Transamination and asymmetry in glutamate transport across the basolateral membrane of frog small intestine

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(Received 20 October 1981)

Transport of L-glutamate across the basolateral membrane of frog small-intestinal epithelium, unlike that of L-alanine, is highly asymmetric; thus the rate constant ($K_{entry}$) describing the entry of glutamate into the epithelium from the vascular bed across this membrane is one order of magnitude greater than the rate constant ($K_{exit}$) describing its exit. This asymmetry, which appears not to depend upon the Na gradient, may be important in maintaining a high intracellular concentration of glutamate relative to alanine thereby favouring the production of alanine from glutamate by transamination.

It has long been recognized that the small intestine is a major site of metabolism of $\alpha$-amino N (Dent & Schilling, 1949; Wiseman, 1953; Windmueller & Spaeth, 1974, 1975; Hanson & Parsons, 1977). Thus for example transamination of glutamate, catalysed by the enzyme glutamic-alanine transaminase (EC 2.6.1.2), results in the production of alanine through the reaction

$$\text{glutamic acid + pyruvate } \xrightleftharpoons{K_1}{K_2} \text{alanine + } \alpha\text{-ketoglutarate.} \quad (1)$$

This reaction is readily reversible ($K_1/K_2 = 1.6$; Boyer, 1973) and the forward reaction will be favoured by a high cellular concentration of glutamate relative to alanine. As the intraepithelial amino acid concentrations will be determined, in part, by their cellular entry and exit mechanisms, we have investigated the transport systems available for glutamate and alanine entry and exit across the basolateral membrane of frog ($Rana ridibunda$) intact small intestine.

Methods

Evidence that frog small intestine converts glutamate to alanine in vitro

Everted rings of $R. ridibunda$ small intestine were incubated for 60 min at 20°C in Frog Ringer solution containing 10 mM L-glutamate, 1 mM D-glucose, and $[14C]$inulin (an extracellular marker); as a control, rings were incubated in a similar medium from which
glutamate had been omitted. After incubation, the tissue was removed from the solutions and extracted in 0.04 M HNO₃ overnight at 80°C. Samples of the extract were analysed by thin-layer chromatography (Brenner et al., 1962). Extracts of the control tissue gave rise to two barely visible Ninhydrin-positive spots (Rf values 0.19 and 0.27); in contrast, extracts of the tissue incubated with glutamate produced seven spots after development with Ninhydrin, including two prominent, purple-staining spots with Rf values 0.19 and 0.37, which were shown by comparison with amino acid 'standards' to be glutamate and alanine respectively.

**Measurement of basolateral membrane transport of glutamate and alanine**

The vascularly perfused preparation of *R. ridibunda* small intestine (Boyd et al., 1975) is ideally suited to the study of basolateral-membrane solute transport. Washout of solute from the luminally or vascularly preloaded epithelium into the vascular bed is characterized by a rate constant, $K_{\text{exit}}$. This describes exit from the epithelium into the vascular bed across the basolateral membrane. Wash-in to the tissue from the vascular bed (Boyd & Parsons, 1979) enables a further rate constant, $K_{\text{entry}}$, to be calculated. Rate constants describing glutamate and alanine entry and exit across the basolateral membrane are presented in Table 1.

**Results and Discussion**

**Exit from the luminally preloaded epithelium**

Fig. 1 shows the vascular appearance of alanine and glutamate during washout from the luminally preloaded epithelium. It is clear that alanine washout is biphasic (as is that of leucine; cf. Cheeseman, 1979), the initial phase being characterized by $K_{\text{exit}}$ (fast,

<table>
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<tr>
<th></th>
<th>$K_{\text{exit}}$ (min⁻¹) for washout from epithelium into vascular bed following loading from Lumen</th>
<th>$K_{\text{exit}}$ (min⁻¹) for washout from epithelium into vascular bed following loading from Vascular bed</th>
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<tbody>
<tr>
<td><strong>Alanine</strong></td>
<td>0.150 ± 0.008 (14) fast (unstripped)</td>
<td>0.181 ± 0.051 (8) fast (unstripped)</td>
</tr>
<tr>
<td></td>
<td>0.044 ± 0.004 (14) slow</td>
<td>0.031 ± 0.007 (8) slow</td>
</tr>
<tr>
<td><strong>Glutamic acid</strong></td>
<td>0.027 ± 0.003 (12)</td>
<td>0.155 ± 0.032 (10) fast (unstripped)</td>
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<td></td>
<td></td>
<td>0.026 ± 0.005 (10) slow</td>
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Fig. 1. Vascular appearance of glutamate and alanine during washout from the luminally preloaded epithelium of *R. ridibunda* small intestine. Both experiments were carried out on the same animal, and in each the epithelium was loaded from the lumen with 1 mM amino acid until the rate of vascular appearance reached a steady state; after the removal of solute from the lumen, washout was followed for the subsequent 30 (alanine) or 35 (glutamate) min.

unstripped) = 0.165 min⁻¹; glutamate exit is monophasic and slow, with $K_{\text{exit}} = 0.036$ min⁻¹.

