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The Plasma Membrane and Cellular Handling of Proteins

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The present study has shown that molecular conformation and charge determine how proteins are handled in the renal proximal tubule and the efficiency with which they are reabsorbed by the proximal tubule cells. Furthermore it is shown that in that in these cells there is a very extensive recycling of membranes directly from endocytic vacuoles and back to the luminal cell membrane.

(Key Words: Renal proximal tubule, Hydrolysis, Endocytosis, Membrane recycling)

INTRODUCTION

The cellular handling of proteins and peptides has been extensively studied in recent years. In the renal proximal tubule there appears to be two different main routes for the catabolization of proteins, one is extracellular degradation by hydrolytic enzymes located on the luminal cell membrane (contact hydrolysis) and the other, endocytosis followed by lysosomal degradation.

The aims of this study were

1. To study which characteristics of a protein that determine whether it is degraded by contact hydrolysis or taken up by endocytosis by proximal tubule cells.
2. To determine if the isoelectric point of a protein influences the efficiency of its binding and subsequent endocytic uptake in the proximal tubule.
3. To determine whether or not the dense apical tubules in the proximal tubule cells are responsible for the recycling of membrane between the endocytic vacuoles and the luminal cell membrane.

EFFECTS OF MOLECULAR STRUCTURE

The concept of contact hydrolysis of proteins, polypeptides and other substances in the small intestine has been reviewed by Ugolev *et al.* (22, 23). Recently it was shown that the brush border in the kidney proximal tubule contained hydrolytic enzymes which degraded different peptides such as angiotension II and bradykinin to amino acids (2, 19) whereas oxytocin (17)

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and vasopressin (10) were resistant to degradation. The authors concluded that probably the s-s bridges in oxytocin and vasopressin were responsible for the resistance to degradation. Since these peptides consisted of only 8-10 amino acids were decided to evaluate if there was also a difference in the renal tubular handling of larger proteins (18).

Glucagon (linear protein, mol wt 3,600) and insulin (two peptides joined by s-s bridges, mol wt 6,000) were labeled with ^{125}I and microinfused *in vivo* into surface proximal tubules and the urine was recovered and analyzed. In other experiments the tubule which had been microinfused was recovered at different time intervals after microinfusion and analyzed for radioactivity. The experiments showed that virtually all radioactivity appearing in the urine after microinfusion of glucagon consisted of degradation products whereas insulin was recovered intact. Glucagon did not accumulate in the tubule whereas insulin was sequestered by the proximal tubule and then slowly disappeared indicating an endocytic uptake and subsequent lysosomal degradation, which has also previously been shown for insulin (1). Furthermore we showed (18) that isolated brush border membranes from rabbit kidney cortex degraded glucagon but not insulin. It thus appears that it is not the size of the molecule but rather the molecular structure of proteins that determines if the proteins are degraded by enzymes in the plasma membrane or are taken up by endocytosis. Linear molecules seem to be susceptible to the proteolytic enzymes on the luminal cell membrane whereas more complex molecules instead are taken up by the cells by endocytosis.

EFFECTS OF CHARGE

The endocytic uptake of proteins has been studied in a variety of cells including the proximal tubule cells. In the proximal tubule relatively little is known about the specificity of protein binding to the luminal cell surface. However, Mogensen and Sølling (14) found that systemic infusion of positively charged amino acids increased the urinary excretion of several proteins. It is well known that cell surfaces carry a net negative charge (24), which has also been demonstrated on the surface of proximal tubule cells (8, 20). Since the protein, lysozyme (isoelectric point about 11) is reabsorbed by the proximal tubule to nearly 100% (4) whereas peroxidase (isoelectric point about 7) is only reabsorbed to about 10% (21) this prompted us to study the effect of varying the isoelectric point of proteins on their binding and subsequent endocytic uptake by proximal tubule cells (6). After reabsorption into the endocytic vacuoles the proteins are transferred into lysosomes (12) and degraded to aminoacids (3) which has also been shown for a variety of other cell types.

To investigate the effects of charge anionic native ferritin (pI, 4.3) and cationized ferritin (pI, about 10) was microinfused into surface proximal convoluted tubules of rats and the tubules were fixed with glutaraldehyde at different time intervals after the infusion and prepared for electron microscopy (6).

