Polarized Amino Acid Transport by an Epithelial Cell Line of Renal Origin (LLC-PK₁)

THE BASOLATERAL SYSTEMS*

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The absorption of neutral amino acids across the renal proximal tubule and intestinal epithelia involves at least two sets of transport systems arranged in series. Absorption is achieved by the entry of the amino acids into the epithelial cells through the brush border membrane by an uphill, saturable, stereospecific, and Na⁺-dependent transport system (1-3). The amino acids which accumulate within the cell by the luminal Na⁺-co-transport mechanism, leave the cell at the contraluminal side by other carrier-mediated mechanisms. In contrast to the Na⁺-dependent sugar absorption, the active Na⁺-dependent step could be localized not only in the apical but also in the basolateral membrane of epithelial cells depending on the amino acid in consideration. For instance, studies performed with plasma membrane vesicles (2, 4) and measurements of the unidirectional fluxes from the lumen into the epithelial cell (5), have shown that, in the brush border membrane of the proximal tubular cells and intestinal epithelia, there is a transport system for the neutral amino acids alanine and phenylalanine in which the specific substrate is co-transported with Na⁺. The transport of amino acids from the same group across the basolateral membrane of intestinal cells occurs through three distinct transport systems (6). These systems, originally designated A (for alanine), ASC (for alanine, serine, and cysteine), and L (for leucine), are distinguished by their dependence or independence on sodium and by the nature of the amino acids which they transport (7). System A is Na⁺-dependent and transports most strongly neutral amino acids with short, polar, or linear side chains. System ASC is also Na⁺-dependent, but excludes N-methylated derivatives including the nonmetabolizable model substrate MeAIB. System L is sodium-independent and does not tolerate a highly branched side chain or N-methyl group. In the kidney, basolateral amino acid transport by systems A and ASC has been suggested by experiments with the multiple indicator-dilution technique (8) and with isolated perfused proximal tubules (9). However, a complete characterization of the amino acid transport systems in this membrane is not presently available. In addition, it remains uncertain whether transport into renal epithelium meets the A/ASC/L pattern and whether other systems contribute to neutral amino acid transport.

The LLC-PK₁ cells grown on a permeable support provide a cell culture model that exhibits several characteristics of in vitro epithelial membranes. For instance, we have demonstrated that this epithelial cell line from a pig kidney has a Na⁺-dependent hexose (10) and phosphate (11) transport system with characteristics similar to those observed in the proximal tubule. Further studies have localized the uphill, saturable, and Na⁺-dependent sugar transport in the apical membrane of these cultured epithelial cells (12). The simplicity of the model and the possibility to reconstitute a complete monolayer from isolated cells makes it an ideal tool for studying the development of the epithelial cell polarization. On the basis of these observations, we decided to investigate whether other transport systems were also specifically localized in the basolateral membrane of LLC-PK₁ cells. The presence of these transport systems will be used as functional marker of this membrane during the study of events associated with the genesis and development of epithelial cell polarization.

MATERIALS AND METHODS

Cell Culture and Monolayer Preparation—LLC-PK₁ cells obtained from The American Type Culture Collection (CRL 1392) were

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1 The abbreviations used are: AIB, 2-aminoisobutyric acid; MeAIB, 2-(methylamino)isobutyric acid; cycloleucine, L-amino-cyclopentane-1-carboxylic acid; BCH, 2-aminobicyclo(2,2,1)heptane-2-carboxylic acid; EBSS, Earle's balanced salt solution.
maintained by serial passage in 10-cm diameter plastic tissue culture dishes (Costar, Cambridge, MA). The cells were fed with Dulbecco's modified Eagle's medium with 1-glutamine and streptomycin (100 µg/ml). All cultures were maintained in an atmosphere of 5% CO₂ in air at 37°C. When cell growth reached saturation, subcultures were prepared using a 0.02% EDTA, 0.05% trypsin solution. The cells were used between the 209th and the 240th passage.

Monolayers on a permeable support were prepared using a polycarbonate filter membrane of 25 mm diameter, and with 5-µm pores (Nucleopore Corp., Pleasanton, CA). The filters were covered with a very thin layer of 0.5% collagen dispersion (BioWhittaker) and applied to a standard microscope slide. The methods for collagen aggregation and sterilization of the collagen-coated membrane, were described elsewhere (13). The total thickness of this permeable support is 12 µm.

