Biochem. J. (1975) **146**, 133–139
Printed in Great Britain

Studies on a Wide-Spectrum Intestinal Dipeptide Uptake System in the Monkey and in the Human

By MANJUSRI DAS and A. N. RADHAKRISHNAN*
Wellcome Research Unit, Christian Medical College Hospital, Vellore 632004, *Tamil Nadu, India*

(*Received* 24 *June* 1974)

1. The intestinal transport of glycine and leucine residues of glycyl-L-leucine was studied in the monkey and in the human *in vitro*. Uptake of both [^{14}C]glycyl-L-leucine and glycyl-L-[^{14}C]leucine show similar K_t values, but there is a marked difference in the $V_{max.}$ values. Preliminary studies suggest that this anomalous difference in the $V_{max.}$ values may be due to the greater efflux rate of glycine from the tissue. 2. Arrhenius plots of both [^{14}C]glycyl-L-leucine uptake and glycyl-L-[^{14}C]leucine uptake in the monkey intestine show a discontinuity at about 20°C. The activation energies above and below the discontinuity are similar for both [^{14}C]glycyl-L-leucine uptake and glycyl-L-[^{14}C]leucine uptake. These similarities in uptake characteristics suggest that the dipeptide glycyl-L-leucine is transported as one unit. 3. In the monkey intestine, glycyl-L-leucine uptake is inhibited by a wide variety of dipeptides, including those containing acidic and basic amino acids. The inhibition was shown to be competitive by using four representative dipeptides namely: L-alanyl-L-alanine, L-alanyl-L-leucine, L-glutamyl-L-glutamic acid and L-lysyl-L-lysine. The results strongly suggest that in the monkey intestine there may be a dipeptide-uptake system with an extremely broad specificity. These results were also confirmed in the human in a limited way.

Long neglected since the original observation of Newey & Smyth (1959), the role of peptide absorption in the terminal stages of protein digestion in the small intestine is now being increasingly recognized. In recent years intestinal dipeptide absorption has been studied in various mammalian species, including the human (Lis *et al.*, 1971; Adibi, 1971; Hellier *et al.*, 1972*a*; for a review, see Matthews, 1972). A peptide-transport system distinct from the amino acid-transport systems has been shown to be present in most animal species, but the actual mechanism of uptake is still largely unknown. A preliminary study (Das & Radhakrishnan, 1974*b*) on glycyl-L-leucine uptake in monkey intestine showed that the specificity of the uptake system was similar to the substrate specificity of the monkey intestinal glycyl-L-leucine hydrolase (Das & Radhakrishnan, 1972, 1973) in its broad scope, but was different in some crucial respects. This indicated that the initial entry process for dipeptides may be independent of the action of the 'master' dipeptidase. Although the dipeptidases may not be directly involved in the uptake process, an indirect role was suggested by the observation that the V_{max}/K_t value for uptake was the highest in the jejunal region, which was shown earlier to be the site of maximal activity of the glycyl-L-leucine hydrolase (Das & Radhakrishnan, 1974*a*). The studies of Das & Radhakrishnan (1974*b*) in monkey intestine also showed that the properties of the glycyl-L-leucine-uptake system are quite different from that of free amino acid uptake. Later, when radioactive glycyl-L-leucine, labelled in either the glycine or the leucine residue, became available, a more detailed study of the characteristics of the uptake process was made both in the monkey and in the human. The results of these studies are reported in the present paper.

*Present address: Roche Institute for Molecular Biology, Nutley, N.J, 07110, U.S.A.

Experimental

Chemicals

[^{14}C]Glycyl-L-leucine and glycyl-L-[^{14}C]leucine were from The Radiochemical Centre, Amersham, Bucks., U.K. The unlabelled peptides were obtained as indicated. L-Glutaminyl-L-glutamine was kindly donated by Professor Alton Meister (Cornell University, New York, N.Y., U.S.A.). L-Glutamyl-L-glutamic acid, L-tyrosyl-L-lysine, and glycyl-L-leucyl-L-glycine were from Schwarz/Mann, Orangeburg, N.Y., U.S.A. All other peptides were from Sigma Chemical Co., St. Louis, Mo., U.S.A. The peptides were all chromatographically pure.

All other chemicals were of analytical grade.

Uptake experiments

Adult male monkeys (*Macaca radiata*) in the weight range 4–6kg were used. The region of intestine at 35–40% distance of the total length of intestine from the pyloric end was used in all these experiments.

