Thiamine pyrophosphatase activity in secretory cells of the lateral prostate and seminal vesicle of normal and castrated guinea pigs and castrates treated with oestradiol

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Summary

Ultrastructural localization of thiamine pyrophosphatase (TPPase) activity was studied in secretory cells of the lateral prostate and seminal vesicle of normal and castrated guinea pigs and castrates treated with 17β-oestradiol benzoate. The present study has demonstrated that TPPase reaction product is consistently localized in the three to four trans cisternae of Golgi complexes in both the lateral prostate and the seminal vesicle. The reaction was intense and the reaction product often filled the cisternae completely.

After castration there was a decrease in TPPase activity in both glands as revealed by the reduction in the amount of the reaction product which was found mainly in one to two trans cisternae of the regressed Golgi complex. The reaction product changed from a dense to a more particulate or granular pattern or to discrete deposits of high electron-density.

Administration of 17β-oestradiol benzoate to the castrates caused changes in the localization and patterns of distribution of TPPase. In the lateral prostate there was an apparent increase in TPPase activity. The reaction product was found in two to four trans cisternae and occasionally in the trans-most cisternae of the dilated Golgi complex. The reaction product appeared as discrete, dense coarse precipitates. In the seminal vesicle TPPase reaction product was consistently found in one to two trans cisternae in cells with larger Golgi complexes. However, almost all cisternae of the smaller Golgi complexes were TPPase-positive. The cytochemical results of the present study suggest that TPPase activity and possibly the process of glycosylation in secretory cells of the lateral prostate and seminal vesicle may have been affected after castration and after oestradiol administration.

Introduction

Almost all eukaryotic proteins destined for secretion and for insertion in the plasma membrane are glycoproteins, having galactose residues at the ultimate position of their N-linked oligosaccharide chains (Strous, 1986). During glycosylation, galactose residues are transferred to glycoprotein or glycolipid at the non-reducing N-acetylglucosamine, or in some cases N-acetylgalactosamine, end of the carbohydrate side-chain by the action of galactosyltransferase using uridine diphosphate galactose (UDP-GAL) as a donor. Thiamine pyrophosphatase (TPPase) (EC 3.6.1.-) works in concert with galactosyltransferase in the trans stacks (Roth & Berger, 1982; Fleischer, 1983; Pavelka, 1988) degrading the uridine diphosphate (UDP) to uridine monophosphate to avoid end-product inhibition. TPPase can therefore serve as a marker enzyme to monitor the process of glycosylation in the Golgi complex.

The androgen-dependency of the male accessory sex glands for differentiation and maintenance of their structure and function has been well documented (Brandes, 1974). They contribute to the complex content of the seminal fluid which is essential for the nourishment and transport of the mature sperms. In addition to testosterone, other hormones such as oestrogen and prolactin have been implicated in the growth and function of these organs (Mawhinney & Neubauer, 1979; Sandberg, 1981). We have also demonstrated that administration of pharmacological doses of oestradiol to castrated guinea pigs induced appreciable changes in the secretory function of the guinea-pig lateral prostate (Tam & Wong, 1991). The present study was undertaken to examine the effects of castration alone, and in conjunction with oestradiol administration, on the localization of TPPase activity in secretory cells of the guinea-pig lateral prostate and seminal vesicle.
Materials and methods