**Uptake Assay**—Amino acid uptake was measured in Earle's balanced salt solution. Under an atmosphere of approximately 5% CO₂ in air the pH of the solution was 7.4. For sodium-free assays the NaHPO₄, NaCl, and NaHCO₃ were replaced by K₂HPO₄, choline chloride, and choline bicarbonate, respectively. The monolayers grown on collagen-coated Nucleopore filter membranes were removed from the microscope slide to provide free access to the amino acid from both sides of the membrane. They were washed for 15 s with Earle's solution and then allowed to take up the labeled amino acid for various times at 25°C.

The uptake medium with the labeled amino acid also contained methoxy-[3H]inulin or L-[3H]glucose as extracellular markers. The unlabeled amino acid influx was determined under conditions approaching initial entry rates in the first 1 to 5 min of incubation. At the end of each uptake period, the monolayers were washed for 15 s with ice-cold sodium-free Earle's solution. After dissolving the samples with tissue solubilizer (NCS tissue solubilizer Amersham), the radioactivity was measured by liquid scintillation using Dimilume 30 (Packard Instrument Co.) scintillation fluid. Corrections for interstitial trapping were made by measuring the amount of methoxy-[3H]inulin or L-[3H]glucose associated with each sample. After a 15-s wash, there was a 97% reduction in the L-[3H]glucose count. However, even after 10 min of washing in ice-cold EBSS solution, no further changes were observed in the amino acid content other than those observed in the first 15 s indicating that the intracellular content was not affected by this procedure. Rates of amino acid uptake were normalized for the DNA content of each sample.

**Measurement of Cellular Water**—Cell volume measurements were conducted by a modification of the method described by Kletzen et al. (14). 3-O-Methyl-d-glucose seems to inhibit the active transport of α-methylglucoside (10), and it is possible that this inhibition could also represent active transport. Therefore, to avoid this possibility we utilized L-arabinose, another nonmetabolizable sugar for eukaryotic cells, which is transported into LLC-PK₁ monolayers only by a facilitated diffusion mechanism. In contrast to 3-O-methyl-d-glucose, L-arabinose required a longer incubation to reach equilibrium between intracellular and extracellular concentrations. The amount of L-[3H]arabinose retained by the intracellular compartment was determined following 3 h incubation in tracer amounts of the labeled sugar. In the same experiments, the volume of cellular water determined by this method was compared with the cell volume estimated from the difference between total water (1H2O) area space) and the extracellular fluid volume (L-[3H]glucose space). Almost identical results were obtained by the two methods. Cellular water was normalized for the DNA content of each sample.

**Electrolyte Determination**—Confluent monolayers of LLC-PK₁ cells were incubated for 5 min in Earle's solution containing L-[3H]glucose (1 µCi/ml) as extracellular marker. After equilibration, the incubation medium was removed and the monolayer washed with 0.2 M cold sucrose solution. Previous studies indicate that the intracellular electrolyte content is not affected by this washing procedure. The monolayers were extracted overnight (18 h or more) in 1 ml of 0.1 M nitric acid. Sodium and potassium were measured in the acid extract by an ion-sensitive electrode (Radiometer, Copenhagen, Denmark) using external standard preparations in 0.1 M nitric acid. L-[3H]Glucose was measured in 0.1-ml samples by liquid scintillation counting after neutralization with 0.1 M NaOH. After correcting for the extracellular fluid, the results were normalized for the DNA content of each sample.

**DNA Assay**—DNA was measured by a modification of the fluorometric micromethod of Switzer and Summer (15).

**Materials**—All sera, growth media, antibiotics, and trypsin-EDTA solutions were from Gibco (Grand Island, NY), 2-[methyl-3H]aminosyrutic acid (specific activity 10 Ci/mmol), N-methyl-2-amino-[1-3H]isobutyric acid (specific activity 53.5 µCi/mmol), L-1-amino-cyclopentane-[1-14C]carboxylic acid (specific activity 48.5 µCi/mmol), [methoxy-3H]inulin (specific activity 200 µCi/g), and [carboxy-14C]lactose (specific activity 1.91 mCi/g) were purchased from New England Nuclear. L-[1-14C]Alanine (specific activity 40 mCi/mmol) was obtained from ICN (Irvine, CA). All of the unlabeled amino acids, as well as L-arabinose and calf thymus deoxyribonucleic acids were obtained from Sigma. 3,5-Diaminobenzoic acid hydrochloride and BCH were obtained from Aldrich.