An earlier study (Das & Radhakrishnan, 1974*b*) had shown this region to be the most active site for peptide uptake. Small rectangular strips of intestine were used in all the uptake experiments.

The surgical specimen of the human intestine (7.6cm long) was resected from the jejunal region of a patient undergoing corrective surgery for complications arising after a gastroenterostomy. It was immediately transferred into ice-cold oxygenated Krebs–Ringer bicarbonate buffer (Umbreit *et al.*, 1964) containing 0.5% glucose. Pieces of the mucosal layer were carefully removed and small bits weighing between 15 and 20mg were used for the uptake studies, the procedure for which was the same as for the monkey intestinal strips.

Uptake experiments were carried out under the same conditions as described previously (Das & Radhakrishnan, 1974*b*), except for a few modifications. Each intestinal strip (10–20mg) was pre-incubated at the same temperature as the incubation step, with shaking for 5min in 2ml of oxygenated Krebs–Ringer bicarbonate buffer, containing 0.5% glucose. Afterwards the medium was aspirated and fresh medium (0.5ml of Krebs–Ringer bicarbonate buffer containing 0.5% glucose) containing the peptide solution was added and the mixture was then incubated for 2min at 37°C except where otherwise stated. After incubation, the intestinal strip was gently blotted on filter paper, weighed, and then digested in a scintillation vial with 0.5ml of 2M-NaOH at 75°C for 2h. After digestion, 0.5ml of water and 10ml of the scintillation mixture were added. The scintillation mixture contained 10% naphthalene, 0.5% 3,5-diphenyloxazole and 3% Cab-o-Sil in dioxan. The radioactivity was measured in a liquid-scintillation counter (Beckman model LS-100).

For the determination of extracellular space, the tissue was incubated with [^{3}H]inulin under the same conditions as for incubation with peptide solution. The radioactivity was determined as described above. The extracellular fluid for 2min incubation was found to be 5% of the wet wt. of tissue. The total water content determined separately by drying the tissue at 110°C for 12h was found to be 78% of the wet wt. of tissue.

During uptake glycyl-L-leucine was completely hydrolysed so that only the free amino acids glycine and leucine could be detected in the tissue. The tissue uptake of [^{14}C]glycyl-L-leucine and glycyl-L-[^{14}C]-leucine was calculated from the radioactivity present in the tissue after correcting for the extracellular space. The uptake was calculated from the following equation:

$$\text{Uptake}\ (\mu\text{mol/min per g of tissue}) = \frac{1}{T}\left[\left(\frac{P_t^*}{W}\times\frac{[P]_m}{[P^*]_m}\right)-\left(\frac{ECV}{100}\times[P]_m\right)\right]$$

where subscripts t and m refer to tissue and medium, respectively; $[P]_m$ is the concentration of glycyl-L-leucine in μmol/ml in the medium; P_t^* is the c.p.m. due to glycyl-L-leucine in the tissue; $[P^*]_m$ is the c.p.m./ml due to glycyl-L-leucine in the medium; W is the weight of the tissue in grams; ECV is the extracellular volume expressed as ml of water present/100g of tissue; T is the time of incubation in minutes. All values for intestinal uptake were expressed per g wet wt. of intestine.

In separate experiments it was found that the uptake rate with the tissue strips with both mucosal and serosal sides exposed was not significantly different from the rate obtained with everted segments of intestine with only the mucosal side exposed. The everted sacs were prepared by the method of Malathi *et al.* (1973).

All the incubations were carried out in duplicate with the tissue from the same animal. In some experiments (Figs. 1 and 2*a*) the experiments were repeated in a second animal. The variations were not more than ±15% between animals and often within ±5% in the same animal.

All the incubation experiments were started immediately after the animal was killed and the experiments were completed within 1h. However, it was found by separate experiments that the tissue maintained in oxygenated Krebs–Ringer buffer showed no deterioration in the uptake property almost up to 3h after killing the animal. Longer storage of the tissue in the oxygenated Krebs–Ringer buffer at 0°C resulted in gradual loss of dipeptide uptake. It is noteworthy that the dipeptide-uptake rate decreased drastically when younger monkeys below about 2kg weight were used.

