Kinetics of mucosal influx of glycylsarcosine, glycine and leucine into hamster jejunum and ileum in vitro

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Summary

1. This paper describes an investigation of the kinetics of influx of the dipeptide glycylsarcosine and the amino acids glycine and L-leucine into rings of everted hamster small intestine in vitro, in proximal and distal small intestine (jejunum and ileum). Results were expressed per unit wet weight of intestine.

2. At all concentrations studied (0.1–100 mmol/l), influx of glycylsarcosine was more rapid in the jejunum than in the ileum. In contrast, at all concentrations studied, influx of glycine and leucine was more rapid in the ileum than the jejunum.

3. Estimates of the simple diffusion component in total influx were made. This component became increasingly large as the substrate concentration was raised. After correction for simple diffusion, transport of all three substrates conformed to Michaelis–Menten kinetics in both jejunum and ileum. Values for simple diffusion, apparent $K_t$ and $V_{max}$ are reported.

4. Possibly physiological implications of the results are discussed, and it is pointed out that under experimental conditions similar to our own, simple diffusion is too large a component in total influx to be ignored.

Key words: amino acid absorption, intestine, peptide absorption, simple diffusion, transport kinetics.

Introduction

In the rat, and also in the hamster, the sites of maximal absorption of small peptides and of free amino acids appear to be different along the length of the small intestine, whether absorption is expressed on the basis of unit length or of unit weight. Thus, in the rat, investigations of absorption from tied loops of small intestine in vivo showed that maximal absorption of a partial hydrolysate of casein consisting mainly of small peptides was in the proximal third of the small intestine, whereas the site of maximal absorption of the equivalent mixture of free amino acids was in the middle third of the small intestine (Lis, Matthews & Crampton, 1972). The site of maximal absorption of free L-methionine was in the ileum, whereas that of the equivalent L-methionyl-L-methionine was in the jejunum (Crampton, Lis & Matthews, 1973). In the hamster, Matthews & Laster (1965) found that the site of maximal absorption of glycine, L-alanine, L-valine, L-leucine and $\alpha$-aminoisobutyric acid was in the ileum, whereas D.M. Matthews & D. Burston (unpublished work) found that the site of maximal absorption of the hydrolysis-resistant peptides glycylsarcosine and glycylsarcosylsarcosine was in the jejunum. The results in the rat have been quoted as evidence supporting the hypothesis of the independence of mucosal uptake of peptides and amino acids (Matthews, 1975). Nevertheless, all these observations have been made at single arbitrary concentrations, and no investigation of the kinetic characteristics of absorption of amino acids or peptides at different sites in the small intestine of rat or hamster has yet been made. In this paper we report the results of an investigation
of the kinetic characteristics of mucosal uptake of a dipeptide and two amino acids at two sites (proximal and distal) in the small intestine of the hamster. The dipeptide chosen for study was glycylsarcosine, a neutral dipeptide which is so resistant to hydrolysis that mucosal uptake is entirely or almost entirely the result of uptake of intact peptide (Matthews, 1975). This avoids complications arising from the partial hydrolysis in the brush border, which occurs with many peptides, so that total uptake from them is the result of a mixture of uptake of intact peptide and amino acids released in the brush border. The amino acids used were glycine and L-leucine, two neutral amino acids with very different absorption kinetics (Matthews & Laster, 1965). The investigation included measurements of the part played by simple diffusion in total uptake.

Materials and methods

Standard abbreviations are used for amino acids and glycylsarcosine. Leucine was in the L-form. [14C]Gly-Sar was synthesized as described earlier (Addison, Burston, Dalrymple, Matthews, Payne, Sleisenger & Wilkinson, 1975). Labelled amino acids were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Unlabelled Gly-Sar and amino acids were obtained from the Sigma Chemical Co., St. Louis, MO, U.S.A. All other reagents were analytical or scintillation grade.

