Lysosomal enzymes in cells separated from rat testis

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Summary. Fractions enriched with Sertoli cells (S), germ cells (G), and interstitial cells (I) were separated from rat testis after enzymic treatment and double filtration through nylon meshes. The fractions were analysed for protein content and for enzymic activity of 4 acid hydrolases known to be of lysosomal nature in other tissues. Acid phosphatase activity was preferentially recovered in Fraction G, the highest activity of β -glucuronidase was found in Fraction I while the activity of aryl sulphatase and β -N-acetyl-D-glucosaminidase was prominent in Fraction S. With the exception of acid phosphatase, the enzymes were mostly recovered in a subcellular fraction of whole testis homogenate separated between 600 and 27 000 g. The results may reflect the peculiar enzyme composition of the lysosomal apparatus of each cell type.

Introduction

Biochemical study of the lysosomal apparatus of the testis is hampered by the cellular complexity of the organ. The testicular parenchyma is composed of interstitial tissue and seminiferous tubules: the former contains various connective tissue cellular elements, especially macrophages and clusters of Leydig cells; the latter consist of Sertoli cells and a differentiating population of germ cells. Ultrastructural studies using acid phosphatase activity as a marker have shown that lysosomal-like bodies are localized in Sertoli and germ cells (Posalaki, Szabó, Bácsi & Ökrös, 1968; Chemes & Fawcett, 1978). Acid phosphatase is by far the most studied acid hydrolytic enzyme in the rat testis. Males & Turkington (1971) found by differential centrifugation of testis homogenates that the subcellular distribution of acid phosphatase and β -glucuronidase is of lysosomal nature. Vanha-Perttula (1971) and Vanha-Perttula & Nikkanen (1973) have suggested that 2 of the 4 types of testicular acid phosphatase are preferentially localized within the interstitial tissue while the others are within the seminiferous tubules. One of these latter (phosphatase III) is related to cells present in every stage of the seminiferous wave, possibly the Sertoli cells (Parvinen & Vanha-Perttula, 1972). The cellular origin of acid phosphatase and other hydrolytic enzymes was also inferred from the changes of activity after suppression of germ cells in rat (Pecora & Arata, 1964; Ahlquist, 1966; Males & Turkington, 1971) and human (Guha & Vanha-Perttula, 1980) testis.

To obtain more direct evidence of the cellular localization of several lysosomal enzymes in rat testis we assayed them in fractions enriched in Sertoli cells, germ cells and cells from the interstitium.

Materials and Methods

Testes of albino rats, of the Holtzman strain and aged 3 months, were removed under anaesthesia with pentobarbitone sodium. For each cell separation experiment the testes of one

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animal were decapsulated and a portion of the tissue was set apart for chemical analysis (whole tissue). The rest was incubated at 31°C in a flask containing 30 ml calcium-free Krebs–Ringer bicarbonate buffer (KRBB: Umbreit, Burris & Stauffer, 1964) with 0.1% collagenase (Sigma type IV) for 15 min. The flask was shaken at 100 cycles/min. During the incubation the seminiferous tubules became dissociated; they were allowed to settle on the bottom of the flask while the supernatant, containing extratubular cells, was centrifuged at 50 *g* for 5 min. The sediment (Fraction I) was rinsed three times with 10 ml KRBB, then 0.25% trypsin (Sigma type III) and 0.1% DNAase (Sigma type I) in 10 ml KRBB were added to the flask; this was incubated as described above for 12 min. After the incubation, fragments of tissue were disintegrated by gently pipetting them for several times into and out of a fire-polished Pasteur pipette. The resulting cell suspension was filtered through a 100 µm nylon mesh to eliminate remaining fragments of tissue, and then through a 60 µm nylon mesh to retain Sertoli cells (modified from the method of Steinberger *et al.* 1975). The retained cells were rinsed three times with 10 ml KRBB (Fraction S); the filtered cell suspension, enriched with germinal cells, was centrifuged and the sediment was washed as described above (Fraction G). The cells of each fraction and the portion of whole tissue were suspended in 0.01 M Tris–acetate buffer (pH 7.4) and sonicated for 1 min before the chemical analysis.

