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Delineation of Sodium-Stimulated Amino Acid Transport Pathways in Rabbit Kidney Brush Border Vesicles

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Summary. We have confirmed previous demonstrations of sodium gradient-stimulated transport of L-alanine, phenylalanine, proline, and β-alanine, and in addition demonstrated transport of N-methylamino-isobutyric acid (MeAIB) and lysine in isolated rabbit kidney brush border vesicles. In order to probe the multiplicity of transport pathways available to each of these ^{14}C-amino acids, we measured the ability of test amino acids to inhibit tracer uptake. To obtain a rough estimate of nonspecific effects, e.g., dissipation of the transmembrane sodium electrochemical potential gradient, we measured the ability of D-glucose to inhibit tracer uptake. L-alanine and phenylalanine were completely mutually inhibitory. Roughly 75% of the ^{14}C-L-alanine uptake could be inhibited by proline and β-alanine, while lysine and MeAIB were no more effective than D-glucose. Roughly 50% of the ^{14}C-phenylalanine uptake could be inhibited by proline and β-alanine; lysine was as effective as proline and β-alanine, and the effects of pairs of these amino acids at 50 mM each were not cumulative. MeAIB was no more effective than D-glucose. We conclude that three pathways mediate the uptake of neutral L, α-amino acids. One system is inaccessible to lysine, proline, and β-alanine. The second system carries a major fraction of the L-alanine flux; it is sensitive to proline and β-alanine, but not to lysine. The third system carries half the ^{14}C-phenylalanine flux, and it is sensitive to proline, lysine, and β-alanine. Since the neutral, L, α-amino acid fluxes are insensitive to MeAIB, we conclude that they are not mediated by the classical *A* system, and since all of the L-alanine flux is inhibited by phenylalanine, we conclude that it is not mediated by the classical *ASC* system. L-alanine and phenylalanine completely inhibit uptake of lysine. MeAIB is no more effective than D-glucose in inhibiting lysine uptake, while proline and β-alanine appear to inhibit a component of the lysine flux. We conclude that the ^{14}C-lysine fluxes are mediated by two systems, one, shared with phenylalanine, which is inhibited by proline, β-alanine, and L-alanine, and one which is inhibited by L-alanine and phenylalanine but inaccessible to proline, β-alanine, and MeAIB. Fluxes of ^{14}C-proline and ^{14}C-MeAIB are completely inhibited by L-alanine, phenylalanine, proline, and MeAIB, but they are insensitive to lysine. Proline and MeAIB, as well as alanine and phenylalanine, but not lysine, inhibit ^{14}C-β-alanine uptake. However, β-alanine inhibits only 38% of the ^{14}C-proline uptake and 57% of the MeAIB uptake. We conclude that two systems mediate uptake of proline and MeAIB, and that one of these systems also transports β-alanine.

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Introduction

The discrete nature of the malabsorption syndromes in cystinuria, prolinuria, β-aminoaciduria, and Hartnup disease has led to the conclusion that the epithelial cells of the proximal tubule and the small intestine contain a multiplicity of systems for transport of amino acids. Amino acid absorption in these tissues involves two steps: sodium gradient-dependent accumulation across the brush border membrane and amino acid gradient-driven facilitated diffusion across the basal lateral membrane. In principle, a defect in either membrane would result in a transport deficiency, and in no case has the lesion responsible for a malabsorption syndrome been localized.

Christensen (1969, 1975) has developed a general strategy for delineating parallel transport pathways which may be available for an amino acid. The limiting value by which increasing concentrations of an amino acid inhibit the flux of a test amino acid is equal to the fraction of the test amino acid flux which is carried by a system capable of interacting with both amino acids. Following this approach, Mircheff, van Os, and Wright (1980) identified four distinct alanine-transporting systems in the basal lateral plasma membranes of intestinal epithelial cells, and they showed that three of these had structural specificities similar to systems *A*, *ASC*, and *L*, which are found in the plasma membranes of a variety of nonepithelial, i.e., nonpolarized, cells. We now apply the same strategy in an attempt to delineate the amino acid transport systems in rabbit kidney brush border membranes and to identify systems which, when damaged, could produce defects characteristic of the amino acid malabsorption syndromes.

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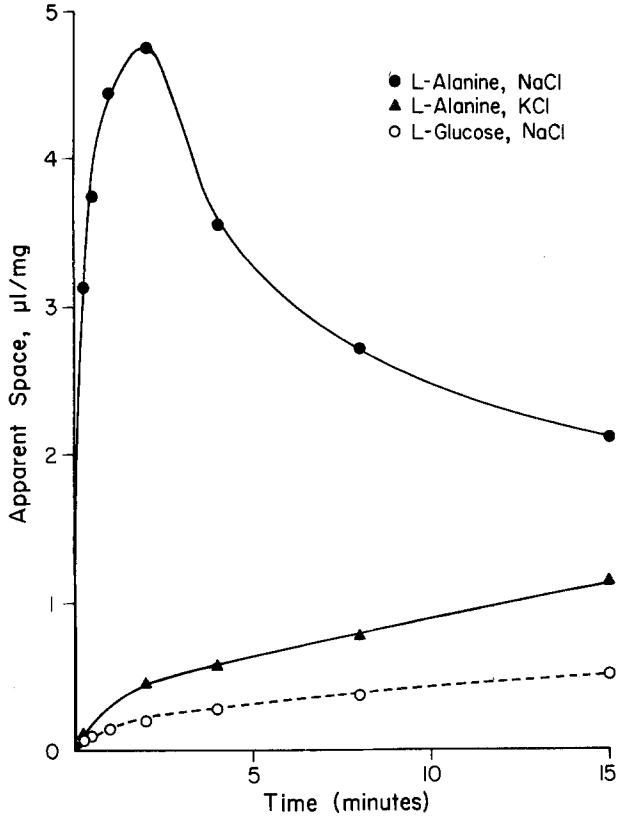

Fig. 1. Time course of L-alanine uptake. Uptake of ^{14}C-L-alanine (6.2 µM) was measured essentially as described under Methods. The intravesicular medium contained 50 mM KCl and 300 mM mannitol, and the final reaction media contained either 50 mM KCl or 50 mM NaCl and 16.7 mM KCl in 300 mM mannitol. Replacement of NaCl by KCl had no effect on the time course of L-glucose uptake; for simplicity, only the time course in external NaCl has been reproduced. The quantities of ^{14}C-L-alanine and ^{3}H-L-glucose measured on each filter were corrected by subtraction of a time = 0 blank, and each point is the mean of duplicate determinations.