Experimental procedure

The experimental procedure and measurement of uptake into rings of everted hamster (Mesocricetus auratus) small intestine were previously described (Matthews, Addison & Burston, 1974). Incubations were carried out for 2 or 20 min at 37°C in 3 ml of Tris-phosphate saline medium, pH 7.2 (Reiser & Christiansen, 1973). After removal from the incubation medium, each ring was rinsed in chilled NaCl solution (154 mmol/l) at 4°C and, after blotting on hard filter paper (Whatman no. 50), was eluted for 5 min in 1 ml of sulphosalicylic acid (60 g/l) at 100°C. After centrifugation, 0-5 ml of supernatant was added to 15 ml of 1,4-dioxan-based scintillation fluid and radioactivity was measured in a liquid-scintillation spectrometer (Packard Tricarb model 3380).

Uptake was expressed as μmol/g initial wet wt. after correction for peptide or amino acid in the inulin space. Inulin space, determined as described by Cheng, Navab, Lis, Miller & Matthews (1971), was 4% of initial wet weight for jejunum and 3.3% for ileum at 2 min and 8% and 7% at 20 min respectively. The SEM of influx was usually about 10%.

Results

A preliminary experiment was carried out in which the small intestine of six animals was divided into six equal segments throughout its entire length, and everted rings were prepared from around the midpoint of each segment. These were incubated in [14C]Gly-Sar (10 mmol/l) for 20 min. The mean uptake of [14C]Gly-Sar, from proximal segment 1 to distal segment 6, in μmol 20 min⁻¹ g⁻¹ was (±SEM): (1) 10.9 ± 1.4; (2) 14.3 ± 0.7; (3) 11.7 ± 1.0; (4) 8.6 ± 1.1; (5) 5.6 ± 0.4; (6) 5.3 ± 0.3. The results showed that, under these conditions, accumulation of the peptide was greater in the proximal intestine than in the distal intestine. Site 2, in the proximal intestine, and site 5, in the distal intestine, were chosen for further experiments, and will be referred to as ‘jejunum’ and ‘ileum’ respectively. All further experiments were carried out with 2 min incubations, i.e. under conditions approximating influx (Sleisenger, Burston, Dalrymple, Wilkinson & Matthews, 1976).

Influx in jejunum

Influxes of [14C]glycine, [14C]leucine and [14C]Gly-Sar over the concentration range 0.1–100 mmol/l are shown in Figs. 1 and 2. In the lower part of the concentration range (Fig. 1), which probably includes physiological concentrations, leucine is taken up most rapidly, Gly-Sar slightly less rapidly, and glycine very much less rapidly than the other two compounds. At high concentrations (Fig. 2), the order of rates of uptake is altered, and above 50 mmol/l, Gly-Sar is taken up most rapidly, glycine less rapidly and leucine least rapidly.

Influx in ileum

At low concentrations (Fig. 3), the order of rates of uptake of the three compounds studied is similar to that in the jejunum. At high concentrations (Fig. 4) the order of uptake is altered, as in the jejunum, but not in the same way; in the ileum, at concentrations of 40 mmol/l and above, glycine is taken up most rapidly, Gly-Sar less rapidly, and leucine least rapidly.
Comparison of results in jejunum and ileum

At all concentrations, leucine is taken up slightly more rapidly in ileum than in jejunum. The same applies to glycine, only with glycine the difference in rates of uptake between ileum and jejunum is rather greater. Gly-Sar behaves differently from the amino acids: at all concentrations the peptide is taken up more rapidly in the jejunum than in the ileum.

Estimation of diffusion component and of kinetic parameters of mediated influx

Values for influx of $[^{14}\text{C}]$Gly-Sar in the jejunum will be used to illustrate how the component of simple diffusion in influx was estimated, and how estimates of apparent $K_t$ and $V_{\text{max}}$ for the mediated component were derived. An estimate of the part played by simple diffusion in influx of Gly-Sar was made by considering this compound as a competitive inhibitor of its own mediated transport (Neame & Richards, 1972) as described in the preceding paper (Matthews, Gandy, Taylor & Burston, 1979). Extrapolation of the inhibitory effects of a range of higher concentrations of Gly-Sar on influx of lower concentrations of this compound to infinitely high inhibitor concentrations by means of the Preston–Schaeffer–Curran plot (Preston, Schaeffer & Curran, 1974) showed that there was a component in influx which was not susceptible to competitive inhibition. This component, which amounted to 0.026 μmol min$^{-1}$ g$^{-1}$ at a substrate concentration of 1 mmol/l (Table 1), was taken to represent simple diffusion. Since simple diffusion is directly proportional to substrate concentration, it was then possible to correct all observed values for influx for the simple diffusion component over the entire concentration range. Having subtracted values for the simple diffusion component, the remainders represented mediated transport of the peptide. Uncorrected values for jejunal influx of $[^{14}\text{C}]$Gly-Sar, together with corrected values and the line representing simple diffusion, are shown in Fig. 5.

