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Research Article

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The Number of Glycine Residues Which Limits Intact Absorption of Glycine Oligopeptides in Human Jejunum

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ABSTRACT Studies were performed to determine whether glycine peptides of four or more glycine residues can be transported by the peptide carrier system, previously shown to transport diglycine and triglycine. When human jejunum was perfused with tetraglycine solutions, the rate of tetraglycine disappearance increased linearly as the concentration was increased over the range of 12.5–50 mM, however, the rate was slow in comparison to diglycine and triglycine disappearance rates.

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The rates of glycine uptake were always markedly greater from diglycine and triglycine solutions than from corresponding glycine or tetraglycine solutions; rates of glycine uptake from tetraglycine solutions were either similar to or greater than rates from glycine solutions, depending on the infusion concentration. When the number of glycine residues was increased to hexaglycine, the phenomenon of a greater rate of glycine uptake from a peptide versus a free amino acid solution was no longer apparent. In vitro assay of peptide hydrolase activity of the luminal fluid revealed no activity against diglycine and triglycine and only trace activities against tetraglycine, pentaglycine, and hexaglycine.

The above observations suggest the following conclusions: (*a*) the disappearance of tetraglycine in the human jejunum is accomplished principally by hydrolysis by brush border oligopeptidases; (*b*) the rate limiting step in the uptake of glycine from tetraglycine or higher peptides is due to hydrolysis of these peptides to absorbable products.

INTRODUCTION

Studies over the past several years have established the presence of a peptide carrier system in human intestine (1–2). The essential features of this carrier system are as follows (1–4): (*a*) it does not take up amino acids but does transport dipeptides and some tripeptides; (*b*) it has a higher maximal rate of uptake than the amino acid carrier system; (*c*) it prefers peptides with lipophilic amino acids in both the N- and C-terminal positions; (*d*) it fulfills the criteria of active transport in animal intestine; (*e*) it is an important, if not the predominant, uptake mechanism for amino acid constituents of dietary protein; and (*f*) it is less affected by malnutrition and mucosal disease than is the amino acid carrier system.

The present studies were undertaken to determine the capacity of the peptide carrier system for transporting peptides consisting of four or more amino acids. Among the 160,000 theoretically possible tetrapeptides containing the 20 amino acids found in protein, we chose tetraglycine because it is highly soluble and because we had previously investigated the intestinal absorption of diglycine and triglycine (5–7). After finding that intestinal disappearance of tetraglycine is chiefly due to hydrolysis by the brush border peptide hydrolases rather than to uptake by the peptide carrier system, we proceeded to investigate the rate of intra-

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luminal hydrolysis of a series of glycine oligopeptides (tetraglycine, pentaglycine, and hexaglycine) in the jejunum of healthy human volunteers. Additional studies were performed to investigate the rate of glycine uptake from glycine oligopeptides that are taken up intact (diglycine and triglycine) and from glycine oligopeptides that are mostly hydrolyzed before absorption (tetraglycine and hexaglycine).

METHODS

Perfusion studies were carried out in the jejunum of 28 healthy human volunteers by the methods previously described (5, 8). The subjects consisted of 8 women and 20 men, ranging in age from 19 to 23 yr. The distance between the port of infusion and the port of aspiration in the double lumen tube was 30 cm. The rate of infusion of test solutions was 15 ml/min. The subjects were divided into six groups. There were four subjects in group A, seven in group B, four in group C, four in group D, four in group E, and five in group F. The composition of test solutions used for the perfusion studies in each group of volunteers is detailed in Table I.

All the test solutions contained 0.4% polyethylene glycol as a nonabsorbable marker, and between 85 and 140 mM sodium chloride. The pH of the test solutions varied between 6.8 and 7.2. Previously published and unpublished studies from this laboratory have shown that the above variations of sodium concentration and pH of the test solutions do not significantly alter the rates of amino acid and peptide absorption in the jejunum of healthy human volunteers (9–11).

Ion exchange chromatography technique was used for the analysis of glycine and glycine peptides in the intestinal aspirate. With an automated amino acid analyzer (model 120-C, Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.), 0.20 N sodium citrate buffer (pH 3.70), a column pressure of 150–200 lb/in^2, and a column temperature of 62°C, we determined the elution times for glycine, diglycine, triglycine, tetraglycine, pentaglycine, and hexaglycine as 75, 175, 190, 165, 150, and 135 min, respectively.

The peptide hydrolase activity of intraluminal fluid against glycine peptides was measured by the method previously described (5, 7).

Glycine absorption rates from free glycine solutions and peptide disappearance rates were calculated by the following formula:

Absorption (G_1) or disappearance ($G_{n>1}$) rate

$$= \left([G_n]_p - [G_n]_a \frac{M_p}{M_a}\right) R$$

where the rate is expressed as micromoles per minute per 30-cm segment; G_n = glycine compound of n number of residues in length, for example, G_1 denotes free glycine, G_2 denotes diglycine, etc.; concentrations are expressed as micromoles per milliliter; p and a denote measurements made on perfusate and aspirate, respectively; M denotes concentration of polyethyleneglycol; and R is the rate of perfusion in milliliters per minute.

