *et al.*, 1976). Although there is a general reduction in anabolic processes during prostatic atrophy, the rate of cell loss after castration occurs much more rapidly than can be accounted for by normal cell turnover and suggests that involution of the prostate is an active process (Bruchovsky *et al.*, 1975).

Specific proteolytic enzymes which have been implicated in processes involving extensive tissue remodelling are those which catalyse the conversion of plasminogen to the active proteinase, plasmin (Beers *et al.*, 1975; Ossowski *et al.*, 1979;

Peltz *et al.*, 1983). Plasminogen activators have been measured in extracts from normal and neoplastic prostatic tissue of the rat (Gotterer *et al.*, 1956; Rennie *et al.*, 1980b; Strickland *et al.*, 1983) and the human (Nijs *et al.*, 1973; Camiolo *et al.*, 1981; Rennie *et al.*, 1982). Although plasminogen activator activity has not been studied extensively in normal prostate, two classes of plasminogen activator enzymes have been detected in prostatic tumours, a tissue type and a urokinase type (Strickland *et al.*, 1983). Furthermore, the concentration of plasminogen activators in prostatic tumours is higher in lysosomal fractions than in cytosol and is inversely correlated with the nuclear concentration of androgen receptors (Rennie *et al.*, 1980a,b, 1982).

The purpose of the present investigation was to determine whether alterations in plasminogen activator activity were related to the involution process in rat ventral prostate. It was found that while plasminogen activator activity was low in the prostates from non-castrated rats, within 2 days following castration the enzyme activity increased several-fold in the involuting prostates. Most of the plasminogen activator activity was recovered in the cytosolic fraction, which upon gel electrophoresis was found to contain at least two major forms of the enzyme. Treatment with the potent androgen, dihydrotestosterone, reversed the involution process and reduced the plasminogen activator activity in the prostates to levels near those in the samples from non-castrated animals.

Materials and methods

Experimental animals

Male Wistar rats (250–300 g), purchased from Charles River (St. Constant, Quebec, Canada), were castrated through a scrotal incision while under diethyl ether anaesthesia. Dihydrotestosterone (17 β -hydroxy-5 α -androstane-3-one) was injected subcutaneously at a dosage of either 40 or 400 μ g/100 g body wt. in a 0.6 ml solution of 10% (v/v) ethanol and 1% (v/v) polyoxyethylene sorbitan monopalmitate. After periods of treatment ranging from 1 to 7 days the rats were decapitated and the ventral prostates were removed.

Preparation of tissue extracts

All preparative procedures were carried out at 4°C. The ventral prostates from 4–16 rats (0.6–1.5 g) were minced with scissors, forced through a stainless steel screen with a Teflon pestle to remove stromal material (Wilkin *et al.*, 1980; Filipenko *et al.*, 1981), and homogenized in 20 ml of 10 mM-Tes (2-[[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]-amino]ethanesulphonic acid) / NaOH buffer

(pH 7.0)/1.5 mM-CaCl₂/0.5 mM-2-mercaptoethanol/0.25 M-sucrose (Tes/sucrose buffer) by using a Dounce apparatus with 25 strokes of a loose-fitting pestle and 15 strokes of a tight-fitting pestle. The homogenate was centrifuged at 800g for 15 min (Sorvall RC5 centrifuge, SS-34 rotor, r_{av} 10.7 cm) to yield a crude nuclear pellet and a supernatant fraction, which contained both intraluminal and intracellular material. The nuclear pellet was resuspended in 10 mM-Tes/NaOH buffer (pH 7.0) and aliquots were taken for DNA determinations and for counting in a haemocytometer following staining with aqueous 1% (w/v) Methylene Blue.

The 800g supernatant (cytoplasm) was centrifuged at 18000g for 20 min and the resulting supernatant (cytosolic extract) was set aside for enzyme and protein analysis. The pellet fraction (crude lysosomal pellet) was resuspended in 1 ml of Tes/sucrose buffer with or without 0.5% (v/v) Triton X-100, sonicated with three 7 s pulses at a scale setting of 40 (yellow code) with a Biosonik III apparatus (Bronwill Scientific, Rochester, NY, U.S.A.), and centrifuged at 6000g for 2 min in an Eppendorf microcentrifuge. The supernatant was assayed for protein and plasminogen activator. The inclusion of Triton X-100 in the extraction buffer did not alter the yield of plasminogen activator activity in the final supernatant and subsequently it was deleted from the procedure.