Fig. 6 is a Hofstee plot of influx of $[^{14}\text{C}]$Gly-Sar into the jejunum ($V$) against $V/S$, where $V =$ influx
(μmol min⁻¹ g⁻¹) and \( S = \) substrate concentration (mmol/l), showing the curve uncorrected for the diffusion component and the line corrected for the diffusion component. The uncorrected plot is obviously curved, i.e. bi- or multi-phasic. In the corrected plot, the points are scattered about a straight line. This suggests that mediated influx of Gly-Sar in the jejunum conforms to Michaelis-Menten kinetics, apparent \( K_t \) being 6.0 mmol/l and \( V_{\text{max}} \) 1.7 μmol min⁻¹ g⁻¹ (Table 1).

The data for influx of [¹⁴C]Gly-Sar in the ileum, for leucine in the jejunum and ileum and for glycine in the jejunum and ileum were treated similarly. In all cases, correction of the values for influx for the simple diffusion component converted the Hofstee plot from a curved into a linear form, estimates of \( K_t \) and \( V_{\text{max}} \), being derived from the linear plots. Estimates of the simple diffusion component in influx and \( K_t \) and \( V_{\text{max}} \) for all three compounds in jejunum and ileum are given in Table 1.

**Discussion**

The results show that the kinetics of influx of glycine and leucine are such that, at all concentrations studied, influx is more rapid in the ileum than in the jejunum, whereas the kinetics of influx of Gly-Sar are such that, at all concentrations studied, influx is more rapid in the jejunum than in the ileum. This is true both before and after correction for the simple diffusion component in influx. The findings indicate that results obtained at single concentrations, quoted in the Introduction, did not give a misleading impression. It does appear that the site of maximal uptake of Gly-Sar, a dipeptide which shares an uptake system with very many other di- and tri-peptides (Matthews, 1975), is in fact more proximal than that of two representative neutral amino acids. It would be premature to ascribe any definite physiological

![Fig. 3. Influx of [¹⁴C]Gly-Sar (●), [¹⁴C]Gly (○) and [¹⁴C]Leu (▲) into rings of everted hamster ileum over the substrate concentration range of 0.1-3 mmol/l. Each point is the mean of influx into 9-15 rings, one from each of 9-15 animals.](image)

![Fig. 4. Influx of [¹⁴C]Gly-Sar (●), [¹⁴C]Gly (○) and [¹⁴C]Leu (▲) into rings of everted hamster ileum over the substrate concentration range 1-100 mmol/l. Each point is the mean of influx into 9-15 rings, one from each of 9-15 animals.](image)
TABLE 1. Values for apparent \( K_s \), \( V_{\text{max}} \), and for simple diffusion for glycine, leucine and Gly-Sar in jejunum and ileum of hamster small intestine in vitro

These values are uncorrected for the ‘unstirred layer’ and \( K_s \) values may therefore be overestimates (Sleisenger et al., 1976).

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Site</th>
<th>( K_s ) (mmol/l)</th>
<th>( V_{\text{max}} ) (( \mu \text{mol min}^{-1} \text{g}^{-1} ))</th>
<th>Simple diffusion at substrate concen. 1 mmol/l (( \mu \text{mol min}^{-1} \text{g}^{-1} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gly</td>
<td>Jejunum</td>
<td>5.0</td>
<td>0.30</td>
<td>0.027</td>
</tr>
<tr>
<td>Gly</td>
<td>Ileum</td>
<td>25</td>
<td>1.7</td>
<td>0.034</td>
</tr>
<tr>
<td>Leu</td>
<td>Jejunum</td>
<td>2.0</td>
<td>1.1</td>
<td>0.010</td>
</tr>
<tr>
<td>Leu</td>
<td>Ileum</td>
<td>1.9</td>
<td>1.3</td>
<td>0.014</td>
</tr>
<tr>
<td>Gly-Sar</td>
<td>Jejunum</td>
<td>6.0</td>
<td>1.7</td>
<td>0.026</td>
</tr>
<tr>
<td>Gly-Sar</td>
<td>Ileum</td>
<td>6.4</td>
<td>1.5</td>
<td>0.022</td>
</tr>
</tbody>
</table>