Glycine absorption rates from tetraglycine solutions were calculated by the following formula:

$$^{A}G_4 = \left(4[G_4]_p - \{[G_1] + 2[G_2] + 3[G_3] + 4[G_4]\}_a \frac{M_p}{M_a}\right) R$$

where the rate is similarly expressed as micromoles absorbed per minute per 30-cm segment.

TABLE I
Protocol for Perfusion Studies

Group of subjects	Composition of test solutions
A	150 mM NaCl, 12.5 mM tetraglycine,* 25 mM tetraglycine, 50 mM tetraglycine
B	20 mM tetraglycine, 20 mM tetraglycine + 50 mM glycyl-L-leucine,‡ 20 mM tetraglycine + 50 mM L-leucine‡
C	20 mM tetraglycine, 20 mM tetraglycine + 50 mM triglycine,§ 20 mM tetraglycine + 50 mM glycine‡
D	100 mM glycine, 50 mM diglycine,‡ 33.33 mM triglycine, 25 mM tetraglycine
E	200 mM glycine, 100 mM diglycine, 66.67 mM triglycine, 50 mM tetraglycine
F	18 mM glycine, 3 mM tetraglycine, 3 mM pentaglycine,§ 3 mM hexaglycine*

* Bachem, Inc., Marina Del Rey, Calif.
‡ Grand Island Biological Co., Grand Island, N. Y.
§ Sigma Chemical Co., St. Louis, Mo.

Glycine absorption rates (micromoles per minute per 30 cm) from hexaglycine solutions were calculated as follows:

$$^{A}G_6 = \left(6[G_6]_p - \{[G_1] + 2[G_2] + 3[G_3] + 4[G_4] + 5[G_5] + 6[G_6]\}_a \frac{M_p}{M_a}\right) R$$

Luminal appearance rates of the products of hydrolysis were calculated as follows:

$$\textit{Luminal appearance rate} = [G_n] \frac{M_p}{M_a} \cdot R$$

where the rate is expressed as micromoles per minute per 30-cm.

The method of paired t test was used for the statistical analysis of the data (12).

In vitro transport studies. Jejunal rings have been used extensively for studying dipeptide and tripeptide transport by the intestinal mucosa (13–15). We used this technique to investigate tetraglycine transport by the mucosal cells. The experimental procedure was essentially similar to that previously described by Matthews et al. (14). Everted rings of jejunum were prepared from male Sprague-Dawley rats (250–300 g body wt). Single rings weighing 15–30 mg were incubated for 30 min at 37°C in 1 ml Krebs-Ringer phosphate saline (16) containing half the standard concentration of calcium. In separate experiments, the incubation medium contained varying concentrations of glycine and tetraglycine. In some experiments the incubation medium contained 0.2 μCi of [^{14}C]-inulin (spec act of 2.5 mCi/g, New England Nuclear, Boston, Mass.), and the inulin space was determined as described previously (17). After incubation, the rings were removed, rinsed, blotted, and eluted in 1 ml 6% sulfosalicylic acid for 5 min at 100°C. Media were diluted 1:1 with 6% sulfosalicylic acid. Both eluted rings and media were centrifuged at 500 g for 10 min; supernates were stored at −20°C until analysis.

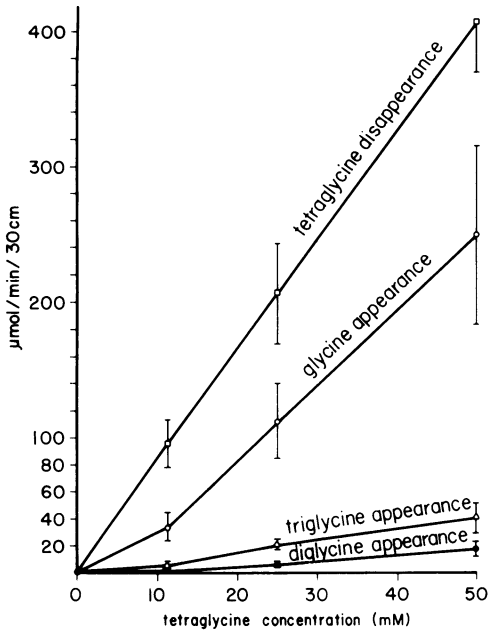

FIGURE 1 Tetraglycine disappearance rates and appearance rates of hydrolytic products (mean±SEM in four subjects).

Total tissue water was determined by drying overnight at 100°C. The concentration of glycine and glycine peptides in the tissue and medium were measured by the ion-exchange chromatography technique described above. The intracellular concentrations of glycine and glycine peptides were calculated by the formula previously published (18).

Hydrolase activity of brush border and cytosol fraction against tetraglycine. Specimens of human jejunum obtained at autopsy were processed immediately by the method of Welsh et al. (19) to yield brush border membranes. Briefly, approximately 2 g of villi were dissected free of the underlying musculature and homogenized in 60 ml of 5 mM EDTA-5mM Tris buffer (pH 7.3) for 15 s in a Waring blender with a Powerstat variable transformer set at 90. The homogenate was filtered through nylon mesh of 40-μm pore size (TETKO, Inc., Emsford, N. Y.). The filtrate was centrifuged at 19,200 *g* for 20 min at 4°C. The pellet, resuspended in 14% glycerol, was retained for the assay of peptide hydrolase activity.

