Uptake of L-valyl-L-valine and glycylsarcosine by hamster jejunum *in vitro*

D. BURSTON¹, R. A. WAPNIR², E. TAYLOR¹ AND D. M. MATTHEWS¹

¹ Department of Experimental Chemical Pathology, Westminster Medical School, London, and ² Department of Pediatrics, North Shore University Hospital, Manhasset, New York, and Department of Pediatrics, Cornell University Medical College, New York, USA.

(Received 21 April/9 November 1981; accepted 12 January 1982)

Summary

1. Preliminary observations concerned with the effect of the lipophilic properties of the amino acid side-chains of peptides on their apparent affinity for uptake by rings of everted hamster jejunum showed that of the series glycylglycine, L-alanyl-L-alanine, L-valyl-L-valine and L-leucyl-L-leucine, with increasingly lipophilic side-chains, L-valyl-L-valine, not L-leucyl-L-leucine, was the most powerful inhibitor of uptake of the hydrolysis-resistant dipeptide glycylsarcosine. This apparently anomalous observation indicated a need for further investigation, and this paper reports investigations of the kinetics of uptake of L-valyl-L-valine and of competition for uptake between L-valyl-L-valine and glycylsarcosine.

2. L-Valyl-L-valine was capable of complete competitive inhibition of mediated uptake of glycylsarcosine. Free L-valine did not inhibit mediated uptake of glycylsarcosine. Glycylsarcosine could inhibit mediated uptake of L-valyl-L-valine only partially, but a mixture of glycylsarcosine and L-valine was capable of producing complete inhibition of mediated uptake of L-valyl-L-valine.

3. Investigation of the kinetics of uptake of L-valyl-L-valine indicated two mediated components. Component (a), which disappeared in the presence of free L-leucine, probably represented uptake of free L-valine after hydrolysis of the peptide. Component (b) probably represented peptide uptake.

4. The estimates of $K_t$ obtained for uptake of intact L-valyl-L-valine were many times greater than $K_i$ for inhibition of uptake of glycylsarcosine by L-valyl-L-valine. A possible explanation of the discrepancy is the existence of two pathways for uptake of L-valyl-L-valine and glycylsarcosine, for one of which L-valyl-L-valine has a low $K_i$ (i.e. a high affinity) not readily demonstrable by kinetic analysis.

5. The results suggest that mediated uptake of L-valyl-L-valine is the result of at least two processes, uptake of intact peptide by a mechanism or mechanisms shared with glycylsarcosine and also hydrolysis followed by uptake of free L-valine; estimates of the proportions of intact valine and of free valine taken up by mediated transport suggest that at pH 5 uptake of intact peptide varies from 25% at low concentrations to 55% at high concentrations. They do not explain why L-valyl-L-valine is a stronger inhibitor of uptake of glycylsarcosine than the more lipophilic L-leucyl-L-leucine, but do suggest how such a situation could arise.

Key words: absorption kinetics, competition for absorption, intestine, peptide absorption.

Introduction

It is believed that the apparent affinity of neutral amino acids for uptake by the absorptive cells of the small intestine is related to the lipophilic properties of the side-chain [1–3]. Preliminary observations concerned with the effect of the lipophilic properties of the amino acid side-chains of peptides on their apparent affinity for uptake...
by hamster jejunum in vitro showed that of the series glycylglycine, L-alanyl-L-alanine, L-valyl-L-valine and L-leucyl-L-leucine, with increasingly lipophilic side-chains, L-valyl-L-valine (Val–Val) was unexpectedly the most powerful inhibitor of uptake of the hydrolysis-resistant dipeptide glycylsarcosine (Gly-Sar). This observation indicated a need for investigation of the kinetics of uptake and inhibitory ability of each of the peptides in the series. This paper reports an investigation of the kinetic characteristics of uptake of Val–Val and of competition for uptake between Val–Val and Gly-Sar. As in our previous investigations [4, 5], the present work was carried out at pH 5 instead of the traditional 7.2 or 7.4. The lower pH reduced hydrolysis by the everted ring system employed, and uptake was not much reduced from its peak value at pH 6–7.

Materials and methods

[U-14C]Gly-Sar was kindly supplied by Dr S. Wilkinson of the Wellcome Research Laboratories, Beckenham, Kent, U.K. Val-[14C]Val was synthesized as described below. Uniformly labelled L-[14C]valine was obtained from The Radiochemical Centre, Amersham, Bucks, U.K. Unlabelled Gly-Sar, Val–Val, L-valine (Val) and L-leucine (Leu) were obtained from the Sigma Chemical Company, St Louis, Mo, U.S.A.

