Electrogenic Na\(^+\)-dependent L-alanine transport in the lizard duodenum. Involvement of systems A and ASC

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Medina, Virtudes, Antonio Lorenzo, and Mario Díaz. Electrogenic Na\(^+\)-dependent L-alanine transport in the lizard duodenum. Involvement of systems A and ASC. Am J Physiol Regulatory Integrative Comp Physiol 280: R612–R622, 2001.—L-Alanine transport across the isolated duodenal mucosa of the lizard Gallotia galloti has been studied in Ussing chambers under short-circuit conditions. Net L-alanine fluxes, transepithelial potential difference (PD), and short-circuit current (Isc) showed concentration-dependent relationships. Na\(^+\)-dependent L-alanine transport was substantially inhibited by the analog \(\alpha\)-methyl aminoisobutyric acid (MeAIB). Likewise, MeAIB fluxes were completely inhibited by L- 

amino acid transport. System A transport activity was electrophysiological and exhibited hyperbolic relationships for net MeAIB fluxes, PD, and Isc, which displayed similar apparent \(K_m\) values. Na\(^+\)-dependent L-alanine transport, but not MeAIB transport, was partially inhibited by L-serine and L-cysteine, indicating the participation of system ASC. This transport activity represents the major pathway for L-alanine absorption and seemed to operate in an electroneutral mode with a negligible contribution to the L-alanine-induced electronegativity. It is concluded from the present study that the active Na\(^+\)-dependent L-alanine transport across the isolated duodenal mucosa of Gallotia galloti results from the independent activity of systems A and ASC for neutral amino acid transport.

neutral amino acid absorption; Na\(^+\)-coupled amino acid transport; reptilian intestine

TRANSPORT PROCESSES ACROSS the small intestine enterocytes involve the uptake from the gut across the brush-border membrane and the exit to the portal blood across the basolateral membrane. It is generally accepted that the uphill transport of amino acids in intestinal cells occurs secondarily to the coupling of amino acid transfer to the metabolic energy stored in the transmembrane Na\(^+\) electrochemical gradient, which is maintained by the activity of the basolateral Na\(^+\)-K\(^-\)-ATPase. This vectorial movement of sodium occurs with a concomitant change in the transepithelial potential difference and has led researchers to establish that the Na\(^+\)-coupled amino acid cotransport across the apical membranes of small intestine is both rhogenetic and conductive (22, 29).

It has been shown that amino acid transport across the small intestinal epithelium comprises several Na\(^+\)-dependent and -independent transport systems (11, 29, 30). For neutral amino acids, the existence of systems that transport [NBB system (neutral brush border system, transports most neutral amino acids but excludes MeAIB); system A; system ASC; Phe system (primarily phenylalanine, glycine, and methionine); \(\beta\)-amino (\(\beta\)-amino acids such as taurine and \(\beta\)-alanine); Imino (imino acids such as proline, hydroxyproline); and the Na\(^+\)-independent system L] have been reported in isolated cells or membrane vesicles obtained from the small intestine of different species of mammals (11, 19, 30). Recently, we have demonstrated the presence both of Na\(^+\)-dependent and -independent active-transport mechanisms for L-alanine in the duodenum of the lizard Gallotia galloti (13).

In the present study, with the use of the short-circuit technique together with radioisotope fluxes, we aimed at determining the characteristics of the Na\(^+\)-dependent L-alanine transport in the isolated lizard duodenum to identify the possible transport systems involved as well as their individual contribution to the overall amino acid absorption. Finally, we explored the electrophysiological correlations associated with the activity of the different Na\(^+\)-dependent L-alanine transport systems.

MATERIAL AND METHODS

Animals and solutions. Adult male lizards (Gallotia galloti) weighing 25–40 g were killed by spinal transection, and the duodenum was removed and rinsed in ice-cold bathing solution. The standard bathing solution contained (in mM) 107 NaCl, 4.5 KCl, 25 NaHCO\(_3\), 1.8 Na\(_2\)HPO\(_4\), 0.2 NaH\(_2\)PO\(_4\), 1.25 CaCl\(_2\), and 1.0 MgCl\(_2\) and had a final pH of 7.3. The intestinal segments were mounted in water-jacketed Ussing chambers with exposed area of 0.21 cm\(^2\) and bathed on both sides with 4 ml of Ringer solution. Chambers were continuously gassed with 5% CO\(_2\) and 95% O\(_2\), and the temperature was maintained at 27°C. In some experiments, choline was used to replace sodium ions in the bathing solutions.