**RESULTS**

**AIB Uptake**—In the presence of sodium, the amino acid is accumulated by the monolayers (figure not shown). Apparent intracellular concentrations of AIB were calculated after correcting for the extracellular space and by using intracellular water values of 110 ± mg DNA⁻¹ and 125 ± mg DNA⁻¹ measured in AIB concentrations of 1 and 10 mM, respectively. The intracellular AIB concentration calculated after 90 min incubation were 4.7 mM and 16.6 mM, respectively.

**AIB Medium Concentration and Initial Uptake Velocity**—Initial uptake velocity was determined during a 1- to 5-min uptake over a 40-fold range of AIB concentration (from 0.1 to 40 mM) with or without Na⁺ in the incubation medium. The characteristic of the uptake in the presence of Na⁺ suggests that entry of the analog could be mediated by both saturable and nonsaturable transport processes. In the absence of Na⁺, however, the initial entry rates at concentrations between 0.1 to 40 mM show only a linear component. After subtracting the Na⁺-independent component from the total entry in presence of Na⁺, it appeared that the remaining component was a saturable process (Fig. 1a). This latter process displayed a single component when analyzed by an Eadie-Hofstee representation (Fig. 1b). Thus, kinetic analysis has resolved the curvilinear characteristic of the total entry in presence of Na⁺ into at least two components: one which is Na⁺-dependent with a Kₐ of 4.7 mM and a Vₘₐₓ of 3.95 pmol h⁻¹ mg DNA⁻¹, and one which is Na⁺-independent and apparently nonsaturable.

**External Medium Sodium Concentration and AIB Uptake**—The results presented in Fig. 1 indicate that a large fraction of AIB uptake occurs by a Na⁺-dependent process.
This effect is quite specific for Na+ since other alkali-cation are unable to induce similar effects. The uptake in a medium in which Na+ was replaced by an equivalent amount of cation (i.e. Li+, Rb+, Cs+, or K+) was 0.016 ± 0.003, 0.021 ± 0.002, 0.021 ± 0.003, and 0.016 ± 0.002 μmol h⁻¹ mg DNA⁻¹, respectively. The control value in Na+-containing medium was 0.069 ± 0.002 μmol h⁻¹ mg DNA⁻¹. Each value represents the mean of five to eight monolayers ± S.E. p was <0.001 in all cases. The effect of Na⁺ concentrations on the initial entry rates of AIB were measured using amino acid concentrations of 0.5 to 10 mM in the presence of 28.6 and 143 mM Na⁺ (Fig. 2). Increases in sodium concentration in the external medium caused an increase in Vmax, from 1.15 to 2.14 μmol h⁻¹ mg DNA⁻¹. The apparent Kₙ for AIB, however, was not affected (5.41 mM at 28.6 mM sodium and 5.49 mM at 143 mM sodium).

Fig. 3 shows that the apparent Kₙ of sodium ranges only between 7.55 to 5.49 mM while the apparent Vmax range between 0.15 to 1.38 μmol h⁻¹ mg DNA⁻¹ when these parameters are determined at AIB concentration of 0.5, 1, 4, and 10 mM. Ouabain at a concentration of 5 μM produces a partial inhibition of AIB uptake. No further effect was observed, on increasing the ouabain concentration to 1 mM. The partial inhibition of AIB uptake induced by the glucoside is associated with a complete dissipation of the electrolyte gradients across the cell membrane (Table I). Removal of Na⁺ from the incubation medium seems to be more effective than ouabain in blocking the uptake of AIB. This effect, however, is not associated with significant changes in the intracellular electrolyte concentration. The 45% reduction in the potassium content (Table I) was associated with a reduction in the cell volume from 119 ± 9 μl mg DNA⁻¹ (n = 8) to 69.0 ± 6 μl mg DNA⁻¹ (n = 6) such that there was no change in the intracellular potassium concentration.

