A Comparison of the Intestinal Absorption of Amino Acids in Piglets When Provided in Free Form or as a Dipeptide*

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ABSTRACT : Three 28 day-old Duroc×Large White×Landrace litter mate gilts weighing an average of 6.5 kg were used to study the intestinal absorption of amino acids when provided in dipeptide form or in the form of a free amino acid mixture. The pigs were given one of three treatments. The control involved a duodenal infusion containing no amino-acids (phosphate buffer plus 5% sorbitol) while the remaining two treatments involved either a duodenal infusion containing a glycine-lysine dipeptide (1 g) or a mixture of the free amino acids glycine and lysine at the same concentration as in the dipeptide. Blood was drawn from a cannula inserted in the portal vein, at 5 to 20 minute intervals, for two hours following infusion. The concentration of intact dipeptide as well as free glycine and lysine in the portal blood was determined by high performance liquid chromatography. The intact dipeptide was never detected in the portal blood at any time after infusion. Lysine appeared in the portal blood more rapidly after infusion of dipeptide than after infusion of free lysine and the concentration of lysine in portal blood was higher in the pig infused with the dipeptide than after infusion of free lysine at almost all time points measured. The cumulative absorption of lysine and glycine from the intestine during the two hour period after infusion was greater in the pig infused with dipeptide than in the pig infused with free amino acids. The results suggest that although intact dipeptide did not reach the portal circulation, a special transport mechanism for absorption of dipeptide by intestinal cells appears to be present in pigs similar to that observed in other species. (*Asian-Aus. J. Anim. Sci. 1999. Vol. 12, No. 6 : 939-943*)

Key Words : Dipeptide, Free Amino Acids, Piglets, Portal Vein, Absorption

INTRODUCTION

Studies of the absorption of small peptides in the intestine of different species of animals have shown that intact small peptides are taken up by intestinal mucosa cells and are absorbed more rapidly than free amino acids (Newey et al., 1960; Adibbi and Phillips, 1968; Burston et al., 1972; Matthews et al., 1974). This implies that an independent transport system for small peptides exists in the intestine of animals. Therefore, competition between amino acids for absorption can be avoided when small peptides are absorbed.

It is not clear whether small peptide absorption properties differ among animal species. Most studies on peptide absorption have focused on rats (Burston et al., 1972; Hara et al., 1984), hamsters (Mathews et al., 1974; Burston et al., 1980), guinea pigs (Himukai et al., 1983), rabbits (Ganapathy et al., 1984) and humans (Addibi, 1971; Grimble and Silk, 1989). More recently research interest has focused on ruminants (Kolen and Webb, 1982; Webb et al., 1992, Webb et al., 1993). However, studies have not been conducted to determine whether or not pigs have the ability to absorb dipeptide. Therefore, the present experiment was conducted to monitor changes in the concentration of amino acids in the portal blood of piglets following duodenal infusion of either a dipeptide or an amino acid mixture and to detect whether or not intact dipeptide enters the blood circulation. In addition, the intestinal absorption of the amino acids from a dipeptide were compared with that of a free amino acid mixture with an identical amino acid composition.

MATERIALS AND METHODS

Experimental animals and surgical procedures

Three 28 day-old Duroc×Large White×Landrace litter mate gilts weighing an average of 6.5 kg were fasted for 24 hours prior to portal vein cannulation. The piglets were anaesthetized with ketamine hydrochloride (25 mg/kg, i.m.) and placed in dorsal recumbancy and the skin and linea alba were incised. The portal vein was visualized in the mesenteric ligament, dorsolateral to the proximal duodenum and pancreas. The mesenteric tissues overlying the portal vein were carefully incised and the portal vein was isolated for a length of about 1.5 cm by blunt dissection. A strand of light silk suture material was passed around the portal vein and a 0.5 cm length of tubing was threaded over both ends of the silk suture to serve as an occluding device. This suture was drawn to the proximal part of the exposed vein and a second suture was installed at the distal part. The two

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sutures were then drawn tight with the tubing to briefly occlude the portal vein. The 1.0 mm O.D. tubing (previously introduced into the peritoneal cavity via a stab incision in the left abdominal wall) was then immediately passed into the portal vein via a nick in the vein and the occluding sutures released. The cannula was secured by sutures in the tissues near the portal vein and tacked down to the dorsal abdominal wall with silk sutures. The cannula was immediately flushed with heparinized saline solution and was kept filled with heparinized saline solution except at sampling times.