Adult male guinea pigs (Dunkin Hartley) aged 12 weeks were used in the present study. Anaesthesia was induced by intramuscular injections of sodium pentobarbitone (Sagatal, England) at a concentration of 43 mg kg⁻¹ bodyweight. Bilateral orchidectomy was performed on six guinea pigs, and the animals were then returned to the holding room for six weeks to allow involution of the accessory sex glands as described elsewhere (Tam & Wong, 1991). 17β-Oestradiol benzoate (Sigma) was dissolved in ethyl oleate and benzyl alcohol (4:1, v/v) at a concentration of 25 mg ml⁻¹. It was injected subcutaneously to three of the castrated animals at a dosage of 5 mg day⁻¹. The castrated controls were given vehicle only. Twenty hours after the last injections, the normal, castrated and the oestradiol-treated castrated guinea pigs were killed with an overdose of sodium pentobarbitone (60 mg). The animals were cannulated at the thoracic aorta with the incision of inferior vena cava. The vascular system was then flushed with 200 ml heparinized saline. This was followed by 300 ml fixative containing 2 mM MgCl₂, 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M cacodylate buffer at pH 7.2. For cytochemical demonstration of thiamine pyrophosphatase activity, tissues were cut with a tissue chopper (Mickle Laboratory Engineering Co., England) to a thickness of 50–70 μm immediately after perfusion. The sections were incubated for TPPase activity in a medium prepared according to the methods of Novikoff and Goldfischer (1961), using cocarboxylase as substrate for 2 h at 37°C. Substrate omission in the incubating medium served as control for TPPase activity. After incubation, the tissue was rinsed and post-fixed in 1% osmium tetroxide for 1 h and stained en bloc with uranyl acetate before embedding for examination by electron microscopy. At least five blocks from each animal were cut and examined. No further staining was performed on these sections.

Results

With the cytochemical method used in the present investigation, thiamine pyrophosphatase reaction product appears as an electron-dense, coalescent precipitate of lead phosphate. Non-enzymatic artefactual lead binding appears as a fine precipitate with a particle size of not more than 0.025 μm in diameter distributed randomly in the cytoplasm of the epithelial cells.

Lateral prostate

The Golgi complex of secretory cells of the normal guinea pig consists of five to six cisternae (Fig. 1). In normal controls, three to four trans cisternae displayed TPPase reaction product (Fig. 2). The reaction was intense and the reaction product often filled the cisternae completely. The trans-most cisternae were devoid of reaction product.

After castration, there was a reduction in the size of individual Golgi complex when compared with the normal controls. The cisternae at the cis side were frequently dilated, while those on the trans side were narrower and flattened (Fig. 3). There was an apparent increase in the number of rigid lamellae within the Golgi region after castration when compared with the normal control. The morphology of the rigid lamellae differs from that of the Golgi cisternae in that they are straight tubules with relatively thick walls and with spaces of definite width. TPPase activity was markedly reduced as revealed by the qualitative decrease in amount of reaction product, which was mainly confined to one to two trans cisternae of the regressed Golgi complexes (Fig. 4). The reaction product changed from a dense to a more particulate or granular pattern (Fig. 5). In general, the trans-most cisternae were devoid of reaction product, although weak TPPase activity was occasionally observed (Fig. 6).

After administration of 17β-oestradiol benzoate to the castrated guinea pigs, the Golgi complex was well organized, being composed of five to six slightly dilated cisternae (Fig. 7). An apparent increase in TPPase activity was observed. The reaction product appeared as discrete, dense coarse precipitate found within the cisternae (Fig. 8). Reaction product was observed consistently not only in the two to four trans cisternae but occasionally in the trans-most cisterna. The condensing granules were TPPase-unreactive.

Seminal vesicle

In the normal control animals, the secretory cells of the seminal vesicle were characterized by the presence of multiple Golgi complexes consisting of five to six cisternae (Fig. 9). Each individual Golgi complex was comparatively smaller than those of the lateral prostate under normal condition. Electron-dense secretory material was often observed within the cisternae. TPPase reaction product was highly electron-dense and was found in two to four trans Golgi cisternae (Fig. 10). The reaction was intense, and the reaction product completely filled the lumen of the cisternae concerned. Other sites of positive activity were found associated with the lateral membranes and the luminal membrane of secretory cells, particularly at the microvilli (Fig. 11). Reaction product was also found associated with the periphery of the secretory granules in some secretory cells. The localization of TPPase activity in secretory cells of the seminal vesicle is essentially similar to that reported by Wong and Tam (1988).

After castration, there was a reduction in both the size and number of the Golgi complexes when compared with the normal control. TPPase reaction product was found within one to two trans cisternae of the Golgi stacks (Fig. 12). The reaction product changed from a continuous pattern to discrete deposits of high electron-density.