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Electrical measurements. The electrical measurements were made as described previously (using calomel (for voltage sensing) and Ag/AgCl electrodes (for current passage) connected to the bathing solutions through 4% (vol/wt) agar bridges (4, 13)). Electrical measurements were continuously monitored with an automatic computer-controlled voltage-clamp device (AC-microclamp). The tissues were first incubated under open-circuit conditions for 20 min and then short-circuited; the potential difference (PD) and the short-circuit current (Isc) were determined every minute. Every 5 s the tissues were pulsed with ±10 μA pulses of 1-s duration, and from the displacement of the PD, the tissue conductance (Gt) was derived. Corrections for electrode offset potential and solution resistance were determined at the beginning of every experiment and stored in the computer-controlled voltage-clamp device.

Transepithelial fluxes. Unidirectional amino acid fluxes were measured under short-circuit conditions using the procedure described in detail by Bolaños et al. (4). Briefly, 20 min after the tissues were properly mounted in the chamber, 5.0 μCi of the appropriate labeled substrate [3H-labeled L-alanine or 14C-labeled methylaminoisobutyric acid (MeAIB)] were added to the serosal or mucosal sides of the tissue. After an additional 20 min, by which isotope fluxes had reached the steady state, duplicate 200-μl aliquot samples were taken from the unlabeled side at regular 20-min intervals for 1 h and replaced by an equal volume of Ringer solution. Isotope radioactivity was measured in a liquid scintillation spectrometer (LKB-1209, Rackbeta), and the unidirectional and net fluxes were determined using a computer program written in our laboratory (12) that also provided the statistical tools required for data analysis. Inhibition experiments were carried out by adding small volumes (100 μl) of concentrated stock solutions, containing the amino acids or analogs, to the mucosal and/or serosal compartments.

Statistical and mathematical analysis. Results are expressed as means ± SE. Statistical comparison of mean values was made using one-way ANOVA, and two-tailed Student’s t-test were appropriate. Curve fitting of experimental data was performed by nonlinear regression analysis by using a computer-iteration procedure provided in the SigmaPlot package (Jandel Scientific). Experimental PD and Isc data were fitted to the following equations:

\[
PD = PD_0 + \frac{PD_{max} \times [S]}{K_m + [S]}
\]

And

\[
Isc = Isc_0 + \frac{Isc_{max} \times [S]}{K_m + [S]}
\]

where \(K_m\) is the apparent Michaelis-Menten constant, \(PD_{max}\) and \(Isc_{max}\) are the PD and Isc estimates at saturation, and \(PD_0\) and \(Isc_0\) are the background PD and Isc (offset) values obtained in the absence of substrate, respectively. The normalized PD (δPD) and Isc (δIsc) parameters were calculated by subtracting the average offset values from the corresponding electrical parameters.

Materials. MeAIB, L-alanine, L-cysteine, and L-serine were obtained from Sigma-Aldrich. [3H-labeled L-alanine and [14C]-labeled MeAIB were purchased from Amersham Ibérica. All reagents used were analytical grade.

RESULTS

Transepithelial L-alanine fluxes. Figure 1A illustrates the results of unidirectional and net fluxes of L-alanine (1 mM) across the isolated lizard duodenum measured under short-circuit conditions. With the use of Na+-containing Ringer solutions, the mucosal-to-serosal flux (Jms) was 50.9 ± 4.9 nmol/cm²·h, whereas the serosal-to-mucosal flux (Jsm) was 35.8 ± 5.8 nmol/cm²·h, resulting in a statistically different from zero net absorptive flux (Jnet) of 15.2 ± 1.7 nmol/cm²·h (P < 0.01). Replacement of the standard Ringer solu-
tion with an Na\(^+\)-free solution brought about a considerable reduction of \(J_{ms}\) compared with the value in the presence of Na\(^+\), with no change in \(J_{sm}\), which significantly decreased \(J_{net}\) to 7.8 ± 0.1 nmol/cm\(^2\)/h (\(P < 0.01\)), still significantly different from zero (\(P < 0.05\)). This is in agreement with our previous observations demonstrating that the lizard duodenum displays Na\(^+\)-dependent and -independent l-alanine transport pathways (13).

**Kinetic analysis of l-alanine fluxes.** The kinetic characteristics of l-alanine transport in the presence of sodium were further assessed under short-circuit conditions and in the presence of identical l-alanine concentrations (50 \(\mu\)M to 10 mM) on both the mucosal and serosal reservoirs. Thus the changes on the net transepithelial fluxes are entirely attributable to active-transport processes, because the diffusive components are presumably identical in the two opposite directions (mucosa-to-serosa and serosa-to-mucosa).