Efflux of AIB from Monolayers of LLC-PK, Cells—The monolayers were incubated for 60 min at 10 mM concentration of [³H]-labeled AIB. After three 30-s washes in cold Earle's solution, the monolayers were transferred to flasks containing the incubation solution at 37 °C, with or without unlabeled amino acids. Results showing the effect of AIB and proline on the [³H]AIB efflux are presented in Fig. 4. The presence of AIB in the incubation medium not only does not stimulate, but apparently inhibits the efflux of [³H]AIB from the intracellular compartment as shown by a reduction in the efflux constant from 0.0037 min⁻¹ under control conditions (no amino acid present) to 0.0029 min⁻¹ (10 mM AIB in the incubation medium). Proline, on the contrary, also at a concentration of 10 mM, produces a clear stimulation of AIB efflux as shown by the increase in the efflux constant to 0.0108 min⁻¹.

MeAIB Uptake—As occurs with AIB, the uptake of MeAIB was also diminished in absence of Na⁺. An analysis similar to the one performed with AIB shown in Fig. 1 indicates that the total uptake of MeAIB can be resolved into two components: an apparently nonsaturable Na⁺-independent component, and a saturable Na⁺-dependent component with a Kₙ of
with or without Na+. The uptake of AIB was then followed in EBSS alone (C—O) or EBSS with 10 mM AIB (—o) or 10 mM proline (Δ—Δ). Each value is the mean of six determinations and are accurate to within 10% S.E. Best fitting lines were determined by linear regression analysis ($r^2 = 0.94, 0.95, and 0.98$).

Fig. 5 (right). Inhibition of AIB influx by MeAIB. a, inhibition of AIB influx at a concentration of 0.1 mM Na+-containing media. The broken line represents the nonsaturable influx measured in Na+-free medium. b, plot to determine portion of the uptake of AIB subject to competitive inhibition by MeAIB. $V_o$ is the rate of uptake of AIB without MeAIB. $V_i$ is the rate of uptake at a given concentration of MeAIB. The intercept on the ordinate will give the reciprocal of the velocity that is subject to inhibition. Best fitting line was determined by linear regression analysis ($r^2 = 0.98$). Each value is the mean of nine monolayers ± S.E.

Table II: Effect of various amino acids on influx of AIB in LLC-PK1 monolayers

<table>
<thead>
<tr>
<th>Amino acid group</th>
<th>Inhibitor (10 mM)</th>
<th>AIB influx</th>
<th>Per cent change as compared to control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uncharged</td>
<td>None + Na</td>
<td>0.051 ± 0.005</td>
<td>-89</td>
</tr>
<tr>
<td></td>
<td>Na</td>
<td>0.016 ± 0.003</td>
<td>-50</td>
</tr>
<tr>
<td>Nonpolar</td>
<td>Alanine</td>
<td>0.026 ± 0.001</td>
<td>-84</td>
</tr>
<tr>
<td></td>
<td>Isoleucine</td>
<td>0.048 ± 0.001</td>
<td>-80</td>
</tr>
<tr>
<td></td>
<td>Phenylalanine</td>
<td>0.036 ± 0.001</td>
<td>-65</td>
</tr>
<tr>
<td></td>
<td>Proline</td>
<td>0.029 ± 0.004</td>
<td>-40</td>
</tr>
<tr>
<td></td>
<td>Valine</td>
<td>0.044 ± 0.001</td>
<td>-40</td>
</tr>
<tr>
<td></td>
<td>MeAIB</td>
<td>0.031 ± 0.001</td>
<td>-40</td>
</tr>
<tr>
<td>Uncharged</td>
<td>Cysteine</td>
<td>0.006 ± 0.002</td>
<td>-39</td>
</tr>
<tr>
<td></td>
<td>Glutamine</td>
<td>0.020 ± 0.004</td>
<td>-61</td>
</tr>
<tr>
<td></td>
<td>Glycine</td>
<td>0.036 ± 0.002</td>
<td>-29</td>
</tr>
<tr>
<td></td>
<td>Serine</td>
<td>0.016 ± 0.003</td>
<td>-65</td>
</tr>
<tr>
<td>Charged</td>
<td>Aspartic acid</td>
<td>0.172 ± 0.004</td>
<td>+237</td>
</tr>
<tr>
<td></td>
<td>Glutamic acid</td>
<td>0.260 ± 0.006</td>
<td>+409</td>
</tr>
<tr>
<td></td>
<td>Charged Lysine</td>
<td>0.260 ± 0.005</td>
<td>+409</td>
</tr>
<tr>
<td></td>
<td>Arginine</td>
<td>0.304 ± 0.005</td>
<td>+452</td>
</tr>
</tbody>
</table>