In similar experiments Fass, Hammerman and Sactor (1977) observed, at the peak of the overshoot, an alanine content of 11 pmol/mg when the reaction contained 20 µM ^{14}C-L-alanine. Multiplication of the maximum alanine space, 4.7 µl/mg by 10 µM would yield an alanine content of 94 pmol/mg

Materials and Methods

Renal brush border membrane vesicles were prepared by the previously described procedure (Kippen et al., 1979) which uses differential and density gradient centrifugation, and calcium precipitation. Purity of the membrane vesicles was routinely determined by assay of trehalase, a marker for brush border membranes, ($Na^+ - K^+$) ATPase, a marker for basal-lateral membranes, and succinate dehydrogenase, a marker for mitochondria. Protein concentration was estimated by the BioRad protein assay (BioRad Laboratories). Brush border membrane vesicles were consistently enriched at least 10-fold and were essentially free of basal-lateral membranes and mitochondria.

The buffer solution used throughout the experiments was 1 mM Tris-Hepes (1 mM Hepes brought to pH 7.5 with Tris) containing various amounts of salts (NaCl or KCl) and/or D-mannitol, as indicated. Total osmolarity was maintained constant at either 400 or 450 mOsm. Under most conditions, a sodium gradient (extravesicular to intravesicular) was present at time zero.

All experiments were done using a double label isotope procedure in which tracer amounts of both the ^{14}C-labeled substrate and either ^{3}H-L-glucose or ^{3}H-D-mannitol were present. In each experiment, appropriate amounts of both isotopes were added to a test tube and dried under a stream of nitrogen. The isotopes were resuspended in water, and 10 µl of the isotope mixture were added to 90 µl of uptake buffer in a 6-ml polypropylene test tube. Uptake into the membrane vesicles was begun by addition of 50 µl of brush border membrane suspension containing 0.4–0.6 mg of membrane suspension to the uptake buffer. Uptake was terminated by addition of 850 µl of ice cold stop buffer consisting of 1 mM Tris-Hepes, pH 7.5, containing sufficient NaCl to make it isosmotic with the uptake buffer. The suspension was rapidly filtered through a Millipore filter (HAWP 0.45 µm) and washed with 4 ml of the stop buffer. The filter was placed in a liquid scintillation vial with 1 ml of ethyl acetate. After the filter was dissolved, 10 ml of Formula 963 (New England Nuclear) were added and the radioactivity was counted by liquid scintillation spectrometry. The stopping, filtration, and washing procedures took less than 10 sec. All uptake experiments were conducted at 22 °C.

The ^{3}H-labeled markers, L-glucose and D-mannitol, provided a dual correction for excess reaction medium not washed from the filters and for uptake of the amino acid substrates which could be attributed to simple permeation. The ^{14}C and ^{3}H retained on the filters were expressed as the apparent intravesicular spaces in units of µl/mg protein (*see* Mircheff et al., 1980). Background, i.e., $t = 0$, values were obtained by adding ice-cold stopping solution to reaction tubes immediately before addition of the brush border membranes. In presenting the time courses of ^{14}C-amino acid and ^{3}H-marker uptake, we have subtracted background values from each time point. In analyzing inhibition of ^{14}C-amino acid transport by test amino acids and D-glucose we corrected ^{14}C spaces at both $t = 0$ and $t = 0.25$ min by subtracting the simultaneously measured ^{3}H-marker spaces, and we expressed initial amino acid uptake as the change in the specific ^{14}C space between 0 and 0.25 min.

The following isotopes were obtained from Amersham: ^{14}C-L-proline, ^{14}C-L-phenylalanine, ^{14}C-L-alanine, ^{14}C-L-lysine and ^{3}H-beta-alanine. Obtained from New England Nuclear were: ^{14}C-methylaminoisobutyric acid, ^{14}C-D-mannitol, ^{3}H-L-glucose. All other chemicals were obtained from Sigma.

Results

Uptake of Amino Acids

In most experiments brush border vesicles were preloaded with 50 mM KCl, and they were diluted into reaction media to yield initial extravesicular concentrations of either 50 mM NaCl or 50 mM KCl. The time courses for uptake of L-alanine (Fig. 1) and L-proline (Fig. 2) showed Na^+ gradient-dependent overshoots characteristic of sodium-coupled transport into an intravesicular space. In the presence of inwardly directed sodium gradients uptake of alanine was maximal at 2.0 min and uptake of proline was maximal within 0.5 min. In the absence of external Na^+, proline was taken up with a time course similar to L-glucose, a solute for which there should be no

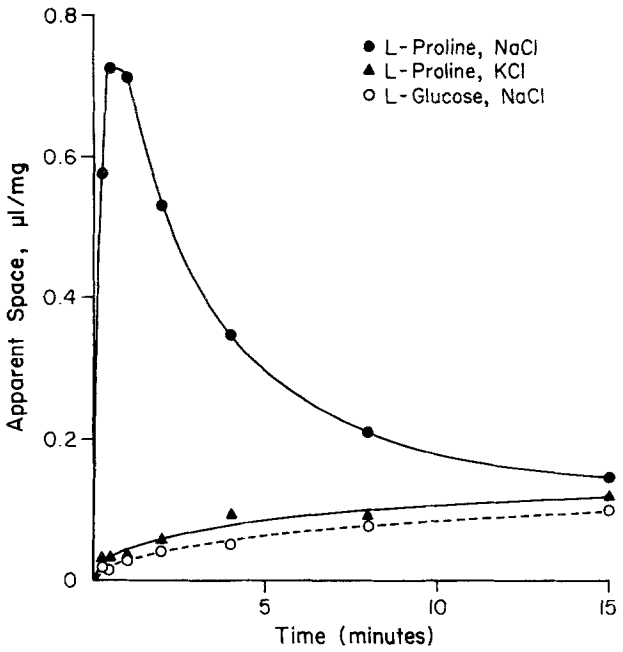

Fig. 2. Time course of L-proline uptake. ^{14}C-L-proline (5.8 µM) uptake was measured as described under Methods. Ionic conditions and blank corrections were as described in the legend to Fig. 1. Replacement of NaCl by KCl in the external medium had no effect on the time course of L-glucose uptake.