All other reagents were analytical or scintillation grade.

Experimental procedure

The experimental procedure and measurement of 2 min uptake (approximating influx) into rings of everted jejunum were as previously described [4–6].

All incubations were carried out in 0–5 ml of medium under conditions exactly as described by Taylor et al. [5]. Briefly, rings were incubated in 0–5 ml of substrate for 2 min at 37°C under O₂, with shaking at 100 strokes/min. After removal from the incubation medium the rings were rinsed in NaCl solution (154 mmol/l) at 4°C, blotted and eluted for 5 min in 1 ml of sulphosalicylic acid (60 g/l) in a boiling-water bath.

After centrifugation 0.5 ml of supernatant was added to 15 ml of 1,4-dioxane-based scintillation fluid and radioactivity was measured in a liquid scintillation spectrometer (Packard Tricarb model 3380).

The determination of the effect of pH on uptake and hydrolysis by intact rings was as previously described [5]. Free valine in the incubation medium was analysed by ion-exchange chromatography on a Locarte automatic loading amino acid analyser.

Uptake was expressed as μmol min⁻¹ g⁻¹ initial wet weight after correction for substrate in the inulin space [5] as previously described [7]. All estimates of uptake were the mean value of measurements of uptake into at least six rings from at least three animals.

Preparation of Val-[14C]Val

Uniformly labelled [14C]valine (50 μCi), in 1 ml of water containing 2% (v/v) ethanol, was evaporated to dryness in a 15 ml conical centrifuge tube. To the dried [14C]amino acid was added 20 μl of triethylamine (2.5 mol/l) in 1,2-dimethoxyethane and 20 μl of deionized water. After mixing, 50 μmol of t-butoxycarbonylvaline hydroxysuccinimide ester (Bachem Fein-chemikalien AG, Leistral, Switzerland) in 200 μl of 1,2-dimethoxyethane was added and the mixture allowed to stand at room temperature for 20 h to allow all the [14C]amino acid to react. The solution was evaporated to dryness on a rotary evaporator. Deionized water (200 μl) was added, the solution acidified with HCl (1 mol/l), and extracted three times with 200 μl of ethyl acetate. The ethyl acetate extract, after being washed with 200 μl of water containing 20 μl of HCl (1 mol/l), was evaporated to dryness. Trifluoroacetic acid (500 μl) was added and the solution allowed to stand at room temperature for 90 min. The trifluoroacetic acid was evaporated to dryness, starting at 20°C and then by increasing the temperature to 45°C, both in vacuo. The final residue was dissolved in 100 μl of ethanol/water (1:1, v/v) and applied to washed Whatman 3 MM chromatography paper and run with valine and Val-Val standards in butanol-acetic acid/water (120:30:50 by vol.) for about 8 h. The paper was dried and the standards were sprayed with ninhydrin. With the position of the peptide standard used as a guide, strips of paper were cut horizontally across the area of the chromatogram carrying the synthesized material. The strips were extracted with 1 ml of ethanol/water and 1 μl of each extract was counted by liquid scintillation counting. The strips containing the major radioactivity were extracted repeatedly with 1 ml aliquots of ethanol/water until the extract contained only a low level of radioactivity. The extracts were pooled, dried down and dissolved in 100 μl of ethanol/water, which was applied to a thin-layer chromatographic plate (10 cm × 10 cm cellulose-coated aluminium sheets, E. Merck Laboratory Chemicals, Darmstadt,
West Germany). A Val-Val standard was applied adjacent to the synthesized material and the plate was run in butan-1-ol/acetic acid/water (50:50:15:30, by vol.). The plate was dried and placed on X-ray film in the dark, wrapped in aluminium foil and left overnight. The cellulose layer corresponding to the darkened area on the developed X-ray film was scraped off into a tube. This area also corresponded to the position of the peptide standard. It was extracted three times with 1 ml of ethanol/water and the extract was dried down and taken up in 1 ml of water. A small amount (about 1 \( \mu l \)) was added to a solution of unlabelled Val-Val and run in thin-layer chromatographic plates with the valine and Val-Val standards in four different solvent systems:

1. butan-1-ol/water/acetic acid (12:5:3, by vol);
2. butan-1-ol/pyridine/water (1:1:1, by vol);
3. ethanol/aq. ammonia (sp. gr. 0-88)/water (80:4:16, by vol);
4. \( t \)-butanol/methyl ethyl ketone/25% (w/v) ammonia/water (70:40:15:30, by vol).