Results

Time-course of uptake

The time-course of uptake of [^{14}C]glycyl-L-leucine and glycyl-L-[^{14}C]leucine by monkey intestine is shown in Fig. 1. The uptake is linear up to about 2min. Within this time about 10% of the peptide in the medium is hydrolysed. The marked difference in the uptake rates of glycine and leucine residues of glycyl-L-leucine is noticeable throughout the 20min time-course. After the initial 5min glycyl-L-leucine may be transported mainly in the form of amino acids, since there is extensive hydrolysis of the peptide in the medium. However, it should be emphasized that the bulk of the uptake as measured in the 2min period was due to uptake of intact dipeptide, since only dipeptides but not free amino acids effectively inhibited this uptake (Das & Radhakrishnan, 1974*b*). Secondly, the existence of extremely powerful dipeptidases leads to total hydrolysis of the dipeptides entering the cell, even with the very short incubation period employed.

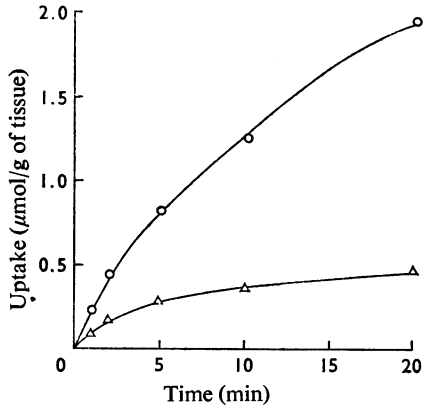

Fig. 1. *Time-course of uptake of* [^{14}C]*glycyl-*L*-leucine* (△) *and glycyl-*L-[^{14}C]*leucine* (○) *in the monkey intestine*

The concentration of peptide in the medium was 0.5mM. The specific radioactivity of [^{14}C]glycyl-L-leucine and glycyl-L-[^{14}C]leucine in the medium varied between 2×10^5 and 3×10^5c.p.m./μmol of glycyl-L-leucine. Extracellular space was determined for the time-periods shown in the Figure.

Kinetic characteristics of [^{14}C]*glycyl-*L*-leucine uptake and glycyl-*L-[^{14}C]*leucine uptake*

The effect of concentration of glycyl-L-leucine in the medium on the rate of accumulation of glycine and leucine residues is shown in Fig. 2. In the monkey intestine (Fig. 2*a*), the K_t values for uptake of both glycine and leucine residues are similar, but there is a marked difference in the $V_{max.}$ values. In the human intestine also the situation is somewhat similar (Fig. 2*b*). Both [^{14}C]glycyl-L-leucine uptake and glycyl-L-[^{14}C]leucine uptake have similar K_t values. The difference in the $V_{max.}$ values in this case are not so marked as in the case of monkey.

If the dipeptides are transported intact into mucosal cells, it would be expected that the two amino acid residues of the peptide would be accumulated inside the cell to the same extent. But in actual experiments in the present study a marked difference in the uptake rates of glycine and leucine residues of glycyl-L-leucine was observed (Fig. 2*a*). Such differences have also been noticed by earlier workers (Burston *et al.*, 1972) although an explanation is not readily available for this anomalous result. To understand this phenomenon, a study was made on the rates of efflux of glycine and leucine from monkey intestinal strips. The intestinal strips were preloaded with [^{14}C]glycine or [^{14}C]leucine by incubating with these amino acids (0.5mM) for 5min. After washing the preloaded tissue with ice-cold Krebs–Ringer bicarbonate buffer, these were incubated at 37°C for 3min in the same buffer to

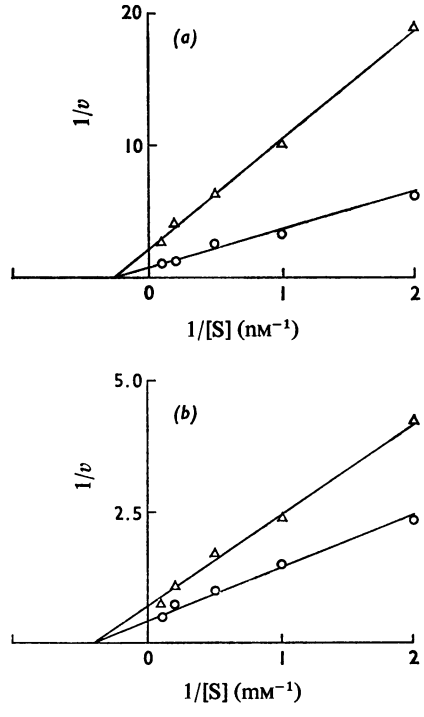

Fig. 2. *Lineweaver–Burk plots for* [^{14}C]*glycyl-*L*-leucine uptake* (△) *and glycyl-*L-[^{14}C]*leucine uptake* (○) *in the monkey* (*a*) *and in the human* (*b*)

