Age-Dependent Change in Intestinal Absorption of Dipeptides and Their Constituent Amino Acids in the Guinea Pig

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Summary

The influxes from the mucosal solution to the enterocytes were investigated for glycylglycine, glycyl-L-leucine, and their constituent amino acids in isolated intestinal segments from suckling (3 to 4 days), weanling (10 to 14 days), and adult guinea pigs. Glycine and L-leucine influxes from the mucosal solution containing free glycine or L-leucine were not significantly different between sucklings and adults in either the jejunum or the ileum. On the other hand, glycylglycine and glycyl-L-leucine influxes were significantly greater in the following sequence: sucklings > weanlings > adults. Such age-dependent difference in dipeptide influx was more prominent in the jejunum than in the ileum. Kinetically, such developmental change in dipeptide influx was found to be related to the change in the value of $J_{\max}$ (the maximum influx). The data indicated that the intact-peptide transfer mechanism has more important significance in protein hydrolysate absorption in immature stages of animals than in adults.

Speculation

Age-dependent change in dipeptide influxes into the enterocytes may be related to the change in the density of carriers for intact peptides at the brush border membranes. It is also speculated that the greater importance of the intact-peptide transport may have some relation to immature intracaval (intermediate) digestion or less effective functional linkage between membrane surface hydrolysis and amino acid transport in suckling and weanling stages.

Recent studies on intestinal absorption of protein hydrolysates have disclosed that there exist specified carrier system(s) for intact di- and tripeptides at the brush border membrane of the small intestine (12). The transferred peptides are hydrolyzed to free amino acids by the intracellular peptidases (6, 9, 14, 15). It has also been shown that the rate of absorption is frequently, although not always, greater when certain amounts of amino acids are given in the mucosal solution in the forms of peptide than in the form of free amino acid (1, 5).

The developmental patterns of transport of monosaccharides and amino acids have been studied in various animal species (3, 4, 7). These previous observations agree in that the transport activities for these nutrients exhibit a striking peak at the perinatal period, and then they decrease slowly with increase in age. On the other hand, little is known about the developmental change in intact-peptide transport. Only available data are those reported by Rubino and Guandalini (15), who demonstrated that, in rabbit jejunum, the influxes of glycyl-L-proline and glycyl-L-phenylalanine were much greater in sucklings than in adults although glycine influxes were not significantly different among fetuses, newborns, sucklings, and adults.

The present study aimed at investigating the age-dependent change in relative importance of the intact-peptide transport and membrane surface hydrolysis (amino acid transport) for glycylglycine and glycyl-L-leucine which have been well studied in adult guinea pigs (8, 9).

MATERIALS AND METHODS

The procedures of preparation of isolated everted intestinal segments and methods of uptake measurements were mostly the same as those described in our previous papers (8, 9, 17). Guinea pigs of three different age groups were examined; sucklings (3 to 4 days after the birth) weighing from 80 to 120 g, weanlings (10 to 14 days) weighing from 160 to 220 g, and adults weighing from 350 to 500 g. No discrimination was made for sex. After more than 3 hr fasting, the animals were anesthetized with urethane (1 g/kg body weight IP injection). The proximal portion of the jejunum and the distal ileum were excised and divided into small segments of about 2.5 cm length. Each segment was everted in a usual way and fixed over a polyethylene tube. The polyethylene tubes of 3 mm outer diameter were used for suckling intestines, those of 3.5 mm diameter were used for weanlings intestines, and those of 5 mm diameter were used for adult intestine. The everted segments thus prepared were preincubated for 30 min at 37°C in 20 ml oxygenated buffer solution (pH 7.4) containing 100 mM NaCl. Osmolality was adjusted to 290 mOsm/kg H₂O with D-mannitol.