In similar measurements of proline transport by isolated kidney brush border vesicles, Hammerman and Sacktor (1977) observed a maximum L-proline content of 40 pmol/mg, when the reaction proline concentration was 25 µM. For comparison, multiplication of the peak proline space in Fig. 2, 0.73 µl/mg, by 25 µM would yield a proline content of 18.3 pmol/mg

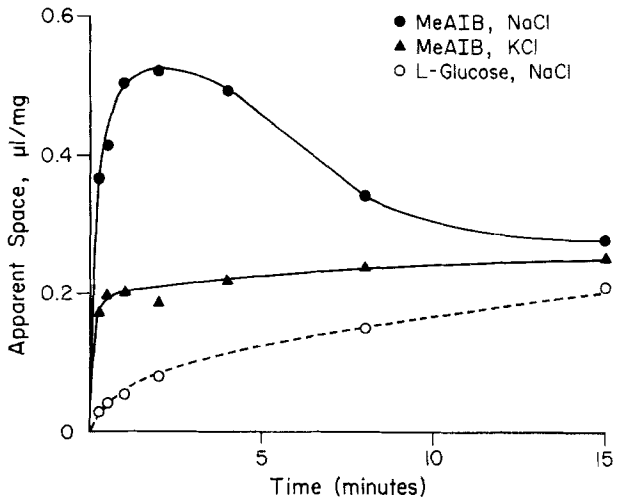

Fig. 3. Time course of MeAIB uptake. ^{14}C-MeAIB (14.4 µM) uptake was measured as described under Methods. Ionic conditions and blank corrections were as described in the legend to Fig. 1. Replacement of NaCl by KCl in the external medium had no effect on L-glucose uptake

mediating transport system. Although replacement of sodium by potassium in the extravesicular medium reduced the rate of alanine uptake more than 60-fold, alanine transport remained more rapid than L-glucose or D-mannitol uptake. In a preliminary experiment with KCl-loaded membranes in a KCl medium, the excess of alanine uptake over L-glucose uptake was abolished by 50 mM phenylalanine but not by proline or MeAIB[1]; that is, the system mediating the small sodium-independent flux of alanine has a structural specificity similar to system *L* of nonpolarized cells (Oxender & Christensen, 1963) and intestinal basolateral membranes (Mircheff et al., 1980).

Uptake of MeAIB (Fig. 3) and β-alanine (Fig. 4) was accelerated roughly twofold by an inwardly directed Na^+ gradient, and both time courses exhibited sodium-dependent overshoots. In contrast to alanine and proline, however, MeAIB and β-alanine were taken up quite rapidly, with half times less than 0.25 min, in the absence of external sodium.

Uptake of lysine (Fig. 5) was four times more rapid than uptake of L-glucose in the presence of external K, and it was accelerated twofold by inwardly directed Na^+ gradients. However, the time course of lysine uptake showed no sodium-dependent overshoot.

Indirect Inhibition of Amino Acid Uptake

Uptake of alanine, proline, MeAIB, β-alanine, and lysine is accelerated by external Na^+. We measured time courses of uptake of alanine, proline, and β-alanine by vesicles that had been preloaded with 100 mM NaCl rather than with KCl (data not shown). Substitution of the intravesicular cation had no effect on the rate of L-glucose or D-mannitol uptake, but it decreased the rate of amino acid uptake from Na-containing media to nearly the rates of uptake from K-containing media. That is, uptake of the amino acids tested depended on inwardly directed Na gradients rather than simply the presence of Na^+ in the extravesicular medium. This result carries the implication that sodium coupled fluxes of two solutes may be mutually inhibitory, because they tend to dissipate the transmembrane sodium gradient, even when the fluxes are mediated by distinct transport systems. Initially we attempted to eliminate this potential artifact by carrying out competition experiments with equal intravesicular and external Na concentrations. This strategy was adequate in the case of β-alanine uptake, but it was unsatisfactory for measurement of proline, alanine, and phenylalanine uptake, since it was difficult to obtain accurate measure-

[1] *Abbreviation*: MeAIB, N-methylaminoisobutyric acid.

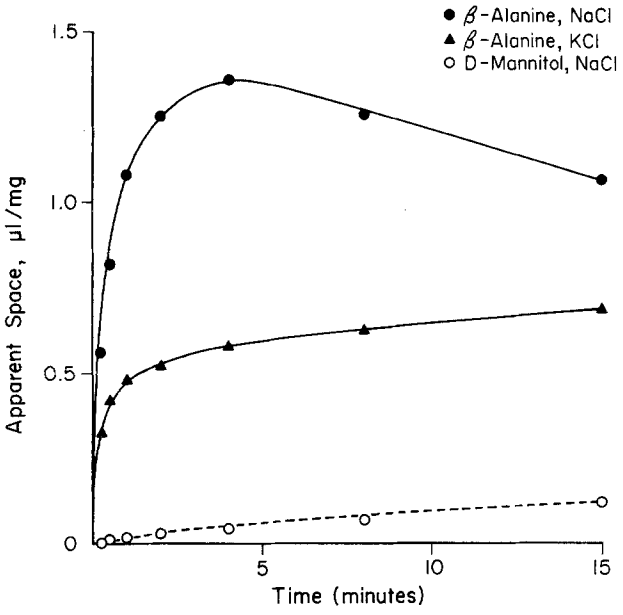

Fig. 4. Uptake of ^{14}C-β-alanine (0.09 µM) was measured as described under Methods. Ionic conditions and blank corrections were as described in the legend to Fig. 1, and replacement of external NaCl by KCl had no effect on the time course of D-mannitol uptake. In similar experiments with rabbit kidney brush border vesicles, Hammerman and Sacktor (1978) observed maximum β-alanine uptake of 20 pmol/mg when the medium β-alanine concentration was 20 µM. Multiplication of the maximum β-alanine space in Fig. 4, 1.35 µl/mg, by 20 µM would yield a β-alanine content of 27 pmol/mg