All incubation media were adjusted to an osmolarity of 346mosmol/litre with appropriate additions of mannitol. The specific radioactivity of [^{14}C]glycyl-L-leucine and glycyl-L-[^{14}C]leucine in the medium varied between 1×10^5 and 3×10^5c.p.m./μmol of glycyl-L-leucine. v is expressed as μmol absorbed/min per g of tissue. The kinetic constants for the monkey intestinal uptake system are: $V_{max.}$ for [^{14}C]glycyl-L-leucine = 0.50μmol/min per g of tissue; $V_{max.}$ for glycyl-L-[^{14}C]leucine = 1.43μmol/min per g of tissue; K_t for [^{14}C]glycyl-L-leucine = 4mM; K_t for glycyl-L-[^{14}C]leucine = 4mM. The kinetic constants for the human intestinal uptake system are: $V_{max.}$ for [^{14}C]glycyl-L-leucine = 1.4μmol/min per g of tissue; $V_{max.}$ for glycyl-L-[^{14}C]leucine = 2.2μmol/min per g of tissue; K_t for [^{14}C]glycine-L-leucine = 2.5mM; K_t for glycyl-L-[^{14}C]leucine = 2.5mM.

measure the efflux of radioactivity into the medium. The endogenous amounts of glycine and leucine in the tissue were determined by using an alcoholic [80% (v/v) ethanol] extract of the tissue. The amino acids were measured by using an automatic amino acid analyser. The endogenous tissue amount of glycine was found to be about 3 times the tissue amount of leucine. The efflux rates of glycine and leucine were calculated by using these data. The rate of efflux of glycine from the tissue was found to be at

Table 1. *Specificity of the monkey intestinal glycyl-L-leucine uptake system*

In these experiments the concentration of ^{14}C-labelled glycyl-L-leucine was 0.5 mM and the concentration of the unlabelled peptides was 10 mM in all cases. The specific radioactivity varied between 2×10^5 and 2×10^6 c.p.m./μmol of glycyl-L-leucine. All the peptides, excepting glycyl-D-leucine, contained amino acids of the L configuration. Different animals were used for a given number of dipeptides and the values are expressed as a percentage of the observed glycyl-L-leucine uptake in each experiment. Z, *N*-benzyloxycarbonyl; Gly-γ-Abu, glycyl-γ-aminobutyric acid.

Peptide	% inhibition of uptake of [^{14}C]Glycyl-L-leucine	% inhibition of uptake of Glycyl-L-[^{14}C]leucine	Peptide	% inhibition of uptake of [^{14}C]Glycyl-L-leucine	% inhibition of uptake of Glycyl-L-[^{14}C]leucine
Gly-Gly	24	20	Gly-Asp	38	50
Gly-Ala	42	50	Gly-Glu	49	42
Gly-Val	70	65	Gly-Asn	50	63
Gly-Ile	80	78	Ala-Asp	52	62
Gly-Leu	82	86	Met-Glu	62	64
Gly-Ser	53	70	Asp-Gly	43	40
Gly-Phe	51	73	Glu-Ala	60	54
Gly-Trp	44	67	Glu-Val	79	58
Gly-His	45	63	Glu-Glu	57	54
Gly-Met	70	86	Gln-Gln	70	76
Ala-Gly	82	70	Tyr-Lys	72	77
Ala-Ala	73	73	Lys-Phe	70	67
Ala-Leu	89	92	Lys-Lys	64	62
Ala-Ser	88	74	Gly-D-Leu	12	10
Ala-Phe	80	86	Z-Gly-Leu	15	30
Ala-Met	85	86	Gly-β-Ala	15	20
Leu-Gly	74	87	Gly-γ-Abu	25	32
Ser-Leu	84	85	Gly-Gly-Leu	60	58
Met-Leu	70	91	Gly-Leu-Gly	62	58
Gly-Pro	76	69	Gly-Gly-Gly	26	20
Pro-Gly	63	51			

least twice the rate of efflux of leucine. Supporting this line of evidence was the observation that when labelled glycyl-L-leucine was used, the specific radioactivity of glycine in the medium was diluted about twice, although there was no dilution of leucine radioactivity. These results suggest that the higher efflux rate of glycine may be partly responsible for the lower uptake of glycine residue compared with the uptake of leucine residue.