As previously reported (8, 9), the Na⁺ dependence of Gly-Gly uptake is very weak, and transport of intact Gly-Leu is Na⁺ independent. Also, glycine uptake, which is absolutely dependent on the presence of Na⁺, is almost saturated at 100 mM-Na⁺ in regard to Na⁺ effect. Therefore, the data obtained at 100 mM-Na⁺ are comparable with those obtained at normal Na⁺ concentration (150 mM). The main reasons for the use of 100 mM-Na⁺ are as follows: (1) spontaneous decline of transport function is much slower in the 100 mM-Na⁺-medium than in normal Ringer solution; (2) greater electrical responses can be recorded than in 150 mM-Na⁺ (10); and (3) the solutions of high concentrations of test substances can be prepared without changing Na⁺ concentration and osmolality. For these reasons, all our previous studies on adult guinea pigs were carried out in 100 mM-Na⁺. For the sake of direct comparison of the data obtained in younger animals with those in adults, the same medium conditions were used in the present study.

The tissue uptake of the dipeptides and the amino acids was examined by incubating the preparations for 1 min in 10 ml test buffer solutions containing the test substance and its tracer. The tracers used were [1-¹⁴C]glycylglycine, [1-¹⁴C]glycyl-L-leucine, glycyl-L-[U-¹⁴C]leucine, [2-¹⁴C]glycine, and L-[U-¹⁴C]leucine. D-[1-³H]Mannitol was used for correcting the amounts of the mucosal bathing fluid adhering the mucosal surface of the preparations.

All radioactive compounds were purchased from Radiochemical Center, Amersham. Upon completion of the test incubation, the preparation was washed for 3 to 5 sec in ice-cold isotonic mannitol solution, removed from the supporting tube, blotted on filter paper, and then put into 3 ml of 3% trichloroacetic acid solution. The extraction was carried out at room temperature for more than 3 hr during which the extraction tubes were shaken continuously. The radioactivities of the extraction fluids and the incubation media were counted in a liquid scintillation counter (Aloka LSC-602). All uptake data were presented as mean ($\pm$ S.E.) (nmoles $\cdot$ cm⁻² serosal surface area $\cdot$ min⁻¹) of observations from different animals. The time course of uptake of the dipeptides and the amino acids was found to be linear with time at least up to 2 min after the start of incubation. Therefore, the values of 1-min uptake were taken as the initial influxes across the mucosal border of the preparations.

RESULTS

UPTAKE RATES FOR GLYCINE AND L-LEUCINE

The rates of uptake of glycine and L-leucine from the mucosal solutions containing respective free amino acids at 5 mM were compared between suckling and adult intestines. The data obtained are summarized in Figure 1, which shows that there is no significant difference between sucklings and adults in either glycine or L-leucine uptake rate. In both age groups, significantly greater uptake was seen in the ileum than in the jejunum. The mean values of rates of glycine uptake (nmoles $\cdot$ cm⁻² $\cdot$ min⁻¹) were 7.5 ± 4.2 in suckling jejunum, 9.2 ± 1.1 in adult jejunum, 45.1 ± 5.7 in suckling ileum, and 44.2 ± 4.5 in adult ileum. For L-leucine, the uptake rates were 59.8 ± 12.3 in suckling jejunum, 60.0 ± 7.5 in adult jejunum, 90.6 ± 18.1 in suckling ileum, and 97.1 ± 10.0 in adult ileum.

UPTAKE RATES FOR GLYCYLGLYCINE AND GLYCYL-L-LEUCINE

Uptake rates for glycylglycine and glycyl-L-leucine measured in sucklings and adults are summarized in Figure 2. Glycyl-L-leucine influx was examined with two different tracers; glycine-labeled (Gly*-Leu) and leucine-labeled (Gly-Leu*) compounds. In adult guinea pigs, Gly*-Leu influx was shown to be transferred only by the intact-peptide transport mechanism (9). In contrast, Gly-Leu* influx, usually about twice the Gly*-Leu influx in adult guinea pig intestine, consists of two components; one is the component transferred by the intact-peptide transport mechanism, and the other the component transferred as leucine after the membrane

Fig. 1. Rates of uptake of glycine and L-leucine by the small intestines isolated from sucklings and adults. The everted preparations were incubated for one min in media containing either of two amino acids at 5 mM. Each value is the mean of data obtained from 5 different animals. Bars, S.E.

surface hydrolysis. Glycylglycine is known to be transferred totally by the intact-peptide transport mechanism independent of glycine transport (1, 9).

