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The role of the Golgi apparatus during terminal differentiation of mouse urothelial surface cells

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SUMMARY

The development of the Golgi apparatus in the surface cells of mouse urinary bladder during embryonic development was investigated by electronmicroscopic cytochemistry. The distributions of NADPase and TPPase activities were studied in the urinary bladder during day 15 to day 18 of gestation. At the early embryonic stage, the products of the NADPase and TPPase reactions were visible exclusively in 1 to 2 medial and/or trans Golgi saccules. The strongest increment of NADPase and TPPase positive Golgi cisternae was detected at day 17 when the activity of the urothelial cells was very prominent. At this age, NADPase activity was detected also in lysosomes and on the apical surface of the urothelial cells. The highest distribution pattern of NADPase and TPPase activities observed at this stage rapidly decreases at day 18 of fetal life. The results suggest that the organization of the Golgi apparatus reflected the intensity of the processes occuring in the urothelial cells during gestation.

INTRODUCTION

During fetal development, the morphologically poorly differentiated epithelium of the urinary bladder in mouse undergoes dynamic modifications (Ayres *et al.*, 1985). The rapid developmental changes lead to the formation of the transitional epithelium of adult animals built-up from basal, polygonal intermediate and terminally differentiated superficial cells (Cano *et al.*, 1986; Cohen *et al.*, 1988). In previous studies, it was reported that the formation of tight junctions, fusiform vesicles and asymmetric apical plasma membrane takes place in late days of embryonic life. The blood-urine permeability barrier is also formed before the excretion of urine (Hoyes *et al.*, 1972; Jezernik and Pipan 1993). The structural reorganization of plasma membrane into highly specialized membrane plaque regions, containing specific proteins - uroplakines, and the formation of numerous fusiform vesicles in the cytoplasm of surface cells indicates extensive biosynthetic and endocytotic activity during this period. From the morphological point of view, the urinary bladder assumes the adult morphologic appearance just before the birth (Cano *et al.*, 1986). Desquamation of urothelial cells into the bladder lumen (Jezernik *et al.*, 1997) is another significant feature during embryogenesis of the urothelium. It has been found that the mouse urothelium is characterized by a short lived generation of superficial cells (Ayres *et al.*, 1985; Jezernik *et al.*, 1995) during the late fetal period,

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while the superficial cells of the adult organism under normal conditions are extremely stable (Hicks 1975; Hicks and Chowaniec 1978; Watanabe and Sasaki 1992). This finding is also confirmed by the dynamics of cell detachment just before, and immediately after, birth (Jezernik and Pipan 1993). The phenomenon of uroepithelial shedding occurs only sporadically in normal, undamaged urothelium. However, this process can be induced and intensified also under different stress conditions (Watanabe and Sasaki 1992; Dalal *et al.*, 1994; Stein *et al.*, 1996). The Golgi apparatus plays a central role in protein processing and is a major sorting compartment for proteins destined for lysosomes, endosomes, plasma membrane and secretory granules (Lussier *et al.*, 1995; Matsuo *et al.*, 1995). However, it has been suggested that the Golgi complex is also the critical site for the morphogenesis of the asymmetric unit membrane of the superficial cells of the urinary bladder (Zhang and Seguchi 1994). In spite of the importance of the Golgi apparatus in the different activities of urothelial cells, little is known about morphological and functional development of this organelle during particular differentiation stages of the epithelial cells of the developing urinary bladder. Using cytochemical methods, we have studied the structural arrangement of the Golgi apparatus in the epithelial cells of the developing mouse urinary bladder. To define the correlation between the development of the Golgi apparatus and the differentiation step of superficial cells, we have examined the distribution of NADPase and TPPase activity in the urinary bladder during day 15 to day 18 of mouse fetal life. NADPase is not located exclusively in the medial region of the Golgi stack in the cells. It is also found in many different intracellular locations including trans Golgi saccules, trans Golgi network, lysosomes, or any combination thereof (Smith *et al.*, 1990). As observed by Grondin and Beaudoin (1996), NADPase is secreted by the cells of the exocrine pancreas. This lysosomal enzyme can be released via a secretory pathway which is neither regulated nor constitutive (Beaudoin *et al.*, 1984; Grondin and Beaudoin 1996). It was found that the uroepithelial cells are programmed to respond by shedding to different stimuli (Nativ *et al.*, 1996). Because the epithelial cells secrete proteolytic enzymes in this process, we proposed that certain lysosomal enzymes could play a similar role in desquamation of cells during embryonic development.