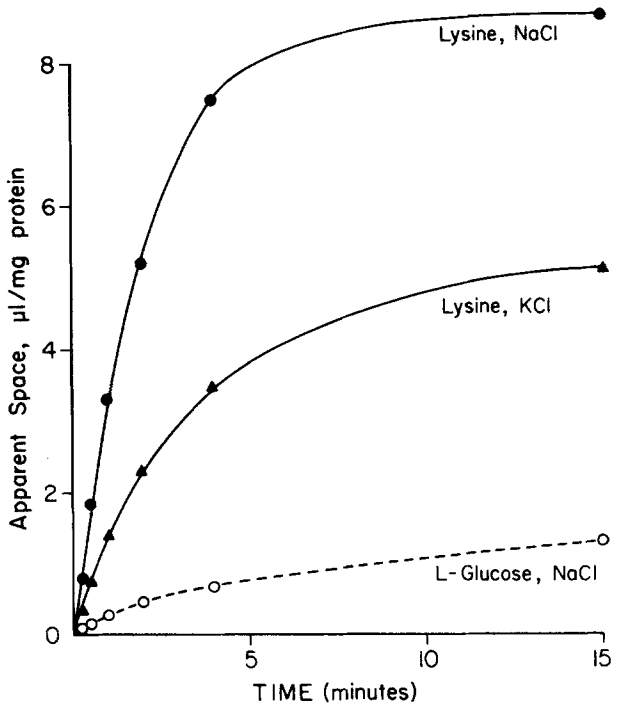

Fig. 5. Time course of L-lysine (5.4 µM) uptake. Uptake was measured as described under Methods. The intravesicular medium contained 300 mM mannitol, and the final uptake reaction media contained 100 mM mannitol plus either 100 mM NaCl or 100 mM KCl. Replacement of NaCl by KCl had no effect on the time course of L-glucose uptake. Blank corrections were made as described in Fig. 1

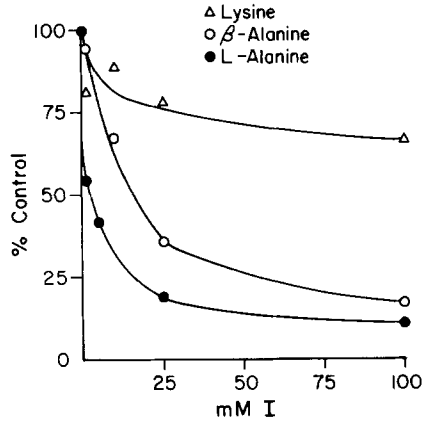

Fig. 6. Inhibition of ^{14}C-L-alanine uptake. Membranes were preloaded with a medium containing 450 mM D-mannitol. The reaction medium contained 100 mM NaCl, the specified concentrations of the test amino acids, designated I on the abscissa, and sufficient D-mannitol to maintain a constant milliosmolarity of 450. Uptake of ^{14}C-L-alanine (6.2 µM) after 0.25 min reaction was measured as described under Methods. The quantity of ^{14}C-L-alanine measured on each filter was corrected for background binding and for uptake attributable to nonmediated permeation as described under Methods. Each point represents the mean of triplicate determinations. In their study of alanine transport by rabbit kidney brush borders, Fass et al. (1977) obtained a value of 400 µM from extrapolation of a Lineweaver-Burke plot. While we do not have sufficient data for such a kinetic analysis, we note from Fig. 6 that the concentration for half maximal inhibition of ^{14}C-L-alanine uptake by L-alanine is approximately 1 mM

ments of partial inhibition of the fluxes which remained in the absence of Na gradients. Thus, in the competition experiments to be described below we measured the ability of D-glucose to inhibit amino acid fluxes in order to estimate the extent to which amino acids may inhibit sodium-coupled transport by acting to dissipate the Na gradient.

We also measured the ability of glucose to inhibit uptake of L-alanine, phenylalanine, proline, and MeAIB in the presence and absence of the protonophore FCCP. FCCP somewhat decreased uptake of the test amino acids in the absence of D-glucose; however, FCCP also decreased the fraction of the amino acid uptake which was sensitive to 100 mM D-glucose. This result tends to validate our conclusion that D-glucose reduces the rate of amino acid uptake by driving sodium fluxes which dissipate and possibly reverse the sodium electrochemical potential gradient.

Inhibition of L-*alanine Transport.* Concentration dependences for inhibition of ^{14}C-L-alanine uptake by lysine, β-alanine, and L-alanine are presented in Fig. 6. Inhibition by lysine appeared to approach its limiting value at 100 mM lysine. Similarly the curves for L-alanine and β-alanine appeared to approach li-

Table 1. Inhibition of alanine transport

Inhibitor	Inhibition of ^{14}C-L-alanine uptake
D-glucose	33.7±10.5%
MeAIB	10.4± 2.9%
L-lysine	33.7± 3.9%
L-proline	74.8± 1.3%
β-alanine	83.0± 2.1%
L-phenylalanine	96.3± 2.2%
L-alanine	89.6± 1.9%

Initial uptake of ^{14}C-L-alanine was measured, as described in the legend to Fig. 6, in the presence of 100 mM concentrations of D-glucose and the test amino acids. Values presented are the percentage reductions ±SD ($n=3$) from a control containing only tracer alanine.

Table 2. Inhibition of phenylalanine transport

Inhibitor	Inhibition of ^{14}C-L-phenylalanine uptake
D-glucose	14.5±12.2%
MeAIB	22.5± 3.9%
L-lysine	52.9± 1.1%
L-proline	58.0± 3.0%
β-alanine	45.6± 6.1%
L-phenylalanine	96.8± 1.2%
L-alanine	92.4± 0.9%

Initial uptake of ^{14}C-L-phenylalanine, as described in the legend to Fig. 6, in the presence of 100 mM concentrations of D-glucose and the test amino acids. Values presented are the percentage reductions ±SD ($n=3$) from the control containing only tracer phenylalanine.

miting values at 100 mM concentrations. Table 1 summarizes inhibition by 100 mM concentrations of D-glucose and the six test amino acids. MeAIB was less effective than D-glucose in inhibiting L-alanine uptake; thus, no significant fraction of the alanine flux can be ascribed to a system which interacts with MeAIB. Similarly, lysine was only as effective as D-glucose in inhibiting L-alanine transport, and it appears that the alanine transporting systems are unable to interact with lysine. L-alanine and phenylalanine inhibited 90 and 96%, respectively, of the initial alanine uptake. Proline and β-alanine inhibited, respectively, 75 and 84% of the L-alanine uptake. Thus, most of the L-alanine flux is mediated by a system which is also capable of interacting with phenylalanine, proline, and β-alanine. While data from Fig. 6 do not provide firm estimates of the limiting values for inhibition by β-alanine and proline, they suggest that a minor fraction of the alanine flux may be mediated by a system which is unable to interact with proline or β-alanine.