As can be seen in Fig. 1B, the increase on the external l-alanine concentration was followed by a hyperbolic rise on the calculated net l-alanine fluxes. The kinetic constants describing this saturable transport were computed by nonlinear regression and were as follows: apparent \(K_m\) = 0.18 ± 0.01 mM; maximum flux (\(J_{max}\)) = 47.6 ± 2.9 nmol/cm\(^2\)/h. Interestingly, the \(J_{max}\) value determined under these conditions was about threefold that obtained in the absence of sodium (14.8 ± 1.3 nmol/cm\(^2\)/h) (13) and strongly indicates the presence of an Na\(^+\)-dependent carrier-mediated pathway being responsible for the majority of transepithelial l-alanine transport.

**Kinetic analysis of l-alanine-induced electrical responses.** To determine the possible relationship between the concentration of l-alanine in the bathing solutions and the bioelectrical parameters, we accomplished the analysis of the variations on the PD and Isc in response to changes in the external l-alanine concentration. The concentration-response curves for the PD and Isc elicited in the range of l-alanine concentrations tested (Fig. 2) demonstrated that both PD and Isc displayed hyperbolic relationships. The kinetic parameters that were computed from nonlinear regression-fitting analysis to the saturation equations (see METHODS) showed apparent Michaelis-Menten constants for both PD and Isc, respectively, which were not statistically different. The PD\(_{max}\) and Isc\(_{max}\) estimates, representing the l-alanine-induced increase on PD and Isc values at saturation, were 1.39 ± 0.16 mV and 8.89 ± 1.14 \(\mu\)A/cm\(^2\), and the values of PD\(_o\) and Isc\(_o\), obtained in the absence of l-alanine were 2.49 ± 0.11 mV and 22.07 ± 0.85 \(\mu\)A/cm\(^2\), respectively. The values of PD\(_o\) and Isc\(_o\) were similar to those reported earlier for the lizard duodenum in the presence of sodium (24) and are consistent with the existence of a basal electrogenic Na\(^+\) absorption that accounts for ~50% of the total sodium uptake (24).

In addition, the regression analysis performed on the results of the experiments in which the electrical PD, Isc, and amino acid fluxes were measured simultaneously (Fig. 3) revealed the existence of a positive correlation between \(\delta\)PD or \(\delta\)Isc and \(J_{net}\) or \(J_{ms}\) (\(P < 0.05\), \(r^2 > 0.81\) in all cases). The fact that the slope constants for each dependent variable (\(\delta\)PD or \(\delta\)Isc) were similar for \(J_{ms}\) or \(J_{net}\) indicate that the magnitude of l-alanine fluxes may be estimated from the change of either \(\delta\)PD or \(\delta\)Isc.

**Effects of MeAIB on transepithelial l-alanine fluxes, PD and Isc.** In an attempt to identify different transport systems carrying l-alanine in the isolated lizard duodenum, we used well-known specific analogs and substrates to emphasize the differences in molecular traits.
recognition by each transport system. It has been described that amino acid analogs with \(N\)-methyl group on \(\alpha\)-amino \(N\) are recognized by system A and are excluded by other systems. The unidirectional and net fluxes of L-alanine (1 mM) across the isolated lizard duodenum in the absence and in the presence of saturating concentrations of MeAIB (20 mM) in the bathing solution are given in Fig. 4A. Under control conditions, using \(Na^+\)-containing Ringer solutions, \(J_{ms}\) was 33.1 ± 3.4 nmol/cm\(^2\)/hr and \(J_{sm}\) was 25.4 ± 2.9 nmol/cm\(^2\)/hr, resulting in a statistically different from zero net absorptive \(J_{net}\) of 7.7 ± 0.5 nmol/cm\(^2\)/hr (\(P < 0.01\)). Once the analog was added to the bathing solutions, both \(J_{ms}\) and \(J_{net}\) were considerably decreased compared with control conditions (Fig. 4A). Thus \(J_{ms}\) was reduced to 24.8 ± 2.9 nmol/cm\(^2\)/hr and the calculated \(J_{net}\) was decreased by 41.3% down to 4.2 ± 0.1 nmol/cm\(^2\)/hr (\(P < 0.01\)). The finding that this \(J_{net}\) was significantly different from zero (\(P < 0.05\)) strongly indicates that the lizard duodenum possesses different \(Na^+\)-dependent L-alanine transport pathways. Interestingly, despite the dramatic reduction of L-alanine \(J_{ms}\) and \(J_{net}\) caused by the addition of MeAIB, neither the transepithelial PD nor the Isc were affected by the addition of the amino acid analog (Table 1, L-alanine experiments).

![Image](http://ajpregu.physiology.org/)

Fig. 3. Regression analyses for the L-alanine fluxes and normalized PD (\(\delta PD\), A) or Isc (\(\delta Isc\), B) simultaneously determined in the same tissues. Linear regression equations and correlation coefficients are indicated. \(J_{\text{unip}}\), L-alanine flux.