MATERIALS AND METHODS

Female mice (Albany strain) were mated and at day 15, 16, 17 and 18 of gestation. The animals were sacrified under anesthesia with ether. The urinary bladders were removed from the fetuses and used for cytochemical studies.

NADPase

The bladders were fixed for 1 hr in 1.5% glutaraldehyde, 2% dextran and 0.005% $CaCl_2$ in 0.1 M Na-cacodylate buffer (pH 7.4) and rinsed overnight in the same buffer. After 30 min washing in the 0.1 M Na-acetate buffer with 5% sucrose (pH 5) the tissue was incubated in a medium containing 4 mM beta $NADP^+$ (Sigma), 0.02 M Pb-acetate, 4% sucrose in 0.02 M Na-acetate buffer (pH 5.0) (Smith 1980).

TPPase

After fixation in 2.5% glutaraldehyde in 0.1 M Na-cacodylate buffer the tissue was washed overnight in 0.1 M Na-cacodilate buffer containing 10% DMSO and 7.5% sucrose. The criosections were incubated in the incubation medium composed of 2.2 mM TPP-chloride (Sigma), 4 mM $Pb(NO_3)_2$, 5 mM Mn-chloride and 5% sucrose in 0.08 M Tris-malate buffer (pH 7.2) for 1 hour.

The tissues were postfixed in an aqueous solution of 1% OsO_4 and 3% potassium ferrocyanide for 1 hour at 4°C, dehydrated, and embedded in Epon. Ultrathin sections were examined in a Jeol 100CX and Philips 100EM electron microscope.

RESULTS

The undifferentiated surface cells of urinary bladder on day 15 of gestation have relatively poorly developed Golgi stacks containing only few cisternae localized in the basolateral region of the cells. Predominantly NADPase activity is observed in 1 to 2 medial saccules showing focal accumulation of reaction product (Fig. 1a). In the vicinity of these saccules, tubulovesicular structures are observed. The individual vesicles contain

Fig. 1 - Bladder epithelium on day 15 of gestation. **(a)** NADPase cytochemical localization is observed in medial saccules of poorly developed Golgi stacks (G). **(b)** Precipitate of TPPase activity is dispersed in two trans Golgi cisternae. x 36000 (a), x 37000 (b). Bars: 0.5 μm

the reaction product of NADPase activity. Most of the TPPase activity was concentrated in two trans Golgi cisternae (Fig. 1b).

At day 16 of gestation, the number of NADPase and TPPase positive Golgi saccules is not significantly changed. The reaction product of NADPase and TPPase activity is found in 1 to 3 medial and trans Golgi cisternae. Many tubulovesicular structures are adjacent to the Golgi stacks (Fig. 2a and 2b).