FIG. 5. Influx of \([^{14}C]\)Gly-Sar into rings of everted hamster jejunum over the concentration range 1–100 mmol/l (●) and after correction for the non-mediated component (○). The simple diffusion component determined as described in the text is shown by the line without points. Each point is the mean of influx into 11 rings, one from each of 11 animals.

significance to the findings, though it may be that the proximal small intestine is best adapted to large-scale uptake of peptides, whereas the distal small intestine is best adapted to ‘mopping up’ amino acids which have been released into the intestinal lumen by back-diffusion from brush-border or intracellular sites of peptide hydrolysis.

The only other study known to us of the kinetics of influx of a dipeptide along the length of the small intestine is that of Das & Radhakrishnan (1974) in the monkey, Macaca radiata. These authors concluded that, at low concentrations, influx of Gly-Leu was most rapid in the proximal small intestine, but that the same applied to free leucine. It has previously been pointed out that there may be species differences in the kinetic characteristics of absorption of peptides and amino acids along the length of the small intestine (Matthews, 1975).

Two points concerning the relative rates of uptake of the compounds studied deserve mention. One is that, at low concentrations, leucine is taken up much more rapidly than glycine. At high concentrations, the situation is reversed, glycine being taken up more rapidly than leucine. This phenomenon results from the kinetic characteristics of mediated transport of each substrate, as shown by the values for \( K_s \) and \( V_{\text{max}} \), and the large part...
played by simple diffusion in total influx at high concentrations of glycine. A similar reversal of relative rates of intestinal transport of glycine and leucine at low and high concentrations was observed by Matthews & Laster (1965). The second point concerns the very slow influx of glycine in the jejunum in relation to that of Gly-Sar in the low concentration range. This suggests that, if absorption in vivo is related to influx in vitro, jejunal absorption of free glycine compared with that of peptide-bound glycine may be of relatively little physiological importance.

The values obtained for the simple diffusion component in influx of the compounds studied show that this is not a negligible component in total influx even at low concentrations of glycine and Gly-Sar, and at high concentrations makes a very large contribution to total influx of all three compounds studied (Table 2). With the preparation we have used, and possibly with other preparations of small intestine in vitro, the simple diffusion component cannot be ignored in studies of the kinetics of influx. If it is ignored, there may be a tendency to interpret results as indicating the operation of more than one mediated uptake mechanism, where perhaps only one such mechanism exists (Atkins & Gardner, 1977; Matthews et al., 1978).

Some features of the values for simple diffusion indicate a need for further investigation. Since in man, at least, the ‘aqueous pores’ of the small intestinal mucosa are believed to have a much larger radius in proximal than in distal small intestine (Fordtran, Rector, Locklear & Ewton, 1967; Soergel, Whalen & Harris, 1968), we had foreseen the possibility of higher values for simple diffusion in the jejunum than in the ileum. There is also no obvious explanation for why the values for simple diffusion of leucine are considerably less than those for Gly-Sar, since the molecular volumes of the amino acid and the peptide are unlikely to differ greatly.

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References


Table 2. Simple diffusion as a percentage of total influx of glycine, leucine and Gly-Sar in the jejunum and ileum of hamster small intestine in vitro, at three different substrate concentrations

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Site</th>
<th>Diffusion (% of total influx)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gly</td>
<td>Jejunum</td>
<td>35  58  77</td>
</tr>
<tr>
<td>Gly</td>
<td>Ileum</td>
<td>34  58  65</td>
</tr>
<tr>
<td>Leu</td>
<td>Jejunum</td>
<td>3-0 10  52</td>
</tr>
<tr>
<td>Leu</td>
<td>Ileum</td>
<td>2-8 11  50</td>
</tr>
<tr>
<td>Gly-Sar</td>
<td>Jejunum</td>
<td>10  20  60</td>
</tr>
<tr>
<td>Gly-Sar</td>
<td>Ileum</td>
<td>11  20  67</td>
</tr>
</tbody>
</table>
Jejunal and ileal dipeptide and amino acid influx