The cytosol fraction was prepared according to the method of Kim et al. (20).The mucosa was scraped and homogenized in 14% glycerol (4 ml/g) in a Potter-Elvehjem tissue homogenizer with a Teflon pestle. The homogenate was centrifuged at 20,000 *g* for 20 min. The supernate was centrifuged at 105,000 *g* for 1 h. The resultant supernate was termed cytosol fraction and was assayed for hydrolase activity.

The peptide hydrolase activity of brush border and cytosol fraction against tetraglycine was assayed by the following method. The standard assay mixture contained 8 μmol tetraglycine, 0.2 ml 0.05 M KCl-borate buffer (pH 7.6), and 0.2 ml brush-border or cytosol fraction (0.5–2.5 mg protein). The mixture was incubated for 30 min at 37°C in a shaking water bath. Under these conditions, the rate of reaction was linear. The reaction was terminated by the addition of 0.5 ml 7.5% sulfosalicylic acid. The sample was stored at −20°C until analysis. The concentration of protein in both brush border and soluble fractions was determined by the method of Lowry et al. (21).

RESULTS

Rates of tetraglycine disappearance and appearance of its digestive products. The rate of luminal disappearance of tetraglycine and the rates of appearance of its digestive products (triglycine, diglycine, and glycine) in the gut lumen during a jejunal perfusion with tetraglycine test solutions are summarized in Fig. 1. Over the range of concentrations infused (12.5, 25, and 50 mM), the rate of tetraglycine disappearance increased linearly. The highest concentration of tetraglycine that could be readily dissolved in water was approximately 50 mM. At each infusion concentration of tetraglycine, glycine, diglycine, and triglycine appeared in the luminal fluid. Among the products of tetraglycine hydrolysis, the rate of appearance of glycine was always considerably greater than those of diglycine and triglycine (Fig. 1). Furthermore, with each increase in concentration of tetraglycine in the infusion solution, there was a more pronounced difference between the luminal appearance rates of glycine and either diglycine or triglycine.

Effect of dipeptide and tripeptide on tetraglycine disappearance. The effect of glycylleucine (50 mM) on the disappearance rate of tetraglycine during the infusion of 20-mM test solution and the appearance rates of the products of its hydrolysis (triglycine and diglycine) are shown in Fig. 2. The concentration of glycylleucine was chosen on the basis of previous studies which showed that at this concentration glycylleucine markedly inhibits the intestinal absorption rate of

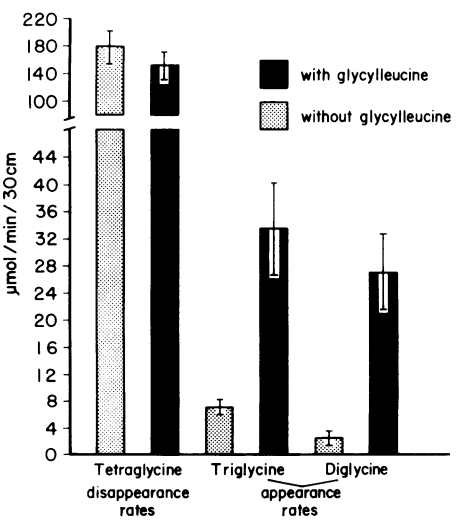

FIGURE 2 Rates of tetraglycine disappearance and triglycine and diglycine appearance from test solutions containing 20 mM tetraglycine and 20 mM tetraglycine plus 50 mM glycylleucine. Mean±SEM values were determined from perfusion studies in seven subjects. Accumulation rates of diglycine and triglycine are significantly greater in the presence of glycylleucine ($P < 0.01$).

triglycine (7). The disappearance rate of tetraglycine was not significantly affected by the addition of glycylleucine to the test solution, but there were significant increases ($P < 0.01$) in the appearance rates of both triglycine and diglycine (Fig. 2). Similarly, the addition of 50 mM triglycine to a 20-mM solution of tetraglycine had no significant effect on the rate of tetraglycine disappearance.

Effect of amino acids on tetraglycine disappearance. The effect of leucine on the rate of tetraglycine disappearance, rate of glycine uptake from the tetraglycine solution, and appearance rates of glycine, diglycine, and triglycine are summarized in Fig. 3. Addition of leucine (50 mM) to the tetraglycine solution (20 mM) significantly decreased ($P < 0.01$) rates of tetraglycine disappearance and of glycine uptake. Both of these rates were decreased by about 50% compared to rates without added leucine. The appearance rates of glycine and diglycine were not significantly affected, but that of triglycine was significantly ($P < 0.01$) reduced by the addition of leucine to the tetraglycine solution. In contrast to leucine, the addition of 50 mM glycine to the 20-mM solution of tetraglycine was without significant effect on the disappearance rate of tetraglycine.

Effect of amino acids on mucosal tetraglycine hydrolysis. To investigate a possible site of action of leucine on tetraglycine disappearance (Fig. 3), the activity of brush border and cytosol peptide hydrolases in the presence and absence of leucine was assayed in vitro. Subcellular fractionation studies of the hydrolase activity against tetraglycine in human intestinal mucosa showed that all of the activity was confined to the brush border fraction (26±6 μmol tetraglycine hydrolyzed per minute per milligram protein,mean±SEM). The cytosol fraction lacked any detectable hydrolase activity against this tetrapeptide. These results are in agreement with those of Kim et al. (20). Addition of leucine in a concentration used for the perfusion studies (50 mM, Fig. 3) totally abolished the hydrolytic activity of the brush border fraction against tetraglycine. Glycine in a similar concentration (50 mM) was without significant effect on tetraglycine hydrolysis by the brush border enzymes.