Preparation of solutions for infusion

The glycine-lysine dipeptide and the free amino acids glycine and lysine used in this experiment were purchased from Sigma Chemical Company (St. Louis, MO). The basal (control) infusion solution was 25 mM pH 7.0 phosphate buffer plus 5% sorbitol. For the dipeptide infusion, 1 g glycine-lysine was added to the control solution to give a concentration of 0.017 g/ml while the free amino acid infusion contained glycine and lysine at a concentration equivalent to the dipeptide solution.

Infusion procedures and collection of blood

Immediately after portal cannulation, piglets were infused intravenously with a 5% glucose solution by slow drip to support their circulatory volume, and a single 60 ml bolus of test solution was slowly injected into the lumen of the proximal duodenum with a sterile 100 cc glass syringe via a 20 gauge needle inserted at the antimesenteric border. Portal blood (3 ml) was collected from the indwelling catheter at 5 to 20 minute intervals, for the 2 hour period immediately following infusion of the test solution (i.e. at 0, 5, 10, 20, 30, 45, 60, 80, 100 and 120 minutes after infusion). Blood samples were kept refrigerated for 4 hours and were then centrifuged (Heraeus Biofuge 22R Centrifuge) for 10 minutes at 2000×g in a refrigerated centrifuge maintained at 4℃. The supernatant was deproteinized with an equal volume of 0.6 M perchloric acid, the mixture was centrifuged again at 10,000×g for 20 minutes at 4℃, and the clear supernatant was stored at -20℃ until analyzed.

Measurement of dipeptide and amino acids

The dipeptide concentration was determined using a Beckman Gold System 338 (Beijing, China) high performance liquid chromatograph (HPLC) with UV detector. It was found that the largest UV absorbance value for the glycine-lysine dipeptide was at about 210 nm as determined by UV spectrochromatography, therefore a UV wave length of 213 nm was used for HPLC when the dipeptide was tested. Elution was performed with a flow rate of 0.9 ml/min employing a 0.1% trifluoric acid 0.1% trifluoric acid-acetonitrile gradient.

Free amino acids were estimated by employing an automated post column derivitization (0-phthaldialdehyde) using a Shimadzu LC 10 liquid chromatograph (Kyoto, Japan). The formula for calculating the concentration of amino acids (AA) was:

$$\frac{\left[\text{Area of Blood Sample} \times \text{Concentration of Standard Sample} \times \text{Dilution Times of Blood Sample}\right]}{\text{Area of Standard Sample}}$$

Statistical analysis

Regression analysis was performed using a least-squares method to fit a cubic polynomial modeling the absorption data (SPSS, 1993). Total absorption amounts were calculated by integration of the cubic equations obtained from the curve-fitting procedure (Jacques and Judd, 1987). Portal blood flow rates were assumed to be constant over time and among pigs (Meulen, et al., 1997). Absorption rate means were compared using a paired sample Student's T-test.

RESULTS

Intact glycine-lysine dipeptide was not detected in the portal vein at any time during the two hour period following duodenal infusion of the dipeptide solution. The concentrations of free glycine and lysine in the portal vein at each time point are shown in table 1.

Table 1. The portal concentration of glycine and lysine during the two hour period after infusing a dipeptide of free amino acids

Minutes	Glycine (μ mole/ml)			Lysine (μ mole/ml)		
	Di-peptide	Free amino acid	Control	Di-peptide	Free amino acid	Control
0	0.47	0.89	0.80	0.17	0.24	0.25
5	1.60	-	0.88	0.40	-	0.25
10	1.30	1.72	0.90	0.39	0.41	0.34
20	1.53	2.10	0.87	0.52	0.43	0.22
30	1.36	1.88	0.92	0.37	0.39	0.45
45	1.40	1.94	0.71	0.74	0.43	0.25
60	1.01	1.94	0.43	0.39	0.45	0.24
80	1.03	1.42	0.78	0.44	0.41	0.24
100	1.15	1.32	0.77	0.47	0.44	0.34
120	3.20	1.45	0.82	2.70	0.43	0.33

* Blood sampling was not possible at five minutes in the free amino acid pig.

Glycine and lysine concentrations from the dipeptide glycine-lysine and from free glycine and

lysine were higher than those in the control at all time points after infusing the test solutions with the exception of the lysine concentration determined 30 minutes after infusion in both the pig infused with dipeptide and the pig infused with free amino acids. Lysine concentrations in the portal vein from the pig infused with the glycine-lysine dipeptide were higher at 20, 45, 80, 100 and 120 minutes than the values from the pig infused with free glycine and lysine but were lower at 10, 30, and 60 minutes. Glycine concentrations were lower at each time point in the pig infused with dipeptide than in the pig infused with free amino acids with the exception of the sample taken at 120 minutes. The concentration of amino acids in the portal blood of the control pig did not vary significantly among samples taken during the 120 minute collection period.