Initially the ferritin, anionic and cationized ferritin bound to the brush border and apical invaginations (Fig. 1). At later time periods the proteins

were found in endocytic vacuoles as has also been found for a variety of other proteins. Qualitatively there seemed to be no difference in the uptake however a quantitative analysis revealed that the cationized ferritin was taken up 8-9 times as efficiently as the anionic ferritin. Thus, molecular charge is an important factor determining the efficiency of binding and subsequent endocytosis of proteins in the proximal tubule.

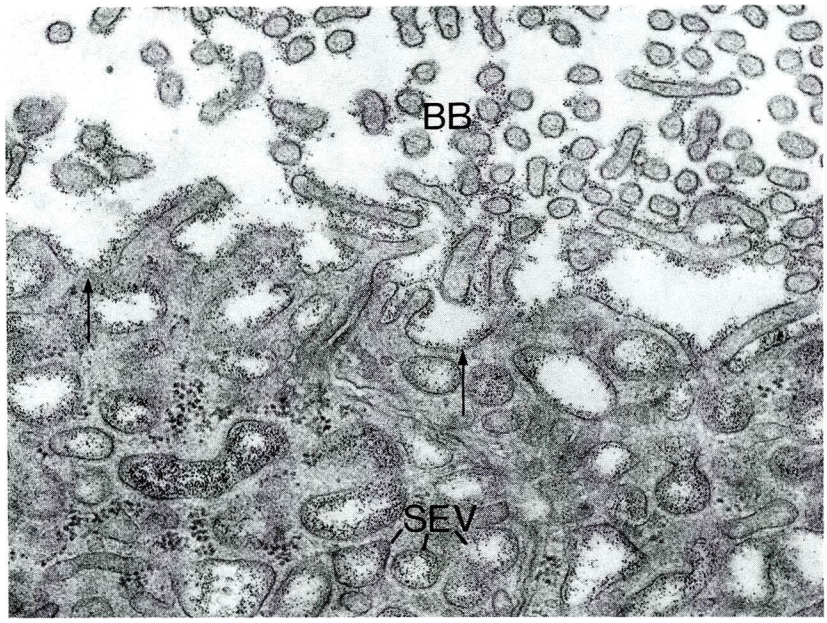

Fig. 1 Part of proximal tubule cells from tubule microinfused with cationized ferritin 20 seconds prior to fixation. The ferritin is localized on the brush border (BB) in apical invaginations (arrows) and in small endocytic vesicles (SEV). ×38,000.

MEMBRANE RECYCLING

One of the main questions in cell biology has been how cells are able to produce the membrane necessary for the endocytic process which in the proximal tubule is quite extensive. As suggested by Mata and David-Ferreira (11) and Farquhar *et al.*, in a variety of cells (7, 9, 16) there appears to be a recycling of membrane between the Golgi region and the cell membrane. As demonstrated elegantly by Muller *et al.* (15) this process of recycling of membrane in macrophages seems to be directly from the phagolysosomes to the cell membrane however the structures carrying the membrane back to the cell membrane were not identified.

Maunsbach (13) suggested that the dense apical tubules which are often seen connected to endocytic vacuoles in the proximal tubule cells of the kidney might be responsible for the recycling of membrane in these cells, and this suggestion was later supported by a morphometric study on proximal tubules from rats intoxicated with sodium maleate (4).

In this study cationized ferritin was microinfused into proximal convoluted tubules which were fixed at different time intervals after infusion,