Rates of glycine uptake from glycine and its oligopeptide solutions. In Fig. 4, the rates of glycine uptake from tetraglycine solutions are compared to the rates of glycine uptake from test solutions containing glycine, diglycine, or triglycine. The test solutions were designed to contain an equivalent amount of glycine, either 100 or 200 mM. At the glycine equivalent

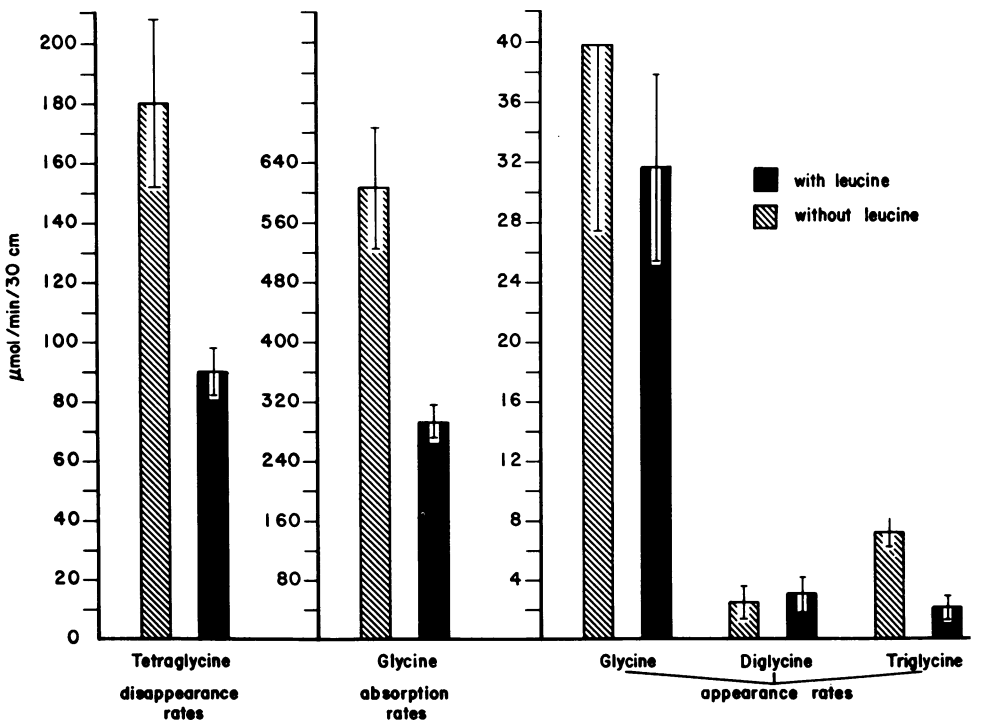

FIGURE 3 Rates of tetraglycine disappearance, glycine absorption, and appearance of glycine, diglycine, and triglycine from test solutions containing either 20 mM tetraglycine or 20 mM tetraglycine plus 50 mM leucine (mean±SEM in seven subjects). Rates of peptide disappearance, glycine absorption, and triglycine appearance were significantly reduced in the presence of leucine ($P < 0.01$).

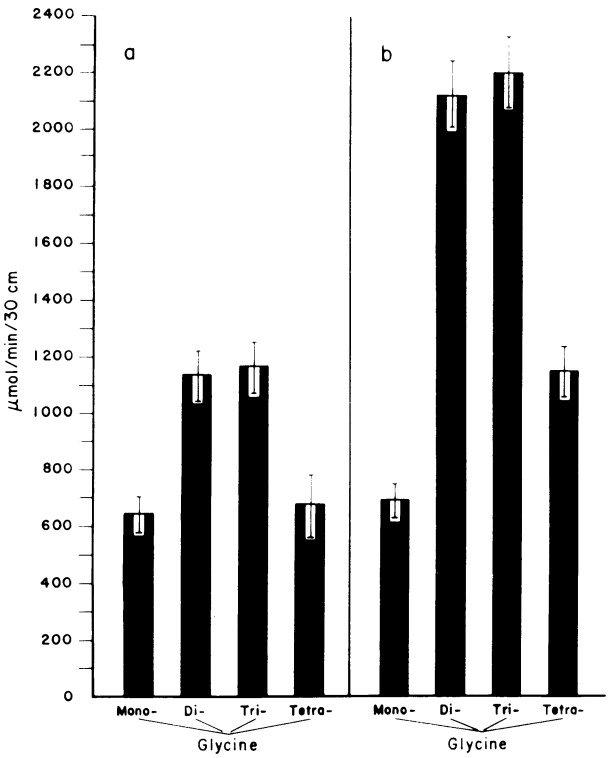

FIGURE 4 Rates of glycine uptake from solutions containing equivalent amounts of glycine in free or peptide form (mean ±SEM in four subjects). (*a*) Rates from 100 mM glycine equivalent solutions. (*b*) Rates from 200 mM glycine equivalent solutions.

concentration of 100 mM, the rate of glycine uptake from the tetraglycine solution was similar to that from the glycine solution, but it was considerably smaller (P values < 0.01) than the rates of glycine uptake from either diglycine or triglycine solutions (Fig. 4 *a*). When the concentration of glycine equivalent in the test solution was increased to 200 mM, the rates of glycine uptake from all three peptide solutions were increased, but the rate of glycine uptake from the glycine solution remained unchanged. Therefore, the rate of glycine uptake from the tetraglycine solution was significantly greater ($P < 0.01$) than from the corresponding glycine test solution, but it was still smaller than those from diglycine and triglycine solutions (Fig. 4 *b*).