Inhibition of Phenylalanine Transport. Table 2 and Fig. 7 summarize inhibition of ^{14}C-L-phenylalanine

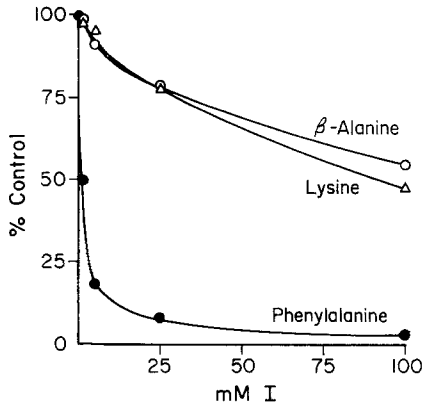

Fig. 7. Inhibition of ^{14}C-L-phenylalanine uptake. This experiment was performed exactly as described in the legend to Fig. 6. In an analogous study of phenylalanine transport by brush border membranes from rat kidney, Evers, Murer and Kinne (1976) calculated a Michaelis constant of 6 mM. With a caution about the significance of such a value when transport is mediated by two parallel systems, as discussed in the text, we note that the phenylalanine concentration yielding half maximal inhibition of ^{14}C-L-phenylalanine uptake was approximately 1 mM

Table 3. Inhibition of phenylalanine transport by combinations of amino acids

I	50 mM I	50 mM I + 50 mM L-proline	50 mM I + 50 mM L-proline	100 mM I
β-alanine	43.9±1.7%	56.5±2.3%	58.6±3.3%	47.8±6.9%
L-proline	51.3±0.9%	–	62.4±0.5%	55.6±6.6%
L-lysine	42.1±6.8%	–	–	55.5±2.3%

Initial uptake of ^{14}C-L-phenylalanine was measured essentially as described in the legends to Fig. 6 and Table 2. Each inhibitor was present at either 50 or 100 mM, and osmolarity was kept constant by adjustment of the concentration of D-mannitol. Values presented are the percentage reductions ±SD ($n=7$) from a control containing only tracer L-phenylalanine.

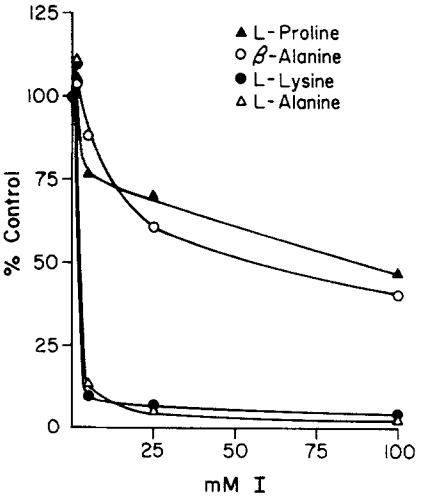

Fig. 8. Inhibition of ^{14}C-L-lysine uptake. *See* legend to Fig. 6

Table 4. Inhibition of L-lysine transport

Inhibitor	Inhibition of ^{14}C-L-lysine uptake
D-glucose	43.4±9.5%
MeAIB	49.9±7.8%
L-proline	58.2±6.2%
β-alanine	62.1±7.2%
L-phenylalanine	94.0±7.0%
L-alanine	98.2±1.9%
L-lysine	81.3±4.3%

Initial uptake of ^{14}C-L-lysine was measured, as described in the legend to Fig. 6, in the presence of 100 mM concentrations of D-glucose and the test amino acids. Values presented are the percentage reductions ±SD ($n=3$) from a control containing only tracer ^{14}C-L-lysine.

Table 5. Inhibition of L-proline transport

Inhibitor	Inhibition of ^{14}C-L-proline uptake
D-glucose	11.6±6.9%
L-lysine	(+14.3±1.8%)
β-alanine	38.6±2.4%
L-alanine	72.7±1.6%
L-phenylalanine	95.8±6.4%
MeAIB	92.0±7.0%
L-proline	97.8±0.4%

Initial uptake of ^{14}C-L-proline was measured, as described in the legend to Fig. 6, in the presence of 100 mM concentrations of D-glucose and the test amino acids. Values presented are the percentage reductions ±SD ($n=3$) from a control containing only tracer ^{14}C-L-proline.

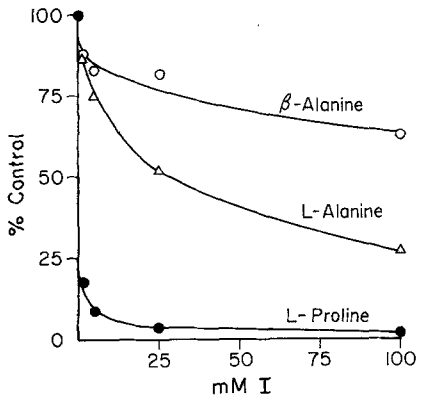

Fig. 9. Inhibition of ^{14}C-L-proline uptake. *See* legend to Fig. 6. In their study of proline transport by brush border vesicles from rabbit kidney, Hammerman and Sacktor (1977) calculated a Michaelis constant of 220 μM. Although the lowest proline concentration we tested was 1.0 mM, we can interpolate from the curve in Fig. 9 that the proline concentration giving half maximal inhibition of ^{14}C-L-proline uptake is less than 500 μM

