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Research Article

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Evidence for Two Different Modes of Tripeptide Disappearance in Human Intestine

UPTAKE BY PEPTIDE CARRIER SYSTEMS AND HYDROLYSIS BY PEPTIDE HYDROLASES

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ABSTRACT The intestinal fate of two tripeptides (triglycine and trileucine), which differ markedly in solubility and molecular weight, have been investigated by jejunal perfusion in healthy human volunteers. Rates of glycine or leucine uptake from test solutions containing triglycine or trileucine were greater than from test solutions containing corresponding amounts of free glycine or free leucine, respectively. The rate of glycine uptake from a 100 mM triglycine solution was greater than that from a 150 mM diglycine solution. At each infused load of triglycine (e.g., 1,000 μmol/min) the rates (micromoles/minute per 30 cm) of either triglycine disappearance (810±40) or glycine absorption (2,208±122) were markedly greater than the luminal accumulation rates of either diglycine (56±10) or free glycine (110±18). The luminal accumulation rate of free leucine during infusion of a 5 mM trileucine solution was over threefold greater than that of free glycine during the infusion of a 5 mM triglycine solution. Luminal fluid exhibited no hydrolytic activity against triglycine, but contained some activity against trileucine. Saturation of free amino acid carrier system with a large load of leucine did not affect glycine absorption rate from a triglycine test solution, but isoleucine markedly inhibited the uptake of leucine from a trileucine solution. When the carrier system for dipeptides was saturated with a large amount of glycylleucine, the disappearance rate of triglycine was considerably reduced while that of trileucine remained unaffected. After addition of glycylleucine to tripeptide solutions, there was a minimal increase in the luminal accumulation of diglycine, while dileucine accumulation was increased by 62-fold.

These studies suggest that the modes of intestinal disappearance of the above two tripeptides are different. Triglycine is taken up intact by human jejunum; this uptake is mediated totally or partially by the carrier system which also transports dipeptides. In contrast, trileucine is hydrolyzed to leucine and dileucine mostly on the cell surface and a small fraction is hydrolyzed in the gut lumen; these hydrolytic products are then taken up by the free amino acid and dipeptide carrier systems, respectively.

INTRODUCTION

Studies of the intestinal absorption of free amino acids and dipeptides in human intestine (1-13) have suggested that mucosal uptake of these substrates is mediated by carrier systems located on the brush border membrane. Furthermore, there appear to be separate carrier systems for free amino acids and dipeptides (6, 12). In comparison to free amino acids and dipeptides, the intestinal fate of tripeptides has not been extensively studied whether in man or in experimental animals. In the present studies we have investigated the intestinal transport and hydrolysis of tripeptides.

Dietary proteins usually contain 20 different amino acids. Among the 8,000 possible tripeptides, triglycine and trileucine were chosen as model substrates for the

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present studies on the basis of marked differences in their molecular weights and water solubilities. Among tripeptides, triglycine has the smallest molecular weight and is highly soluble. In contrast, trileucine is a bulky tripeptide with very poor solubility.

Amino acid absorption from any tripeptide solution may be accomplished by one or more of the following mechanisms: (*a*) complete hydrolysis by luminal and/or brush border peptide hydrolases to free amino acids and then transport by free amino acid carrier systems; (*b*) partial hydrolysis to free amino acid and dipeptide and subsequent uptake of these products by free amino acid and dipeptide carrier systems, respectively; (*c*) transport into the mucosal cell without prior hydrolysis. Our present studies have been designed to investigate the importance of each of the above pathways for the mucosal uptake of amino acids from triglycine and trileucine in the intestine of healthy human volunteers.

METHODS

In situ perfusion studies were carried out in the jejunum of 26 healthy human volunteers (ages ranged from 20 to 24 yr) by the method previously described (1, 5, 6). A 30-cm or 15-cm jejunal segment was perfused at a rate of 15 ml/min. One set of test solutions (Table I) was infused on each day of study. The number of subjects used for the investigation of each set of solutions ranged from three to seven with a median of four. Each subject was used for the infusion of one or more sets of test solutions. Whenever indicated, venous blood was collected in heparinized test tubes before and 30 and 60 min after the start of each infusion. Each blood sample was immediately centrifuged, and plasma was separated.

