Amino Acid Transport in Brush-Border-Membrane Vesicles Isolated from Human Small Intestine

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(Received 27 May 1977)

Uptake of L-alanine and L-phenylalanine by purified brush-border-membrane vesicles isolated from human small intestine was investigated by using a rapid-filtration technique. L-Alanine entered the same osmotically reactive space as D-glucose, indicating that transport into the vesicles rather than binding to the membranes was being observed. The uptake rate for L-alanine was higher in the presence of a Na⁺ gradient than in the presence of a K⁺ gradient. In the presence of a Na⁺ gradient, the lipophilic anion SCN[−] caused an increase in L-alanine transport, whereas the nearly impermeant SO₄^{2−} anion decreased the uptake of L-alanine compared with its uptake in the presence of Cl[−]. The uptake of L-phenylalanine into the brush-border-membrane vesicles was also stimulated by Na⁺. The results indicate co-transport of Na⁺ and neutral amino acids in the human intestinal brush-border membrane.

Na⁺-dependent amino acid transport in the small intestine of rats, rabbits and frogs has been demonstrated by many investigators using different methods: Csaky (1961) studied transport in a surviving isolated frog small intestine; Rosenberg *et al.* (1964) used both rat and rabbit jejunal sacs; Munck & Schultz (1969) used short-circuited segments of rabbit ileum for transmural-flux determination; and Rose & Schultz (1971) measured changes of transmural and transmucosal electrical p.d. as a function of Na⁺-dependent amino acid transport in isolated strips of rabbit ileum mucosa. Summarizing these findings, Schultz & Curran (1970) inferred the existence of a Na⁺ amino acid co-transport system in the brush-border membrane. However, in perfusion experiments *in vivo* in man it was not possible to demonstrate Na⁺-dependence of intestinal amino acid transport (Flesher *et al.*, 1966; Cook, 1972). The apparent differences in the results obtained by studies with the intact epithelial tissue *in vivo* (man) and *in vitro* (laboratory animals) might be explained by methodological difficulties in controlling different parameters, such as fluxes through the tight junction and cellular metabolism. In particular, large unstirred layers make it almost impossible to determine substrate and co-substrate concentration at the transport sites, i.e. absence of Na⁺ from the luminal perfusate does not necessarily imply its absence in the micro-environment of the brush-border membrane.

In studies with isolated human brush-border-membrane vesicles we have shown the existence of a Na⁺/glucose co-transport mechanism (H. Lücke, H. Berner, H. Menge & H. Murer, unpublished work). In the present paper, we demonstrate the

presence of a similar transport system for neutral amino acids in human brush-border membranes.

Methods

Pieces (length 25 cm) of histologically normal human upper jejunum obtained from patients in the course of abdominal surgery [kindly provided by Dr. W. Berner, Department of Surgery, University Hospital, Hannover, Germany, and by Dr. H. Menge, Department of Medicine, University Hospital, Marburg (Lahn), Germany] were rinsed with Ringer solution (147 mM-NaCl, 4 mM-KCl and 2.25 mM-CaCl₂) and kept frozen at −40°C. From 20 g of frozen intestine, brush-border-membrane vesicles were isolated by a modification (H. Lücke, H. Berner, H. Menge & H. Murer, unpublished work) of the method of Schmitz *et al.* (1973). Pieces (20 g) of small intestine were immersed in 60 ml of 300 mM-mannitol containing 12 mM-Tris/HCl buffer, pH 7.1. After thawing, the resulting thick suspension was subjected to vibration (100 Hz) by a Vibromixer fitted with a 2 cm-diameter vibrating perforated plate (model E₁; Chemap, Männedorf, Switzerland). This treatment released the mucosal cells into the medium. The sonicated suspension was passed through a Buchner funnel with 1 mm-diameter holes; the large pieces of connective tissue were retained by the funnel. The filtrate was diluted 1:6 with ice-cold distilled water and homogenized in a Waring Blendor mixer at maximum speed for 3 min. After the addition of CaCl₂ (final concn. 10 mM) and then standing the homogenate at 4°C for 15 min it was centrifuged at 7500g for 15 min. The sediment was discarded and the supernatant was centrifuged at 20000g for 30 min. The resulting pellet was resuspended in 70 ml

of 100 mM-mannitol containing 20 mM-Hepes*/Tris buffer, pH 7.4, and homogenized with a glass-Teflon homogenizer (ten strokes at 1400 rev./min). CaCl_2 was added to a final concentration of 10 mM and the two centrifugations (7500g and 20000g) were repeated. The resulting 20000g pellet represented the purified brush-border vesicles preparation and was used after suspension in 100 mM-mannitol containing 20 mM-Hepes/Tris buffer, pH 7.4, for transport experiments.