Additional studies were performed to compare the rate of glycine uptake from a hexaglycine solution and an equivalent glycine solution. Inasmuch as hexaglycine is not soluble in aqueous solutions at concentrations greater than 3 mM, rates of glycine uptake were compared from test solutions containing either 3 mM hexaglycine or 18 mM glycine (Fig. 5 *a*). The rate of glycine uptake was significantly smaller ($P < 0.01$) from the hexaglycine.

Rates of disappearance of tetraglycine, pentaglycine, and hexaglycine and appearance of their digestive products. With the maximal concentration of hexaglycine that is water soluble (3 mM) as the infusion concentration, rates of disappearance of tetraglycine, pentaglycine, and hexaglycine were investigated in the jejunum of the same subjects (Fig. 5 *b*). There were no significant differences between the rates of disappearance of any two of these glycine peptides.

The rates of appearance of the hydrolytic products of the above three peptides are shown in Fig. 6. In the case of hexaglycine and pentaglycine, the first products of their hydrolyses, namely, pentaglycine and tetraglycine, respectively, had the greatest concentration among the peptides appearing in the gut lumen. During the infusion of each of the glycine peptides, the rates of appearance of both triglycine and diglycine were always considerably smaller than those of other hydrolytic products.

Hydrolysis by intraluminal peptide hydrolases. The potential of luminal fluid to hydrolyze homologous glycine oligopeptides in vitro is summarized in Table II. Although luminal fluid lacked any peptide hydrolase activity against diglycine and triglycine, it had peptide hydrolase activity against tetraglycine, pentaglycine, and hexaglycine. There were no significant differences between the rates of hydrolysis of these peptides by the luminal fluid. Although the extrapolation of in vitro data to the in vivo situation may not be appropriate, these results indicate that not more than a minor fraction of the disappearance rate of glycine peptides (Fig. 5 *b*) could be accounted for by hydrolysis by the intraluminal fluid.

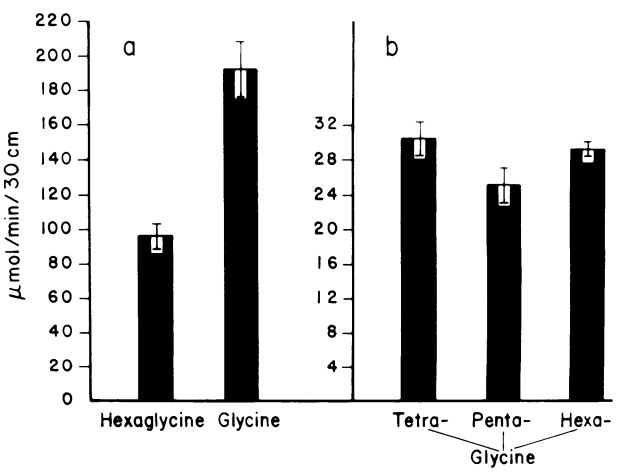

FIGURE 5 (*a*) Rates of glycine absorption from test solutions containing either 3 mM hexaglycine or the equivalent amount of glycine in free form, i.e., 18 mM glycine (mean±SEM, five subjects). Difference is statistically significant ($P < 0.01$). (*b*) Rates of peptide disappearance from solutions of 3 mM tetraglycine, 3 mM pentaglycine, or 3 mM hexaglycine (mean ±SEM, five subjects).

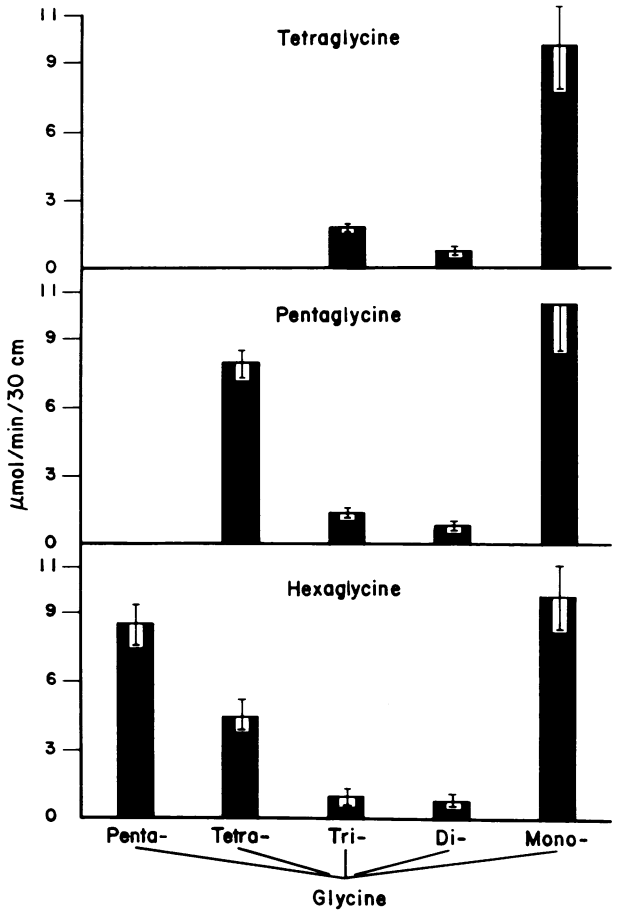

FIGURE 6 Rates of appearance of the hydrolytic products of tetraglycine, pentaglycine, and hexaglycine during infusion of 3 mM of each. Mean±SEM values were determined from measurements made in five subjects.