Fig. 4. A: effects of \(\alpha\)-methyl aminoisobutyric acid (MeAIB) on L-alanine unidirectional and net fluxes under short-circuit conditions in the presence of \(Na^+\) in the bathing solutions. MeAIB was added to both sides of the tissues to yield a final concentration of 20 mM. The concentration of L-alanine was 1 mM throughout the experiment. B: effect of L-alanine on unidirectional and net MeAIB fluxes under short-circuit conditions in the presence of sodium in the bathing solutions. The concentrations of MeAIB and L-alanine were 10 and 20 mM, respectively. L-alanine was added to the bathing solutions 20 min after the control values were obtained. C: concentration dependence for net MeAIB flux under short-circuit conditions. The rectangular hyperbola represents the Michaelis-Menten fit for the data as deduced by nonlinear regression analysis. Results are mean ± SE for 8 (A and B) and 6 (C) different experiments. Statistically different from the control (*\(P < 0.05\) and **\(P < 0.01\)).
As can be seen in Fig. 4C, net MeAIB fluxes followed a saturable transport kinetic with an apparent 
Km of 0.64 ± 0.20 mM and the Jmax was 6.9 ± 0.4 nmol/cm²·h. These results strongly suggest the presence of a 
carrier-mediated system being responsible for net MeAIB transport.

The concentration-response curves for PD and Isc elicited in the presence of different concentrations of 
MeAIB (Fig. 5) also revealed hyperbolic relationships with apparent Michaelis constants of 0.47 ± 0.07 and 
0.45 ± 0.1 mM for PD and Isc, respectively, which were not statistically different. The PDmax and Iscmax estimates, representing the MeAIB-induced increase on PD and Isc values at saturation, were 0.50 ± 0.02 mV and 3.26 ± 0.17 μA/cm², respectively. The offset values, corresponding to the PDo and Isco obtained in the absence of l-alanine, were 1.50 ± 0.02 mV and 20.10 ± 1.37 μA/cm², respectively. Preliminary experiments performed in the absence of sodium showed that MeAIB addition to the bath failed to produce any appreciable effect on either PD or Isc; hence, the electronegenic effects of MeAIB transport are consistent with MeAIB/Na⁺ cotransport activity.

Given the hyperbolic relationship between the electrical parameters and the l-alanine concentration, we 
performed regression analysis to test whether the mag-

<p>| Table 1. Electrical parameters associated with the Na⁺-dependent l-alanine and MeAIB transport |
|---------------------------------------------|------------------|------------------|</p>
<table>
<thead>
<tr>
<th>Experimental Condition</th>
<th>PD, mV</th>
<th>Isc, μA/cm²</th>
<th>Gt, mS/cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.81 ± 0.38</td>
<td>19.87 ± 1.71</td>
<td>9.90 ± 1.00</td>
</tr>
<tr>
<td>MeAIB (20 mM)</td>
<td>2.40 ± 0.29</td>
<td>18.37 ± 1.67</td>
<td>10.01 ± 0.50</td>
</tr>
<tr>
<td>MeAIB experiments</td>
<td>1.47 ± 0.29</td>
<td>31.46 ± 4.26</td>
<td>13.33 ± 1.45</td>
</tr>
<tr>
<td>L-Alanine (20 mM)</td>
<td>2.80 ± 0.26*</td>
<td>21.28 ± 2.00</td>
<td>12.51 ± 1.20</td>
</tr>
<tr>
<td>MeAIB experiments</td>
<td>1.47 ± 0.29</td>
<td>21.28 ± 4.00</td>
<td>12.51 ± 1.20</td>
</tr>
</tbody>
</table>

Values are means ± SE for 16 determinations. Control values were obtained 20 min after the addition of MeAIB (A) or l-alanine (B). The concentrations of l-alanine (in A) and MeAIB (in B) were 1 and 10 mM, respectively, throughout the experiment and were added to both the serosal and mucosal solutions. *P < 0.01. PD, potential difference; Gt, tissue conductance; Isc, short-circuit current; MeAIB, α-methyl aminoisobutyric acid.
magnitude of the unidirectional or net fluxes could provide a prediction of the change on the δPD or δIsc. The results shown in Fig. 6 show that the data could be significantly fitted to linear equations with \( r^2 > 0.84 \) in all cases, indicating that the magnitude of net MeAIB fluxes may be adequately inferred from the change of either δPD or δIsc.