Structural specificity of glycyl-L-leucine uptake in monkey intestine

The uptake of both [^{14}C]glycyl-L-leucine and glycyl-L-[^{14}C]leucine is inhibited by a wide variety of dipeptides, including dipeptides of the composition: neutral–neutral, neutral–acidic, acidic–neutral, neutral–basic, basic–neutral, basic–basic and acidic–acidic (Table 1). As noted in our preliminary study (Das & Radhakrishnan, 1974*b*), the degree of inhibition seems to be dependent on the hydrophobicity of the peptide. Peptides such as glycyl-L-isoleucine, glycyl-L-methionine, L-alanyl-L-leucine, L-alanyl-L-serine, L-alanyl-L-phenylalanine, L-alanyl-L-methionine, L-leucyl-glycine, L-seryl-L-leucine, and L-methionyl-L-leucine are the strongest inhibitors of glycyl-L-leucine uptake. The nature of the *N*-terminal amino acid also appears to determine the inhibitory capacity, since L-alanyl-glycine is a stronger inhibitor than glycyl-L-alanine. The slight inhibition obtained with glycyl-glycine is in contrast with our earlier findings (Das & Radhakrishnan, 1974*b*) in which, by a chemical method, glycyl-L-leucine uptake was shown to be unaffected in the presence of glycyl-glycine. However, considering the higher sensitivity of the present procedure, the results reported here may be more reliable.

The basic dipeptides are comparable with the neutral dipeptides in their inhibitory capacity. The acidic dipeptides are poor inhibitors compared with the neutral and basic peptides. However, amidation of the dicarboxylic amino acid residues results in a higher inhibitory capacity, since L-glutaminyl-L-glutamine and glycyl-L-asparagine are stronger inhibitors than L-glutamyl-L-glutamic acid and glycyl-L-aspartic acid respectively.

To understand the nature of inhibition, four representative dipeptides namely, L-alanyl-L-alanine, L-alanyl-L-leucine, L-lysyl-L-lysine and L-glutamyl-L-glutamic acid were used to study their effects on the kinetic constants of glycyl-L-leucine uptake. As shown in Fig. 3, all these four dipeptides inhibit glycyl-L-leucine uptake by increasing the K_t,

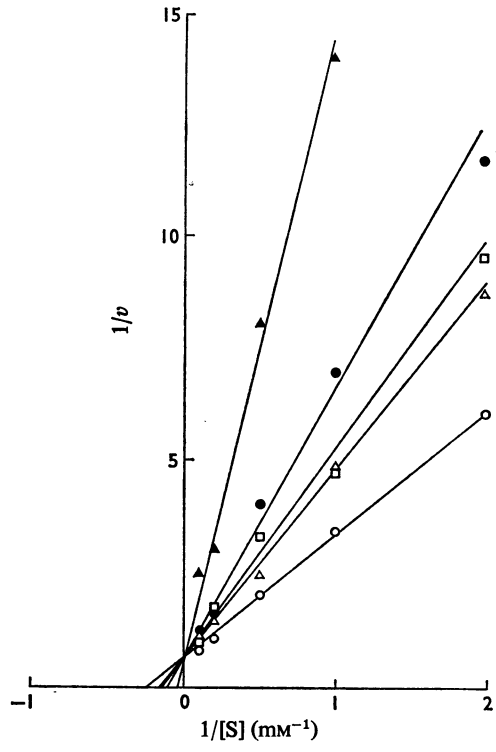

Fig. 3. *Lineweaver–Burk plots for glycyl-L-[^{14}C]leucine uptake in the monkey intestine, in the presence and absence of various inhibitory peptides*

○, Glycyl-L-leucine alone; the other curves refer to incubation of glycyl-L-leucine with 2.5mM-L-lysyl-L-lysine (△); with 5mM-L-glutamyl-L-glutamic acid (□); with 5mM-L-alanyl-L-alanine (●); with 5mM-L-alanyl-L-leucine (▲). The specific radioactivity of glycyl-L-[^{14}C]leucine in the medium varied between 1×10^5 and 1×10^6c.p.m./μmol of glycyl-L-leucine. v is expressed as μmol/min per g of tissue.

whereas the $V_{max.}$ is not altered. This suggests that the dipeptides which inhibit glycyl-L-leucine uptake are also transported by the same glycyl-L-leucine uptake system. On the basis of this assumption the dipeptide uptake system in monkey intestine appears to have a very broad specificity.