The four plates were placed on X-ray film and left overnight. In each solvent system a single zone was found, corresponding in position to the added unlabelled Val-Val and to the Val-Val standard.

### Estimation of hydrolysis by subcellular fractions and postincubation media

Estimates of the hydrolysis of Val-Val by subcellular fractions of the absorptive cells and by postincubation media and of the possible effect of L-leucine (Leu) on these processes were made as follows: 0.5 ml aliquots of Val-\(^{14}\)CVal at a concentration of either 1 mmol/l or 10 mmol/l, and made up in the presence or absence of leucine (25 mmol/l in Tris-phosphate/saline buffer, pH 7.0 or pH 5.0), were preincubated for 5 min at 37 °C in glass centrifuge tubes. To these solutions was added either 10 \( \mu l \) of a soluble jejunal fraction or 50 \( \mu l \) of a jejunal brush border fraction previously shown to display maximum peptidase activity. These fractions were kindly prepared by Dr T. J. Peters from hamster jejunum [8]. After 20 min incubation the reaction was stopped by placing the tubes in a boiling-water bath for 5 min. The tubes were then centrifuged and 0.1 ml of supernatant was applied to Whatman 3 MM chromatography paper, alongside a valine marker. The chromatograms were run in butan-1-ol/acetone/acetic acid/water (120:30:50, by vol.) for 6 h, dried and cut horizontally into 1 cm strips. The strips of paper were placed in radioactivity counting vials, 15 ml of scintillation fluid was added and the samples were counted as described earlier. The hydrolytic ability of postincubation media was measured by incubating rings prepared as usual in Tris–phosphate/saline buffer (0.5 ml) at pH 7.0 or pH 5.0 at 37 °C for 2 min. The rings were then removed and 10 \( \mu l \) of Val-\(^{14}\)CVal (50 mmol/l) was added to the medium to give a final Val-Val concentration of approximately 1 mmol/l. The solutions were incubated for a further 2 min. The tubes were then placed in a boiling-water bath for 5 min. After centrifuging 0.1 ml of supernatant was applied to Whatman 3 MM chromatography paper and the paper treated as described above.

All determinations of hydrolysis were carried out in duplicate, and the mean value was taken. Hydrolysis was expressed as the percentage of the substrate hydrolysed during the experimental period.

### Results

The effects of pH on uptake and hydrolysis of Val-Val by jejunal rings over the range pH 4–8 are shown in Fig. 1. At pH 5, at which subsequent experiments were carried out, uptake was only slightly less than at pH 7, whereas the amount of free valine appearing in the incubation medium was greatly reduced. At pH 7 the soluble fraction hydrolysed 95% of Val-Val (1 mmol/l) and in the presence of leucine (25 mmol/l) it hydrolysed 91%. The corresponding figures for Val-Val (10 mmol/l) were 56% and 53% respectively. At pH 7, the brush border fraction hydrolysed 5.5% of Val-Val (1 mmol/l) and in the presence of leucine (25 mmol/l) it hydrolysed 4.5%. Again, at pH 7, the postincubation fluid hydrolysed 76% of Val-Val (1 mmol/l) and in the presence of leucine (25 mmol/l) it hydrolysed

![Fig. 1. Effects of pH on uptake and hydrolysis of Val-\(^{14}\)CVal (5 mmol/l) by rings of everted hamster jejunum.](image-url)
FIG. 2. Preston–Schaeffer–Curran plots of uptake of Val-[^14]C]Val (1 mmol/l) in the presence of a range of concentrations of Gly-Sar (▲—▲) and Gly-Sar plus free valine (●—●), and uptake of [^14]C]Gly-Sar (1 mmol/l) in the presence of a range of concentrations of Val-Val (O—O). I represents concentration of inhibitor(s), V₀ rate of uptake in the absence of inhibitor(s) and Vₕ rate of uptake in the presence of inhibitor(s). When I is zero, the concentration of inhibitor(s) is infinitely high, and knowing V₀, Vₕ [the transport remaining at an infinitely high concentration of inhibitor(s)] can be calculated from the intercept on the ordinate. In plotting the line for uptake in the presence of Gly-Sar plus free valine, the total concentration of inhibitors was halved. This altered the slope of the line, but made no difference to its intercept on the vertical axis. Lines were drawn to fit by eye.

96%. At pH 5, neither soluble fraction, brush border fraction nor postincubation fluid produced any measurable hydrolysis of Val-Val. (The results are not intended to illustrate relative hydrolytic activities of the fractions used, but were obtained to provide an indication of the possible effects of leucine on hydrolysis of Val-Val.)