Proteinase and plasminogen activator assays

Proteolytic and plasminogen activator activities were measured as reported previously (Rennie *et al.*, 1980b, 1982) and were based on the fluorescamine assays originally described by Kessner & Troll (1976) and Katz *et al.* (1976) respectively. For proteolytic activity 20–50 μ l of the appropriate cellular extracts were adjusted to a final volume of 125 μ l with 50 mM-sodium veronal (diethylbarbiturate)/HCl buffer (pH 8.0)/100 mM-NaCl (veronal buffer) containing 50 mM-CaCl₂ and mixed with 125 μ l (5 mg) of succinylated protamine in the same buffer solution. After incubation at 37°C for 3 and 33 min, 50 μ l aliquots were diluted with 2 ml of 0.2 M-borate/NaOH buffer (pH 8.0) and then mixed with 100 μ g of fluorescamine dissolved in 50 μ l of acetone. The reaction between fluorescamine and the free amino groups generated by proteolytic cleavage, as well as the hydrolysis of excess fluorescamine, were complete within 1 min. The fluorescent products were stable for several hours. With an Aminco-Bowman spectrophotofluorimeter (American Instruments Co., Silver Spring, MD, U.S.A.) the relative fluorescence of each sample was measured at an excitation wavelength of 390 nm and an emission wavelength of 480 nm.

Plasminogen activators were measured in the above system with the following modification. The sample aliquots (20–50 μ l) were first mixed with 45 μ l of veronal buffer containing 0.05 unit (Sigma) of pig plasminogen and then made to 125 μ l with veronal buffer. After incubation for 1 h at 37°C, 125 μ l (5mg) of the succinylated protamine solution was added and the mixture was assayed for proteolytic activity as before. Each sample was assayed at four or more protein concentrations such that the reaction was linear with respect to the amount of proteinase or plasminogen activator activity present. The derivation of optimal assay conditions is described in detail elsewhere (Rennie *et al.*, 1980b). Control samples without either plasminogen or tissue extracts were assayed to correct for non-specific proteinase activity and for plasmin contamination of plasminogen respectively. The relative fluorescent units were expressed in amino equivalents using log-linear regression plots with 0–6.0 μ M-glycine as the reference standard in the fluorescamine assay (Rennie *et al.*, 1982). One Sigma unit of plasminogen (Sigma Chemical Co., St. Louis, MO, U.S.A.) is the amount of plasminogen which after activation with urokinase will produce a change of 1.0 A_{275} unit after incubation for 20 min at 37°C (pH 7.5), when acid-soluble products from α -casein are measured.

Electrophoretic analysis of plasminogen activator activity

Proteins having plasminogen activator activity were also detected by using slab gel electrophoresis techniques similar in format to those described by Granelli-Piperno & Reich (1978) and Danø *et al.* (1980). Samples (75–500 μ g of protein) were applied to polyacrylamide slab gels, composed of 7.5% (w/v) acrylamide/bisacrylamide (37.5:1)/0.375M-Tris/HCl (pH 8.8) in the separating gel and 3% (w/v) acrylamide/bisacrylamide (37.5:1)/0.125M-Tris/HCl (pH 6.8) in the stacking gel, and were electrophoresed at 4°C at a constant 240 V. After approx. 2.5 h, the gels were removed and then overlaid onto 1% (w/v) agarose substrate gels. The substrate gels were prepared from Bio-Rad proteinase substrate gel tablets (Bio-Rad Laboratories, Richmond, CA, U.S.A.) which contain bovine casein and to which pig plasminogen (0.05 unit/ml) was added. The gels were incubated in a moist chamber at 37°C for 3 h during which time plasminogen activators diffuse into the agarose gel and activate plasminogen to plasmin which in turn causes visible bands of lysis. Termination of the plasmin digestion and enhancement of the clear zones was accomplished by immersing the gels in methanol/acetic acid/water (3:1:6, by vol.).