*Significantly different: $p < 0.001$.
*No significant difference.
*Significantly different: $p < 0.01$.

The monolayers were incubated for 60 min at 37 °C in complete EBSS with 10 mM [3H]AIB to accumulate the radioactive analog. The uptake of AIB was then followed in EBSS alone (C—O) or EBSS with 10 mM AIB (—o) or 10 mM proline (Δ—Δ). Each value is the mean of six determinations and are accurate to within 10% S.E. Best fitting lines were determined by linear regression analysis ($r^2 = 0.94, 0.95, and 0.98$).
Amino Acid Transport by a Renal Cell Line

TABLE III
Effect of various amino acids on influx of MeAIB in LLC-PK, monolayers.

<table>
<thead>
<tr>
<th>Amino acid group</th>
<th>Inhibitor (10 mM)</th>
<th>MeAIB influx μmol h⁻¹ mg DNA⁻¹</th>
<th>Per cent change as compared to control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uncharged</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonpolar</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uncharged</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polar</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Charged</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acidic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basic</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Condition</th>
<th>AIB influx</th>
<th>MeAIB influx</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.068 ± 0.004 (8)</td>
<td>0.049 ± 0.004 (8)</td>
</tr>
<tr>
<td>10 mM BCH</td>
<td>0.070 ± 0.004 (8)</td>
<td>0.042 ± 0.004 (8)</td>
</tr>
</tbody>
</table>

For details see legend in Fig. 5b.

rate of uptake of cycloleucine at a concentration of 0.1 mM in Na⁺-free medium. The results show an almost complete inhibition of the Na⁺-independent cycloleucine uptake by BCH. A plot of these data as in Fig. 5b yields a straight line. The ordinate intercept of Fig. 7b indicates that the BCH-inhibitable fraction is 94% of the total influx of cycloleucine in Na⁺-free medium. Fig. 8 represents the inhibition by AIB of the initial rate of uptake of cycloleucine at a concentration of 0.1 mM in Na⁺-containing medium. The residual part of cycloleucine transit after maximal inhibition by AIB is almost identical with the influx of cycloleucine in Na⁺-free medium. Thus, the inhibition produced by the analog agrees quite well with the relative magnitude of the saturable, Na⁺-dependent fraction of cycloleucine influx. MeAIB was completely ineffective in inhibiting the influx of cycloleucine in Na⁺-containing medium.

Polarized Influx of AIB and Cycloleucine in Monolayers of LLC-PK, Cells—Table V shows the influx of AIB at a concentration of 0.1 mM in medium with or without Na⁺ when the uptake is measured from the apical or basolateral side of the monolayer. The results indicate that most (98%) of the Na⁺-dependent influx of AIB occurs from the basolateral side. No difference was observed when the uptake in the presence or absence of Na⁺ was measured from the apical side. From the basolateral side, however, the uptake in the presence of Na⁺ was 1.9 times larger than the uptake in the absence of Na⁺. The uptake from the basolateral side in the presence of Na⁺ is also 2.9 and 3.5 times larger than the uptake from the apical side in presence and absence of Na⁺, respectively.

The influx of cycloleucine also shows a polarized distribu-

Fig. 6. Time course of cycloleucine uptake. Monolayers of LLC-PK, cells were incubated in EBSS containing 1 mM cycloleucine with (—O—O) or without Na⁺ (—O—O) and 10 mM cycloleucine with Na⁺ (—O—O) plus 0.25 μCi/ml of [¹⁴C]cycloleucine and 1 μCi/ml of [¹³H]julin as extracellular marker. The monolayers were allowed to take up the [¹⁴C]cycloleucine for various times at 25 °C. Each point is an average of eight monolayers ± S.E.