After subtracting the initial amino acid concentration in the portal blood (0 minutes), the results presented in figure 1 were obtained. Figure 1 shows that a cubic equation, fit to the data points, simulated very well the absorption changes of amino acids from both the dipeptide glycine-lysine and free glycine and lysine (r^2=0.94 to 0.97).

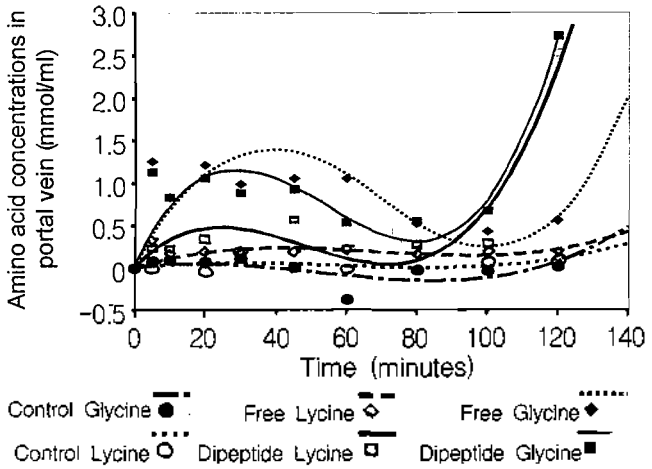

Figure 1. Lysine and glycine concentration in the portal vein of piglets infused with either glycine-lysine dipeptide, free glycine and lysine or a sorbitol sulution

Regression coefficients are presented in table 2. The cumulative absorption of amino acids over the period from 0-120 minutes was calculated by integrating the cubic equations obtained from the cubic regression analysis of the concentrations of amino acids. The general form for the equation modeling the cumulative absorption of amino acids, where k is a constant descriptive of portal blood flow rate, was:

$$\text{Cumulative Absorption} = \int_0^{120} b_0t + b_1t^2 + b_2t^3 = (b_0(x^2/2) + b_1(x^3/3) + b_2(x^4/4))k$$

Table 2. Regression coefficients of cubic equations modeling absorption of glycine and lysine provided as a dipeptide or in free form[1]

	b_0	b_1	b_2	r^2
Peptide glycine	0.0903	-0.0021	1.3×10^{-5}	0.95
Free glycine	0.0749	-0.0014	6.6×10^{-6}	0.97
Peptide lysine	0.044	-0.0012	8.3×10^{-6}	0.94
Free lysine	0.105	-0.0002	7.5×10^{-7}	0.96
Control glycine	0.0018	-0.0001	9.8×10^{-7}	0.35
Control lysine	0.0043	-0.0001	6.0×10^{-7}	0.40

[1] General form of the cubic equations is: Concentration= $b_0t + b_1t^2 + b_2t^3$ where t=time.

Table 3. Cummulative absorption of glycine and lysine from either a dipeptide or as free amino aicds in the portal vein of piglets at each time interval after infusing test solutions

Minutes	Lysine (μ mole)		Glycine (μ mole)	
	Dipeptide	Free amino acid	Dipeptide	Free amino acid
0	0.00	0.00	0.00	0.00
5	0.50	0.12	1.04	0.88
10	1.82	0.47	3.84	0.30
15	3.70	1.00	7.95	6.96
20	5.93	1.68	12.94	11.56
25	8.30	2.49	18.48	16.87
30	10.67	3.39	24.24	22.63
35	12.90	4.35	29.97	28.65
40	14.89	5.36	35.44	34.74
45	16.57	6.39	40.51	40.75
50	17.92	7.43	45.04	46.53
55	18.93	8.47	48.97	51.99
60	19.63	9.49	52.28	57.04
65	20.07	10.48	55.00	61.62
70	20.35	11.44	57.20	65.70
75	20.58	12.37	59.02	69.26
80	20.92	13.26	60.61	72.33
85	21.56	14.12	62.21	74.94
90	22.70	14.95	64.09	77.17
95	24.60	15.77	66.56	79.09
100	27.53	16.58	69.98	80.84
105	31.81	17.40	74.78	82.54
110	37.77	18.24	81.42	84.36
115	45.78	19.12	90.41	86.50
120	56.24	20.07	102.30	89.16

The cumulative absorption of amino acids is presented in table 3 and is also presented graphically in figures 2 and 3. Figure 2 illustrates that there was no difference in the amount of glycine absorbed between the pig infused with the glycine-lysine dipeptide and the pig infused with free glycine and lysine before 50 minutes. During the period between 50 and 110 minutes after infusion, the amount of

glycine absorbed from the dipeptide form was less than that from the free form, but between 110 and 120 minutes, the amount absorbed was greater from the dipeptide than that in free form.