Uptake of [^14]C]Gly-Sar (1 mmol/l) was estimated alone and in the presence of a range of concentrations of Val-Val (20, 30, 50, 75 and 100 mmol/l) and the Preston–Schaeffer–Curran plot [9] (Fig. 2) was used to obtain uptake of [^14]C]Gly-Sar in the presence of an infinitely high concentration of Val-Val. Val-Val was a powerful inhibitor of uptake of Gly-Sar. It was found that uninhibited uptake (V₀) of [^14]C]Gly-Sar (1 mmol/l) was 0.23 μmol min⁻¹ g⁻¹ and Vₕ, uninhibitable uptake in the presence of an infinitely high concentration of inhibitor, was 0.023 μmol min⁻¹ g⁻¹. The value for V₀ was so similar to d (0.030 μmol min⁻¹ g⁻¹ at 1 mmol/l), the value assumed to represent non-mediated uptake of [^14]C]Gly-Sar (possibly by simple diffusion), obtained previously under similar conditions [5] that it was concluded that Val-Val could completely inhibit mediated uptake of Gly-Sar. Kᵢ, an inverse measure of the apparent affinity for transport 110⁻¹ for Val-Val, derived from its inhibitory effect on uptake of [^14]C]Gly-Sar (1 mmol/l) and calculated by the method of Finch & Hird [11], was 0.84 mmol/l. In this calculation, Kᵢ for Gly-Sar was taken to be 6.1 mmol/l (see below).

Uptake of [^14]C]Gly-Sar over a range of concentrations (0, 1, 0.2, 0.5, 1, 2, 5, 10 and 20 mmol/l) was estimated alone and in the presence of Val-Val (1 mmol/l), and the results corrected for d, on the assumption that d was directly proportional to concentration over the entire concentration range [4]. The results were then plotted according to Lineweaver & Burk (1/V against 1/S, where V is velocity of uptake and S is concentration) (Fig. 3). The plot indicated competitive inhibition of uptake of [^14]C]Gly-Sar by Val-Val, since the effect of Val-Val was to increase apparent Kᵢ for Gly-Sar without altering Vₕ max. In this plot, the uninhibited Kᵢ for Gly-Sar was 6.1 mmol/l. A similar experiment using valine (1 mmol/l) as inhibitor and [^14]C]Gly-Sar concentrations 0.1, 0.5, 2, 10 and 20 mmol/l showed that the free amino acid did not inhibit uptake of Gly-Sar.

Next, the kinetic characteristics of uptake of Val-[^14]C]Val were obtained by measuring uptake
Uptake of dipeptides by jejunum

*Uptake of dipeptides by jejunum*

Fig. 4. Plots of uptake of [14C]Val (-----) and Val-[14C]Val (-----) over the concentration range 0:1–100 mmol/l. All values are corrected for d. Lines were calculated from kinetic constants shown in the text.

Fig. 5. Preston–Schaeffer–Curran plots of uptake of [14C]valine (1 mmol/l) (-----) in the presence of a range of concentrations of valine, and uptake of Val-[14C]Val (-----) in the presence of a range of concentrations of Val-Val. F, V, and V, are as given in Fig. 2. Lines were drawn to fit by eye.

Fig. 6. Hofstee plots of uptake from Val-[14C]Val after correction for d, in the absence of leucine (-----) and in the presence of leucine (25 mmol/l) (-----). The curve for uptake in the absence of leucine was calculated from kinetic components (a) and (b) (see the text). Values for uptake in the presence of leucine, assuming uptake of free valine to be completely inhibited, were calculated as described in the text and the line was drawn to fit by eye.

of radioactivity from this peptide over the concentration range 0:1–100 mmol/l (0:1, 0:2, 0:5, 1:0, 2, 5, 10, 20, 30, 50, 75 and 100 mmol/l) (Fig. 4). A Preston–Schaeffer–Curran plot (Fig. 5) of uptake of Val-[14C]Val (1 mmol/l) in the presence of Val-Val over a range of inhibitor concentrations (4, 9, 19, 29, 49, 74 and 99 mmol/l) [4] gave a value for d of 0.006 μmol min⁻¹ g⁻¹, and figures for uptake of Val-[14C]Val were corrected for this as above. Corrected values for uptake of Val-[14C]Val over the concentration range 0:1–100 mmol/l were plotted by using the Hofstee plot (V against V/S). The Hofstee plot (Fig. 6) was clearly biphasic, and resolution into two components as described by Rubino et al. [12] gave (a) K, 0.15 mmol/l, Vₘₐₓ, 0.075 μmol min⁻¹ g⁻¹ and (b) K, 6.9 mmol/l, Vₘₐₓ, 1.8 μmol min⁻¹ g⁻¹.