TABLE I

Protocol for Perfusion Studies

Sets of solutions	Composition of test solutions
I	33.33 mM triglycine, 50 mM diglycine, and 100 mM glycine
II	66.67 mM triglycine and 100 mM diglycine
III	100 mM triglycine and 150 mM diglycine
IV	33.33 mM triglycine and 33.33 mM triglycine plus 100 mM L-leucine
V	33.33 mM triglycine and 33.33 mM triglycine plus 100 mM glycyl-L-leucine
VI	33.33 mM glycine and 33.33 mM glycine plus 100 mM L-leucine
VII	5 mM L-leucyl-L-leucyl-L-leucine and 15 mM L-leucine
VIII	5 mM triglycine and 15 mM glycine
IX	5 mM L-leucyl-L-leucyl-L-leucine and 5 mM L-leucyl-L-leucyl-L-leucine plus 50 mM L-isoleucine
X	5 mM L-leucyl-L-leucyl-L-leucine and 5 mM L-leucyl-L-leucyl-L-leucine plus 50 mM glycyl-L-leucine

All amino acids and dipeptides were purchased from the General Biochemicals Div., Mogul Corporation, Chagrin Falls, Ohio. Triglycine was obtained from Sigma Chemical Co., St. Louis, Mo. Trileucine was purchased from Bachem, Inc., Marina Del Rey, Calif.

All the test solutions contained 0.4% polyethylene glycol as a nonabsorbable marker and between 100 and 150 mM sodium chloride. The pH of test solutions varied between 6.70 and 7.10. Previous published and unpublished studies from this laboratory have shown that the above variations in sodium concentration and in pH of test solutions do not significantly alter the rates of amino acid and peptide absorption in the jejunum of healthy human volunteers (7, 14, 15).

Ion exchange chromatography techniques were used to analyze for free glycine, free leucine, glycylleucine, and diglycine in intestinal aspirates or plasma, as previously described (5). The same methods were also used for the quantitation of triglycine, trileucine, and dileucine in the present studies. Using an automated amino acid analyzer (model 120-C, Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.), 0.20 M sodium citrate buffer (pH 4.26), and a column pressure of 150–200 lb/in^2, we determined the elution times for diglycine and triglycine as 90 and 100 min, respectively. Dileucine and trileucine were eluted with 0.38 M sodium citrate buffer (pH 5.36). Elution times were 105 and 110 min, respectively.

In vitro enzyme assay. Aspirates obtained after the infusion of an intestinal segment with physiological saline were centrifuged at 1,935 *g* for 15 min in a refrigerated centrifuge maintained at 0°C. 3 ml of the resulting supernate was added to test tubes preincubated in a metabolic shaker maintained at 37°C. 0.5 ml of either 33.33 or 100 mM triglycine or physiological saline was added to these tubes, and the mixtures were incubated for 1, 5, 10, and 15 min. When trileucine or dileucine was used as substrate, the same procedure as described above was followed except 2 ml of supernate was used as enzyme source, and 1 ml of 5 mM trileucine or dileucine was added to the incubation mixture. Incubation was terminated by heating the mixture at 95°C for 15 min in a water bath or by addition of 3 ml of 6% sulfosalicylic acid. Preliminary experiments indicated that peptide hydrolase activity of intraluminal fluid was not significantly altered by centrifugation, but the results were more consistent when the luminal fluids were centrifuged before enzyme assay.

Calculations. The disappearance rates of tripeptides were calculated by the following formula:

$$T_d = \left(T_p - T_a \frac{M_p}{M_a}\right) R$$

where T_d is the tripeptide disappearance rate in micromoles/minute per segment; T_p, the concentration of tripeptide in the test solution in micromoles/milliliter; T_a, the concentration of tripeptide in the intestinal aspirate in micromoles/milliliter; M_p, the concentration of polyethylene glycol (a nonabsorbable marker) in the test solution; M_a, the concentration of polyethylene glycol in the intestinal aspirate; and R, the rate of infusion in milliliters/minute.

Amino acid absorption rates from test solutions containing tripeptides were calculated by the following formula:

$$A_a = \left[3T_p - (F_a + 2D_a + 3T_a)\frac{M_p}{M_a}\right] R$$

where A_a is the amino acid absorption rate in micromoles/minute per segment; F_a, the concentration of free amino acid in the intestinal aspirate in micromoles/milliliter; and D_a, the concentration of dipeptide in the intestinal aspirate in micromoles/milliliter.

Amino acid absorption rates from test solutions containing free amino acid or dipeptide were calculated as previously described (5). Accumulation rates of free amino acids and dipeptides in the perfused segment were calculated by the following formula:

$$A = C\frac{M_p}{M_a}R$$

where A is the accumulation rate of either free amino acid or dipeptide in micromoles/minute per segment and C, the concentration of either free amino acid or dipeptide in the intestinal aspirate in micromoles/milliliter.