The glycyl-L-leucine uptake system is highly stereospecific since glycyl-D-leucine does not inhibit uptake to any significant extent. The *N*-terminal α-amino group appears to have an important role in uptake, since *N*-benzyloxycarbonyl-glycyl-L-leucine is a poor inhibitor of uptake. However, substitution of the *N*-terminal α-amino group hydrogen does not always affect inhibitory capacity, as L-prolyl-glycine is a good inhibitor. Thus it appears that the dipeptide uptake system in monkey intestine can tolerate substitution of the peptide α-amino group provided that this can be achieved with retention of the positive charge. Peptides containing β- or γ-amino acids (glycyl-β-alanine or glycyl-γ-aminobutyric acid) are poor inhibitors of uptake.

In the present study, a few tripeptides were also tested for inhibitory activity. All the three tripeptides (glycyl-L-leucyl-glycine, glycyl-glycyl-L-leucine, and glycyl-glycyl-glycine) inhibit glycyl-L-leucine uptake. It is not yet clear whether this inhibition is due to tripeptides *per se* or due to the dipeptides formed by hydrolysis by the brush-border tripeptidases. It is noteworthy that the extent of inhibition by these tripeptides is similar to that of the dipeptides which results from amino-tripeptidase action on the tripeptides.

Although the final uptake value of the two amino acid residues of glycyl-L-leucine was different, the degree of inhibition of the uptake of both glycine and leucine residues in the presence of a particular inhibitory peptide was almost identical.

Specificity of glycyl-L-[^{14}C]leucine uptake in the human

The specificity of glycyl-L-[^{14}C]leucine uptake in the human intestine was studied in a very limited way. All the five dipeptides tested were inhibitory (Table 2). L-Alanyl-L-leucine and L-lysyl-L-phenylalanine were strong inhibitors, whereas L-glutamyl-L-glutamic acid, glycyl-L-proline and L-prolyl-glycine were less potent inhibitors. The results suggest that the specificity of dipeptide uptake in the human may be very similar to that in the monkey.

Effect of temperature on uptake

The effect of temperature on the uptake of glycyl-L-leucine in monkey intestine is shown in Fig. 4. In these experiments a higher concentration of glycyl-L-leucine (40mM) was used to obtain near-maximal velocities. Das & Radhakrishnan (1974*b*)

Table 2. *Specificity of the human intestinal glycyl-L-[^{14}C]-leucine uptake system*

In these experiments the concentration of glycyl-L-[^{14}C]-leucine was 0.5mM and the concentration of the unlabelled peptides was 10mM in all cases. The specific radioactivity varied between 2×10^5 and 2×10^6c.p.m./μmol of glycyl-L-leucine. All the peptides contained amino acids of the L configuration.

Peptide	% inhibition of uptake of glycyl-L-[^{14}C]leucine
Ala-Leu	89
Lys-Phe	69
Glu-Glu	22
Gly-Pro	44
Pro-Gly	32

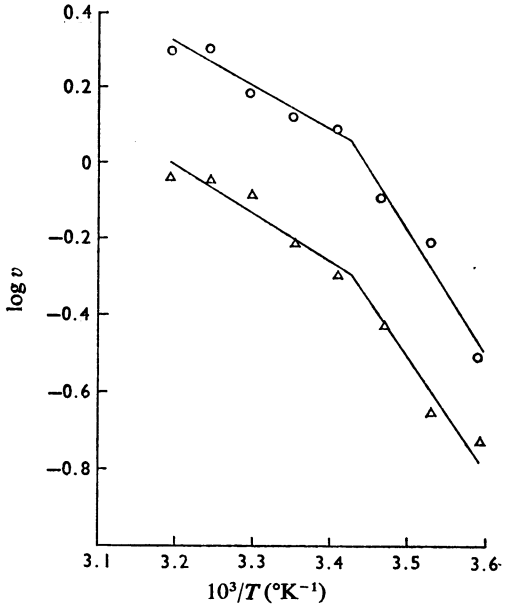

Fig. 4. *Arrhenius plots for [^{14}C]glycyl-L-leucine uptake (△) and glycyl-L-[^{14}C]leucine uptake (○) in the monkey intestine*