Mucosal cell transport of glycine and its peptides in vitro. To investigate further the possibility of the transport of unhydrolyzed tetraglycine, we determined whether there is any intracellular accumulation of this tetrapeptide when an intestinal mucosal cell preparation is incubated with tetraglycine in vitro. The use of rat intestine for such a study was validated by the fact that there are close similarities in peptide transport between human and rat intestine (3). The use of a cellular accumulation technique was validated by the fact that cytoplasmic fraction of rat jejunum, like that of man, lacks any peptide hydrolase activity against tetraglycine (Adibi and Morse, unpublished observations) and, therefore, if tetraglycine were to enter the cell interior, it would not be subject to hydrolysis by an intracellular enzyme.

There was no intracellular accumulation of tetraglycine when this tetrapeptide in concentrations of 5 or 20 mM was added to the jejunal rings. Although neither

TABLE II
Rates of Hydrolysis of Glycine Peptides by the Luminal Fluid

Substrate	Rate of hydrolysis
	μmol/ml luminal fluid/60 min
Diglycine	0
Triglycine	0
Tetraglycine	0.72±0.28
Pentaglycine	0.80±0.26
Hexaglycine	0.84±0.21

Mean±SEM, four subjects.

diglycine nor triglycine was detected in the intracellular and extracellular fluid when jejunal rings were incubated in the buffer, both diglycine and triglycine appeared in both of these fluids when tetraglycine was added to the incubation medium (Fig. 7). In general, the concentration of diglycine was always nearly 10-fold greater than the concentration of triglycine in the tissue or the medium. Furthermore, at each concentra-

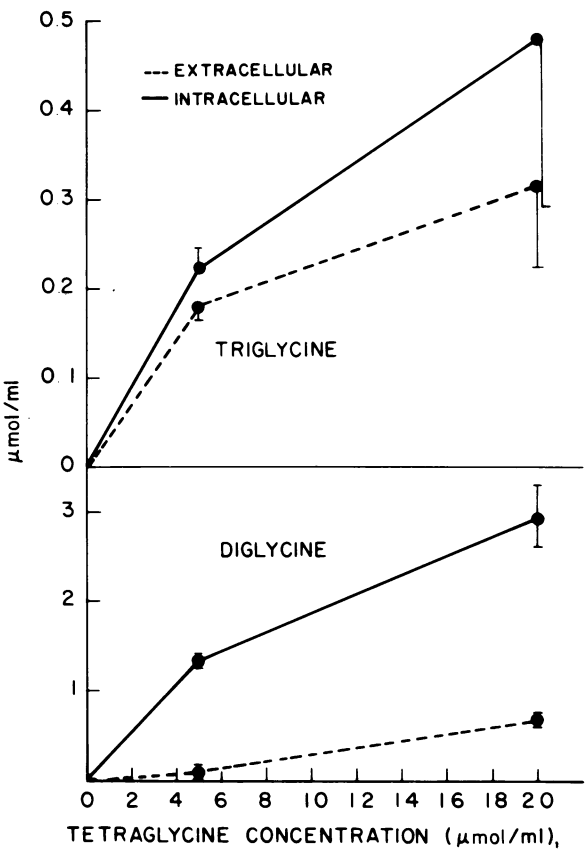

FIGURE 7 Extracellular and intracellular concentrations of diglycine and triglycine. A jejunal ring preparation was incubated for 30 min in a medium that initially contained 5 or 20 mM tetraglycine. Each point represents mean±SEM of four rats.

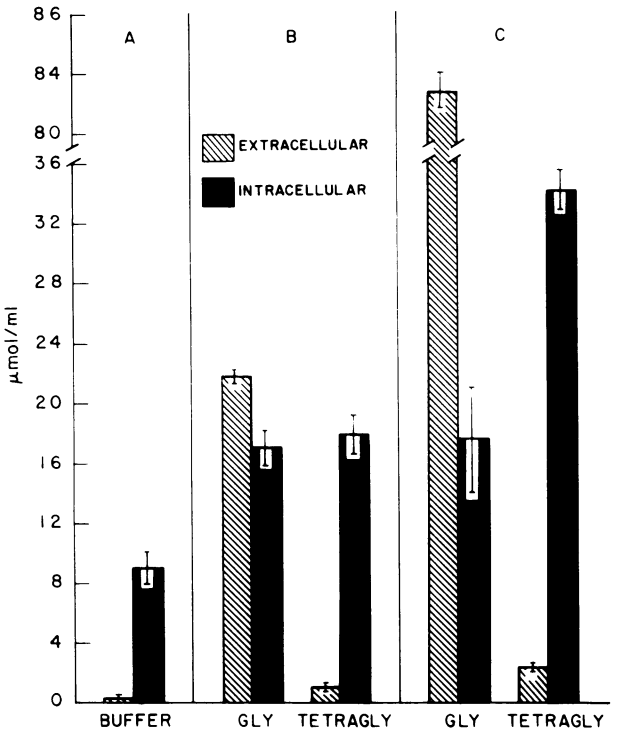

FIGURE 8 Extracellular and intracellular concentrations of glycine. A jejunal ring preparation was incubated for 30 min in a medium that initially contained: (*A*) buffer only, (*B*) 20 mM glycine (gly) or 5 mM tetraglycine, and (*C*) 80 mM glycine or 20 mM tetraglycine. Each bar represents mean±SEM of four rats.