Fig. 7. Inhibition of cycloleucine influx by BCH in Na⁺-free medium. a, inhibition of cycloleucine influx at a concentration of 0.1 mM by BCH in Na⁺-free medium. b, plot to determine the portion of the uptake of cycloleucine subject to inhibition by BCH. V₂ and V₁ have similar meaning as in legend Fig. 5b. The ordinate intercept C gives the reciprocal of the velocity that is subjected to inhibition. Best fitting lines were determined by linear regression analysis (r² = 0.99). Each value is the mean of seven monolayers ± S.E.

Fig. 8. Inhibition of cycloleucine influx by AIB and MeAIB in Na⁺-containing media. a, inhibition by AIB (—O—O) and MeAIB (—O—O) of cycloleucine influx at a concentration of 0.1 mM in Na⁺-containing media. The broken line represents the influx of cycloleucine measured in Na⁺-free medium. b, plot to determine portion of the cycloleucine uptake subject to inhibition by AIB. For details see legend in Fig. 5b.
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TABLE V
Polarized influx of AIB by monolayers of LLC-PK₁, cells

Cells were allowed to grow and form confluent monolayers on the permeable support. Each monolayer was then suspended between two Lucite chambers. Uptake in presence or absence of Na⁺ was performed in 1.0 mM cycloleucine for 5 min at 25 °C from the apical or basolateral side of the monolayer. The integrity of the monolayers during the 5-min uptake was controlled by measuring the transepithelial electrical resistance. A value of 88 ± 3 Ω cm² (n = 22) was obtained that remained constant during the uptake period. Uptake values are the mean of five to six determinations ± S.E.

| Uptake from | Condition | Total influx | Na⁺-dependent influx | p  
|-------------|-----------|--------------|----------------------|-----
| Apical      | +Na⁺      | 0.016 ± 0.001| 0.003 ± 0.001        |     
|             | −Na⁺      | 0.013 ± 0.001|                      |     
| Basolateral | +Na⁺      | 0.046 ± 0.003| 0.022 ± 0.001        | <0.001 
|             | −Na⁺      | 0.024 ± 0.003|                      |     

TABLE VI
Polarized uptake of cycloleucine by monolayers of LLC-PK₁, cells

Cells were allowed to grow and form confluent monolayers on the permeable support. Each monolayer was then suspended between two Lucite chambers. Uptake in presence or absence of Na⁺ was performed in 1.0 mM cycloleucine for 5 min at 25 °C from the apical or basolateral side. The integrity of the monolayers during the 5-min uptake was controlled by measuring the transepithelial electrical resistance. A value of 88 ± 3 Ω cm² (n = 22) was obtained that remained constant during the uptake period. Uptake values are the mean of five to six determinations ± S.E.

<table>
<thead>
<tr>
<th>Uptake from</th>
<th>Condition</th>
<th>Cycloleucine uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>μmol h⁻¹ mg DNA⁻¹</td>
</tr>
<tr>
<td>a. Apical side</td>
<td>+Na⁺</td>
<td>0.26 ± 0.004</td>
</tr>
<tr>
<td>b. Apical side</td>
<td>−Na⁺</td>
<td>0.29 ± 0.024</td>
</tr>
<tr>
<td>c. Basolateral side</td>
<td>+Na⁺</td>
<td>4.01 ± 0.48</td>
</tr>
<tr>
<td>d. Basolateral side</td>
<td>−Na⁺</td>
<td>1.88 ± 0.12</td>
</tr>
</tbody>
</table>

DISCUSSION

The present study provides evidence that monolayers of LLC-PK₁, an epithelial cell line of renal origin, retain the capacity to accumulate amino acids. The different pathways of amino acid influx were analyzed by studying the uptake of three nonmetabolizable analogs: AIB, MeAIB, and cycloleucine.

AIB is accumulated by LLC-PK₁ cells through a mechanism with several features of a carrier-mediated process. The total influx of AIB can be divided into at least two components. The Na⁺-independent component appears to be a nonsaturable process. Although the absence of saturation, the lack of response to structural analogs, and the absence of concentrative capacity suggest a simple diffusion mechanism, this interpretation must be taken with reservation. Na⁺-independent AIB uptake with characteristics similar to those reported here has been observed in a large variety of cells (16-18).