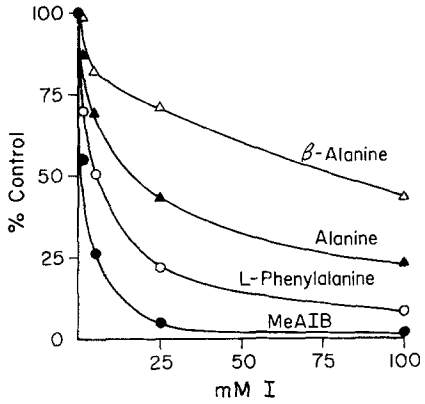

Fig. 10. Inhibition of ^{14}C-MeAIB uptake. *See* legend to Fig. 6. For comparison with the results summarized in Fig. 9, the MeAIB concentration yielding half maximal inhibition of ^{14}C-MeAIB uptake was, roughly, 1.5 mM

uptake by D-glucose and the test amino acids. Inhibition by MeAIB fell within one standard deviation of inhibition by D-glucose; thus, as was the case for L-alanine, it appears that no significant fraction of the phenylalanine flux is mediated by a system which is capable of interacting with MeAIB. Phenylalanine and L-alanine inhibited 97 and 92% of the ^{14}C-L-phenylalanine uptake, and 100 mM concentrations of lysine, proline, and β-alanine inhibited between 46 and 58% of the phenylalanine uptake. This result suggests that two distinct systems mediate roughly equal fractions of the phenylalanine transport. The effects of combinations of proline, β-alanine, and lysine are presented in Table 3. Combinations of two test amino acids at 50 mM concentration were no more effective than each amino acid alone at 100 mM concentration. Therefore, lysine, proline, and β-alanine all interact with one of the phenylalanine transporting systems; the second system appears accessible only to neutral L,α-amino acids.

Inhibition of Lysine Transport. Figure 8 and Table 4 summarize the abilities of D-glucose and the test amino acids to inhibit uptake of ^{14}C-L-lysine. Glucose inhibited a large fraction, 43%, of the lysine uptake, and inhibition by MeAIB fell within one standard deviation of inhibition by glucose. Thus, no significant fraction of the lysine transport can be attributed to a system which is capable of interacting with MeAIB. Proline and β-alanine inhibited somewhat larger fractions of the lysine uptake, while alanine and phenylalanine eliminated ^{14}C-L-lysine uptake. Therefore, it appears that a small fraction, roughly 10%, of the lysine flux may be mediated by a system which is capable of interacting with proline and β-alanine. The remainder of the lysine flux is mediated

Table 6. Inhibition of MeAIB transport

Inhibitor	Inhibition of ^{14}C-MeAIB uptake
D-glucose	28.4±3.4%
L-lysine	4.9±4.3%
β-alanine	57.0±3.2%
L-alanine	68.2±0.8%
L-phenylalanine	91.4±0.9%
MeAIB	98.4±0.5%
L-proline	96.8±0.3%

Initial uptake of ^{14}C-MeAIB was measured, as described in the legend to Fig. 6, in the presence of 100 mM concentrations of D-glucose and the test amino acids. Values presented are percentage reductions ±SD ($n=3$) from a control containing tracer MeAIB.

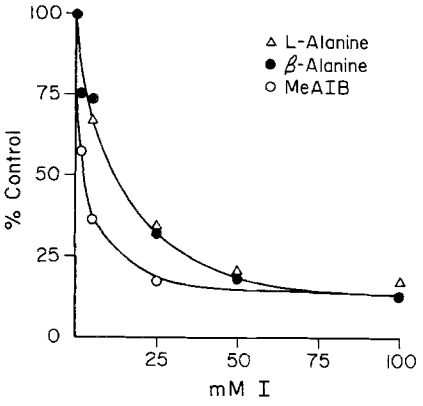

Fig. 11. Inhibition of ^{14}C-β-alanine uptake. This experiment was performed similarly to those described in Figs. 6–11, except uptake was measured in the absence of a NaCl gradient. The intravesicular medium contained 50 mM NaCl and 300 mM D-mannitol, and the extravesicular contained 50 mM NaCl, the test amino acids, and D-mannitol with a constant milliosmolarity of 400. Measuring β-alanine uptake by rabbit kidney brush border vesicles in the presence of an inwardly directed Na-gradient, Hammerman and Sacktor (1978) calculated a Michaelis constant of 30 μM. The β-alanine concentration producing half maximal inhibition of ^{14}C-β-alanine uptake in Fig. 11 is, roughly 10 mM. The reason for this disparity is not apparent, but it is possible that 50 mM NaCl in the intravesicular medium may grossly influence the affinity of the transport system for external β-alanine

by a system which interacts with L-alanine and phenylalanine, but not with the imino acids and β-amino acid.

Inhibition of Proline Transport. Inhibition of ^{14}C-L-proline uptake is summarized in Fig. 9 and Table 5. D-glucose inhibited 12%, and 100 mM lysine resulted in a small, 14%, stimulation of proline uptake. At 100 mM concentration β-alanine inhibited 39% of the proline uptake, and the data in Fig. 9 suggest that this approximates a limiting value. Proline, MeAIB, and phenylalanine virtually eliminated ^{14}C-L-proline uptake. At 100 mM, L-alanine inhibited 73% of the proline uptake; however, the data in Fig. 9 suggest

Table 7. Inhibition of β-alanine transport

Inhibitor	Inhibition of ^{14}C-β-alanine uptake
D-glucose	34.0±8.4%
β-alanine	86.6±8.2%
MeAIB	86.5±5.2%
L-proline	87.5±5.7%
L-alanine	82.4±3.9%
L-phenylalanine	71.7±5.3%

Initial uptake of ^{14}C-β-alanine was measured, as described in the legend to Fig. 11, in the presence of 100 mM concentrations of D-glucose and the test amino acids. Values presented are the percentage reductions ±SD from a control containing only tracer β-alanine.

that this may not be the limiting value. A conservative interpretation of these results is that proline transport is mediated by two systems; both systems interact with phenylalanine, alanine, and MeAIB, but only one is capable of interacting with β-alanine.