The average rate of tripeptide hydrolysis by the intraluminal enzymes in the perfused segment was calculated by the following formula:

$$T_h = \tfrac{1}{2}H_a\frac{M_p}{M_a}R$$

where T_h is the average rate of tripeptide hydrolysis in micromoles/minute and H_a, the amount (micromoles) of peptide that can be hydrolyzed by 1 ml of intestinal aspirate when incubated for 1 min under the above-described in vitro enzyme assay conditions.

The method of paired t test or Student's t test was used for the statistical analysis of the data (16).

RESULTS

Kinetics of glycine absorption from solutions of free, di-, and triglycine. Jejunal absorption rates of glycine from three test solutions containing equivalent concentrations of glycine (100 mM) in the form of free glycine (100 mM), diglycine (50 mM), or triglycine (33.33 mM) are shown in Fig. 1. The glycine absorption rate was significantly greater ($P < 0.01$) from either the diglycine or the triglycine than from the free glycine test solution. At this concentration, there was no significant difference between glycine absorption rates from the diglycine and triglycine test solutions. Even when the infusion concentration of glycine was doubled (200 mM) by increasing the concentration of diglycine to 100 mM and triglycine to 66.67 mM, there was still no significant difference between glycine absorption rates from these test solutions (Fig. 1). However, when the infusion concentration of glycine was tripled (300 mM) by increasing the concentration of diglycine to 150 mM and triglycine to 100 mM, glycine absorption rates were significantly greater from the triglycine than from the diglycine solution ($P < 0.01$, paired t test).

Plasma free amino acid and peptide concentrations during triglycine infusion. Before infusion of triglycine, the average concentration of glycine in the plasma was 221 μmol/liter; no diglycine or triglycine was detected. Infusion of triglycine test solutions caused a marked increase in the concentration of free glycine (Fig. 2), and

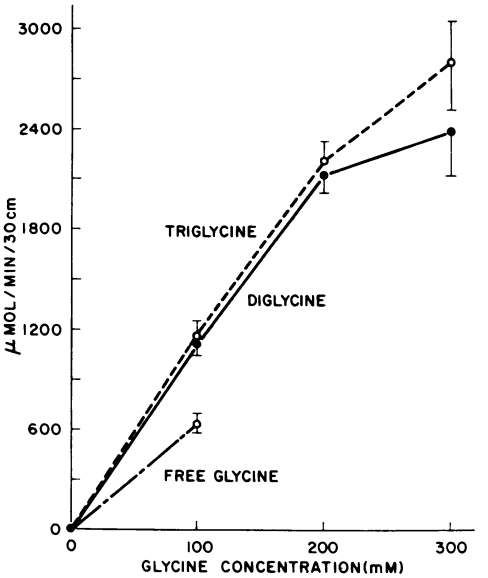

FIGURE 1 Rates of glycine uptake (mean±SEM) from test solutions containing glycine either in the form of free glycine, diglycine, or triglycine. Perfusion studies were performed in the jejunum of each of four subjects. The horizontal axis represents the total glycine content of each test solution. At the 100 mM concentration, the glycine absorption rate from the free glycine test solution was significantly smaller than from either the diglycine ($P < 0.01$) or the triglycine test solution ($P < 0.01$). At the 300 mM concentration, the glycine absorption rate was significantly greater from the solution of triglycine than of diglycine ($P < 0.01$, as compared by the paired t test).

the appearance of a substantial concentration of diglycine (as high as 239 μmol/liter in one individual) in peripheral plasma. Triglycine was never detected in peripheral plasma. Each increase in perfusate concentration of triglycine further elevated the plasma levels of both free glycine and diglycine (Fig. 2). Even at the highest perfusate concentration of triglycine, the increase in plasma concentration (mean±SEM in four subjects) of free glycine was markedly greater than the attained concentration of diglycine (5,722±422 vs. 165±22 μmol/liter).

Rates of luminal disappearance of triglycine and appearance of the products of its hydrolysis. The rates of triglycine disappearance at several concentrations are shown in Fig. 3. The jejunum exhibited a great potential for triglycine disappearance. Even at the highest infusion concentration used (100 mM), 78% of triglycine infused into a 30-cm jejunal segment disappeared.