Other procedures and chemicals

Protein determinations were done by the method of Bradford (1976) with the Bio-Rad assay kit using bovine α -globulin as the reference standard. DNA was measured by the diphenylamine method (Burton, 1956) with calf thymus DNA as the standard.

Dihydrotestosterone was purchased from Steraloids (Pawling, NY, U.S.A.). Fluorescamine (Fluoram) was supplied by Pierce Chemical Co. (Rockford, IL, U.S.A.) and all other reagents were obtained from Sigma. Aqueous solutions were prepared with deionized glass-distilled water.

Results

Plasminogen activator activity in cytosolic extracts from normal, involuting and regenerating prostates

With a fluorometric assay, plasminogen activator activity was determined in cytosolic extracts (18000g supernatants) prepared from the rat ventral prostates of non-castrated, castrated, and androgen-treated rats. While the mean concentration of plasminogen activator activity per mg of protein is relatively low in the cytosolic extracts from non-castrated rats [8.8 ± 2.5 (5) μ mol/30 min per mg of protein, mean $\pm$ S.E.M. (n)], after a 1–2 day latent period following castration, the enzyme activity rises in a near-linear fashion over a 10 day interval and then remains elevated (Fig. 1a). By day 7 after castration the plasminogen activator activity [88.3 ± 14.9 (3) μ mol/30 min per mg of protein] is almost 10-fold higher than that measured in the extracts from the non-castrated rats.

Treatment of the 7-day castrated rats with dihydrotestosterone alters the plasminogen activator activity in a dose-related manner (Fig. 1a). At the low dose (40 μ g/100g body wt. per day), enzyme activity is maintained at approximately the same level as in the cytosolic extracts from the 7-day castrated rats, whereas at the high dose (400 μ g) there is a reduction in plasminogen activator activity such that by day 7 of treatment, the activity [20.2 ± 5.4 (3) μ mol/30 min per mg of protein] is only about twice as high as in the non-castrated samples.

When the data are expressed on a per cell or per mg of DNA basis a pattern similar to the per mg of protein results is observed (Fig. 1b). This indicates that, in addition to castration-induced increases in plasminogen activator activity relative to other prostatic proteins, there is an increase in the per cell concentration of this enzyme activity within 2 days following castration. By comparison, the number of cells per prostate declines most rapidly between 2 and 7 days after castration (Fig. 1b).

Fig. 1. Plasminogen activator activity in cytosolic extracts

At daily intervals after castration, cytosolic extracts (18000g supernatant) were prepared from prostates obtained from groups of 4–16 rats and were assayed for plasminogen activator activity (●). The number of cells per prostate (Δ) was determined by counting the nuclei in the 800g pellet. Beginning 7 days after castration (as indicated by the arrow), dihydrotestosterone was administered subcutaneously in daily doses of either 400 μg (○) or 40 μg (□) per 100g body wt. The enzyme activity in cytosolic extracts from the prostates was determined at the times indicated. Plasminogen activator activity is expressed on the basis of per mg of protein (a) or per mg of DNA (b).

Fig. 2. Plasminogen activator activity in the 18000g pellet extracts

In the context of the experiments described in the legend to Fig. 1, extracts were prepared from the 18000g pellets and assayed for plasminogen activator activity on the basis of per mg of protein (a) or per mg of DNA (b). Plasminogen activator activity in 18000g pellets was measured in the prostates of untreated castrated rats (●) and 7-day castrated rats treated daily with dihydrotestosterone at doses of either 400 μg (○) or 40 μg (□) per 100g body wt. The arrow indicates the beginning of androgen treatment.

Treatment of 7-day castrated rats with a daily high dose of dihydrotestosterone for 7 days raises the number of cells per prostate to approx. 70% of the cell population determined in the prostates of non-castrated rats, while at the same time effectively reduces the cellular concentration of plasminogen activator activity to a level near that of the non-castrated rats.