**MeAIB-resistant, sodium-dependent L-alanine transport.** The next set of experiments was designed to test whether active MeAIB-resistant L-alanine transport could be ascribed to the presence of some other neutral amino acid transport system, using a substrate-discrimination methodology under short-circuit conditions. Figure 7 shows the results of the effects of L-serine, L-cysteine (typical substrates for system ASC), and L-threonine (substrate for system NBB) on L-alanine unidirectional and net fluxes. As can be seen, in the presence of sodium, both L-serine and L-cysteine (Fig. 7, A and B) markedly reduced the mucosa-to-serosa and net L-alanine fluxes, whereas L-threonine was without effect (Fig. 7C). Both amino acids, L-serine and L-cysteine, inhibited L-alanine transport by the same magnitude (49.1% and 54%, respectively), and these effects appear to be Na⁺-dependent because they were not observed in Na⁺-free solutions (Fig. 7D), suggesting that these amino acids interact with the same Na⁺-dependent transport system. The inhibition by L-serine and L-cysteine together with the absolute Na⁺ requirement exhibited by this transport system are compatible with system ASC being responsible for a significant fraction of the transepithelial alanine transport across the isolated duodenum of Gallotia galloti. Interestingly, the addition of L-cysteine, L-serine, or L-threonine failed to induce any change on the transepithelial PD, Isc, or \( G_r \) (Table 2).

To test for the independence of the MeAIB-transporting system (system A) and the L-cysteine- and L-serine-sensitive L-alanine transport system (system ASC) in the lizard duodenum we assessed the effects of L-cysteine on MeAIB transport. The results of these experiments are displayed in Fig. 8A. Clearly, the addition of saturating concentrations of L-cysteine did not alter either the unidirectional or net MeAIB fluxes, indicating that the effects of L-cysteine (and L-serine, not shown) on the L-alanine transport observed above were due to the inhibition of a transport system distinguishable from and independent to that transporting MeAIB.

Strikingly, the addition of L-cysteine to MeAIB-transporting tissues did not affect any of the bioelectrical parameters (Table 3), as it would be expected if the activity of system ASC were electrogenic, and suggests that although the activity of system ASC can be demonstrated under voltage-clamp conditions and in the absence of electrochemical gradients, its activity is clearly not electrogenic but consistent with an active electroneutral process.

**Relative contribution of different pathways.** The evidence presented until now indicates that the Na⁺-dependent L-alanine transport can be explained in terms of the synergistic activity of two different components, systems A and ASC. It was found interesting to assess the relative contribution of each individual transport system to the overall L-alanine transport across the duodenal mucosa of G. galloti using a strategy of combining inhibitors in a series of steps to isolate the different transport systems on the basis of their substrate affinity (7).

As shown in Fig. 8B, L-alanine \( J_{\text{ms}} \) and \( J_{\text{net}} \) were partially inhibited by MeAIB. When L-cysteine was added to the incubation media, net L-alanine fluxes were further inhibited. The percentages of inhibition of net L-alanine fluxes by MeAIB and L-cysteine were 43.6% and 82.2%, respectively, and were caused by the reduction of \( J_{\text{ms}} \); inasmuch, neither MeAIB nor L-cysteine significantly affected the L-alanine \( J_{\text{sm}} \). We have shown previously that MeAIB and L-serine do not affect L-alanine transport in the absence of sodium; thereby these effects are entirely attributable to the inhibition of the Na⁺-dependent transport systems. The remaining L-alanine \( J_{\text{net}} \) (3.2 ± 0.6 nmol/cm²·h) was totally inhibited by the addition of cycloleucine, an amino acid analog that selectively inhibits sodium-
Fig. 7. Effects of L-serine (A, D), L-cysteine (B), and L-threonine (C) on l-alanine unidirectional and net fluxes under short-circuit conditions. Experiments A-C were carried out in the presence of sodium, whereas experiment D was performed under Na⁺-free conditions. The amino acids were added to both sides of the tissue at a final concentration of 20 mM. L-Alanine (1 mM) was present throughout the experiments and was added to the bathing solutions 20 min before the control values were obtained. Results are means ± SE for 8 (A, B, and C) and 6 experiments (D). **Statistically different from the control (P < 0.01).

Table 2. Electrical parameters associated with the Na⁺-dependent l-alanine transport under control conditions and in the presence of different amino acids and analogs

<table>
<thead>
<tr>
<th>Experimental Condition</th>
<th>PD, mV</th>
<th>Isc, μA/cm²</th>
<th>Gc, mS/cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>l-Alanine experiments</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3.25 ± 0.19</td>
<td>18.01 ± 1.43</td>
<td>8.61 ± 1.7</td>
</tr>
<tr>
<td>L-Serine (20 mM)</td>
<td>3.19 ± 0.19</td>
<td>18.22 ± 0.85</td>
<td>9.38 ± 0.48</td>
</tr>
<tr>
<td>Control</td>
<td>2.81 ± 0.44</td>
<td>20.86 ± 1.85</td>
<td>7.16 ± 0.82</td>
</tr>
<tr>
<td>L-Cysteine (20 mM)</td>
<td>3.06 ± 0.40</td>
<td>24.58 ± 2.57</td>
<td>7.53 ± 1.15</td>
</tr>
<tr>
<td>Control</td>
<td>2.40 ± 0.25</td>
<td>17.44 ± 1.85</td>
<td>7.95 ± 0.5</td>
</tr>
<tr>
<td>L-Threonine (20 mM)</td>
<td>2.41 ± 0.20</td>
<td>18.44 ± 2.28</td>
<td>8.45 ± 1.0</td>
</tr>
</tbody>
</table>