Inhibition of MeAIB Transport. MeAIB was similar to proline in its pattern of sensitivity to D-glucose and the test amino acids (Fig. 10 and Table 6). Lysine failed to significantly inhibit uptake of ^{14}C-MeAIB, while D-glucose inhibited 28% of the initial uptake. Phenylalanine and proline virtually abolished ^{14}C-MeAIB uptake, while β-alanine and L-alanine were only partially effective. It is clear that a fraction of the MeAIB flux is mediated by a system which interacts with β-alanine and L-alanine as well as with proline and phenylalanine. Since the curves for β-alanine and L-alanine do not unequivocally approach limiting values, it is not certain to what extent MeAIB flux is also carried by systems which are unable to interact with β-alanine and L-alanine.

Inhibition of β-alanine Transport. The abilities of D-glucose and the test amino acids to inhibit β-alanine was measured in early experiments depicted in Fig. 11 and Table 7, with vesicles that had been preloaded with NaCl. L-alanine, proline, and MeAIB were as effective as β-alanine in blocking ^{14}C-β-alanine uptake, and 100 mM phenylalanine inhibited 72% of the β-alanine uptake. Therefore, it is necessary to postulate only one pathway for transport of β-alanine.

Discussion

By examining the abilities of 100 mM concentrations of six test amino acids to inhibit the initial uptake of ^{14}C-amino acids, we have come to several conclusions about the number of transport pathways available to each of the labeled amino acids. Thus, β-

Table 8. Synopsis of amino acid transport systems in rabbit kidney brush border vesicles

System	Transports	Inhibited by	No interaction with
1.	Phenylalanine Alanine		MeAIB, Lysine Proline β-alanine
2.	Alanine	Phenylalanine Proline β-alanine	MeAIB Lysine
3.	Phenylalanine Lysine	Alanine Proline β-alanine	MeAIB
4.	Lysine	Alanine Phenylalanine	MeAIB Proline β-alanine
5.	Proline MeAIB β-alanine Taurine[a]	Phenylalanine Alanine	Lysine
6.	Proline MeAIB	Phenylalanine Alanine	Lysine β-alanine

[a] *See* Hammerman and Sacktor (1978) and Rozen et al. (1979). As described in the Discussion, these transport pathways are the minimum required by the results of this study. Because of difficulty in discerning maximal levels of inhibition, there remains some uncertainty about the fractions of alanine flux mediated by system *1* and MeAIB flux mediated by system *6*.

alanine flux is mediated by only one pathway. Lysine, alanine, and MeAIB each appear to have available major and minor pathways, while fluxes of phenylalanine and proline each appear to be partitioned equally between two systems.

By comparing the specificities of the amino acid transport pathways, we are able to deduce the mininum number of systems responsible for transport of the six test amino acids.

Alanine and phenylalanine are mutually competitive, and a component of the fluxes of these two amino acids is insensitive to any of the other test amino acids. Therefore, we conclude that a common neutral, L-α amino acid specific system is responsible for the β-alanine- and proline-insensitive fluxes of alanine and phenylalanine; this system is designated system *1* in Table 8. The results with lysine as inhibitor indicate that the proline and β-alanine-sensitive fluxes of alanine and phenylalanine must be mediated by two separate systems.

Lysine interacts with the β-alanine- and proline-sensitive system which transports phenylalanine; this system, designated system *3* in Table 8, cannot carry a significant fraction of the alanine flux, since alanine uptake is insensitive to lysine. Similarly, no significant fraction of the phenylalanine flux may be mediated by the proline- and β-alanine-sensitive, alanine-transporting system, designated system *2*, since lysine is as effective as proline and β-alanine in inhibiting phenylalanine transport. The characteristics of these systems are summarized in Table 8.

Lysine flux is partitioned into two components, one of which is sensitive to proline and β-alanine. Both components are sensitive to alanine and phenylalanine, while neither is sensitive to MeAIB. The most economical conclusion is that the proline- and β-alanine-sensitive component of lysine flux is mediated by the same system that is responsible for the proline-, β-alanine- and lysine-sensitive flux of phenylalanine, i.e., system *3* of Table 8. The second lysine transporting system, that which is insensitive to proline or β-alanine, cannot account for a significant fraction of the alanine flux, since neither of the alanine transport pathways interacts with lysine. Nor can the second lysine-transporting system account for a significant fraction of the phenylalanine flux, since the only component of phenylalanine transport which is sensitive to lysine is equally sensitive to proline and β-alanine. The second lysine transporting system is designated system *4* in Table 8. Christensen and Liang (1966) have also observed that lysine is transported by a multiplicity of systems in Ehrlich cells.

Two separate systems mediate the uptake of proline, one which interacts with, and one which is insensitive to, β-alanine. At least 57% of the initial uptake of MeAIB was also inhibited by β-alanine, but since inhibition of MeAIB uptake by β-alanine showed no approach to an asymptote, it is not clear to what extent MeAIB may be transported by a system which is unable to interact with β-alanine. Proline and MeAIB inhibit each other's fluxes, and both abolish uptake of β-alanine. The fluxes of proline, MeAIB, and β-alanine are also completely inhibited by phenylalanine, and they are insensitive to lysine. Therefore, we conclude that a single system, designated system *5* in Table 8, accounts for both β-alanine transport and for the β-alanine-sensitive components of the fluxes of proline and MeAIB; the second proline-transporting system, that which is insensitive to β-alanine, is designated system *6* in Table 8; it may also mediate a fraction of the MeAIB flux. Since transport of taurine has been shown to be inhibited by β-alanine (Rozen, Tenenhouse & Scriver, 1979), we conclude that the β-amino acid is also transported by system *5*. This system appears to be similar to the β-alanine transporting system of Ehrlich cells (Christensen, 1964). Neither of these systems can account for significant fractions of the fluxes of alanine, phenylalanine, and lysine, since these fluxes are not directly inhibited by MeAIB.