Plasminogen activator activity in the 18000g pellet fraction

In specimens of rat and human tumours of the prostate the highest concentration of plasminogen

activator activity on a per mg of protein basis is recovered in extracts of a crude lysosomal preparation (Rennie *et al.*, 1980, 1982). To determine whether this is also true of the rat ventral prostate, the 18000g pellet fraction, which contains lysosomes, mitochondria and other particulate cellular components, was assayed for plasminogen activator activity. As shown in Fig. 2(a), sonicated extracts of the 18000g pellet possess plasminogen activator activity. In the prostate from non-castrated rats, the concentration of plasminogen activator activity in this fraction [11.8 ± 1.0 (5) μmol/30 min per mg of protein] is nearly equivalent

to that found in the cytosolic fraction. However, 4 days following castration the concentration of this enzyme activity in the 18000g pellet is approximately twice that measured in the comparable cytosolic extracts. With intervals of greater than 4 days after castration, the concentration of plasminogen activator activity in the pellet fraction declines. Administration of dihydrotestosterone to 7-day castrated rats results in only minor alterations in the plasminogen activator activity per mg of protein (Fig. 2a).

On a per mg of DNA basis (Fig. 2b) the plasminogen activator activity associated with the 18000g pellet is highest at 4 and 7 days following castration but declines thereafter. Relative to the cytosolic fraction, at each experimental point only about 10% or less of the total cellular plasminogen activator activity per mg of DNA resides in the 18000g pellet. Thus most of the plasminogen activator activity in rat prostate cells is recovered in the cytosolic extract; furthermore, the cytosolic pattern of increased plasminogen activator activity following castration is more strongly associated through an inverse relationship with the process of prostatic involution.

Non-specific proteolytic activity in prostatic extracts

In the context of the above experiments with plasminogen activator activity, non-specific proteolytic activity at pH 8.0 was also measured. In the cytosolic extract 1 day after castration, the proteolytic activity fell to approx. 35% of the concentration in non-castrated rats and remained at that level over the 14 day period (Fig. 3a). Treatment with dihydrotestosterone restored the activity in a dose-related manner (Fig. 3a). As shown in Fig. 3(b), the proteolytic activity in the extracts from the 18000g pellet fraction also declined following castration although in a more gradual fashion than that observed in the cytosolic extracts. Androgen treatments results in a transient increases in proteolytic activity (Fig. 3b). In all samples the proteolytic activity was always much lower than the comparable plasminogen activator activity (Figs. 1a and 2a).

Gel electrophoretic analysis of plasminogen activators

Proteins with plasminogen activator activity were also detected by using a gel electrophoretic technique in which polyacrylamide slab gels were layered over opaque agarose gels containing plasminogen and casein. During incubation of the gels, the plasminogen activators activate the plasminogen in the agarose gel to plasmin which then hydrolyses the casein to reveal clear bands of lysis. The results in Fig. 4 indicate that the cytosolic extracts from the prostates of 4-day and 7-day castrated rats have two major bands (A and B)

Fig. 3. Proteinase activity in prostatic extracts. The 18000g supernatant (a) and 18000g pellet (b) fractions from the prostates of groups of untreated castrated rats (●), and 7-day castrated rats treated with either 400 μg (○) or 40 μg (□) of dihydrotestosterone per 100g body wt., were assayed for proteinase activity. The beginning of androgen treatments is indicated by the arrow.

of plasminogen activator activity, whereas with longer intervals following castration at least three additional minor bands of activity are also evident. With the prostatic extracts from 18000g pellets of 4-day castrated rats only one major band (band A) is evident, whereas with longer periods of castration the second band appears. Treatment of 7-day castrated rats with dihydrotestosterone did not alter the distribution of plasminogen activators in the gels. No bands of lysis were detected in prostatic extracts from non-castrated rats or in agarose gels not containing plasminogen. Although the gels were not strictly quantitative with respect to plasminogen activator activity, under controlled conditions with the same protein concentrations the presence and size of bands A and B paralleled the amount of plasminogen activator

Fig. 4. Gel electrophoretic analysis of plasminogen activators in prostatic extracts

