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Localization of Testicular Plasminogen Activator in Discrete Portions (Stages VII and VIII) of the Seminiferous Tubule

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ABSTRACT

We have previously reported that rat Sertoli cells in culture produce and secrete plasminogen activator, a highly specific protease, and that FSH stimulates these processes. We have postulated that localized proteolysis elicited by plasminogen activator may be implicated in the restructuring of the seminiferous tubule which occurs when spermatocytes in early prophase of meiosis are translocated from the basal to the adluminal compartments. To test the conjecture further, we isolated tubule segments at different stages of the cycle of the seminiferous epithelium, using the transillumination procedures of Parvinen and Vanha-Perttula, and we determined levels of plasminogen activator in extracts or culture medium in which segments were incubated for 20 h. We found that levels of plasminogen activator were significantly higher in segments at stages VII and VIII of the cycle and that amounts released into the medium by these segments were more than 100-fold greater than those released by segments of seminiferous tubule from any other stage. Segments with seminiferous epithelium at stages VII and VIII are the regions of the tubule in which spermiation occurs, and in which movement of Sertoli cell cytoplasmic processes around leptotene spermatocytes takes place. We advance the hypothesis that plasminogen activator is intimately related to the localized restructuring which takes place as spermatocytes in meiosis are prepared for translocation into the adluminal compartment and as spermiation occurs at stages VII and VIII in the seminiferous epithelium of the tubule.

INTRODUCTION

The seminiferous tubules in testes of sexually mature mammals consist of two functional compartments separated by tight junctional complexes among adjacent Sertoli cells (Fawcett, 1975). In the compartment located next to the basement membrane (the basal compartment), germ cells undergo mitotic divisions until becoming committed to meiosis. In the other compartment, located adjacent to the lumen (the adluminal compartment), germ cells in meiotic prophase complete meiosis, and all spermiogenesis takes place. In the cycle of seminiferous epithelium, which in the rat has been divided into 14 successive stages (Leblond and Clermont, 1952), release of spermatozoa into the lumen (spermiation) and initiation of the complex restructuring process leading to the passage of meiosis-committed germ cells from the basal to the adluminal compartment occur at approximately the same locus of the tubule, corresponding to stages VII and VIII (Clermont, 1972; Russell, 1977). We have recently advanced the hypothesis that plasminogen activator, synthesized and secreted by Sertoli cells, is implicated in the restructuring of the seminiferous tubule which occurs during these processes (Lacroix et al., 1977, 1979). To test this conjecture further, tubule segments with seminiferous epithelium at different stages of the cycle were isolated by transillumination procedures (Parvinen and Vanha-Perttula, 1972) and levels of plasminogen activator activity were determined in extracts or in the culture medium collected after an incubation period of 20 h. We present data demonstrating that the release of plasminogen activator activity by rat

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testis tubules at stages VII and VIII is much greater than that released by tubule segments at any other stage of the seminiferous epithelium.

MATERIALS AND METHODS

Adult Wistar rats were obtained from CBL (Montreal, Quebec). Hanks' balanced salt solution and Eagle's minimal essential medium (MEM) were prepared by the Medical Sciences Central Services (University of Toronto). MEM was supplemented as described (Lacroix et al. 1977). We purchased 96-well culture trays from Becton, Dickinson and Co. (Mississauga, Ontario). 2-Amino-2-hydroxymethyl-propane-1, 3-diol (Tris) and Triton X-100 were obtained from Sigma (St. Louis, MO).