The layer of superficial cells on day 17 of gestation shows some morphologic characteristics of the mature-appearing bladder. The apical membrane of these cells is already seen as the thick asymmetric unit plasma membrane and many fusiform-like vacuoles are found in the cytoplasm. The optimal planes of section through the Golgi apparatus show that as cell differentiation proceeds, the number of the Golgi stacks, as well as the number of its medial and trans saccules, increase. At this stage, 4 to 6 NADPase positive cisternae in each Golgi stack are observed (Fig. 3a). NADPase activity is associated also with the trans Golgi network, and nevertheless, most of the superficial cells show NADPase activity within lysosomes (Fig. 3b). The lysosomes encircled by clusters of small NADPase positive vesicles contain the heaviest deposit of reaction product. But it is not certain which of the two processes is taking place: fusion of these vesicles with lysosomes or pinching-off of the lysosomes (Fig. 3c). Multivesicular bodies are always NADPase negative. Occasionally, some NADPase positive vesicles are observed in the cytoplasm of the surface cells – in their apical region, and patches of NADPase reaction product are seen over particular domains of the apical plasma membrane (Fig. 3d and 3e). In this period of fetal life, the so called dark cells appear among the superficial cells of normal cytoplasmic density. These dark cells contain heavy deposits of NADPase reaction product in the Golgi stacks and lysosomes (Fig. 3f).

At day 17 of gestation, the strong TPPase reaction is observed in 4 to 6 Golgi cisternae while the trans Golgi network is moderately stained (Fig. 4a). The lysosomes and the MVBs are TPPase negative. Autophagosomes, in particular epithelial cells of the urinary bladder containing TPPase positive trans Golgi cisternae, indicate highly dynamic events in the superficial cells during this stage of development (Fig. 4b).

In the epithelial cells of the urinary bladder of the fetal mouse on day 18 of gestation, NADPase is deposited moderately in medial Golgi saccules (Fig. 5a). The NADPase-positive lysosomal population is less frequently present in the cells at this stage. The cytochemical localization of TPPase shows the diminishing of the trans Golgi region (Fig. 5b).

DISCUSSION

During the maturation of the bladder *in utero*, the fusiform vesicles, highly specialized plasma membrane and tight junctional complexes between adjacent cells are visualized late in gestation (Ayres *et al.*, 1985; Cohen *et al.*, 1988). Yet, before the excretion of urine, the asymmetric apical plasma membrane with the proteoglycan layer acts as an effective permeability barrier preventing the exchange of water and ions between hypertonic urine and cell content (Hicks 1975; Jezernik and Pipan 1993; Nikel *et al.*, 1993).

Our cytochemical observations show that the distributions of NADPase and TPPase in the mouse urothelial cells vary during embryonic development. The increased number of cisternae in the Golgi stacks, as well as the density of reaction products of NADPase and TPPase coincides with the appearance of fusiform vesicles, numerous MVBs, in the apical cytoplasm, and with the formation of a thickened plasma membrane. These findings speak for two processes in these cells: firstly, during this period, the processing of the membrane components in the Golgi apparatus could be more effective, and secondly, the endocytotic activity could be connected with the process of removing not-fully differentiated plasma membranes. The development of the Golgi apparatus into a larger and more active structure is documented for different cell types during differentiation. The Golgi apparatus of the inner enamel epithelial cells is smaller then that of the secretory ameloblasts (Matsuo *et al.*, 1995). The number of NADPase-positive cell compartments increases during embryonic development of the small intestine (Pšeničnik and Pipan 1995) as well as in the crypt-villus direction (Ellinger and Pavelka 1982). Glycosaminoglycans, which are produced by the uroepithelial cells, have two main

Fig. 2 - Electron micrographs of urothelial cells on day 16 of gestation. **(a)** reaction product of NADPase activity is found in 1 to 3 medial Golgi cisternae. **(b)** The number of TPPase positive saccules remains the same as in the earlier stage. x 36000 (a), x 18000 (b). Bars: 0.5 μm.