Uptake of Val-[14C]Val (1 mmol/l) was estimated alone (V₀ = 0.46 μmol min⁻¹ g⁻¹) and in the presence of a range of concentrations of Gly-Sar (20, 30, 50, 75 and 100 mmol/l). A Preston–Schaeffer–Curran plot (Fig. 2) showed
that $V_o (0.20 \text{ mmol min}^{-1} \text{ g}^{-1})$ was very substantial, and far greater than $d$ for Val-Val. It seemed that mediated uptake of Val-Val could not be anywhere near completely inhibited by Gly-Sar. A possible reason for the failure of Gly-Sar to cause complete inhibition of uptake of Val-$^{14}$C$\text{Val}$ was that uptake from this peptide was not solely the result of uptake of intact Val-Val, but was in part due to uptake of free valine liberated from the peptide by hydrolysis in the medium and brush border region, even though such hydrolysis had been reduced by working at pH 5. Uptake of free valine would not be expected to be inhibited by the hydrolysis-resistant peptide Gly-Sar, since amino acid and peptide uptake are independent processes [10]. Because of this, uptake of Val-$^{14}$C$\text{Val}$ (1 mmol/l) was measured alone ($V_o, 0.40 \text{ mmol min}^{-1} \text{ g}^{-1}$) and in the presence of a concentration range (20, 30, 40, 50 and 60 mmol/l) of Gly-Sar together with a concentration range of free valine (20, 30, 40, 50 and 60 mmol/l). A Preston–Schaefier–Curran plot (Fig. 2) of the results gave a value for $V_o$ of 0.010 $\text{mmol min}^{-1} \text{ g}^{-1}$. This was sufficiently close to $d$ for Val-$^{14}$C$\text{Val}$ (0.006 $\text{mmol min}^{-1} \text{ g}^{-1}$) to suggest that the peptide Gly-Sar plus the amino acid valine were together capable of complete inhibition of mediated uptake from Val-Val.

It therefore appeared likely that total uptake of valine from Val-Val was in part the result of uptake of intact peptide, and in part the result of hydrolysis of Val-Val followed by uptake of free valine. If this were so, it seemed possible that one of the two components in the Hofstee plot (Fig. 6) represented uptake of peptide, and the other reflected uptake of free amino acid. An investigation of the kinetics of uptake of free $^{14}$C$\text{Valine}$ (0.1, 0.2, 0.5, 1.0, 2.5, 10, 20, 30, 50, 75 and 100 mmol/l) was undertaken. A Preston–Schaefier–Curran plot (Fig. 5) of uptake of $^{14}$C$\text{Valine}$ (1 mmol/l) in the presence of valine over a range of inhibitor concentrations (4, 9, 19, 29, 49, 74 and 99 mmol/l) gave a value for $d$ of 0.016 $\text{mmol min}^{-1} \text{ g}^{-1}$, and figures for uptake of $^{14}$C$\text{Valine}$ were corrected for this as already described. Corrected values (Fig. 4) for uptake of $^{14}$C$\text{Valine}$ were plotted with the Hofstee plot. In this case, the plot was linear: $K_i, 8.4 \text{ mmol/l}$, $V_{\text{max}} 3.9 \text{ mmol min}^{-1} \text{ g}^{-1}$; however, these values did not correspond to those of either component (a) or component (b) of the Hofstee plot for Val-Val. Consequently, an attempt was made to identify the peptide uptake component in the Hofstee plot for Val-Val by minimizing the element of free amino acid uptake in total uptake from the peptide. This was done by studying the kinetics of uptake of Val-$^{14}$C$\text{Val}$ (0.5, 1.0, 2.5, 10, 20, 30, 50, 75 and 100 mmol/l) in the presence of a concentration of leucine (25 mmol/l) which was expected to produce powerful inhibition of uptake of free valine. The result was to reduce mediated uptake, the resulting kinetic curve showing disappearance of component (a) and giving an approximately linear Hofstee plot with $K_i, 8.2 \text{ mmol/l}$ and $V_{\text{max}} 1.3 \text{ mmol min}^{-1} \text{ g}^{-1}$. These values were not widely different from those of component (b) in the kinetics of uptake of Val-Val in the absence of inhibitor, so that component (b) might represent uptake of unhydrolysed Val-Val. Component (a) might then reflect uptake of free valine from Val-Val. Reasons for the great discrepancies between $K_i$ and $V_{\text{max}}$ values for component (a) and those for free valine are considered in the Discussion.