During the infusion of 33.33 and 66.67 mM triglycine solutions, small amounts of free glycine and diglycine appeared in the lumen (Fig. 3). For example, at the initial concentration of 33.33 mM, the luminal accumulation rate of diglycine was 6% of the luminal disappearance rate or 5% of the infused load of triglycine.

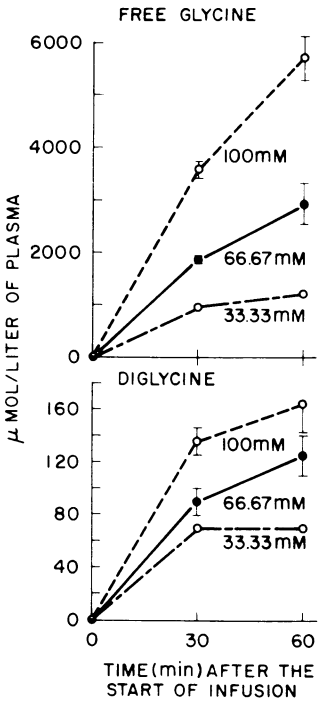

FIGURE 2 Increases in concentration (mean±SEM in four subjects) of free glycine in the peripheral plasma (upper panel) during the infusion of each triglycine test solution. The lower panel represents concentrations (mean±SEM in four subjects) of diglycine in peripheral plasma during the infusion of each triglycine test solution. Diglycine was not detected in the peripheral plasma before the start of any infusion studies.

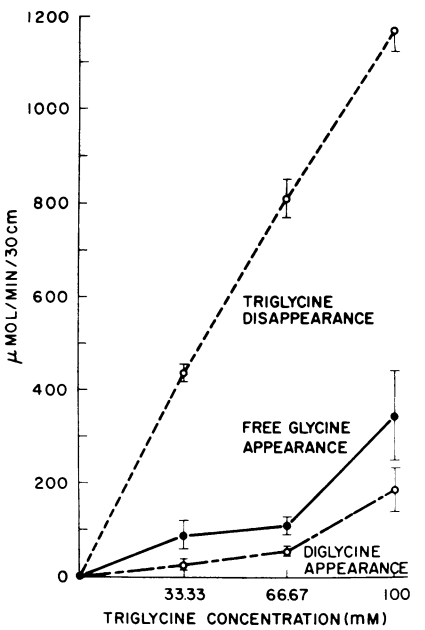

FIGURE 3 Jejunal disappearance rates of triglycine and luminal accumulation rates of free glycine and diglycine (mean±SEM in four subjects).

While the disappearance rate of triglycine was nearly doubled when the perfusate concentration of triglycine was increased from 33.33 to 66.67 mM, accumulation rates of diglycine and free glycine did not increase significantly (Fig. 3). However, when the perfusate concentration of triglycine was increased from 66.67 to 100 mM, both diglycine and free glycine accumulation rates increased significantly ($P < 0.05$, Fig. 3). Even at this increased rate, diglycine accumulation rate was still quite small as compared to triglycine disappearance rate (187±47 vs. 1,168±44 μmol/min per 30 cm).

Effect of leucine and glycylleucine on triglycine disappearance. Addition of 100 mM L-leucine, a concentration five times its K_m value (3, 6), to a 33.33 mM triglycine test solution did not affect the rates of triglycine disappearance and glycine absorption (Fig. 4). Similarly, there were no significant changes in the luminal accumulation rates of either free glycine or diglycine (Fig. 5). On the other hand, addition of 100 mM leucine to a 33.33 mM free glycine test solution markedly reduced the rate of glycine absorption (Fig. 6).

When 100 mM glycyl-L-leucine, a concentration four times its K_m value (6), was added to a triglycine solution, the disappearance rate of triglycine was reduced by 66% (Fig. 7). The glycylleucine disappearance rate from the tripeptide-dipeptide mixture was similar to the rate of glycylleucine disappearance from a test solution containing only this dipeptide in a concentration of 100 mM (5). Therefore, to calculate the rate of glycine uptake from the test solution containing triglycine

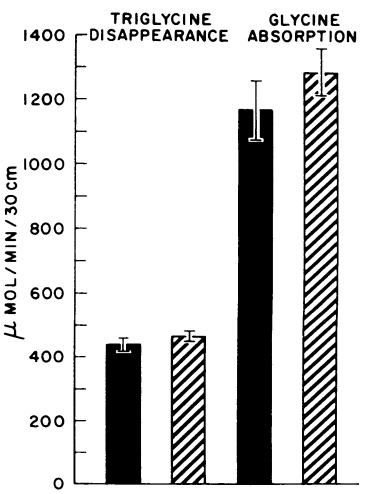

FIGURE 4 Triglycine disappearance rates and rates of glycine uptake (mean±SEM in four subjects) from a 33.33 mM triglycine test solution (solid bar) and a 33.33 mM triglycine solution containing 100 mM free leucine (striped bar).