Previous investigations of the specificities of the systems mediating transepithelial amino acid fluxes

have employed the small intestine from various species. There are striking morphological and functional similarities between epithelial cells of the small intestine and the proximal tubule; for example, glucose transport in both tissues involves sodium gradient driven accumulation across the brush border membrane followed by glucose gradient driven facilitated diffusion across the basal lateral membrane. We are wary of overemphasizing the analogy between small intestine and proximal tubule, but it is worth noting that several studies have produced evidence that intestinal brush border membranes contain at least two distinct pathways for neutral, L,α-amino acids. Schultz and Markscheid-Kaspi (1971) found that alanine and phenylalanine inhibited each other's unidirectional influx in rabbit ileal segments, but the K_m for each amino acid was significantly different from its K_I. More recently Sepulveda and Smith (1978) and Paterson, Sepulveda and Smith (1979) analyzed the kinetics of unidirectional influxes of selected L-α-amino acids and their competition by L-serine in terms of two parallel transport systems, both of which transport long chain and aromatic amino acids but only one of which interacts with serine. Our analysis leads to the conclusion that there are at least three distinct neutral, L,α-amino acid transporting systems in kidney brush borders. It is possible that proximal tubular brush borders may contain systems which are absent from small intestinal brush borders of the same animal. It is also possible that previous analyses have failed to discriminate between systems which are similar according to certain criteria, yet quite distinct when different criteria are applied. For example, systems *2* and *3* from Table 8 are similar in the sense that both interact with alanine and phenylalanine and with β-alanine and proline. However, system *2* does not interact with lysine, while system *3* interacts with, and probably carries a significant fraction of, the flux of lysine; it was precisely this differential sensitivity to lysine which led us to postulate the existence of systems *2* and *3*.

The three neutral L,α-amino acid transporting systems we have delineated appear to be distinct from the classical sodium-stimulated amino acid transport systems found in nonpolarized cells, most notably reticulocytes and ascites cells (Oxender & Christensen, 1963; Christensen, Liang & Archer 1967) and in the basal lateral plasma membranes of intestinal epithelial cells (Mircheff et al., 1980). The classical system *A* transports alanine and phenylalanine, as does system *1* from Table 8; however, system *A* also transports MeAIB, while systems *1, 2* and *3* fail to interact with MeAIB. System *ASC* excludes MeAIB, and it is limited to the short chain, primary L,α-amino acids. System *2* from Table 8 might be expected to have such a specificity, but it, as well as, systems *1* and *3* differ from the classical system *ASC* in that the kidney brush border systems can be completely inhibited by phenylalanine. We have not yet studied amino acid transport in intestinal brush borders or renal basal lateral membranes. However, there is emerging a general picture that the brush border membranes of epithelial cells contain sodium-coupled transport mechanisms for glucose and for neutral, L-α-amino acids which are unqiue to epithelial cells, while the mechanisms which facilitate glucose and L,α-amino acid efflux across the basal lateral membranes are similar, if not identical, to the transport systems of nonpolarized cells.

With the caveat that there may be immense species differences in the complement of brush border amino acid transport systems, it is worthwhile to note that several of the systems delineated in our study have specificities consistent with the systems postulated on the basis of the transport defects observed in the malabsorption syndromes. Perhaps the simplest example is β-amino aciduria (Scriver, Pueschel & Davies, 1966), since only one pathway, system *5* of Table 8, appears to be available for transport of β-alanine across the rabbit kidney brush border membrane. The primary defect in the disease appeared to be a block in β-alanine-α-ketoglutarate transaminase rather than an alteration in a β-amino acid transporting system. However, high concentrations of β-alanine would have saturated a renal transport system, reducing the rate of reabsorption of other β-amino acids. Two pathways, systems *5* and *6*, are available to proline; system *6*, which excludes β-alanine, carries the larger fraction of the tracer flux of proline, and we speculate that a defect in this system would result in prolinuria (Scriver, Efron & Schafer, 1964) and diminished intestinal absorption of proline (Goodman, McIntyre & O'Brien, 1967). Similarly, two pathways, systems *3* and *4*, are available to lysine; system *4* carries the larger fraction of the tracer lysine flux, and damage to this system might markedly impair lysine reabsorption in the kidney or absorption in the small intestine, as is observed in cystinuria (Thier et al., 1965). Cystine and dibasic amino acids share common transport systems in rat renal brush borders (Segal, McNamara & Pepe, 1977). The fact that there are two separate lysine transporting systems also suggests a possible explanation for the existence of three distinct cystinurias (Rosenberg et al., 1966); damage to system *3* alone might produce a moderate decrease in lysine transport, while simultaneous damage to systems *3* and *4* would have the most severe impact on lysine transport.

Hartrup disease is characterized by increased urinary excretion (e.g., Scriver, 1965) and decreased intestinal absorption (Shih et al., 1971) of the neutral, L,α-amino acids. Our data suggest the existence of

three separate neutral, L,α-amino acid transport systems. Our characterization of these systems is not complete enough to sustain firm conclusions about how they may be involved in Hartrup disease, and we have not found in the literature detailed information about intestinal transport of the individual test amino acids we have employed in this study. It is possible, for example, that a defect in system *2*, which carries most of the tracer alanine flux, could, indirectly, by causing elevated luminal concentrations of alanine and other amino acids transported by this system, inhibit transport of phenylalanine via systems *1* and *3*.

Using simple technical and analytical strategies, we have succeeded in delineating six distinct amino acid transport systems in rabbit kidney brush borders. This work provides a sketch of the multiplicity of amino acid transport systems in this membrane; it is far from complete in the sense that application of additional test amino acids may reveal additional heterogeneity in the transport systems, and in the sense that none of the systems so far delineated has been characterized in terms of its kinetic parameters or its ability to carry out amino acid exchange reactions. Analysis of the kinetic characteristics of parallel systems for a single amino acid, e.g., systems *1* and *3*, is complex; however, our present results suggest technical means for simplifying the analytical problem since they show it possible to selectively inhibit certain systems with high concentrations of appropriate amino acids. Our results also open new mechanistic questions. That is, fluxes of lysine, MeAIB, and β-alanine are stimulated by inwardly directed sodium gradients, but substantial fluxes also occur in the absence of sodium. In preliminary experiments the general pattern of sensitivity of lysine uptake was unchanged when KCl replaced NaCl in the external medium, and we tentatively hypothesize that the sodium-stimulated lysine transporting systems are also capable of translocating lysine in the absence of sodium.

Despite the gaps remaining in our understanding of renal and intestinal amino acid transport, we feel that this study will provide a useful paradigm for analysis of the multiplicity of amino and transport systems in human intestinal and renal brush borders, as well as for attempts to understand the mechanistic details of these transport processes.

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