To determine the relationship of the enzymes with subcellular structures, 1 g of decapsulated testis was homogenized in 0.01 M-Tris–acetate buffer containing 0.25 M-sucrose using a glass homogenizer with a teflon pestle. The homogenate was centrifuged at 600 *g* for 5 min, the sediment was suspended in the buffer and homogenized again. The operation was repeated three times using a total of 10 ml buffer. The nuclei-free supernatant was centrifuged at 27 000 *g* for 10 min to obtain a final supernatant and pellets. The 600 *g* and 27 000 *g* pellets were suspended in the sucrose buffer and sonicated for 1 min before being analysed, with the final supernatant, for enzymic activity.

Acid phosphatase (EC 3.1.3.2) activity was measured with β-glycerophosphate or *p*-nitrophenyl phosphate as substrate at pH 5.0. The activity of β-glucuronidase (EC 3.2.1.31) was measured with phenolphthalein glucuronate and that of β-*N*-acetyl-D-glucosaminidase (EC 3.2.1.30) with β-*N*-acetyl-D-glucosaminide (both at pH 5.0). The activity of aryl sulphatase A and B (EC 3.1.6.1) was assayed with nitrocatechol sulphate as substrate at pH 4.7. All substrates were from Sigma (Missouri, U.S.A.) and the reagents were of analytical grade. The methods of enzymic determination were, in essence, those described by Barrett & Heath (1977). The activity was expressed as nmol (or pmol) of substrate degraded/min of incubation at 37°C/mg protein.

The method of Lowry, Rosebrough, Farr & Randall (1951) was used for protein measurement.

The statistical significance of the data was established by analysis of variance and Scheffe's multiple comparison test.

To obtain a total cell suspension, decapsulated testes were dissociated by the enzymic method described above, but omitting the double filtration procedure. The total cell suspension and the cell fractions, conveniently diluted with KRBB, were observed and counted with the phase-contrast microscope to establish the percentage of each cell type. Sertoli cells were identified by their prominent size, a vacuolated cytoplasm with abundant organelles and an indented nucleus of irregular shape. Germ cells were mainly recognized by their high nuclear/cytoplasmic ratio, chromosome condensation for the meiotic stages, and by the presence of acrosomes at the spermatid stage. The cells of the interstitium were characterized for being small, vacuolated and of irregular shape, or for presenting an elongated appearance. They were frequently seen as clusters of cells joined by fibres of connective tissue. It was not possible to identify the different types of interstitial cells. These criteria for cell identification were previously evaluated by a detailed study on isolated testicular cells using light and electron microscopy (Bellvé *et al.*, 1977).

Results

According to the cell counting, Fraction G consisted of 98% germ cells and 2% Sertoli cells. Fraction S contained 40% Sertoli cells; the rest consisted of germ cells including some spermatozoa. In Fraction I 66% of the cells came from the interstitium; the rest were germ cells (33%) and Sertoli cells (1%). The total suspension contained 90% germ cells, 9% Sertoli cells, and only 1% of cells from the interstitium.

The enzyme activity results are shown in Table 1. The acid phosphatase activity in Fraction G was the highest with both substrates, and there was no difference between Fractions I and S. The activity of β -glucuronidase greatly varied among the fractions, with Fractions I and S having 7 and 4 times respectively more activity than did Fraction G. The highest activities of the other two enzymes were recovered in Fraction S, and the lowest in Fraction G.

Table 1. Enzymic activities* in whole tissue and fractions of rat testes (1 rat for each experiment)

Enzyme	No. of experiments	Whole tissue	Fraction		
			I	S	G
Acid phosphatase (PNPP)	5	25.8 $\pm$ 1.6 100%	22.4 $\pm$ 4.5 86.2%	18.4 $\pm$ 3.6 71.3%	30.1 $\pm$ 4.1 ^{a,b,c} 116.7%
Acid phosphatase (GP)	8	25.4 $\pm$ 2.4 100%	16.8 $\pm$ 2.6 ^a 66.1%	16.4 $\pm$ 2.6 ^a 64.6%	30.5 $\pm$ 2.9 ^{a,b,c} 120.1%
β -Glucuronidase	8	308.4 $\pm$ 53.8 100%	650.2 $\pm$ 80.6 ^{a,c} 211.0%	377.5 $\pm$ 74.7 ^b 122.7%	93.1 $\pm$ 16.1 ^{a,b,c} 30.2%
<i>N</i> -Acetyl- β -D-glucosaminidase	8	14.9 $\pm$ 1.4 100%	17.8 $\pm$ 2.3 ^{a,c} 120.1%	22.0 $\pm$ 3.8 ^{a,b} 147.7%	9.4 $\pm$ 1.6 ^{a,b,c} 63.1%
Aryl sulphatase A + B	8	8.9 $\pm$ 1.3 100%	8.2 $\pm$ 0.7 ^{a,c} 92.1%	13.4 $\pm$ 2.2 ^{a,b} 150.6%	7.1 $\pm$ 1.3 ^{a,b,c} 79.8%

PNPP, *p*-nitrophenyl phosphate; GP, β -glycerophosphate.