Values are means ± SE of at least 6 determinations from 3 different sets of experiments. Control values were obtained 20 min after the addition of l-alanine to the bathing solution. The concentration of l-serine, L-cysteine, and L-threonine was 20 mM. The inhibitors were added to the serosal and mucosal solutions, and the electrical measurements were taken 20 min after the addition of the amino acids together with the samples for transport determinations. No significant differences were observed.
transport (Fig. 9, B and C). Interestingly, these analyses revealed that L-alanine-induced electrogenicity (curve 1) may be explained by the activity of two components, namely system A and Na\(^+\)-independent transport (curve 2), with little or no contribution of system ASC (curve 3), which strongly suggest that, at least under short-circuit conditions, this transport system behaves as an electrically neutral transporter.

Table 3. Electrical parameters associated with the Na\(^+\)-dependent MeAIB transport under control conditions and in the presence of L-cysteine

<table>
<thead>
<tr>
<th>Experimental Condition</th>
<th>PD, mV</th>
<th>(I_{sc}), (\mu A/cm^2)</th>
<th>(G_c), mS/cm(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeAIB experiments</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.55 ± 0.19</td>
<td>20.59 ± 2.57</td>
<td>10.64 ± 1.5</td>
</tr>
<tr>
<td>L-Cysteine (20 mM)</td>
<td>1.82 ± 0.10</td>
<td>25.16 ± 2.86</td>
<td>11.15 ± 1.3</td>
</tr>
</tbody>
</table>

Values are means ± SE of 16 determinations. Control values were obtained 20 min after the addition of MeAIB. The concentration of MeAIB was 10 mM throughout the experiment and was added to both sides of the tissue.

DISCUSSION

The present data clearly demonstrate that L-alanine transport across the duodenal mucosa of the lizard *Gallotia galloti* can take place under controlled short-circuit conditions, in the virtual absence of transepithelial electrochemical gradients, and both in the presence and in the absence of sodium in the bathing solution. These data indicate that the isolated lizard duodenum contains active processes for both Na\(^+\)-dependent and -independent L-alanine transport, being the transport in the presence of sodium quantitatively...
much greater than in its absence, a finding that agrees with the results obtained in many other preparations from renal cultured cells to hepatocytes (3, 22, 23). Because the features of the Na\textsuperscript{+}-independent L-alanine transport have been explored in detail in a previous publication (13), the present article focuses on the characterization of the Na\textsuperscript{+}-dependent transport.

In the presence of sodium, duodenal L-alanine net transport displays a clear saturation kinetic that could be described by an apparent $K_m$ of 0.18 mM and a $J_{\text{max}}$ of 47.6 nmol/cm\textsuperscript{2}·h. Because the diffusive pathways for alanine are presumably the same in both directions, $J_{\text{ms}}$ and $J_{\text{sm}}$, the transport kinetic obtained for the net L-alanine transport under the present conditions corresponds to a carrier-mediated transport with no contribution of the diffusive components. On the other hand, the comparison of the kinetic constants calculated in the presence of sodium with the parameters reported for L-alanine transport in the absence of sodium in this same preparation (13) indicate that at saturation, $\sim$31% of the total transport is carried by the Na\textsuperscript{+}-independent system.

The measurements of bioelectrical parameters showed that the addition of L-alanine induced a concentration-dependent increase in transepithelial PD and Isc, without varying $G_t$. Furthermore, L-alanine-induced changes on PD or Isc exhibited a good correlation with $J_{\text{net}}$. These observations strongly suggest that L-alanine has triggered the activation of some electrogenic process, likely carried by the transepithelial movement of sodium toward the serosal compartment, that eventually leads to the development of a serosally positive PD. The concentration-dependence analyses of L-alanine-induced electrogenicity show that the apparent $K_m$ values for PD and Isc are very similar and confirm the observation that the electrical phenomena associated with the amino acid transport occur without changes in the transepithelial $G_t$, which is consistent with the notion that transport of substrates occurs as a secondary process coupled to the influx of Na\textsuperscript{+}. Indeed, electrophysiological studies performed using microelectrode measurements of membrane potential in the lizard duodenum revealed that the presence of a saturating L-alanine concentration in the Na\textsuperscript{+}-containing luminal solution depolarizes the membrane potential by 20 mV and increases the transmural potential with a concomitant increase in the equivalent short-circuit current (17). Similar findings have been demonstrated in other nonmammalian preparations including Necturus intestine (18) and Aplysia californica intestine (16).