tion of tetraglycine the concentration of diglycine was always markedly ($P < 0.01$) greater in the intracellular than in the extracellular fluid. The concentration of triglycine also appeared greater in the intracellular than in the extracellular fluid, but because of wide variation in concentrations, the differences were not statistically significant (Fig. 7).

The intracellular and extracellular concentrations of glycine with or without addition of tetraglycine or glycine to the incubation medium are shown in Fig. 8. The initial concentrations of tetraglycine (5 and 20 mM) and glycine (20 and 80 mM) were designed to be glycine equivalent. Both tetraglycine (5 mM) and glycine (20 mM) were effective in significantly ($P < 0.01$), and similarly, increasing the intracellular concentration of glycine. When the initial concentration of glycine was increased to 80 mM, there was no further increase in the intracellular concentration of this amino acid. In contrast, there was a marked additional increase in the intracellular glycine concentration with 20 mM tetraglycine. There were slight increases in the extracellular glycine concentration with each addition of tetraglycine to the medium, but they were always much smaller than the increments in the intracellular concentration.

DISCUSSION

The principal aim of the present studies was to determine whether the jejunal disappearance of tetraglycine is by transport by the peptide carrier system or by hydrolysis by the intraluminal and membrane-bound enzymes. To distinguish between these two possibilities, it is appropriate to compare the results of the present studies with tetraglycine with those of previous studies with triglycine, which suggested that intact absorption is the main process for jejunal disappearance of this tripeptide (7). Such a comparison leads to the following observations: (*a*) jejunal disappearance of tetraglycine is not affected by glycylleucine, whereas that of triglycine is severely inhibited by this dipeptide; (*b*) the rate of glycine uptake from a tetraglycine (20 mM) solution is similar to that from an equivalent glycine solution (100 mM), whereas the rate of glycine uptake from an equivalent triglycine solution (33.33 mM) is considerably greater (nearly 100%) than that from the glycine or tetraglycine solution; (*c*) jejunal luminal fluid contains hydrolase activity against tetraglycine, whereas it has none against triglycine; (*d*) disappearance of tetraglycine is markedly inhibited by leucine, whereas this amino acid has no effect on the disappearance rate of triglycine.

In view of the sharp differences between the behavior of tetraglycine and triglycine in the gut lumen as summarized above, it appears that tetraglycine is not a substrate for the peptide carrier system which is involved in transport of diglycine and triglycine. Nevertheless, perfusion data do not eliminate the possibility that there is intact absorption of tetraglycine by a mechanism other than that shared by triglycine and diglycine. However, this possibility is not tenable in view of our observation that tetraglycine could not be detected in the mucosal cell interior when jejunal rings were incubated with this tetrapeptide in vitro. The lack of intracellular accumulation was not the result of intracellular hydrolysis because the cytoplasmic fraction was incapable of hydrolyzing tetraglycine and there was marked intracellular accumulation of diglycine and, to a lesser extent, that of triglycine (Fig. 7) in the face of considerable cytoplasmic peptidase activity against these peptides (20).

The present data also show that tetraglycine introduced into the jejunum is hydrolyzed chiefly by the membrane-bound oligopeptidases (20, 22, 23) and to a lesser extent by the luminal enzymes (Table II). The hydrolytic products in turn are taken up by the peptide and amino acid carrier systems, because when the peptide carrier system was saturated with glycylleucine, a considerable fraction of tetraglycine was recovered as triglycine and diglycine (Fig. 2). The increased appearance rate of diglycine probably represents increased

membrane hydrolysis of triglycine, as its absorption has been reduced by glycylleucine (7).

Our conclusion that tetraglycine is not a substrate for the known peptide carrier system is in agreement with observations made by Sleisenger et al. (24). They found that mucosal influx of glycylsarcosine or glycylsarcosylsarcosine was not affected by the addition of glycylsarcosylsarcosylsarcosine to the mucosal fluid of the everted hamster small intestine. They concluded that this tetrapeptide was not transported by the same peptide carrier system for dipeptides and tripeptides.