Protein was determined by the method of Lowry *et al.* (1951) with bovine serum albumin as standard; alkaline phosphatase (EC 3.1.3.1) was measured as described by Berner & Kinne (1976); K^+ -stimulated *p*-nitrophenyl phosphatase (4-nitrophenyl phosphatase, EC 3.1.3.41) was assayed as reported by Murer *et al.* (1976). Alkaline phosphatase, a brush-border marker enzyme, was enriched about 11-fold in the vesicles compared with the initial homogenate, whereas K^+ -stimulated *p*-nitrophenyl phosphatase as a marker enzyme for the contraluminal plasma membrane (Hopfer *et al.*, 1975) was only slightly (1.6-fold) enriched in the final membrane fraction.

Uptake of labelled compounds by isolated brush-border-membrane vesicles was measured by a rapid-filtration technique as described previously (Berner *et al.*, 1976; Evers *et al.*, 1976). The composition of the different incubation media is given in the legend to Fig. 1. For freeze-fracture experiments, vesicles were fixed with 2.5% (w/v) glutaraldehyde, buffered with 0.1 M-sodium cacodylate, pH 7.4, and infiltrated with glycerol up to 30% (v/v). Fracturing was carried out in an EPA 100 freeze-fracture apparatus Leybold, Cologne, Germany). The platinum-carbon replicas were examined in a Philips 300 electron microscope.

Materials

Labelled compounds were purchased from New England Nuclear Corp. (Boston, MA, U.S.A.). Hepes was obtained from Serva (Heidelberg, Germany), and all other chemicals were purchased from Merck (Darmstadt, Germany) and were of the highest purity available.

Results and Discussion

Fig. 1(a) shows the time course of uptake of L-alanine and Na^+ by human intestinal brush-border-membrane vesicles. In the presence of a Na^+ gradient, the uptake of L-alanine was stimulated by approx. 85% compared with the uptake in the presence of a KSCN gradient and showed an overshoot phenomenon. The overshoot indicates an

* Abbreviations: Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid; ATPase, adenosine triphosphatase.

Fig. 1. Time course of (a) L-alanine and $^{22}\text{Na}^+$ uptake and (b) L-phenylalanine uptake by human intestinal brush-border-membrane vesicles

Membrane vesicles, loaded with 100 mM-mannitol containing 20 mM-Hepes/Tris, pH 7.4, were incubated at 25°C in the same medium containing also (a) 0.1 mM-L-[^3H]alanine or (b) 0.1 mM-L-[^3H]phenylalanine and 100 mM-NaSCN (●), 100 mM- $^{22}\text{NaSCN}$ (□) or 100 mM-KSCN (▲). Amino acid (●, ▲) and $^{22}\text{Na}^+$ (□) uptake is expressed as a percentage of the equilibrium uptake by the membranes after 60 min of incubation. The absolute values for L-alanine uptake at equilibrium were 115 and 77 pmol/mg of protein in the NaSCN and KSCN media respectively, that for $^{22}\text{NaSCN}$ was 117 nmol/mg of protein, and those for L-phenylalanine were 136 and 130 pmol/mg of protein in the NaSCN and KSCN media respectively.

intravesicular accumulation of the amino acid driven by the electrochemical p.d. of Na^+ (Murer & Hopfer, 1974). In contrast with the intact cell, Na^+ -dependent

EXPLANATION OF PLATE I

Electron micrograph of freeze-fractured isolated human intestinal brush-border-membrane vesicles
The arrow indicates shadowing direction. The bar represents 0.5 μ m. Magnification: $\times 68\,000$.

accumulation of substrates is only transient in the vesicular system: in the cell, the Na^+ gradient as driving force for Na^+ -coupled movement is maintained by the action of an ATPase that is stimulated in the presence of both Na^+ and K^+ and that is located in the contraluminal membrane (Murer *et al.*, 1976). In the vesicular system, the initial Na^+ gradient is dissipated with time (Fig. 1a) and therefore accumulation can only be transient.

Fig. 1(b) shows the uptake of L-phenylalanine by the vesicles. In the presence of a Na^+ gradient the initial rate of uptake is approximately twice that in the presence of a K^+ gradient. The initial rate of uptake of the Na^+ -dependent system for L-phenylalanine is about one-half that for L-alanine uptake, and only an insignificant overshoot (105% compared with 183% for L-alanine) is observed with L-phenylalanine. The former result agrees with observations made by Schultz *et al.* (1972) for the rabbit small intestine. The latter finding might be due to the fact that the movement of L-phenylalanine is slow compared with the Na^+ movement so that the Na^+ gradient cannot exert its driving force for L-phenylalanine accumulation. Another example of a Na^+ -dependent system that shows no overshoot in the isolated vesicles is the phosphate-transport system in the rat small intestine, which also has a very slow transport rate (Berner *et al.*, 1976).