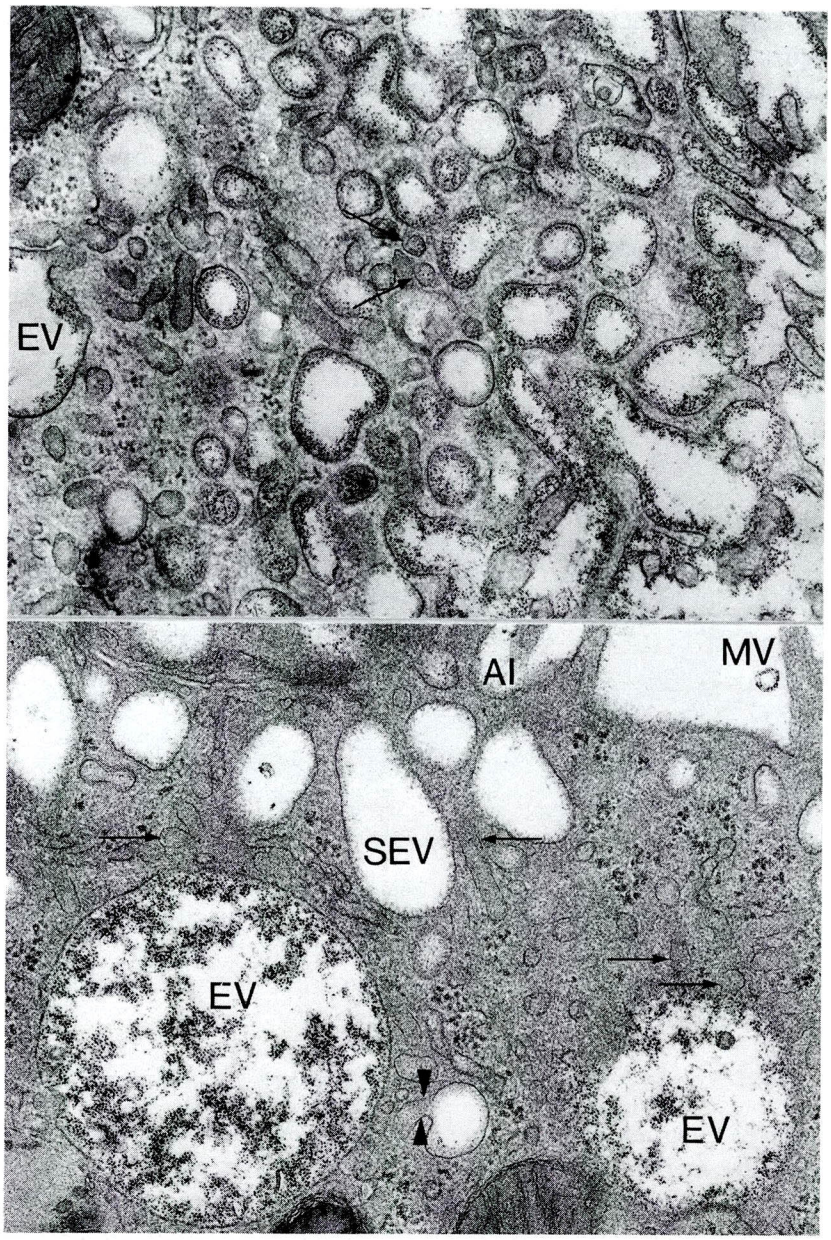

Fig. 2 Part of proximal tubule cells from tubule microinfused with cationized ferritin 20 seconds prior to fixation. The small endocytic vesicles are loaded with ferritin. Only a few dense apical tubules contain ferritin (arrows). A dense apical tubule is connected to an endocytic vacuole (EV). ×34,000.

Fig. 3 Part of proximal tubule cells from tubule microinfused with cationized ferritin 4 minutes prior to fixation. The microvilli (MV), the apical invaginations (AI) and the small endocytic vesicles (SEV) now contain very little ferritin. Considerably more dense apical tubules now contain ferritin (arrows). Two large endocytic vacuoles are filled with ferritin (EV). A dense apical tubule is connected to an endocytic vacuole (arrowheads). ×40,000.

from 20 seconds up to 4 minutes and subsequently prepared for electron microscopy. The experiments revealed that at 20 seconds although the brush border, the apical invaginations and the small endocytic vesicles were loaded with ferritin only few dense apical tubules (15%) contained ferritin and only a few molecules in each profile (Fig. 2). At later time intervals, 90 seconds, the ferritin had moved further down into larger endocytic vacuoles and now considerably more dense apical tubules (50%) contained ferritin but still only a few ferritin molecules in each profile (Fig. 3). The ferritin molecules which are found in dense apical tubules probably represent molecules which are trapped while the apical tubules are being formed from endocytic vacuoles due to binding to the membrane coat. Since with time more and more large endocytic vacuoles contain ferritin, more and more dense apical tubules derived from them contain a few ferritin molecules. The conclusion is that endocytic vacuoles are being formed from the apical invaginations probably with small endocytic vesicles as intermediates. The membrane is then recycled back to the cell surface in the form of dense apical tubules.

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