QUANTITATIVE ANALYSES OF THE DISTRIBUTION OF Na^+, K^+ -ATPASE IN VARIOUS TRANSPORTING EPITHELIAL CELLS

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Na^+, K^+ -ATPase is a pump protein localized on the plasma membrane of the cells in various tissues. In transporting epithelial cells, this enzyme plays definitely important roles in secretion and absorption of Na^+, K^+ and water as well as in the secondary active transport of various substances. We quantitatively analyzed the distribution of Na^+, K^+ -ATPase on many transporting epithelial cells immunoelectron-microscopically, using post-embedding protein A-gold technique.

Na^+, K^+ -ATPase was purified from rat kidney, and antibody against the holoenzyme was raised in rabbit (1). Antibody against the α subunit of this enzyme was affinity-purified (2). For immunoelectron-microscopy, rats were perfused, fixed with a mixed fixative containing 4% paraformaldehyde and 0.1% glutaraldehyde through the hearts for 10 min. The tissues were dehydrated in ethanol series and embedded in Lowicryl K4M, LR Gold, or LR White (Bio Rad, Microscience division, Watford UK) at -20°C using ultraviolet polymerizer (TUV-200; Dosaka EM Co, Kyoto, Japan). Ultra-thin sections were reacted with the antibody against the α subunit of Na^+, K^+ -ATPase at a saturated level for the antibody or control IgG, and then with protein A-gold complex, and were observed under an electron microscope.

For quantitative analyses, gold particles bound to the cross-sectional profiles of the membrane were counted, and the length of each membrane was measured on electron microphotographs by a graphic digitizer (Model G-2; Muto Co, Tokyo, Japan) connected to a personal computer. The average number of gold particles per μm of the membrane (labeling density) was calculated. The specific binding was calculated by subtracting the labeling density of the control from that of the experimental specimens.

Gold particles were exclusively localized on the basolateral plasma membrane in most of the polarized

epithelial cells examined, whether they were absorptive or secretory cells; proximal tubule and distal tubule cells of kidney (3), hepatocytes, exocrine pancreatic acinar cells, acinar cells and striated duct cells of parotid gland, epithelial cells of cochlear duct of inner ear (3, 4), non-pigmented epithelial cells of ciliary body (5), acinar cells and duct cells of exorbital lacrimal gland. Exceptionally, however, the apical distribution of this enzyme was found in the epithelial cells of choroid plexus and in retinal pigmented epithelial cells. Labeling density of gold particles was highest on the basolateral plasma membrane of distal straight tubule and distal convoluted tubule cells of kidney; 3–5 times higher than that of proximal tubule cells. Labeling density was also high on the basolateral plasma membrane of the striated duct cells of parotid gland and of the marginal cells of stria vascularis of inner ear, which was comparable to that of the distal convoluted tubule cells of kidney. We also analyzed the distribution of Na^+, K^+ -ATPase quantitatively on the epithelial cells of ciliary body of which enzyme content was quite low; the labeling density on the basolateral plasma membrane of these cells was only a twentieth of that of distal convoluted tubule cells.

We are trying to develop a more quantitative method to estimate the absolute number of these molecules in the various epithelial cells.

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