As clearly shown in Figure 2, both glycylglycine and Gly*-Leu influxes are significantly greater in sucklings than in adults, particularly in the jejunum. Gly-Leu* influx is also considerably larger in suckling than in adults in the jejunum, but there was no difference in the ileum. Average influxes (nmoles $\cdot$ cm⁻² $\cdot$ min⁻¹) of glycylglycine from 5 mM glycylglycine solution were 29.5 ± 4.7 in suckling jejunum, 10.4 ± 3.5 in adult jejunum, 57.4 ± 9.1 in suckling ileum, and 33.8 ± 2.1 in adult ileum. Gly*-Leu influxes in the jejunum were 42.0 ± 5.0 in sucklings and 10.1 ± 1.6 in adults, and the same influxes in the ileum were 62.6 ± 5.8 in sucklings, and 39.2 ± 2.6 in adults when the preparations were incubated in 5 mM glycyl-L-leucine solutions. Gly-Leu* influxes in the jejunum from the same glycyl-L-leucine solution were 50.7 ± 6.6 in sucklings and 15.2 ± 2.6 in adults, and the same influxes in the ileum were 80.7 ± 18.5 in sucklings and 86.8 ± 8.5 in adults. Thus, glycylglycine influxes in suckling were 2.8 and 1.7 times greater in the jejunum and ileum, respectively, than those in adults. Gly*-Leu influxes were also greater in sucklings than in adults, 4.2 and 1.6 times greater in the jejunum and ileum, respectively.

EFFECT OF L-ISOLEUCINE ON GLY-LEU* INFLUX IN SUCKLING INTESTINES

To know whether Gly-Leu* influx in suckling intestine involves a component transferred as leucine by leucine transport system, as in the case of adult intestines, inhibition experiments with L-isoleucine were carried out. The influx of Gly-Leu* from 5 mM solution was measured in the presence and absence of 10 mM L-isoleucine. The results are summarized in Table 1. In either the jejunum or the ileum, Gly-Leu* influx was significantly reduced by isoleucine. It should be pointed out, however, that the inhibition was significantly smaller in younger animals than in adults (35 to 38% versus 50%).

DIFFERENCE IN KINETIC PROPERTIES OF GLYCYLGLYCINE TRANSPORT BETWEEN SUCKLING AND ADULT JEJUNUM

Double reciprocal plots of glycylglycine influxes versus glycylglycine concentration in the incubation medium fell on a straight line in both sucklings and adults as shown in Figure 3. Our previous study (9) demonstrated that the line for glycylglycine influx was entirely independent of the line for glycine in regard to J_{max} and K_t . The values of J_{max} for the dipeptide were 51 ± 1.9 nmol $\cdot$ cm⁻² $\cdot$ min⁻¹ in the sucklings and 20 ± 2.4 nmol $\cdot$ cm⁻² $\cdot$ min⁻¹ in adults, the value of sucklings being 2.6 times greater than that of adults. On the other hand, the values of K_t were not significantly different; 2.4 ± 0.5 mM in sucklings and 2.1 ± 0.8 mM in adults.

COMPARISON OF DEVELOPMENTAL CHANGES OF INTACT-PEPTIDE TRANSPORT

Figure 4 shows the summary of the data of glycylglycine influxes measured in sucklings, weanlings, and adults under the same medium conditions. In both the jejunum and the ileum, the value of the influx gradually became smaller with age. In the jejunum, the value of adults is about one-third of that suckling, whereas it is about one-half in the ileum.

DISCUSSION

The results of the present study show that, in guinea pigs, there is a marked age-dependent change in intact-peptide transport. Both glycylglycine and Gly*-Leu have been proved to be transferred by the intact peptide transport systems (8, 9), and these dipeptide influxes were much greater in sucklings than in adults. On the other hand, glycine and L-leucine influxes were not significantly different between sucklings and adults. Very similar pattern of developmental change was reported on rabbits by

Fig. 2. Rates of uptake of Gly-Gly, Gly*-L-Leu, and Gly-L-Leu* by the small intestines of sucklings and adults. The everted preparations were incubated for one min with one of the dipeptides at 5 mM. Each value is the mean of data from 5 different animals. Bars, S.E.