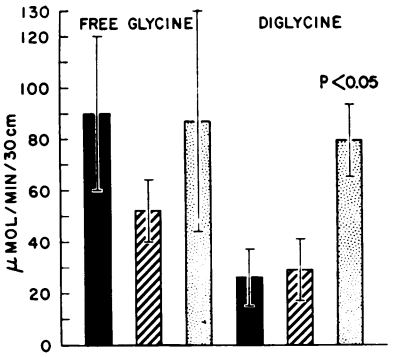

FIGURE 5 Luminal accumulation rates (mean±SEM in four subjects) of free glycine and diglycine during infusion of a 33.33 mM triglycine test solution (solid bar) or the same solution with the addition of either 100 mM free leucine (striped bar) or the addition of 100 mM glycylleucine (stippled bar). The *P* value refers to the difference between luminal accumulation of diglycine before and after addition of glycylleucine. The differences between free glycine accumulation rates were not statistically significant.

and glycylleucine, the rate of glycine uptake from the 100 mM glycylleucine test solution was subtracted from the rate of glycine uptake from the test solution containing both glycylleucine and triglycine. The result of this estimation is shown in Fig. 7. Glycine uptake from triglycine was markedly reduced in the presence of glycylleucine. Addition of glycylleucine to the test solution also resulted in an increase in the rate of diglycine accumulation (Fig. 5), but the rate of free glycine accumulation, calculated on the same basis as described above for glycine absorption, did not significantly change (Fig. 5).

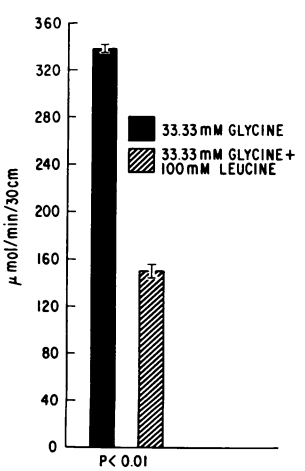

FIGURE 6 Jejunal absorption rates (mean±SEM in three subjects) of glycine from test solutions containing 33.33 mM glycine or 33.33 mM glycine plus 100 mM leucine. The *P* value was calculated by the paired *t* test.

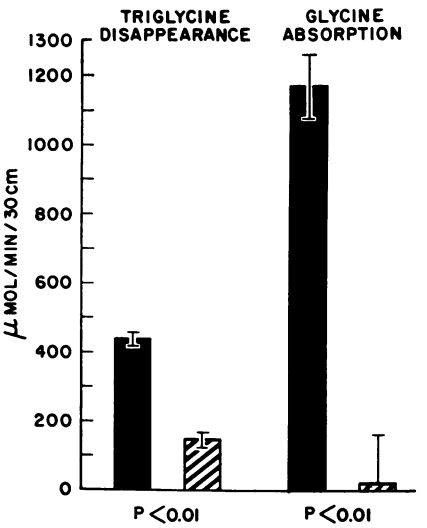

FIGURE 7 Triglycine disappearance rates and glycine absorption rates (mean±SEM in four subjects) from a 33.33 mM triglycine test solution (solid bar) or from a similar solution with the addition of 100 mM glycylleucine (striped bar). The *P* value was calculated by the paired *t* test.

Leucine absorption from solutions of free and trileucine. The highest concentration of trileucine that is soluble in aqueous medium is approximately 5 mM. Due to a rapid rate of disappearance of trileucine from the 30-cm segment in preliminary studies, a 15-cm segment was chosen for experiments with 5 mM trileucine. The results of these studies are summarized in Fig. 8. There was disappearance of 98% of the infused load (75 μmol/min) of trileucine in the 15-cm segment. The intestinal aspirate always contained a trace amount of dileucine. 15% of the infused load of leucine (225 μmol/min) was recovered as free leucine (Fig. 8). Rates of leucine uptake from 5 mM trileucine and 15 mM leucine solutions are compared in Fig. 8. The rate of uptake was significantly greater from the trileucine solution than from the corresponding free leucine solution. For the purpose of comparison, perfusion studies of 5 mM triglycine solution were also carired out in the 15-cm jejunal segment. As shown in Fig. 8, the rate of triglycine disappearance was only slightly smaller than that of trileucine, but the appearance rate of free glycine was less than one-third that of free leucine. As with trileucine, only trace amounts of diglycine appeared in the intestinal aspirates (Fig. 8). However, the difference between the rates of glycine uptake from 5 mM triglycine and 15 mM free glycine solutions was more dramatic than the difference between the rates of leucine uptake from the trileucine and free leucine solutions (95 vs. 29 μmol/min per 15 cm).