The first order dependence of AIB influx with respect to Na⁺ concentration supports the notion that only one Na⁺ ion may serve as a co-substrate for each molecule of amino acid transported. The apparent Kₐ for AIB influx is nearly identical with the Kₐ of the high affinity system reported in rat kidney cortex (19, 20), and appears to be independent of the Na⁺ concentration in the incubation medium. In contrast, the apparent Vₘₐₓ is enhanced when the Na⁺ concentration is increased. This suggests that the stimulatory effect of Na⁺ probably occurs on translocation of the carrier-AIB complex across the cell membrane (16).

The apparent Kₐ for the Na⁺ effect on AIB influx does not appear to be dependent on AIB concentration. The apparent Vₘₐₓ, however, is clearly enhanced by increased AIB concentration. These results are compatible with a model in which the amino acid and Na⁺ combine with the carrier to form a ternary complex (16). In this process, the binding of each substrate occurs independently of the other (14).

The partial inhibition of AIB influx by ouabain at concentrations that completely inhibit the activity of the membrane-bound (Na⁺-K⁺)-ATPase suggests that the uphill transport of AIB is energized, at least in part, by the electrochemical Na⁺-gradient. Further, the inhibition produced by ouabain is only a fraction of the inhibition produced by the removal of Na⁺ from the incubation medium, in contrast to the almost complete inhibition of the Na⁺-dependent hexose and phosphate transport observed in this cell line (10, 11). These results confirm similar observations in rat kidney (21).

The Na⁺ gradient hypothesis proposes that the active transport system for certain solutes in epithelial and nonepithelial cells is driven by the electrochemical Na⁺ gradient generated by the active extrusion of Na⁺ through the (Na⁺-K⁺)-ATPase (22, 23). However, energization by co-transport with Na⁺, although clearly contributory, does not explain totally the mechanism of amino acid transport, even in the Na⁺-dependent systems (24, 25).

Like AIB, the total influx of MeAIB is mediated through a Na⁺-dependent and a Na⁺-independent mechanism. The Na⁺-dependent influx is also the saturable component. Kinetic analysis of the effect of Na⁺ on the MeAIB influx shows an increase in Vₘₐₓ with increasing Na⁺ concentration, but without any appreciable change in Kₐ (data not shown). Therefore, the effect of Na⁺ on this amino acid transport is mediated by a mechanism quite similar to that observed with AIB.

The total influx of cycloleucine also occurs through a Na⁺-sensitive and Na⁺-insensitive component; however, unlike the uptake of AIB and MeAIB, both components are able to operate against a concentration gradient. Cycloleucine transport by a Na⁺-dependent system has also been described in rat hepatocytes (18).

MeAIB, an amino acid analog that was found to be specifically transported by the A system in several cell types (17, 18, 26), inhibits 45% of the Na⁺-dependent influx of AIB in LLC-PK₁ cells; yet the Na⁺-dependent influx of MeAIB was completely inhibited by AIB. MeAIB behaves as a competitive inhibitor of AIB influx suggesting that the MeAIB-inhibitable fraction of AIB influx and MeAIB influx are mediated through the same transport system. From these results, we can conclude that the Na⁺-dependent AIB influx is mediated by at least two transport systems, one of which has similar characteristics to those defined for the A system in Ehrlich cells (7). The selectivity of this system for the naturally occurring amino acids is also quite similar to the A system in Ehrlich ascites tumor cells, and hamster intestine and kidney (7). Since the part of the Na⁺-dependent amino acid influx not inhibited by N-methyl amino acid derivatives has been accounted for by transport through the ASC system (7) we can conclude that this fraction, too, is mediated through the same system. Taken together, the inhibition of AIB and MeAIB influx by naturally occurring amino acids indicates a selectivity similar to the ASC system of Ehrlich cells (7) and intestinal basolateral membrane vesicles (6).
The small inhibition of AIB efflux by the presence of Na+ in the incubation medium agrees with the trans inhibition of AIB influx by internal substrate observed in Ehrlich ascites tumor cells (26). Proline, however, also a substrate for A and ASC systems in this cell line, produces a clear trans stimulation of AIB influx. We can present no arguments to explain these contradictory effects.