An attempt at a more accurate estimate of the kinetic parameters of component (b) was made as follows. Experiments on the inhibitory effect of leucine (25 mmol/l) on uptake of free valine showed that this was not complete at the concentrations studied: mediated uptake was reduced by no more than about 80%. Mediated uptake of valine (0.5 mmol/l) was reduced by leucine (25 mmol/l) by 84% and mediated uptake of valine (5.0 mmol/l) by 75%. From these figures, $K_i$ for leucine was calculated, the mean value being 4.8 mmol/l. From this $K_i$ value, the extent of inhibition of uptake of valine by leucine at any concentration of valine could be calculated on the assumption that leucine and valine shared the same uptake system and that this conformed to Michaelis–Menten kinetics. It was then possible to estimate more closely the proportions of Val-Val taken up as free valine and as intact Val-Val. An approximate estimate of the proportion of Val-Val taken up by mediated uptake of free valine at any given concentration was made from the reduction of total mediated uptake in the presence of leucine. This estimate of uptake of free valine was then multiplied by an appropriate correction factor based on the assumption that leucine was potentially capable of 100% inhibition of uptake of free valine from Val-Val. For example, if the reduction of total mediated uptake (expressed as valine) in the presence of leucine were 0.48 $\text{mmol min}^{-1} \text{ g}^{-1}$, uptake of free valine was taken to be 0.48 x $(100/82) = 0.59 \text{ mmol min}^{-1} \text{ g}^{-1}$. From this figure and that for total mediated uptake, uptake of intact Val-Val could be calculated by difference (Table 1). The example given is taken from data contributing to Table 1 (line 3, initial concentration of Val-Val 2 mmol/l). When the values for mediated uptake of intact Val-Val (Table 1), obtained as described, were plotted they gave an approximately linear
TABLE 1. Mediated uptake of free and peptide valine from Val-Val, and proportions of Val-Val taken up as free amino acid and as peptide

| Initial concn. of Val-Val in medium (mmol/l) | Conc. of free valine appearing in medium at 2 min (mmol/l) | Mediated uptake of Val-Val (μmol min⁻¹ g⁻¹) | Mediated uptake of free valine (μmol min⁻¹ g⁻¹) | Bulk phase concn. to give observed uptake of free valine (mmol/l) | Val-Val taken up by mediated transport as free valine (%) | Val-Val taken up by mediated transport as peptide (%) | Mediated uptake (of valine + Val-Val) as % of total uptake (expressed as Val-Val) (%) |
|---|---|---|---|---|---|---|---|---|
| 0.5 | 0.09 | 0.044 | 0.27 | 0.62 | 75 | 25 | 98 |
| 1 | 0.11 | 0.093 | 0.40 | 0.86 | 68 | 32 | 98 |
| 2 | 0.15 | 0.179 | 0.59 | 1.50 | 62 | 38 | 97 |
| 5 | 0.22 | 0.374 | 0.91 | 2.55 | 55 | 45 | 96 |
| 10 | 0.30 | 0.555 | 1.15 | 3.51 | 51 | 49 | 95 |
| 20 | 0.42 | 0.735 | 1.35 | 4.44 | 48 | 52 | 92 |
| 30 | 0.49 | 0.820 | 1.44 | 4.91 | 47 | 53 | 89 |
| 50 | 0.56 | 0.915 | 1.47 | 5.08 | 45 | 55 | 85 |
| 75 | 0.57 | 0.950 | 1.54 | 5.48 | 45 | 55 | 79 |
| 100 | 0.58 | 0.970 | 1.56 | 5.60 | 45 | 55 | 75 |

Hofstee plot (Fig. 6) with $K_r$ 9.6 mmol/l and $V_{max}$ 1.1 μmol min⁻¹ g⁻¹. These values were similar enough to those of component (b) in the original kinetic plot ($K_r$ 6.9, $V_{max}$ 1.8) to confirm the hypothesis that this component represented the kinetics of uptake of intact Val-Val.

Finally, bulk phase medium concentrations of free valine were estimated on 2 min samples from the experiment on uptake of Val-Val in the presence of leucine. The relationship between these concentrations and initial Val-Val concentration suggested that appearance of free valine in the medium was the result of a saturable hydrolytic process or processes (Table 1). These estimations showed that mediated uptake of free valine from the peptide could not be accounted for by uptake of free valine from the concentrations of this amino acid appearing in the medium. Much higher bulk phase concentrations of free valine would be required to account for the calculated uptake of free valine from the peptide (Table 1), as can be shown from the rate: concentration curve for free valine (Fig. 4).