Prostatic extracts from the 18000g supernatants (lanes 1-4) and pellets (lanes 5-8) were applied to 7.5% (w/v) polyacrylamide gels and electrophoresed at 240 V for 2.5 h. The gels were overlaid onto opaque agarose substrate gels, containing casein and plasminogen, and incubated at 37°C for 3 h. The substrate gels, displaying clear bands of lysis, were fixed and then photographed. Samples from non-castrated rats (lanes 1 and 5) contained 300 µg of protein, whereas samples from 4-day (lanes 2 and 6), 7-day (lanes 3 and 7) and 10-day (lanes 4 and 8) castrated animals contained 150 µg of protein.

activity anticipated from the results with the quantitative fluorometric assay.

Cellular inhibitors of plasminogen activator activity

Since the relatively low concentrations of plasminogen activator activity detected in prostatic extracts from non-castrated and androgen-treated rats could be due to the presence of inhibitors of plasminogen activators, cellular extracts from the prostates of non-castrated rats were mixed in varying proportions with samples containing high levels of plasminogen activator activity. The results shown in Table 1 indicate that even at protein:protein ratios as high as 5:1 the cytosolic extracts from the prostates of non-castrated rats do not inhibit the plasminogen activator activity in the cytosolic extracts from the prostates of 10-day castrated rats. By comparison, prostatic extracts of the 18000g pellet fraction from non-castrated rats do possess factors which will moderately inhibit plasminogen activator activity in similar prostatic extracts from castrated rats. Hence the low concentration of plasminogen activator activity associated with the prostatic extracts from the 18000g pellets of non-castrated rats may be due, in part, to the presence of an inhibitor of this enzyme activity.

At high protein:protein ratios the 18000g pellet fraction from the non-castrated rats will also inhibit the plasminogen activator activity in cytosolic extracts from the prostates of castrated rats.

However, in terms of relative abundance, the mean ratio of protein recovered from the 18000g pellet to that in the 18000g supernatant is approx. 1:16. Since ratio mixtures as high as 1:2 exhibit no inhibitor activity (Table 1), it is unlikely that inhibitors derived from the 18000g pellet fraction contribute significantly to the low plasminogen activator activity associated with the cytosolic extracts from the prostates of non-castrated rats.

Discussion

The finding that plasminogen activator activity in the prostate increases several-fold following castration suggests that increased concentrations of this enzyme are related to the process of glandular involution. This observation is similar in substance to elevations of plasminogen activator activity seen in the uterus and the mammary gland during oestrogen-induced involution of these organs (Katz *et al.*, 1976; Ossowski *et al.*, 1979; Peltz *et al.*, 1983). However, the process in the prostate differs in that the androgen, dihydrotestosterone, is a repressor rather than an inducer of plasminogen activator activity (Figs. 1 and 2).

Most of the plasminogen activator activity (> 90%) on a per cell or per amount of DNA basis is recovered in the cytosolic fraction of the prostate (Fig. 1). Between days 2 and 10 following castration the enzyme activity increases in a near-linear fashion such that by day 10 there is approx.

Table 1. *Inhibition of plasminogen activator activity by prostatic extracts*

Cytosolic (C₀) or 18000g pellet (P₀) extracts from the prostates of non-castrated rats were combined at the protein:protein ratios indicated with cytosolic (C₁₀ and C₁₄) or pellet (P₄) extracts from the prostates of castrated rats (time of castration in days given as subscript). The amount of plasminogen activator activity in the sum of the individual samples (expected) and the mixtures (measured) was determined and the percentage inhibition $\{[(\text{expected} - \text{measured})/\text{expected}] \times 100\%$ was calculated.