Isolated testicular tissue from adult Wistar rats was placed in Hanks' balanced salt solution and long segments of seminiferous tubules were teased apart from the interstitial tissue with fine forceps. Various stages of the seminiferous epithelium cycle were identified in isolated tubules observed with a stereomicroscope using transmitted light at 25- or 50-fold magnification (Parvinen and Vanha-Perttula, 1972). The classification of Leblond and Clermont (1952) was used to identify the stages of the seminiferous epithelium cycle. Because stage VII is longer than stage VIII, it was divided into two sections: portion a-b is adjacent to stage VI and segment VII, c-d precedes stage VIII. Tubule sections of a defined stage of the cycle were cut with iridectomy-type scissors, and 2 mm long segments from the same stage were pooled. Tubule segments from each group were either homogenized or incubated in MEM. Homogenization of the tubule fragments was performed with a conical glass homogenizer in 0.5 ml ice-cold 0.1 M Tris-HCl (pH 8.1), supplemented with 0.01% (v/v) Triton X-100. The homogenates were briefly sonciated (five 0.5 sec ultrasonic pulses delivered by a Branson Sonifier set at 75 W). The extracts were centrifuged at 600 × g for 10 min at 4°C. Supernatant fractions were assayed for levels of plasminogen activator activity (Lacroix et al., 1979) and protein (Lowry et al., 1951). Incubation of the tubule segments was done at 32°C in a humidified atmosphere composed of 5% CO_2:95% air. After 20 h the medium was harvested, centrifuged at 3000 × g for 5 min, and assayed for plasminogen activator activity.

Plasminogen activator was assayed by the [^{125}I]-labeled fibrin plate method previously described (Lacroix et al., 1977, 1979). One unit is the amount of plasminogen activator activity required to generate enough plasmin-dependent proteolytic activity to maintain a rate of 1% fibrinolysis per hour under the conditions described (Lacroix et al., 1977, 1979).

RESULTS

Levels of plasminogen activator activity in extracts of defined segments of seminiferous tubules are significantly higher at stages VII and VIII of the cycle (Fig. 1). Amounts of plasminogen activator activity released into the medium by segments of seminiferous tubules from stages VII and VIII are considerably greater

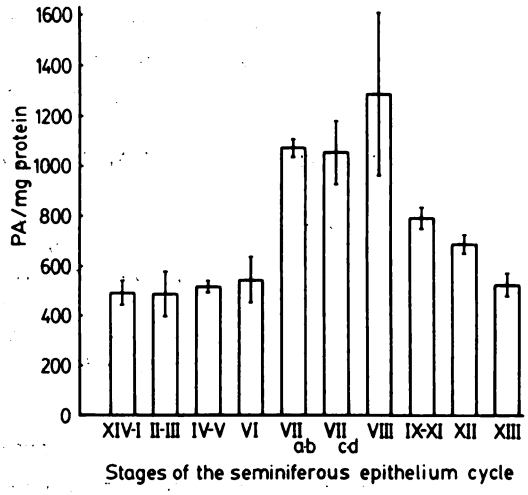

FIG. 1. Levels of plasminogen activator activity in extracts of seminiferous tubule segments. Ten 2 mm long tubule segments were isolated from sections of the seminiferous epithelium corresponding to the indicated stages and were homogenized as described in Materials and Methods. The results shown are the mean values ± SD calculated from four replicates. Similar results were obtained in three other experiments.

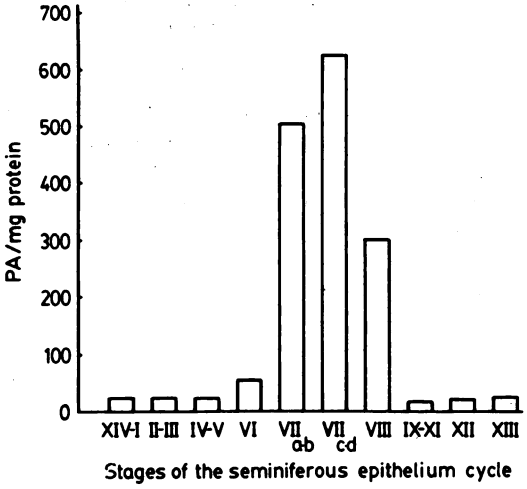

FIG. 2. Levels of plasminogen activator activity released by seminiferous tubule segments into culture medium. Five 2 mm long seminiferous tubule segments of the indicated stages were isolated and incubated in 0.2 ml MEM for 20 h as described in Materials and Methods. The results shown are the mean values calculated from two experiments run in parallel (variation between experiments was never larger than 20%), and are expressed as units of plasminogen activator (PA) released into the medium per milligram tissue protein.