Inhibition of tetraglycine hydrolysis by leucine. One of the unexpected findings of the present studies was the observation that the addition of leucine to tetraglycine solution severely impaired the rate of disappearance of this tetrapeptide. The purpose of this experiment was to investigate the effect of leucine on the rate of glycine uptake from the tetraglycine solution. A comparable experiment investigating the effect of leucine on triglycine disappearance demonstrated that disappearance of this peptide from the gut lumen is chiefly by intact absorption and not by hydrolysis (7). Leucine was without an effect on the rate of glycine uptake from triglycine solution, but markedly inhibited glycine uptake from a glycine solution (7). In the present experiment, leucine markedly reduced the rate of glycine uptake from tetraglycine. This reduction appeared to be chiefly due to inhibition of tetraglycine hydrolysis, inasmuch as both the rate of disappearance of tetraglycine as well as the luminal appearance of triglycine (a product of tetraglycine hydrolysis) were significantly decreased by leucine (Fig. 3). Inhibition of glycine absorption by leucine, as shown previously (7), could not account for this effect, because the rate of glycine accumulation was not increased (Fig. 3). This conclusion was confirmed by the result of the experiment in vitro which showed that leucine abolished the activity of isolated brush border peptide hydrolases against tetraglycine. The specificity of this inhibition was established by showing that glycine had no effect on tetraglycine hydrolysis in vivo or on its hydrolysis by isolated brush borders in vitro. Although much further work remains to be done on the physiological implications of amino acid inhibition of tetrapeptide hydrolysis, the present data suggest that the final products of protein digestion (amino acids) may play a role in the regulation of activity of one or more of the brush border oligopeptidases.

Rates of glycine uptake from glycine peptides. The results of the present experiments confirm those of our previous studies (5-7) that the rates of glycine uptake from diglycine and triglycine are substantially greater than those from corresponding glycine solutions. The present studies extend the previous observation by showing that at a certain range of infusion concentration, the rate of glycine uptake from a tetraglycine solution is also significantly greater than from a corresponding glycine solution (Fig. 4 *b*). Furthermore, under the condition of maximal solubility, when the glycine peptide chain is lengthened to hexaglycine, the phenomenon of a greater rate of glycine uptake from a peptide versus a free amino acid solution is no longer apparent (Fig. 5 *a*).

Several factors appear to govern the rate of glycine uptake from glycine peptide solutions. (*a*) The rates of transport of glycine residues of diglycine and triglycine by the peptide carrier system are considerably faster than the rate of transport of glycine by an amino acid carrier system. (*b*) These differences in the rates of transport become more apparent as the concentration of infusion solution is increased. (*c*) On a molar basis, the rate of tetraglycine hydrolysis by the membrane-bound enzymes is slower than the rate of uptake of triglycine by the peptide carrier system. Under similar conditions of perfusion studies, there is nearly 90% disappearance of triglycine load introduced into the gut lumen (7), whereas there is only 50% disappearance of tetraglycine (Fig. 1).

The above factors need to be taken into account in the interpretation of the results of the present studies dealing with the rate of glycine uptake from glycine peptides and glycine solutions (Figs. 4 and 5). At the lower infusion concentration (Fig. 4 *a*), the amount of triglycine and glycine (theoretically 12.5 mM each), produced as the result of 50% hydrolysis of tetraglycine was probably not sufficiently large to cause a greater rate of glycine uptake from the tetraglycine solution (25 mM) than from the glycine solution (100 mM). At the higher infusion concentration (Fig. 4 *b*), the amount of triglycine and glycine (theoretically 25 mM each) produced as the result of tetraglycine hydrolysis was probably large enough to account for the greater rate of glycine uptake from tetraglycine solution (50 mM) than from the glycine solution (200 mM). It should be noted that when glycine, diglycine, or triglycine are used as substrates, the concentration of 25 mM is far below the saturating concentration for the peptide and amino acid carrier systems, whereas the concentration of 200 mM is far in excess of amino acid carrier system (6, 7). In the case of hexaglycine (Fig. 5 *a*), the time required for the hydrolysis of this oligopeptide to produce absorbable substrates impaired the efficiency of glycine absorption from the hexaglycine solution as compared to that from the glycine solution.

These interpretations of the in vivo data are supported by the result of our in vitro experiments on the effect of tetraglycine and glycine on enriching the intracellular concentration of glycine (Fig. 8). The addition of glycine (80 mM) to the incubation medium containing a jejunal ring preparation increased the intracel-

lular concentration of glycine maximally by twofold, whereas tetraglycine with an equivalent glycine concentration (20 mM) caused a fourfold increase. The mechanism of this greater intracellular concentration appears to be the utilization of two independent transport pathways in the case of tetraglycine versus one pathway in the case of glycine. When the amino acid carrier system has reached a limit in its glycine transport, the peptide carrier system continues the transport of diglycine and triglycine, which are then hydrolyzed by the cytoplasmic peptide hydrolases to expand the intracellular pool of glycine.

In conclusion, the results of the present study establish that the uptake of homologous glycine oligopeptides by the human mucosal epithelium is restricted to diglycine and triglycine. The results of these and previous experiments also show an excellent coordination of labor between peptide transport and hydrolytic systems in intestinal assimilation of homologous glycine oligopeptides. In the case of diglycine and triglycine, where the major peptide hydrolase activity against them resides in cell cytoplasm (20, 25), the transport by the peptide carrier system is the major mode of their luminal disappearance. In the case of tetraglycine, where the peptide hydrolase activity against it resides exclusively in the brush border region, superficial hydrolysis is the major mode of its disappearance from the gut lumen. Based on these considerations, it is reasonable to speculate that future investigations of the biochemical mechanism responsible for this coordinated effort might reveal important information on digestive and absorptive functions of the intestinal epithelium.

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