A number of studies using uptake kinetic techniques in vesicles obtained from mammalian small intestine have demonstrated that neutral amino acid transport by the brush-border membranes is mediated by different processes including Na\textsuperscript{+}-dependent and -independent systems (29, 30). Among the Na\textsuperscript{+}-dependent neutral amino acid transport systems identified in eukaryotic animal cells so far, system A is a ubiquitous carrier that serves mainly the uptake of amino acids with short, polar side chains. System A activity is subject to significant regulation by hormones, but its most relevant characteristic is its tolerance of $N$-methylated substrates such as the nonmetabolizable substrate MeAIB (6, 8).

The results presented here led to the conclusion that a fraction of the total carrier-mediated Na\textsuperscript{+}-dependent L-alanine transport in the mucosal epithelium of Gallotia galloti is actively carried by system A. Several pieces of evidence support this conclusion: 1) unidirectional mucosa-to-serosa and net L-alanine transport are reduced by the addition of MeAIB to the bathing solutions; 2) MeAIB fluxes can be measured under short-circuit conditions and in the absence of electrochemical gradients; 3) MeAIB fluxes were considerably inhibited by the addition of L-alanine to the bathing solution; and 4) MeAIB transport across short-circuited tissues displayed a saturation kinetic. Additionally, our data demonstrate that the activity of system A is clearly electrogenic and displays a hyperbolic relationship between the concentration of MeAIB and the transepithelial PD and Isc. Furthermore, the calculated apparent $K_m$ values for the concentration-response analyses performed on $J_{\text{net}}$, PD, and Isc show a considerable similarity that can only be explained if there is a direct relationship between MeAIB fluxes and the electrophysiological parameters. Supporting this hypothesis, there exists a positive correlation between $J_{\text{net}}$ PD or Isc as measured in the same tissues. Because active MeAIB transport has not been observed under Na\textsuperscript{+}-free conditions, the most straightforward explanation for our results is that an Na\textsuperscript{+}/MeAIB (L-alanine) cotransport drive the active accumulation of both substrates within the absorptive cells, which eventually exit through the plasma membrane toward the serosal solution via undetermined pathways. Studies performed in human fibroblasts (9), mouse erythrocytes (32), hepatocytes (21), rabbit distal ileum (26), and mouse ascites tumor cells (14, 20) have shown that Na\textsuperscript{+}-symport via system A is electrogenic, affected by the transmembrane potential, and exhibits a one-to-one stoichiometry. Additionally, direct electrophysiological measurements have unequivocally demonstrated that MeAIB uptake causes an Na\textsuperscript{+}-dependent membrane depolarization in Ehrlich ascites tumor cells (10) that is consistent with the Na\textsuperscript{+} entry into the cells during the cotransport process.

On the basis of the uptake kinetics and cross-inhibition studies, four different Na\textsuperscript{+}-dependent transport systems for neutral amino acids have been identified in the brush-border membrane and have not been found in other cellular preparations (1, 31): NBB, Phe, Imino, and a $\beta$-system. The MeAIB transport observed here cannot be attributed to the activity of the Imino system because although it accounts for MeAIB uptake in brush-border membranes of several mammalian intestines (1, 31), it is different from system A because it excludes alanine and other short-chained amino acids.

L-Threonine has been shown to be an ideal substrate for the characterization of system NBB in intestinal brush border (22, 25). We have assessed the participation of system NBB in the duodenum of G. galloti by

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measuring the effects of L-threonine on the transepithelial transport of L-alanine. The experiments summarized in Fig. 7 and Table 3 clearly indicate that saturating concentrations of L-threonine failed to produce any appreciable change on L-alanine transport or electrical parameters. These results rule out a significant contribution of system NBB to transepithelial L-alanine transport in the lizard duodenum. On the contrary, addition of either L-serine or L-cysteine to the bathing solutions caused a considerable inhibition of Jnet and Jms. The effects of L-cysteine were specific for a component of L-alanine transport pathways other than system A, because MeAIB fluxes were not affected by the addition of L-cysteine to the bathing solutions (Fig. 8A). Inhibition by L-serine and L-cysteine and resistance to MeAIB confer to this mechanism the characteristics of system ASC. This transport system has been demonstrated in a wide variety of cell preparations (15, 21, 28) and, although the kinetic characterization of system ASC has been hampered because of the lack of a specific substrate, in most untransformed cell types, system ASC is the major Na+-dependent system (6).