The synthetic analog BCH has been shown to react only with the Na+-independent L system in several cell systems, such as Ehrlich cells (31), human fibroblasts (26), and rat hepatocytes (18). In agreement with these observations, BCH does not inhibit the influx of AIB and MeAIB in presence of Na+. This amino acid, however, produced a complete inhibition of the Na+-independent cycloleucine influx by LLC-PK1 monolayers. The Na+-sensitive influx of cycloleucine is totally inhibited by AIB but not by MeAIB, indicating that practically all the cycloleucine in the presence of Na+ occurs through the ASC system. A similar observation has been reported in isolated rat hepatocytes (18).

The heterogeneity of amino acid transport in LLC-PK1 monolayers, reflected by the existence of at least three distinct amino acid transport systems, may be associated with the morphological and functional heterogeneity of the membranes of these cells as shown in previous studies (12, 32). The results presented so far in this study are consistent with a simple two-compartment system where influx and efflux could be measured; however, the extent of intracellular amino acid accumulation gives no information about the localization of these transport systems. Therefore, AIB uptake in the presence or absence of Na+, from the apical or basolateral side was measured and compared as a criteria of localization. The short incubations utilized make it unlikely that an appreciable amount of substrate migrates through the paracellular pathway as shown by a complete absence of the labeled compound transported into the cells mainly from the basolateral side of the plasma membrane monolayer. This polarized uptake is observed either in the presence of Na+, from the apical or basolateral side was measured and compared as a criteria of localization. The short incubations utilized make it unlikely that an appreciable amount of substrate migrates in the paracellular pathway as shown by a complete absence of the labeled compound transported into the cells mainly from the basolateral side of the plasma membrane monolayer. This polarized uptake is observed either in the presence of Na+, from the apical or basolateral side was measured and compared as a criteria of localization. The short incubations utilized make it unlikely that an appreciable amount of substrate migrates through the paracellular pathway as shown by a complete absence of the labeled compound transported into the cells mainly from the basolateral side of the plasma membrane monolayer. This polarized uptake is observed either in the presence of Na+, from the apical or basolateral side was measured and compared as a criteria of localization. The short incubations utilized make it unlikely that an appreciable amount of substrate migrates through the paracellular pathway as shown by a complete absence of the labeled compound transported into the cells mainly from the basolateral side of the plasma membrane monolayer. This polarized uptake is observed either in the presence of Na+, from the apical or basolateral side was measured and compared as a criteria of localization. The short incubations utilized make it unlikely that an appreciable amount of substrate migrates through the paracellular pathway as shown by a complete absence of the labeled compound transported into the cells mainly from the basolateral side of the plasma cell membrane. Basolateral neutral amino acid transport has also been demonstrated in isolated perfused proximal tubules (9), isolated rat hepatocytes (33), and intestinal basolateral membrane vesicles (6). In the latter study, at least three separate basolateral transport systems were identified with similar characteristics to the A, ASC, and L systems reported here. The absence of Na+-dependent AIB uptake from the apical side of LLC-PK1 monolayer, however, appears to be at variance with studies of AIB uptake in isolated renal proximal tubules (9) and with isolated membrane vesicles (2—4). In these studies, the presence of an active Na+-dependent neutral amino acid transport across the apical membrane of the proximal tubular cells has been demonstrated. The occurrence in the apical side of LLC-PK1 monolayers of other transport systems with identical characteristics to those observed in the apical membrane of the proximal tubular cell (12) suggest the presence of a very well developed brush border membrane. Under this circumstance, the absence of the neutral amino acid transport system from the apical membrane of LLC-PK1 cells could be interpreted as (a) that only this transport system has gone unexpressed through a dedifferentiation or transformation process during the establishment of the cell line or (b) that its absence in this particular cell type represents the heterogeneous distribution of the different tubular functions within the different cellular components of the renal proximal tubule. Further studies will be required to discern between these possibilities.

It is not clear from the present and other studies in the literature what the functional implications of the basolateral localization of these transport systems are in the transepithelial transport of amino acid. However, it has been suggested that the active basolateral uptake of amino acid may serve primarily the nutritive requirements of tubular (34) and intestinal cells (6) rather than transepithelial transport.

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