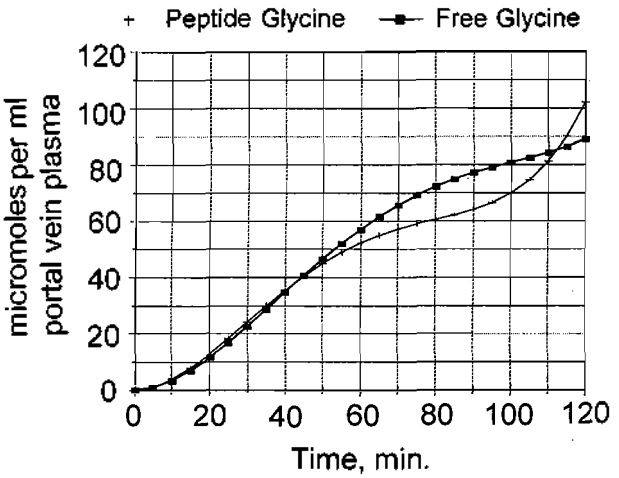

Figure 2. Cumulative absorption of glycine in dipeptide and free form in the portal vein of piglets at different time intervals

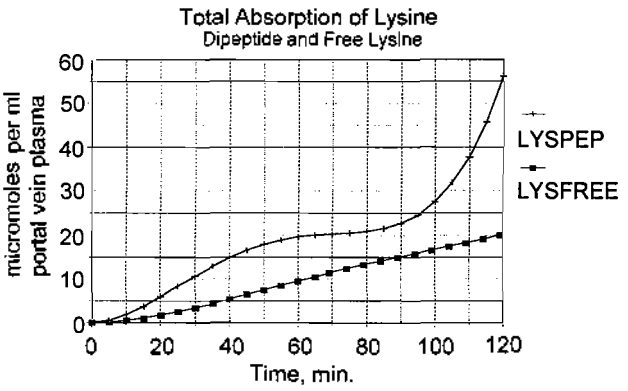

Figure 3. Cumulative absorption of lysine in dipeptide and free form in the portal vein of piglets at different time intervals

Figure 3 illustrates that the amount of lysine absorbed from the dipeptide glycine-lysine was much greater than that from the free amino acid lysine at each time interval in the two hour interval following the infusion of the test solutions. A paired T-test showed that total amount of lysine absorbed in 2 hours was significantly greater from the dipeptide than from the free form ($p<0.001$). In addition, the total amount of glycine absorbed during the two hour test period was also significantly different between the dipeptide-form and free glycine ($p=0.02$).

DISCUSSION

Intact glycine-lysine dipeptide was not detected in the portal vein at any time point in the two hour period following duodenal infusion of the dipeptide solution. This indicates that intact glycine-lysine dipeptide does not enter the blood circulation of piglets after uptake by intestinal cells. However, it does not mean that the glycine-lysine dipeptide shares the same transport system with glycine and lysine in free form.

There were obvious differences in the portal concentrations of amino acids after infusion of the dipeptide glycine-lysine compared with infusion of the free amino acids glycine and lysine. The results of this experiment therefore provide evidence for the existence of an independent transport system for dipeptide in the piglet intestine.

The improved absorption of lysine from the dipeptide form compared with the free amino acid corresponds to reports from other species where amino acid absorption from dipeptide has been reported to be superior to the absorption obtained with amino acids in the free form (Burston et al., 1972, Burston et al., 1980; Cheng et al., 1971; Hara et al., 1984). However, the absorption of glycine from dipeptide and from the free amino acid was not dramatically different. Some researchers have speculated that the location of amino acids (N-terminal residue or C-terminal residue) in the peptide may affect the amino acid absorption behavior which may account for the difference in glycine absorption (Burston et al., 1972).

The more rapid and greater cumulative absorption of lysine from the dipeptide form compared with the free form observed in the present experiment is consistent with the findings of Hansen et al. (1993) who fed starter pigs diets containing either free L-lysine or the dipeptide L-lysyl-glycine and concluded, based on lower serum urea N levels, that lysine was more efficiently utilized when provided as a dipeptide than when provided in the free form.

In conclusion, the overall results of this study demonstrate that pigs are similar to other species and have the ability to absorb amino acids in the form of a dipeptide. The absorption of lysine from the dipeptide form was greater and more rapid than when absorbed as a free amino acid. In contrast, the absorption of glycine did not differ appreciably between the two sources. Further work should determine the factors affecting dipeptide absorption and explore practical ways that the knowledge of dipeptide absorption can be utilized in solving some of the problems in amino acid nutrition of the weaned piglet such as amino acid digestibility continue here.

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