Effect of isoleucine and glycylleucine on trileucine disappearance. Trileucine disappearance was not af-

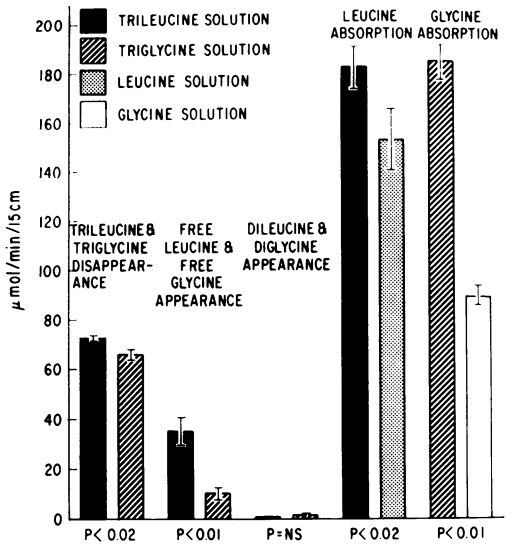

FIGURE 8 Trileucine and triglycine disappearance rates; luminal accumulation rates of free leucine, dileucine, free glycine, and diglycine; and rates of leucine and glycine uptake from 5 mM trileucine and triglycine test solutions, respectively. The mean±SEM values were determined from trileucine and triglycine perfusion studies in the jejunum of seven and four subjects, respectively. The differences between the rates of leucine uptake from 5 mM trileucine and 15 mM leucine solutions and the rates of glycine uptake from 5 mM triglycine and 15 mM glycine solutions were evaluated by the paired *t* test. Other statistical evaluations shown in the figure were done by Student's *t* test.

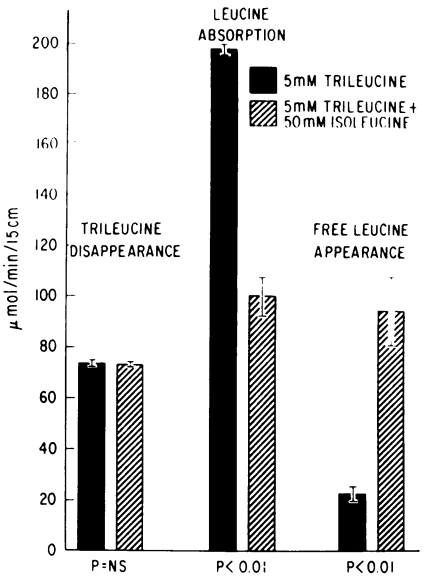

FIGURE 9 Rates of trileucine disappearance, leucine uptake, and luminal accumulation of free leucine from test solutions containing 5 mM trileucine and 5 mM trileucine plus 50 mM isoleucine. Mean±SEM values were determined from perfusion studies in the jejunum of three subjects. The *P* values were determined by the paired *t* test.

fected by isoleucine, but leucine absorption rate was markedly reduced, and free leucine appearance rate was markedly increased (Fig. 9). The same trace amount of dileucine as shown in Fig. 8 was recovered in intestinal aspirates after addition of isoleucine. The rates of trileucine disappearance and dileucine appearance before and after addition of 50 mM glycylleucine to the 5 mM trileucine solution are summarized in Fig. 10. Although addition of glycylleucine did not alter the disappearance rate of trileucine, it markedly increased (by 62-fold) the appearance rate of dileucine.

Triglycine and trileucine hydrolysis by luminal fluid. The jejunal fluid lacked any hydrolytic activity against triglycine regardless of the concentrations used, but exhibited activity against trileucine. The data regarding the potential for trileucine hydrolysis by the intraluminal enzymes of 15-cm segments are summarized in Table II. The average rate of trileucine disappearance during the infusion of a 5 mM solution of this tripeptide was 73 μmol/min per 15 cm. Of this 73 μmol, 3 μmol could be attributed to hydrolysis by intraluminal enzyme (Table II). Under similar conditions of study, the rate of hydrolysis of trileucine by intraluminal enzymes was 10-fold greater than that of dileucine (Table II).