Sample mixture	Ratio	Activity ($\mu\text{mol}/30 \text{ min}$)		Inhibition (%)
		Expected	Measured	
C ₀ :C ₁₀	1:1	79.9	74.4	7
	2:1	54.2	53.0	2
	3:1	50.0	51.6	0
	5:1	28.6	27.6	3
P ₀ :P ₄	1:3	35.5	33.8	5
	1:2	33.3	28.9	13
	2:1	28.5	21.0	26
	3:1	23.7	15.2	36
P ₀ :C ₁₄	1:5	44.3	44.4	0
	1:2	41.3	44.9	0
	2:1	28.4	25.4	10
	4:1	21.9	15.6	29

12 times more plasminogen activator than in prostatic cells from non-castrated rats. A similar magnitude of differences is also observed in the specific activity (per mg of protein) of plasminogen activators in the prostates of castrated and non-castrated rats. Treatment of the castrated rats with dihydrotestosterone reduces the plasminogen activator activity in a time- and dose-related manner.

By comparison, the plasminogen activator activity associated with the 18000g pellet fraction of the prostates increases 4 days after castration but declines with longer intervals (Fig. 2). Treatment with androgens has only minor effects on the amount of activity recovered in this fraction. In tumours of the prostate, the specific activity of plasminogen activators in the pellet fraction is generally several-fold higher than in the supernatant fraction (Rennie *et al.*, 1980b, 1982), although this is apparently not the case in the normal rat ventral prostate. However, leakage of plasminogen activators from this fraction into the supernatant cannot be entirely discounted.

Electrophoretic analysis of plasminogen activators indicate that both the 18000g supernatant and the pellet fraction contain two major bands of activity (Fig. 4). Cytosolic extracts from the prostates of 10-day and 14-day castrated rats contain three additional minor bands of activity that are not observed in the 18000g pellet fractions from these prostates. These minor bands may represent distinct species of plasminogen activators or partially degraded forms of the two major bands. While one may speculate that the two major bands represent the urokinase-like and tissue-type of plasminogen activators observed in carcinomas of the rat ventral and human prostate (Strickland

et al., 1983; Camiolo *et al.*, 1981), no exact designation can be made at this time.

The elevation in plasminogen activator activity following castration may occur as a consequence of decreased cellular concentrations of an inhibitor or increased synthesis of plasminogen activators. Proteinase inhibitors have been measured in human prostate (Nijs *et al.*, 1973) and inhibitors of plasminogen activators have been detected in serum and cell culture supernatants (Granelli-Piperno & Reich, 1978). Furthermore, glucocorticoids have been shown to inhibit plasminogen activator activity in rat hepatoma cells through the induction of an inhibitor (Seifert & Gelehrter, 1978; Barouski & Gelehrter, 1980). In the present study an inhibitor of plasminogen activator activity was found in the 18000g pellet fractions isolated from the prostates of non-castrated rats (Table 1). However, since the relative cellular concentration of this inhibitor(s) is very low, it is unlikely that the castration-induced increase in plasminogen activator activity in cytosolic fractions is due solely to the disappearance of an inhibitor(s).

A more likely explanation for the rise in plasminogen activator activity is the synthesis *de novo* of additional enzyme molecules. The two major bands of plasminogen activator activity seen after electrophoresis experiments (Fig. 4) are undetectable in the prostatic extracts from non-castrated rats, but increase in prominence (relative to protein concentration) with the duration following castration. Whether increased production of plasminogen activators is due primarily to translation of pre-existing mRNA for these enzymes or, as in the case for calcitonin induction of urokinase-

like plasminogen activator in pig kidney cells (Nagamine *et al.*, 1983), a result of increased gene transcription, is uncertain. In support of the latter, Stanicic *et al.* (1978) reported that RNA synthesis remains relatively high in the prostate during involution although the net RNA content decreases as a result of higher rates of degradation.

It is tempting to speculate that the mechanism of androgenic regulation of plasminogen activator activity involves repression of the plasminogen activator genes in the non-castrated rat through direct binding of androgen receptors to the specific regions of the genome which control these genes. Within 24 h after castration, over 90% of the androgen receptors bound to the chromatin and nuclear matrix are detached and translocated into the cytoplasm, where they are eventually catabolized (Bruchovsky & Craven, 1975; Van Doorn *et al.*, 1976; Rennie *et al.*, 1983). Thus, in the context of this model, the loss of androgen receptors causes de-repression of the plasminogen activator genes and accounts for the subsequent increase in the activity of this enzyme.

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