Fig. 4 - Cytochemical localization of TPPase in the bladder epithelium on day 17 of gestation. **(a)** Reaction product of TPPase activity is heavily deposited in the trans-side saccules of Golgi stacks. **(b)** TPPase positive saccule (arrow) is found within the autophagosome (A) of particular epithelial cell. X 27000 (a), x 34000 (b). Bars: 0.5 μm.

functions: creating the barrier between the epithelial cells and urine, and preventing bacterial adherence (Lilly and Parsons 1990; Stefanelli *et al.*, 1990). WGA binding to the asymmetric, thickened cell membranes of the cells of the developing urinary bladder indicates that the glycoconjugates are already present in late fetal period (Jezernik and Pipan 1993). Our results of the cytochemical staining pattern in the developing Golgi stacks suggest that the increased number of cisternae in each Golgi stack at day 17 of gestation coincides also with the intensified synthesis of proteoglycans in the urothelial cells. The accumulation of small vesicles near the Golgi stack at day 16 of gestation could be considered as a preliminary step in the formation of the new Golgi cisternae and stacks, respectively. In the presecretory ameloblast, the new Golgi stacks seem to be formed by the fusion of small vesicles located near the pre-existing trans Golgi network (Matsuo *et al.*, 1995). Elec-

Fig. 3 - Distribution of NADPase in the superficial cells on day 17 of gestation. **(a)** The number of Golgi stacks (arrows) as well as the number of highly NADPase reactive cisternae (arrowhead) is increased. **(b)** Strong reaction of NADPase activity in lysosomes (Ly). **(c)** Notice small positive vesicles (arrows) that encircle the lysosome (Ly). **(d)** Some slightly NADPase-positive vesicles (arrows) are located in the apical region of the superficial cell. **(e)** Patches of NADPase reaction product are seen over particular domains of the apical plasma membrane (arrows). **(f)** The intensity of the reaction precipitate in dark cell is very high in the Golgi stacks (G) and lysosomes (Ly). x 22000 (a), x 36000 (b), x 35000 (c), x 43000 (d), x 49000 (e), x 12000 (f). Bars: 0.5 μm (a-c), 0.2 μm (d,e), 1 μm (f).

Fig. 5 - Superficial cells on day 18 of gestation. **(a)** The reaction product for NADPase is observed most frequently in 2-3 medial saccules of Golgi stack (G). **(b)** Cytochemical localization of TPPase indicates the diminishing of the trans Golgi region (G). x 38000 (a), x 40000 (b). Bars: 0.2 μm.

tron microscopic studies have also confirmed the fragmentation and reassembly of the Golgi apparatus during mitosis (Zeligs and Wollman 1979).

The characteristic feature of NADPase is the variation in its cytochemical localization in various cell types. Hepatocytes and proximal convoluted tubule cells are examples of cells having intense NADPase activity not only within the medial Golgi cisternae but also within compartments being operational for processing and delivery of NADPase to lysosomes (Smith 1990). The strong NADPase activity in the lysosomes of the urothelial cells on day 17 of embryonic life may point to the important role of the NADPase-containing lysosomal population in the remodeling of the surface of the epithelial cells of the urinary bladder. It may be concluded that the increased processing and delivery of NADPase from the Golgi apparatus to lysosomes takes place during the period of intensive turnover of membrane. By day 18 of gestation, the distribution of NADPase has essentially changed, and the reaction product of NADPase activity is found only in 1 to 2 Golgi cisternae. Although the metabolism of the superficial cells of adult organisms is orientated towards the turnover of luminal plasma membrane (Hicks and Chowaniec 1978). NADPase activity is found only in medial cisterne of the Golgi stack. Considering our cytochemical observations which show traces of NADPase dispersed on the apical surface of the epithelial cells, it seems possible that this enzyme is released from the urothelial cells and participates in cell detachment at this developmental stage. This result is

consistent with recent findings (Dalal *et al.*, 1994; Jezernik *et al.*, 1997) showing that the shedding of the uroepithelial cells is mediated by the release of proteolytic enzymes. In summary, our results reveal that the number of NADPase and TPPase positive saccules forming the Golgi stacks changes with cell differentiation and attains the highest level at day 17 of fetal life. These findings suggest that the organization of the Golgi apparatus reflects the intensity of the processes in the urothelial cells during embryonic development

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