* Values (mean $\pm$ s.d.) are pmol (β -glucuronidase) or nmol (other enzymes) of substrate degraded/min of incubation at 37°C/mg protein. The % values are with respect to 100% for the whole tissue.

^{a,b,c} Values significantly different at $P < 0.01$: *a*, compared with whole tissue; *b*, compared with Fraction I; *c*, compared with Fraction S.

After subcellular fractionation of testis homogenates, the activity of aryl sulphatase, β -glucuronidase, β -*N*-acetyl-D-glucosaminidase and acid phosphatase (glycerophosphate substrate) recovered in the 27 000 g particles was 81.0% ($\pm$ 1.7), 78.9% ($\pm$ 4.2), 84.1% ($\pm$ 5.5) and 40.1% ($\pm$ 1.3) respectively (mean $\pm$ s.d.). From 5 to 10% of the activity of each enzyme was recovered in the 600 g particles; the rest was found in the supernatants. This subcellular distribution of acid phosphatase did not change when *p*-nitrophenyl phosphate was the substrate.

Discussion

The distribution of enzymic activities among the fractions was not homogeneous: each fraction was characterized by prominent specific activities. The results indicate that germ cells are particularly rich in acid phosphatase, the cells of the interstitium have a prominent β -glucuronidase activity, and the Sertoli cells have more β -*N*-acetyl-D-glucosaminidase and aryl sulphatase than do the other cell types.

Fractions I and S contained large numbers of germ cells and this may cast doubts upon the interpretation of the preferential localization of 3 of the 4 enzymes. However, the Sertoli cells

and interstitial cells in these fractions were increased by more than 4- and 60-fold respectively compared with the numbers in the total cell suspension, while germ cell numbers were substantially decreased.

The cellular distribution of enzymic activity indicates that the lysosomal apparatus of each cell type differs from that of the others, although not all the enzymic activity we measured is necessarily of lysosomal nature. Acid phosphatase is partly lysosomal in the rat epididymis (Nikkanen & Vanha-Perttula, 1977) and prostate (Serrano *et al.*, 1976), and β -glucuronidase is known to be partly microsomal in rat liver (Barrett & Heath, 1977). In this work, acid phosphatase was recovered in 27 000 g particles to a significantly smaller extent than were the other hydrolases, suggesting that the enzyme is partly unrelated to lysosomes in rat testis, in agreement with the observations of Vanha-Perttula (1971) on the presence of a soluble acid phosphatase within seminiferous tubules.

Males & Turkington (1971) found that acid phosphatase activity rises in maturing rat testis when germinal cells steadily increase in number; hypophysectomy of 28-day-old rats restores the activity to prepubertal levels, and this change is prevented by LH and FSH treatment. Guha & Vanha-Perttula (1980) found that 3 of the 4 types of acid phosphatase characterized in human testis decrease in activity during oestrogen treatment that causes depletion of germ cells. Thus, in agreement with our results, acid phosphatase appears to be mainly linked to germ cells, since its activity depends on the existence of this cell type in the gland. The activity of β -glucuronidase, however, increased in homogenates of testes deprived of germ cells and *vice versa* (Males & Turkington, 1971). This may be explained as follows: the loss of germ cells provokes a significant loss of proteins from the gland, but not of β -glucuronidase activity that was found by us to be low in this type of cell. A similar explanation may account for the results of Pecora & Arata (1964).

Sertoli cells appear to be rich in β -N-acetyl-D-glucosaminidase and aryl sulphatase whose activities are mostly lysosomal since they are recovered in the 27 000 g particles. The function of these enzymes is uncertain but they may be engaged in the hydrolysis of complex carbohydrates from phagocytosed residual bodies and degenerating germ cells (Fawcett, 1975).

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