

23. In Caco-2 Cells, Most of the “Apical” SGLT1 Resides in Intracellular, Microtubuli-associated Vesicles  
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We investigated the distribution of the endogenous sodium/D-glucose cotransporter (SGLT1) in polarized Caco-2 cells, a model for enterocytes. A cellular organelle fraction was separated by free flow electrophoresis and subjected to the analysis of endogenous and exogenous marker enzymes for various membrane vesicle components. Furthermore, the presence of SGLT1 was tested by an ELISA assay using newly developed epitope-specific antibodies. Thereby it was found

that the major amount of SGLT1 resided in intracellular compartments and only a minor amount in apical plasma membranes. The distribution ratio between intracellular SGLT1 and apical membrane-associated SGLT1 was  $\sim 2:1$ . Further immunohistochemical investigation of SGLT1 distribution in fixed Caco-2 cells by epifluorescence and confocal microscopy revealed that the intracellular compartments containing SGLT1 were associated with microtubuli. Elimination of SGLT1 synthesis by incubation of cells with cycloheximide did not significantly reduce the size of the intracellular SGLT1 pool. Furthermore, the half-life of SGLT1 in Caco-2 cells was determined to be 2.5 d by metabolic labeling followed by immunoprecipitation. Our data suggest that most of the intracellular SGLT1 are not transporters en route from biosynthesis to their cellular destination, but represent an intracellular reserve pool. We therefore propose that intracellular compartments containing SGLT1 are involved in an endo-/exocytosis process, which regulates SGLT1 abundance at the apical cell surface.