The concentration of glycyl-L-leucine in the medium was 40mM. The specific radioactivity of [^{14}C]glycyl-L-leucine and glycyl-L-[^{14}C]leucine in the medium varied between 1×10^5 and 1×10^6c.p.m./μmol of glycyl-L-leucine. The extracellular space was determined at the temperatures shown in the figure. v is expressed as μmol absorbed/min per g of tissue.

showed that glycyl-L-leucine uptake at 37°C is saturated at 40mM concentration of the peptide. The Arrhenius plots for both [^{14}C]glycyl-L-leucine uptake and glycyl-L-[^{14}C]leucine uptake show a discontinuity at about 20°C. The activation energies for [^{14}C]glycyl-L-leucine uptake above and below the discontinuity are 24.7kJ/mol and 49.8kJ/mol respectively. The corresponding values for glycyl-L-[^{14}C]-leucine uptake are 23kJ/mol and 57.4kJ/mol. Since there is a sharp increase in the activation energy of the uptake system below 20°C, it would appear that the fluidity of the membrane lipid components may have an important role in the uptake process. These results also suggest that the same uptake mechanism may be responsible for the uptake of both [^{14}C]glycyl-L-leucine and glycyl-L-[^{14}C]leucine.

Discussion

To study the early event in the intestinal absorption of a dipeptide a sensitive method is necessary. A direct measurement of the uptake of each dipeptide is not possible in view of the high cost or paucity of some of the peptides especially with ^{14}C labelling. In the present study, these difficulties were circumvented by studying the inhibition of uptake of a single ^{14}C-labelled dipeptide, namely glycyl-L-leucine, by a wide range of judiciously selected unlabelled peptides. Although the method is indirect it is considered that the data obtained provide adequate proof for a general dipeptide uptake system.

The specificity of the intestinal dipeptide uptake system has been studied earlier by Rubino *et al.* (1971) and Das & Radhakrishnan (1974*b*). After submission of this manuscript we have seen the papers by Matthews and his co-workers on the uptake of carnosine in rings of everted hamster jejunum *in vitro* (Matthews *et al.*, 1974). Since carnosine is very slowly hydrolysed the intact peptide was detectable in the intestine wall. They have also found that there is competition between carnosine and other dipeptides and triglycine (Addison *et al.*, 1974). In these studies the uptake of neutral dipeptides was shown to be inhibited by other neutral dipeptides, but not by the constituent amino acids. In the present paper a more detailed study was made on the specificity of dipeptide uptake by using a wider variety of peptides, including those containing acidic or basic amino acids. The results strongly suggest that in the monkey intestine there is a dipeptide uptake system with an extremely broad specificity. In this respect the dipeptide uptake system in the monkey appears to resemble the bacterial system (Payne & Gilvarg, 1971). The neutral, basic and acidic dipeptides all appear to share the same uptake system. In the case of free amino acid transport, there is evidence for separate uptake systems for basic and neutral amino acids, and acidic amino acids do not compete with neutral amino acids for transport (Wilson, 1962). Thus the nature and specificity of dipeptide transport is entirely different from that of free amino acid transport. There may be only one uptake system for transporting all types of dipeptides derived from dietary proteins. A relatively non-specific uptake system for dipeptide can be expected when it is considered that the number of theoretically possible dipeptides exceeds 400. The uptake mechanism operating for such a large group would be expected to have a rather broad specificity.

In earlier studies on dipeptide absorption in the human, uptake was measured by the perfusion technique (Adibi, 1971; Hellier *et al.*, 1972*a*; Hellier *et al.*, 1972*b*). The method of measurement *in vitro* adopted in the present work, presumably gives a more accurate measure of the rate of initial entry of the peptide into the mucosal cell, but it does not give an idea of the overall absorption rate. The present study on glycyl-L-leucine uptake in the human is somewhat

limited, but it throws some light on the kinetics of the initial entry process which has never been studied before. Also the inhibition of glycyl-L-leucine uptake by dipeptides which are structurally unrelated to glycyl-L-leucine indicates that the uptake system in the human may also have a broad specificity.