**Discussion**

The present results are most readily interpreted by the hypothesis that in hamster jejunum Val-Val and Gly-Sar share a common peptide uptake system or systems and that whereas Gly-Sar is taken up entirely as intact peptide, uptake from Val-Val is the result of two processes, (1) uptake of intact peptide and (2) hydrolysis, probably mainly in the brush border, followed by uptake of free valine. It was suggested some years ago that uptake of most peptides might be the result of such a dual mechanism [7, 13, 14] and Himukai & Hoshi [15] have recently shown that this is true of uptake of Gly-Leu by guinea-pig ileum. Component (b) in the kinetics of uptake of Val-Val is likely to be attributable to peptide uptake. Component (a) which disappears in the presence of leucine, a powerful inhibitor of uptake of free valine, may reflect uptake of free valine from Val-Val. However, this process, even if it follows Michaelis–Menten kinetics, is not, with our data, susceptible to analysis according to such kinetics because the relationship between the effective concentrations of free valine at the uptake sites and the concentrations of Val-Val in the bulk phase of the incubation medium is not known. Consequently, no significance can be attached to the ‘kinetic constants’ obtained for component (a).

An interesting feature of the results is that the $K_r$ values obtained for Val-Val (6.9–9.6 mmol/l) are many times greater than the $K_r$ for Val-Val (0.84 mmol/l) derived from its inhibitory effect on Gly-Sar uptake. This difference is the more striking when it is remembered that if $K_f$ and $K_r$ for Val-Val were equal, as they should be if Val-Val shared a single common uptake system with Gly-Sar, observed $K_f$ would be expected to be higher than ‘actual’ $K_f$ and $K_r$ owing to partial hydrolysis of Val-Val before uptake reducing its effectiveness as inhibitor. Large discrepancies between $K_f$ and $K_r$, which have been described in the field of amino acid transport, may suggest the existence of more than one carrier system [10, 11], and the present results could be explained if the unexpectedly powerful inhibitory effect of Val-Val on uptake of Gly-Sar were the result of a second component in uptake of intact Val-Val, with a low $K_r$, which our kinetic analysis has been unable to resolve. However, this is not the only possible explanation of the discrepancy and at the
present stage we should prefer not to commit ourselves to a final explanation of this result, the reason for which must await further investigation. It should be pointed out that if there are two pathways for uptake of Val-Val, then Gly-Sar must be expected to share both these pathways, otherwise it could not, even in the presence of valine, completely inhibit uptake of Val-Val. Rubino et al. [12] reported that two saturable processes were involved in uptake by rabbit ileum of Gly-Pro, a peptide which undergoes little or no hydrolysis in the brush border [16], but using Gly-Sar, L-glutamyl-L-glutamic acid and L-lysyl-L-lysine [4, 5], we have not previously found evidence for uptake of peptides by more than one common mediated process, though the involvement of multiple processes has not been excluded. In interpreting the data, we have to bear in mind that complex kinetic analysis is subject to possible serious error.

The estimates of the proportions of Val-Val taken up by mediated transport as intact peptide and in the free form are of interest, though it must be stressed that they are likely to be approximate, depending as they do on the assumptions that leucine is capable of completely abolishing mediated uptake of free valine while leaving extracellular hydrolysis and uptake of intact Val-Val not significantly affected. They suggest that, at low concentrations, most of the mediated uptake from Val-Val is in the form of free valine, whereas at high concentrations most of the uptake is in the form of intact peptide. The cause of this effect has not been investigated, though it might occur, for example, if brush border hydrolysis of the peptide approached saturation at lower levels than peptide transport. The observation fits well with the results of earlier experiments with a variety of peptides, in which clear evidence of uptake of intact peptide was obtainable only at the higher concentrations studied [13]. The possible physiological significance of the finding remains to be determined, and in this connection it must not be forgotten that in our experiments hydrolysis was deliberately minimized by working at pH 5 instead of the customary pH 7-7-4, whereas intrajejunal pH may be about 6. Himukai & Hoshi [15], working at pH 7-4, reported that in the guinea-pig intestinal uptake of Gly-Leu was about 50% in the form of intact peptide at several concentrations covering a narrower range than those used in the present work. Previously Adibi [14] concluded that in man Gly-Gly was taken up almost entirely as intact peptide. It has already been pointed out that intestinal handling of different peptides is likely to differ widely, since individual peptides differ greatly in their susceptibility to hydrolysis [13, 17].