The rates of peptide hydrolysis in vivo as determined by our method should be considered as rough estimates. There are a number of problems in estimating in vivo hydrolysis from in vitro determinations of enzyme activity. The problems include proper selection of concentration, cofactors, pH, time of incubation, and expression of unit of enzyme activity. To approximate the in vitro condition to the in vivo condition, 2–3 ml of in-

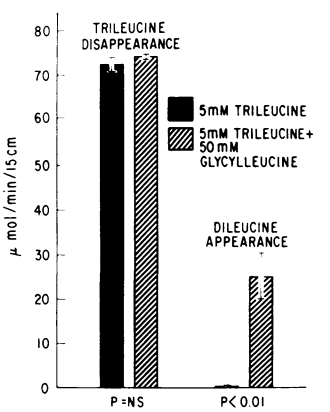

FIGURE 10 Rates of trileucine disappearance and luminal accumulation of dileucine from test solutions containing 5 mM trileucine and 5 mM trileucine plus 50 mM glycylleucine. Mean±SEM values were determined from perfusion studies in the jejunum of three subjects. The *P* values were determined by the paired *t* test.

TABLE II

Average Rates of Peptide Hydrolysis by Luminal Enzymes in a 15-cm Jejunal Segment

Substrate	Rate of hydrolysis*
	μmol/min
L-Leucyl-L-leucyl-L-leucine	3.0±0.5 (range, 6.45–1.50)
L-Leucyl-L-leucine	0.39±0.09 (range, 0.78–0.03)

* Each value represents the mean±SEM of eight separate studies. The difference between the rates of hydrolysis of trileucine and dileucine was statistically significant ($P < 0.01$).

testinal aspirate was added to 0.5–1 ml of test solution containing either triglycine or trileucine. Poor solubility of trileucine limited the range of concentration for assessment of peptide hydrolase activity against this tripeptide. Nevertheless, addition of 1 ml of 5 mM trileucine to 2 ml of intestinal aspirate allowed estimation of peptide hydrolase activity against this tripeptide in a situation of substrate excess.

DISCUSSION

Triglycine, like diglycine (5), appears to be taken up by the mucosal cells without prior hydrolysis. In contrast, the intraluminal disappearance of trileucine appears to be chiefly the result of hydrolysis of this tripeptide by intraluminal and membrane-bound enzymes. The following discussion examines the evidence to support these points.

Triglycine disappearance. (*a*) Intraluminal hydrolysis of triglycine can be dismissed as a possibility since the intraluminal fluid lacked hydrolytic activity against triglycine. (*b*) Since free glycine and diglycine accumulate in the gut lumen and in plasma during the infusion of triglycine solutions, it is necessary to consider the possibility of mucosal surface hydrolysis (17, 18) of triglycine to diglycine and free glycine followed by transport of the products of hydrolysis into the cell by the carrier systems for amino acids and dipeptides, respectively. To determine whether the carrier system for neutral free amino acids is involved in the transport of the amino acid constituent of triglycine, this carrier system was saturated with a large load of free leucine (100 mM). Free leucine markedly inhibits uptake of glycine from a 33.33 mM solution of free glycine (Fig. 6). Under this condition, the rate of uptake of glycine from a 33.33 mM triglycine solution was not significantly altered (Fig. 4). (*c*) To investigate whether the dipeptide carrier system was being utilized for the transport of the diglycine constituent of triglycine, the dipeptide carrier system was saturated with 100 mM glycylleucine. Glycylleucine markedly inhibits diglycine absorption (6). In this experiment, the uptake of glycine from triglycine solution was considerably reduced (Fig. 7). This reduction appeared to be principally a function of a decreased triglycine absorption rather than increased accumulation of diglycine. In the presence of glycylleucine, the triglycine disappearance rate was reduced by 292 μmol/min per 30 cm while diglycine accumulation rate was increased by only 53 μmol/min per 30 cm. These observations suggest that a small fraction of triglycine is hydrolyzed by the brush border peptide hydrolases, but most triglycine is absorbed intact. Furthermore, the uptake of triglycine by the brush border membrane of the jejunal mucosa is mediated partially or totally by the dipeptide carrier system previously characterized (6). Intact absorption does not appear to be unique to triglycine since unhydrolyzed glycylprolylhydroxyproline has been recovered in the urine of human subjects after ingestion of meals containing gelatin (19).