In control experiments (results not shown) we compared the equilibrium spaces occupied by glucose and the different amino acids. These calculations were based on the assumption that at equilibrium the intravesicular substrate concentrations were equal to the concentrations in the incubation media. The space calculated to be occupied by alanine amounted to $1.2 \mu\text{l}/\text{mg}$ of protein and was identical with the space calculated to be occupied by glucose. Glucose has been shown to be taken up into an osmotically reactive space and not to be bound to human intestinal brush-border membranes (H. Lücke, H. Berner, H. Menge & H. Murer, unpublished work). From these experiments we concluded that the same effect occurs in L-alanine uptake. Determined within the same vesicle preparation, the space occupied by phenylalanine at equilibrium was twice that for glucose or alanine. The most likely explanation for this discrepancy is that phenylalanine, owing to its lipophilic properties, is partially incorporated into the membrane lipids. Similar findings were also obtained in transport studies with brush-border-membrane vesicles isolated from renal proximal tubule (Evers *et al.*, 1976) and in transport studies with intact small-intestinal preparations (Lin *et al.*, 1962).

The effect of anion replacement on Na^+ -dependent alanine transport is given in Table 1. Replacement of Cl^- in the presence of a Na^+ gradient by the highly permeant lipophilic anion SCN^- (Mitchell & Moyle,

Table 1. *Effect of anion replacement on L-alanine uptake into brush-border-membrane vesicles (amount taken up during the first 60 s)*

The experiments were carried out in an incubation medium containing 100 mM-mannitol, 20 mM-Hepes/Tris, pH 7.4, 0.1 mM- $[\text{L}^3\text{H}]$ alanine and different salt gradients as given in the Table.

Salt in the incubation medium	L-Alanine uptake (pmol/mg of protein)
0.1 M-NaSCN	193.3
0.1 M-NaCl	130.2
0.05 M- Na_2SO_4 +0.05 M-mannitol	49.8

1969) stimulated the transport of L-alanine; on the other hand, the transport rate was strongly decreased by replacing Cl^- by the practically impermeant SO_4^{2-} . In the presence of Na^+ the diffusion of SCN^- into the vesicles results in an inside-negative membrane potential (Murer & Hopfer, 1974; Evers *et al.*, 1976). This membrane potential provides an additional driving force for rheogenic Na^+ -coupled uptake of neutral amino acids. As a consequence of coupling a non-electrolyte flux with an electrolyte flux, a charge movement seems to be involved in Na^+ -dependent neutral amino acid transport across human brush-border membranes. Since the effect of anion replacement is only observed on Na^+ -coupled, potential-sensitive co-transport mechanisms and not on Na^+ -independent or electroneutral transport processes, the stimulation of Na^+ -dependent alanine transport cannot be attributed to osmotic effects.

These results lead to the conclusion that amino acid transport across human brush-border membranes is Na^+ -dependent and potential-sensitive, as demonstrated in intact rabbit intestinal preparations (Rose & Schultz, 1970; Schultz *et al.*, 1972; Danisi *et al.*, 1976) and in brush-border-membrane vesicles isolated from rat small intestine (Sigrist-Nelson *et al.*, 1975; Evers *et al.*, 1976). With respect to a physiological interpretation of results obtained in studies with isolated membranes it would be useful to know the orientation of the vesicles, i.e. whether the direction of the observed flux is identical with that occurring in the intact epithelium. Whether membrane vesicles are orientated right side out or inside out can be answered by electron microscopy using the freeze-fracture technique (Plate 1). In an electron micrograph of a freeze-fractured brush-border membrane preparation, spherical and elongated structures can be seen. The convex fracture faces obtained show in general a higher particle density than the concave ones. A similar asymmetric distribution (particle-rich cytoplasmic leaflet and particle-poor outer membrane leaflet) was observed in the microvillus membrane when intact intestinal epithelium was freeze-fractured (Haase *et al.*, 1977). Therefore one might conclude that the orientation

of the membrane in the isolated vesicles is in the majority right side out, and we can assume that the influx of amino acids in isolated membrane vesicles occurs in the same direction with respect to membrane surface as in the intact cell.

The flux-coupling mechanism between Na^+ and amino acids located in the brush-border membrane is part of an active transport system that proceeds apparently by the following steps: neutral amino acids are accumulated within the cells driven by the electrochemical gradient for Na^+ ions; the high intracellular concentration of the substrates then provides the driving force for the downhill efflux across the basolateral plasma membrane into the interstitium via facilitated diffusion (Hopfer *et al.*, 1976). The electrochemical p.d. for Na^+ across the brush-border membrane is maintained by an ATPase that is stimulated in the presence of both Na^+ and K^+ and that is located in the contraluminal membrane.

We are grateful to Professor Dr. K. J. Ullrich and to Professor Dr. R. Kinne for valuable discussion during the preparation of the manuscript.

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