In monkey intestine the uptake of glycine and leucine residues of glycyl-L-leucine show great similarities in several properties like K_t value and activation energy. Also in the Arrhenius plots, both show discontinuities at 20°C. The similarities in the characteristics of uptake of the two amino acid residues of a mixed dipeptide support the hypothesis that dipeptides are transported as one unit. However, the marked difference in the uptake rates of [^{14}C]-glycyl-L-leucine and glycyl-L-^{14}[C]leucine apparently contradicts this idea. This anomalous absorption of mixed dipeptides has also been observed by Burston *et al.* (1972). In the present study the observed higher efflux rate of glycine can partly explain this phenomenon. The observed lower efflux of leucine may be due to a more efficient recapture of free leucine from the lumen. Also it should be noted that the brush-border dipeptidases will hydrolyse glycyl-L-leucine to a certain extent, and the liberated amino acids may be taken up at different rates.

Discontinuities in the Arrhenius plots of several membrane-associated functions have been interpreted in terms of a phase change in the membrane lipids (Wilson *et al.*, 1970; Esfahani *et al.*, 1971). The increase in activation energy below the discontinuity is ascribed to close packing of the hydrocarbon chains of membrane lipids. The discontinuities observed in the Arrhenius plots for [^{14}C]glycyl-L-leucine uptake and glycyl-L-[^{14}C]-leucine uptake strongly suggest that the physical state of the membrane lipid components is an important factor in determining peptide uptake.

We thank Professor S. J. Baker and Dr. Prakash Khanduri for providing the surgical specimen of human intestine and Dr. M. D. Hellier (now visiting our laboratory) for his criticisms of the manuscript. The project was supported in part by the Council of Scientific and Industrial Research, New Delhi, India.

References

Addison, J. M., Matthews, D. M. & Burston, D. (1974) *Clin. Sci. Mol. Med.* **46**, 707–714

Adibi, S. A. (1971) *J. Clin. Invest.* **50**, 2266–2275

Burston, D., Addison, J. M. & Matthews, D. M. (1972) *Clin. Sci.* **43**, 823–837

Das, M. & Radhakrishnan, A. N. (1972) *Biochem. J.* **128**, 463–465

Das, M. & Radhakrishnan, A. N. (1973) *Biochem. J.* **135**, 609–615

Das, M. & Radhakrishnan, A. N. (1974*a*) *Clin. Sci. Mol. Med.* **46**, 501–510

Das, M. & Radhakrishnan, A. N. (1974*b*) *Indian J. Biochem. Biophys.* **11**, 12–16

Esfahani, M., Limbrick, A. R., Knutton, S., Oka, T. & Wakil, S. J. (1971) *Proc. Nat. Acad. Sci. U.S.* **68**, 3180–3184

Hellier, M. D., Holdsworth, C. D., Perrett, D. & Thirumalai, C. (1972*a*) *Clin. Sci.* **43**, 659–668

Hellier, M. D., Holdsworth, C. D., McColl, I. & Perrett, D. (1972*b*) *Gut* **13**, 965–969

Lis, M. T., Crampton, R. F. & Matthews, D. M. (1971) *Biochim. Biophys. Acta* **233**, 453–455

Malathi, P., Ramaswamy, K., Caspary, W. F. & Crane, R. K. (1973) *Biochim. Biophys. Acta* **307**, 613–626

Matthews, D. M. (1972) in *Peptide Transport in Bacteria and Mammalian Gut; Ciba Found. Symp.* pp. 71–88, Elsevier, Excerpta Medica, North-Holland, Amsterdam

Matthews, D. M., Addison, J. M. & Burston, D. (1974) *Clin. Sci. Mol. Med.* **46**, 693–705

Newey, H. & Smyth, D. H. (1959) *J. Physiol. (London)* **145**, 48–56

Payne, J. W. & Gilvarg, C. (1971) *Advan. Enzymol. Relat. Areas Mol. Biol.* **35**, 187–244

Rubino, A., Field, M. & Schwachman, H. (1971) *J. Biol. Chem.* **206**, 3542–3548

Umbreit, W. W., Burris, R. H. & Stauffer, J. F. (1964) *Manometric Techniques and Tissue Metabolism*, 4th edn., p. 132, Burgess Publishing Co., Minneapolis

Wilson, G., Rose, S. P. & Fox, C. F. (1970) *Biochem. Biophys. Res. Commun.* **38**, 617–623

Wilson, T. H. (1962) *Intestinal Absorption*, pp. 110–133, W. B. Saunders Company, Philadelphia