Our present data also indicate that the active Na+-independent L-alanine transport, described earlier in this same preparation (13), might coexist with the two Na+-dependent systems and, more interestingly, that the combined activity of the three transport systems may explain the whole L-alanine transport under short-circuit conditions. This has been demonstrated by using a partition strategy combining a series of substrates and analogs in excess to isolate components of transport one by one, as suggested by Christensen (7). Thus the sequential addition of MeAIB and L-cysteine served to inhibit the Na+-dependent components of L-alanine transport attributed to systems A and ASC. The final incorporation of cycloleucine, a nonhydroxylizable amino acid analog, which was shown to be a potent inhibitor of the Na+-independent H+-coupled L-alanine transport in the lizard duodenum (13), vanished the remaining net L-alanine transport (Fig. 8B). Although alternative explanations are possible, the most plausible hypothesis is that the Na+-independent L-alanine transport might coexist with the Na+-dependent transport systems.

In an attempt to characterize system ASC, we have included a mathematical discrimination by nonlinear regression under the assumption that the combined activity of systems A and ASC and the Na+-independent system accounts for the total L-alanine transport under short-circuit conditions. The results of these analyses revealed a hyperbolic relationship with a Jmax of 25.6 nmol/cm²-h, which represents 79% of the total Na+-dependent L-alanine transport, and an apparent Kₘ of 0.16 mM (Fig. 9). As judged by the relevant kinetic parameters of transport obtained here, the apparent affinity for alanine of the putative system ASC carrier is higher than for system A, suggesting that at low luminal L-alanine concentrations, the major transport component corresponds to an Na+-mediation endowed with characteristics of system ASC. The finding that system ASC predominates over other neutral amino acid transport pathways has been observed in different preparations including human fibroblasts (15), renal LLC-PK1 cells (23), Chinese hamster ovary cells (3, 28), and skate hepatocytes (2).

The electrical measurements obtained under short-circuit conditions point out that system ASC behaves as an electroneutral transporter. In support of this hypothesis, the observations that PD or Isc were not altered when L-cysteine was added to tissues transporting MeAIB and that addition of saturating concentrations of either L-serine or L-cysteine to L-alanine transporting tissues also failed to induce any electrical change. These findings were corroborated by a mathematical approach to the experimental data aimed at determining the fraction of transepithelial PD or Isc remaining after subtracting the contribution of both the electrogenic Na+-independent and system A components from the total L-alanine-induced electrogenic- ity. The results of these analyses clearly showed that L-alanine-induced electrogenicity could be adequately explained in terms of the synergistic actions of system A and the Na+-independent system with no contribution of system ASC. This finding is in agreement with the previous studies on human fibroblasts demonstrating that ASC activity accommodates a nonrhoeogenic model in which an electrically silent translocation, with no net movement of charge during a complete cycle of the carrier, is dominant (5).

To our knowledge, this is the first report demonstrating multiple pathways for L-alanine transport in the small intestine of reptiles. Comparison of the data reported here with those of mammalian tissues indicates that the distribution of neutral amino acid transport systems in the lizard duodenum closely resembles that proposed for nonepithelial cells (22, 29). The difference with regard to the small intestine of mammals, like rabbit, rat, or pig, is striking, since system NBB is the main transporter serving neutral amino acids (1, 25), while systems A and ASC activities are small or nonexistent. Nevertheless, in agreement with our findings, detailed studies performed in guinea pig small intestine have unambiguously concluded that the concentrative L-alanine transport in the presence of an Na⁺ gradient is carried out by systems A, ASC, and L both in isolated cells (11) and brush-border membranes (19, 27). The reasons for the different predominance of the various neutral amino acid transport pathways between the same tissues in different species are not known. The fact that the different transport systems display different substrate specificities as well as kinetic features (transport capacity and substrate affinity) allows the intestinal absorption of amino acids taking place under a wider range of dietary conditions, which is especially important for omnivorous species (as is the case of G. galloti). Presumably, evolutionary pressure may select transport systems that are most compatible with the nutritional requirements and food availability.
Perspectives

It has been ascertained that some transport systems (like system A) are clearly subject to adaptive regulation, a phenomena by which the expression levels of a particular transport protein are influenced by the availability of substrates (22). Although the regulation of amino acid transport systems has not been explored in reptiles, this mode of regulation is likely to be relevant in this group of vertebrates inasmuch as the metabolic needs vary during hibernation, starvation, or reproduction periods. Indeed, we have observed a dramatic reduction of transepithelial 1-alanine transport in animals captured in midwinter, where starvation is common (unpublished observations). At present, we are undertaking new experiments to ascertain the possible short- and long-term regulation of the different transport systems demonstrated in the duodenal mucosa of Gallotia galloti.

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