TABLE 1. Plasminogen activator activity released by seminiferous tubule segments.[a]

	Experiment				
	1	2	3	4	5
	Plasminogen activator activity (Units/mg tissue proteins)				
Stage					
Others[b]	91 ± 13	5 ± 41	40 ± 14	0 ± 24	0 ± 8
VII	4187 ± 213	4112 ± 81	1467 ± 27	2454 ± 80	4481 ± 267
VIII	4253 ± 173	1563 ± 211	3579 ± 280	1787 ± 93	2355 ± 117

[a]In each experiment, three seminiferous tubule segments belonging to each of the three indicated groups were incubated in 0.2 ml MEM. After 20 h, the medium was collected and assayed for plasminogen activator activity as described in Materials and Methods. Results are expressed as the mean values ± SD calculated from four replicates.

[b]Others: segments of tubules at stages of the cycle of the seminiferous epithelium other than VII or VIII.

than those released by segments from any other stage of the cycle (Fig. 2). In confirming these results, we simplified experimental procedures by pooling segments from three groups: stage VII, stage VIII, and segments from all other stages. In each of five separate experiments, plasminogen activator activity released during the initial 20 h of culture by tubule segments at stages VII and VIII is at least 40 times higher than levels released by pooled segments from all other stages (Table 1). Although absolute levels of activity are variable in different experiments, the plasminogen activator activity never exceeds 100 units/mg protein in segments other than from stages VII and VIII of the cycle. In contrast, levels of plasminogen activator activity released by segments taken from stages VII and VIII of the seminiferous epithelium cycle range from 1467 to 4480 units/mg protein. Average levels of plasminogen activator activity released by segments from stages VII and VIII (3340 and 2707 units/mg protein, respectively) are more than 100-fold greater than those released by segments taken from other portions of the seminiferous tubule (27 units/mg protein).

DISCUSSION

In several systems besides spermatogenesis, cell migration and tissue restructuring have been correlated with high levels of plasminogen activator production and secretion (Beers et al., 1975; Ossowski, 1979; Strickland et al., 1976). Observations presented in this communication show that plasminogen activator activity in the tubule is highest at stages VII and VIII of the cycle. These are the regions in which spermiation occurs, and in which movement of Sertoli cell cytoplasmic processes around leptotene spermatocytes also takes place.

The control of testicular levels of plasminogen activator is most probably mediated via modulation of the production of this protease by Sertoli cells (Lacroix et al., 1979). Sertoli cells engaged in releasing sperm into the lumen may have metabolic properties different from those of Sertoli cells at other stages of the cycle of the seminiferous epithelium. If so, Sertoli cells at stages VII and VIII may have a lower threshold to agents such as follicle-stimulating hormone, which stimulates the production of plasminogen activator activity by Sertoli cells in primary culture (Lacroix et al., 1977), or testosterone, which enhances the production of androgen-binding protein (ABP) (Louis and Fritz, 1977, 1979). Basal rats of ABP secretion by tubules in culture were reported to be highest in stages VIII through XI, and minimal in stages IV and V (Ritzen et al., 1980). Combined data indicate that Sertoli cells at various stages of the cycle in vivo probably have differing rates of synthesis of some of the protein products secreted into tubular fluid. This differential responsiveness could be dependent on the population of germinal cells associated with the Sertoli cells at a given stage.

We suggest that hormones act in conjunction with signals from adjacent germinal cells to control the synthesis and secretion of plasminogen activator by Sertoli cells at stages VII and VIII, and that this protease is intimately related to the localized restructuring taking place in these portions of the seminiferous tubule.

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