A Study of nonelectrolyte transport in isolated intestinal epithelial cell membranes

Author(s): Sigrist-Nelson, Kristine

Publication Date: 1975

Permanent Link: https://doi.org/10.3929/ethz-a-000099070

Rights / License: In Copyright - Non-Commercial Use Permitted
A STUDY OF NONELECTROLYTE TRANSPORT IN ISOLATED INTESTINAL EPITHELIAL CELL MEMBRANES

A Dissertation submitted to the SWISS FEDERAL INSTITUTE OF TECHNOLOGY for the degree of Doctor of Natural Sciences

Presented by KRISTINE SIGRIST-NELSON Master of Science, Univ. of Maine, USA born on July 20th 1945 from Eschenbach (Lucern)

Accepted on the recommendation of Prof. G. Semenza Prof. E. Carafoli

Clausthal-Zellerfeld Bönecke-Druck 1975
VI. SUMMARY

Highly purified rat intestinal epithelial cell brush border and lateral-basal membranes were isolated by various techniques. The vesiculated membrane preparation was such that an intravesicular aqueous space was separated from the medium by the membrane, making possible transport studies. Nonelectrolyte transport in the membrane preparations was investigated by a Millipore filtration technique.

In brush border membrane two separate transfer agencies for sugars were characterized - a D-glucose transport system, shared with D-galactose and a separate D-fructose transport system. An intact glucose carrier system was demonstrated by the following observations: (a) D-glucose was both taken up and released faster than L-glucose. (b) Sodium ions specifically increased the initial rate and extent of D-glucose uptake 3 to 5-fold. (c) When a sodium gradient was present (medium-vesicle) a transitory glucose transport against a concentration gradient was observed. When the Na⁺ gradient was abolished by either monactin or preincubation with Na⁺, the "overshooting" phenomena disappeared. (d) D-glucose uptake and release was inhibited by phlorizin. (e) Counter transport of D-glucose was demonstrated. (f) At equilibrium both D- and L-glucose reached the same level. (g) D-glucose entry was inhibited by D-galactose and vice versa.

D-fructose transport in brush border membrane was shown to be slower than D-glucose but faster than either L-glucose or D-mannitol. Fructose uptake was linear up to and saturable above concentrations of 200 mM while D-mannitol uptake remained linear. Counter transport of fructose, with fructose-preloaded membranes could also be demonstrated. Neither Na⁺ nor phlorizin exerted any influence on fructose transport. Finally, none of the sugars tested (D-glucose, D-galactose, L-sorbose or D-tagatose) were able to decrease D-fructose entry.
Amino acid entry into the vesiculated brush border membrane preparation was additionally studied. An alanine transport system, displaying the following characteristics, was demonstrated: (a) L-alanine was taken up and released faster than D-alanine. (b) Na\(^+\) as well as Li\(^+\) stimulated the uptake of both stereoisomers. (c) The uptake of L- and D-alanine showed saturation kinetics. (d) Counter transport of L-alanine was demonstrated. (e) Other neutral amino acids inhibited L-alanine but not D-alanine entry when an electrochemical Na\(^+\) gradient across the membrane was present initially during incubation. No inhibition occurred in the absence of a Na\(^+\) gradient. (f) By the use of ionophores the electrogenic activity of L-alanine transport was established.

Transport of the dipeptide, glycyl-L-leucine, into the isolated brush border membrane vesicles was investigated. On the basis of the following observations it was postulated that glycyl-L-leucine was transported intact by a specific dipeptide mechanism: (a) The differing time course and sodium stimulation of glycine, L-leucine and glycyl-L-leucine. (b) The failure of glycine and L-leucine to inhibit glycyl-L-leucine transport. (c) Initial presence of dipeptide within the vesicles. (d) Inhibition of glycyl-L-leucine uptake by other dipeptides. (e) The occurrence of accelerated amino acid uptake in the presence of the dipeptide.

Sugar (D-glucose) and amino acid (L-valine) transport was also studied in isolated lateral-basal membrane. Nonelectrolyte transport in brush border and lateral-basal membranes differed in the following ways: (a) Both D-glucose and L-valine uptake was stimulated by the presence of sodium in the incubation medium, 4-fold and 2-fold, respectively. Nonelectrolyte transport in the lateral-basal membrane was not stimulated by sodium. (b) D-glucose and L-valine transport saturated at higher concentrations in lateral-basal membrane (30-50 mM) than in brush border membrane (10-20 mM). (c) D-galactose strongly inhibited D-glucose transport in brush border membrane. Only minimal inhibition was appar-
-ent with lateral-basal membrane. Phlorizin inhibited D-glucose transport only in the brush border membrane, whereas phloretin inhibited D-glucose transport to a greater extent in lateral-basal preparations. (d) Inhibition of L-valine transport by amino acids followed a similar pattern. Amino acids strongly inhibiting L-valine transport in brush border membrane effected only minimal inhibition in lateral-basal membrane.