Table 1. Inhibitory effect of L-isoleucine on the influx of leucine-residue of glycyl-L-leucine in the small intestine of suckling guinea pigs¹

Sample	Inhibitor (mM)	n ²	Influx (nmoles · cm ⁻² · min ⁻¹)	% of control	P (paired t test)
Jejunum	None	4	57.1 ± 8.5		
	L-Isoleucine (10)	4	39.1 ± 8.1	68.5	P < 0.01
Ileum	None	4	90.7 ± 2.9		
	L-Isoleucine (10)	4	55.9 ± 2.9	61.6	P < 0.001

¹ The everted preparations were incubated for one min in 5 mM glycyl-L-leucine solutions containing Gly-Leu*.

² n, number of measurements.

Fig. 3. Double reciprocal (Lineweaver-Burk) plots for Gly-Gly transport by the jejunum of suckling (●) and adult (○) guinea pigs. Points, mean of data from 5 different animals. Bars, S.E.

Rubino and Guandalini (15), who demonstrated that the uptake of glycyl-L-proline and glycyl-L-phenylalanine was much greater in newborns than in adults though glycine uptake was not significantly different.

Fig. 4. Comparison of uptake rates for Gly-Gly among intestinal preparations to suckling, weanling, and adult guinea pigs. The uptake rates were determined by incubating the preparations in media containing 5 mM glycylglycine. Each value is the mean of the results from 5 different animals. Bars, S.E.

Previous observations in guinea pigs by Butt and Wilson (4) and in rats by Fitzgerald *et al.* (7) showed that tissue uptake of amino acids was greatest immediately after the birth and exhibited an age-dependent decrease. They normalized the data by wet weight of the preparations. The wet weight of the suckling ileum per unit serosal surface area is 39 ± 2.2 mg/cm² ($n = 8$), the value being about 1/4 of that of adults. Therefore, the influx values for glycine and L-leucine become slightly greater in sucklings when the values are expressed in terms of wet weight.

Age-dependent change in Gly*-Leu influx was very similar to that of glycylglycine influx. However, Gly-Leu* influx showed

somewhat different pattern; namely, in the ileum, there was no difference between sucklings and adults. As demonstrated in a previous study (8), Gly-Leu* influx has two influx components, one is transferred by intact peptide transfer mechanism, and the other is transferred as leucine after the membrane digestion. Inhibition experiments with L-isoleucine showed that, also in suckling intestines, the influx component transferred as leucine was present. However, the extent of inhibition by the addition of isoleucine was significantly smaller in sucklings than in adults. This can simply be interpreted by a relatively greater contribution of intact peptide transport and a less contribution of hydrolysate transport in younger animals than in adults. However, other possibilities cannot be excluded that younger animals have a less activity on glycyl-L-leucine dipeptidase at the outside surface of brush border membrane and/or a less effective functional linkage between the membrane digestion and free amino acid transport at the brush border structure.

The age-dependent changes of intact-peptide uptake observed in this study lead us to a speculation that the intracellular dipeptidase activities for glycylglycine and glycyl-L-leucine also decline age-dependently in parallel with decrease in capacity of intact-peptide transport. Unfortunately, to our knowledge few reports have been published concerning developmental change in dipeptidase activities of guinea pig enterocytes. Lindberg and Owman (11) showed that in rats the enterocyte dipeptidase activities including glycylglycine and glycyl-L-leucine dipeptidase rapidly declined to an adult level after the parturition when the newborns had suckled. Also, Rubino (14) showed almost parallel age-dependent changes in both the transport capacity for glycyl-L-proline and its intracellular dipeptidase activity in rabbit intestine. These studies indicate that the developmental pattern of intact peptide transport has an intimate relation to that of respective intracellular hydrolase activity.

From the clinical point of view, several authors have stressed the advantage of administration of peptides over the per oral nutrition with free amino acid mixtures in patients with a reduced absorptive surface area (2, 13, 16). The results of the present study indicate that such an advantage is more greater in younger infants. The greater relative importance of intact-peptide transport should be taken into consideration in treatments of the infant with malabsorption.

CONCLUSION

The rates of uptake of glycine and L-leucine by the small intestine were not significantly different between sucklings and adults in terms of nmols per cm² serosal surface area per min. On

the other hand, the rates of uptake of glycylglycine and glycyl-L-leucine were greater in sucklings than in adults.

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