SHORT COMMUNICATION

Kinetics of uptake of lysine and lysyl-lysine by hamster jejunum in vitro

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Summary

1. The kinetics of 2-min uptake of L-lysine and L-lysyl-L-lysine have been studied by using rings of everted hamster intestine in vitro, and values for $K_t$ and $V_{max}$ established.

2. On a molar basis, mediated uptake was more rapid for the amino acid than for the peptide. Non-mediated uptake was more rapid for the peptide than for the amino acid.

3. Comparison of relative rates of uptake of lysine from equivalent solutions of lysine and lysyl-lysine showed that at low concentrations, uptake of lysine was less rapid from the peptide than from the amino acid, whereas at high concentrations, uptake of lysine was more rapid from the peptide than from the amino acid. This type of effect of concentration on relative rates of uptake from equivalent solutions of amino acid and peptide has not previously been described.

Key words: amino acid absorption, dipeptide absorption, intestinal transport kinetics, jejunum.

Introduction

Since its first description in 1968, the phenomenon of more rapid intestinal absorption of amino acids from small peptides than from the equivalent free amino acids has been reported frequently by numerous investigators, with a variety of techniques, including small intestinal perfusion in man, and with a wide variety of di- and tri-peptides. Nevertheless, there have been scattered reports of certain small peptides being absorbed more slowly than the equivalent amino acids, and it is well known to investigators in the field that, at low concentrations, many peptides are absorbed at approximately the same rate as the equivalent amino acids. Most investigations have been carried out at one concentration only, or at a very limited number of concentrations. Adequate investigations of the kinetics of intestinal uptake of small peptides and the equivalent amino acids are still too few to make useful generalizations possible. Moreover, such investigations have so far been limited to neutral peptides and amino acids. The subject has been comprehensively reviewed by Matthews (1975) and Matthews & Payne (1980).

This paper reports the results of studies of the kinetics of uptake of L-lysine, a basic amino acid which is absorbed relatively slowly from free solution, and L-lysyl-L-lysine (Lys-Lys), the corresponding dipeptide. The work was carried out with hamster jejunum in vitro, at pH 5, a pH at which brush border hydrolysis of Lys-Lys is much less than at the traditional pH of 7.2–7.4 (Taylor, Burston & Matthews, 1980).

Materials and methods

Lysine was obtained from the Sigma Chemical Company, St Louis, Missouri, U.S.A. and Lys-Lys from Bachem Feinchemikalein AG Liestral, Switzerland. [14C]Lysine was obtained from The Radiochemical Centre, Amersham, Bucks., U.K. All other reagents were analytical or scintillation grade.

The experimental procedure and measurement of 2-min uptake of labelled and unlabelled substrates into rings of everted hamster jejunum were as previously described (Matthews, Addison & Burston, 1974; Sleisenger, Burston, Dalrymple, Wilkinson & Matthews, 1976; Matthews, Gandy, Taylor & Burston, 1979).
Briefly, incubations were carried out at 37°C in 0.5 ml of Tris/phosphate/sodium chloride (118 mmol/l) medium adjusted to pH 5-0 with isotonic citric acid. After removal from the incubation medium rings were rinsed in sodium chloride solution (154 mmol/l) at 4°C and after blotting on Whatman no. 50 filter paper, were eluted for 5 min in 1 ml of sulphasalicylic acid (60 g/l) at 100°C. Measurements of uptake were made over a wide range of concentrations (for lysine at 0-1, 0-2, 0-5, 1, 2, 5, 10, 20, 30, 50, 75 and 100 mmol/l and for Lys-Lys at 1, 2, 5, 10, 20, 30, 50, 75 and 100 mmol/l).

In experiments with lysine the radioactivity in the supernatant was measured by scintillation counting as previously described (Matthews et al., 1979). In experiments with Lys-Lys the supernatant was analysed for peptide and amino acid with a Locarte automatic-loading amino acid analyser. Lysine and Lys-Lys were eluted with a sodium citrate/borate buffer, pH 9-35. Some intact peptide as well as a large increase in free lysine was found in the supernatants from rings incubated in the higher peptide concentrations (above 10 mmol/l). Values for uptake of lysine and of Lys-Lys were corrected for substrates in the inulin space as described by Cheng, Navab, Lis, Miller & Matthews (1971). Uptake was expressed as µmol min⁻¹ g⁻¹ initial wet wt.

An estimate of the non-mediated component in uptake was made by the method of self-inhibition described from this laboratory (Matthews et al., 1979; Burston, Gandy, Matthews, Schedl & Taylor, 1979). In short, non-mediated uptake (taken to represent simple diffusion) was estimated from the uptake remaining at an infinitely high concentration of inhibitor, each substrate being treated as a competitive inhibitor of its own mediated transport, with extrapolation to an infinitely high concentration of inhibitor by using the plot described by Preston, Schaeffer & Curran (1974).

Discussion

The results show that in the case of the amino acid and dipeptide studied, uptake of amino acid units from equivalent solutions is more rapid from the amino acid at lower concentrations and more rapid from the peptide at higher concentrations. There is also, of course, a point at which uptake from amino acid and peptide occurs at equal rates. Kinetic behaviour of this kind has not, as far as we know, been previously described for any example of an amino acid and the equivalent peptide. It illustrates particularly clearly the dangers of drawing conclusions based on one set of concentrations only; if investigators do this, they may reach apparently opposite conclusions which are not, in fact, mutually exclusive. The mass of investigations already carried out leaves little doubt that small peptides in general do tend to be absorbed more rapidly than the equivalent amino acids and may in some circumstances be absorbed many times more rapidly (Matthews, 1975; Matthews & Payne, 1980), but the present results show that it is still desirable to compare more extensively and systematically the kinetic behaviour of individual peptides and the equivalent amino acids.

It may be noted that d for Lys-Lys was greater than that for lysine, and that this contributed appreciably to the relative rapidity of total uptake from the peptide in the higher part of the concentration range. We do not think that the larger d value for the peptide than for the amino
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FIG. 1. Uptake of lysine (μmol min⁻¹ g⁻¹) from equivalent solutions of lysine and Lys-Lys over a wide range of concentrations up to 100 mmol of lysine/l and 50 mmol of Lys-Lys/l. Main graph: •—•, mediated uptake of lysine from free lysine and O—O, mediated uptake of lysine from Lys-Lys; lysine content of medium up to 20 mmol/l. Points are corrected for non-mediated uptake, and standard errors are shown where space permits. Inset: total uptake of lysine from free lysine and •—•, total uptake of lysine from Lys-Lys; lysine content of medium up to 100 mmol/l. Curve 1, mediated uptake of lysine from Lys-Lys and curve 2, mediated uptake of lysine from free lysine. Ordinate and abscissa in the inset are in the same units as in the main Figure. Each point on the Figure represents the mean of 9–12 observations in 9–12 animals. Curves are calculated from values for $K_t$, $V_{max}$, and $d$ as given in the text.

acid was likely to be due to experimental error, because multiple estimates of $d$ for glycyl-sarcosine corresponded well (Matthews et al., 1979), and because over- or under-correction for simple diffusion leads to failure to reach a plateau in plots of $V$ against [S] for mediated transport, accompanied by non-linearity of linearizing plots of mediated transport. The larger $d$ value for Lys-Lys might be associated with the fact that at pH 5 Lys-Lys has two net positive charges whereas lysine has only one, and that the interior of the absorptive cells is negatively charged with respect to the incubation medium. However, the possibility that a contribution to what is estimated as uptake by simple diffusion is in fact made by a mediated transport system of unmeasurably high $K_t$ and $V_{max}$ is one that cannot be excluded.

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References