Whether an active process is involved in the mucosal uptake of triglycine cannot be determined from the present data. However, Addison and co-workers (20), using an in vitro preparation of hamster jejunum, have recently found intracellular accumulation of glycylsarcosylsarcosine against an electrochemical gradient. This tripeptide, an analog of triglycine, is poorly hydrolyzed by mucosal enzymes and is, therefore, suitable for the studies of cellular transport.

In contrast to a steep and almost linear increase in triglycine disappearance rates with each increase in triglycine concentration, the accumulation rates of both free glycine and diglycine showed saturation over the initial range (33.33–66.67 mM) of concentrations (Fig. 3). This observation suggests that the potential for uptake may exceed the potential for hydrolysis or that the affinity of triglycine for transport sites is much higher than its affinity for hydrolytic sites on the brush border membrane. The reason for modest increases, after reaching a plateau, in luminal accumulation rates of free glycine and diglycine at the highest triglycine concentration used (100 mM) in the present experiment is not yet clear. Due to an increased intraluminal concentration of triglycine at an infusion concentration of 100 mM, possibly more substrate becomes available for the membrane-bound peptide hydrolases. Alternatively, the increased rates might suggest that a second transport system within the mucosal cells involved with the forward transfer of the products of triglycine hydrolysis is reaching saturation. Indeed, a second transport mechanism for free glycine has been postulated by Newey and Smyth (21) and Matthews and his co-workers (22) to account for the increasing concentration of free glycine in the rat gut lumen with increases in glycine peptide concentration. Whether one or both of the above explanations are valid cannot be decided on the basis of the

available evidence. Nevertheless, progressive increases in the plasma concentrations of both free glycine and diglycine after each increment in triglycine concentration in the infusion solution (Fig. 2) do not indicate that a limiting rate in the mucosal transport of either triglycine or its digestive products has been reached within the range of concentrations used in our studies.

Trileucine disappearance. Four major differences in hydrolysis and transport characteristics indicated that there is a fundamental difference in the intestinal fate of triglycine and trileucine. (*a*) The intraluminal fluid contained peptide hydrolase activity against trileucine but not against triglycine. (*b*) During the perfusion studies, the concentration of free leucine was greater than free glycine by fourfold (Fig. 8). (*c*) The disappearance rate of trileucine was not affected by glycylleucine (Fig. 10), but this dipeptide markedly reduced the disappearance rate of triglycine (Fig. 7). (*d*) Isoleucine reduced the mucosal uptake of leucine from trileucine by over 50% (Fig. 9), but leucine was without effect on uptake of glycine from triglycine (Fig. 4). These observations suggest that a major portion of trileucine is hydrolyzed to free leucine and dileucine on the cell surface and a small fraction is hydrolyzed in the gut lumen. The rate of trileucine hydrolysis by intraluminal enzymes would account for only a small portion of trileucine disappearance rates (Table II and Fig. 8). The greater rate of uptake of leucine from 5 mM trileucine than from 15 mM leucine solution (Fig. 8) indicates that there is uptake of either some intact trileucine or its dileucine constituent, or both. The marked increase (62-fold) in the rate of dileucine accumulation in the gut lumen after saturation of dipeptide carrier system with glycylleucine (Fig. 10) is supportive of intact absorption of dileucine released by superficial hydrolysis of trileucine.

It is now well established that the amino acid absorption rates from a wide variety of dipeptides are significantly greater than from the corresponding amino acid mixtures in human intestine (5, 10–13). Indeed, the data presented in Fig. 1 once again show this phenomenon. Similarly, rates of uptake of amino acids from three tripeptides (triglycine, trileucine, and alanylglycylglycine) studied thus far are greater than from corresponding free amino acid mixtures (Figs. 1 and 8 and reference 23). Matthews and co-workers (24) have shown that in rat intestine rates of amino acid uptake are greater from triglycine, trimethionine, and methionylglycylmethionine than corresponding free amino acid mixtures. The rate of glycine uptake from triglycine is even greater than from diglycine when high concentrations of these peptides are infused (Fig. 1). The greater uptake of amino acids from peptide than from free amino acid solutions may have relevance to amino acid transport under physiological conditions. Although the concentrations of individual oligopeptides in the gut lumen have not yet been well established, the 5 mM concentration of triglycine and trileucine in the present studies falls within the range of postprandial concentrations of peptide amino acids in the intestine of healthy human subjects (25).

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