**Wash-in to the epithelium from the vascular bed**

The rate constants for amino acid entry into the epithelium from the vascular bed across the basolateral membrane (Table 1) indicate that alanine entry and exit are remarkably similar, there being no significant difference between $K_{\text{exit}}$ (fast, unstripped) and $K_{\text{entry}}$. In contrast, $K_{\text{entry}}$ for glutamate is one order of magnitude greater than $K_{\text{exit}}$, indicating that glutamate transport is highly asymmetric.
Exit from the vascularly preloaded epithelium

Alanine washout from the vascularly preloaded epithelium (mean $K_{\text{exit}} \text{[fast, unstripped]} = 0.181 \pm 0.051 \text{ min}^{-1}$) is similar to exit from the epithelium after loading from the lumen (mean $K_{\text{exit}} \text{[fast, unstripped]} = 0.150 \pm 0.008 \text{ min}^{-1}$). However, glutamate washout from the vascularly preloaded epithelium is described by an initial fast phase of vascular appearance (mean $K_{\text{exit}} \text{[fast, unstripped]} = 0.155 \pm 0.032 \text{ min}^{-1}$) and a subsequent slower phase of washout described by rate constant, $K_{\text{exit}} \text{(slow)} = 0.026 \pm 0.005 \text{ min}^{-1}$, similar to that describing the slow monoexponential washout of glutamate from the luminally preloaded epithelium.

This suggests that the initial rapid phase of washout represents vascular appearance of radioactive alanine (shown to be a major product of glutamate metabolism in everted rings) derived by transamination from glutamate that was originally loaded into the epithelium from the vascular side. The second phase of washout may represent vascular appearance of unmetabolized glutamate.

Asymmetric glutamate transport

Glutamate exit across the basolateral membrane is extremely slow, while entry is rapid; the biochemical basis for this asymmetry remains unclear. The rates of glutamate (and alanine) entry and exit across the basolateral membrane are not significantly changed by lowering the external Na concentration from 123 mM to 5 mM (Table 2); thus this marked asymmetry in glutamate transport is maintained in the absence of a sodium concentration gradient. Burckhardt et al. (1980) and Sacktor et al. (1981) have demonstrated that potassium is involved in energizing glutamate uptake into vesicles prepared from rat-kidney proximal-tubule basolateral and brush-border membranes. Our findings with intact tissue would fit nicely with such a mechanism. Mircheff et al. (1980) have identified both Na-dependent and Na-independent pathways for alanine in basolateral membrane vesicles from rat small intestine; our evidence suggests that the Na-independent pathway predominates in the frog small intestine.

The relation between basolateral transport and metabolism

The asymmetry in glutamate basolateral transport is likely to maintain a relatively low plasma concentration of the potentially toxic amino acid glutamate (the concentration of glutamate in the plasma of *Rana catesbeiana* is about 90 µM; Boyd, 1976), while the high intracellular level of glutamate will favour transamination to alanine. Since the basolateral membrane transport of alanine appears to be symmetrical, both exit and entry being fast, any alanine produced from glutamate will be removed rapidly from the cells of the epithelium, thereby favouring the forward reaction in equation 1.

Acknowledgement

V. S. P. thanks the M.R.C. for a research studentship.
Table 2. The effect of removing vascular Na (choline substitution) upon rate constants describing glutamate and alanine movements between the epithelium and the vascular bed of *R. ridibunda* small intestine expressed as a ratio of the rate constant measured in the absence of vascular Na to that measured in the presence of vascular Na.

Values are means ± S.E.M., with the number of samples shown in parentheses.

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<th>$k_{entry}^{-Na^+}$</th>
<th>$k_{entry}^{+Na^+}$</th>
<th>$k_{exit}^{-Na^+}$</th>
<th>$k_{exit}^{+Na^+}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>0.8 ± 0.4 (3)</td>
<td>1.1 ± 0.6 (2)</td>
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<tr>
<td>Glutamic acid</td>
<td>0.7 ± 0.1 (3)</td>
<td>1.2 ± 2.8 (3)</td>
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