Why free valine appeared in the medium in the intact ring-incubation medium system whereas no hydrolysis of Val-Val was demonstrable at pH 5 with either soluble fraction, brush border fraction or postincubation medium, we are at present unable to explain. It might be because the pH microclimate of the brush border is partially maintained even in the presence of a lowered external pH. It is unlikely that appreciable amounts of free valine would back-diffuse from the interior of the absorptive cells (the pH of which is probably little altered by incubation at pH 5) in so short an incubation period as 2 min, at which peptide uptake is linearly related to incubation time [6, 15, 18]. Comparison of the amount of free valine appearing in the medium of the intact system (Fig. 1), at an initial Val-Val concentration of 5 mmol/l in the absence of leucine, with the amount of free valine appearing in the medium at an initial Val-Val concentration of 5 mmol/l, in the presence of leucine (25 mmol/l), suggests that leucine does not substantially alter the rate of hydrolysis of Val-Val at pH 5 in the intact system. The experiments on the possible effects of leucine on hydrolysis of Val-Val were carried out because it has been reported that amino acids may either stimulate or inhibit hydrolysis of peptides [13, 19].

The observation that the concentrations of free valine appearing in the medium during incubation with Val-Val were much too low to account for uptake of free valine from the peptide was not unexpected. Burston et al. [20] found evidence that the effective concentration of an amino acid released from a peptide, as judged by amino acid uptake, was much higher than the concentration appearing in the bulk phase of the medium, and Himukai & Hoshi [15] obtained similar findings. Burston et al. [20] suggested that this phenomenon might be accounted for either by the diffusion barrier of the unstirred layer [21] or, possibly, by Ugolev’s [22] hypothesis of ‘membrane hydrolysis’, according to which amino acids released by hydrolysis of peptides at the absorptive surface are taken up by a pathway or pathways inaccessible to free amino acids. The potentially complete inhibition by free valine of mediated uptake of valine released by hydrolysis of Val-Val does not support Ugolev’s hypothesis, and the unstirred layer effect seems a more likely explanation. Ganapathy & Radhakrishnan [23] apparently overlooked this phenomenon in a paper concluding that there were two distinct pathways for uptake of Gly-Leu by monkey small intestine.
A point requiring discussion is that of the validity or otherwise of the Preston–Schaeffer–Curran plot in a situation in which uptake system is inhibited by more than one inhibitor, each using one of the systems, as when uptake of intact Val-Val and of free valine derived from hydrolysis of Val-Val from valine is inhibited by Gly-Sar and free valine respectively. It can be shown that in this situation the plot becomes non-linear, but the deviation from linearity is so small that it is undetectable graphically and of no practical importance.

Another point concerns the value of \( d \) for Val-Val, used in correcting the uptake/concentration curve for this peptide. This would appear to represent uptake, probably by simple diffusion, of a mixture of valine and Val-Val of uncertain composition, which is likely to vary with peptide concentration, and it may not be justifiable to assume that the value obtained at substrate concentration 1 mmol/l can be used on a basis of simple proportion to give a correction applicable over the entire concentration range. Nevertheless, over much of the concentration range, correction for \( d \) represents only a small proportion of total uptake, and we have not attempted any more elaborate procedure than that described.

Finally, it is of interest to compare rates of uptake, expressed as valine, from the peptide Val-Val and equivalent solutions of free valine (1 mmol of Val-Val = 2 mmol of Val). Over the wide concentration range studied it was found that above a valine concentration of about 0.2 mmol/l, whether or not uptake was corrected for \( d \), uptake of valine from the free amino acid was always more rapid than from the equivalent peptide. This contrasts with the large number of reports, reviewed by Matthews [13], of more rapid absorption of amino acids from peptides than from the equivalent free amino acid(s).

The observations reported here do not explain why Val-Val is a stronger inhibitor of uptake of Gly-Sar than the more lipophilic L-leucyl-L-leucine, though they suggest a possible reason. Such a situation could arise if L-leucyl-L-leucine had a higher affinity or affinities for uptake than Val-Val, yet was much more extensively hydrolysed before uptake than Val-Val, so that its potentially powerful inhibitory effect on uptake of other peptides was largely nullified. This possibility will be explored in subsequent investigations.

Acknowledgments

This work was supported by the Medical Research Council of Great Britain. R.A.W. took part in it while holding a Wellcome Research Travel Grant. We are grateful to Mr T. Sopanen of the Technical Research Centre of Finland for supplying details of the method of peptide synthesis.

References


