Effects of Dietary Proteins on Absorption and Gastrointestinal Movement of p-Aminobenzoic Acid in Conscious Rats

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Summary We monitored the absorption and movement of dietary soluble components along the gastrointestinal tract of rats by using p-aminobenzoic acid (PABA) as a marker after feeding 8 and 16% casein or soybean protein isolate (SPI) diets containing 1% PABA. The portal concentration of PABA, as an index of absorption, increased rapidly and reached the same high level 10 min after the feeding of all four diets, and the increased level of portal PABA was maintained for 30–80 min in each group. The increased levels of the SPI-fed groups continued longer than those of the casein-fed groups. In contrast, the gastric emptying rate slowed after 20 min in all the groups, and the gastric emptying of PABA for the initial 60 min in the 8% casein group was significantly faster than that in the 8% SPI group. The PABA content of the first small intestinal segment, which may be influenced by small intestinal transit, was higher in the casein group. These results indicate that the absorptive rate of PABA is determined not only by gastric emptying but also by small intestinal transit. The gastric emptying and the content of PABA in the first segment of the small intestine was not correlated in 8% protein groups. This suggests that the effect of SPI on gastrointestinal movement is different from that of casein.

Key Words absorption, gastric emptying, small intestinal transit, rats, p-aminobenzoic acid, casein, soybean protein, portal blood

The absorption rate of dietary components is affected by the rate of their outflow from the stomach and small intestinal transit. Lipids in the intestine are well known to inhibit gastric emptying (1, 2). Dietary proteins are also known to affect gastric emptying, but the effects depend on the increment of nutrient density or of osmotic pressure in the duodenum (3, 4). Read et al. reported that dietary protein also affects small intestinal motility or transit (5), but Spiller et al. failed to show the same result (6).

In the present study, we measured the absorption and gastrointestinal move-
ment of \( p \)-aminobenzoic acid (PABA) after feeding a meal of casein or soybean
protein isolate (SPI) containing PABA in conscious rats in order to examine the
influence of different kinds of dietary protein on the gastrointestinal movement of
PABA, and to determine whether the portal absorption of PABA reflects the
movement of PABA from the stomach to the intestine or the movement along the
small intestine.

We previously observed that the absorption of oligo-L-methionine, which is a
slightly digestible peptide, is faster in the casein diet than that in the SPI diet, and
the absorptive rate of casein itself is much higher than that of SPI (7). These results
indicate that the effect of casein on the gastrointestinal function is different from
that of SPI.

\( p \)-Aminobenzoic acid was used as a marker of soluble components in diets.
The absorption was examined by the changes in PABA concentrations in portal
blood after the feeding of test diets. \( p \)-Aminobenzoic acid is known to be absorbed
by a passive diffusion mechanism and also to be acetylated in liver; therefore the
portal absorption rate of PABA depends on the distribution and concentration of
PABA along the intestinal tract (8), and PABA absorbed in portal blood is not
recycled to the intestinal blood flow as such. We previously confirmed that the
PABA was not detectable in superior vena cava blood after oral feeding of 1%
PABA containing diet (unpublished data).

The gastrointestinal movement of PABA was examined by the longitudinal
distribution of a radio-labeled PABA in the gastrointestinal tracts.

MATERIALS AND METHODS

_Diets._ The composition of test diets is shown in Table 1. The test diets were
prepared by the addition of 1% PABA to an 8% protein diet and a 16% protein
diet. To estimate gastrointestinal movement, 7.4 kBq [carboxy-\(^{14}\)C]-PABA was
added to 2 g of both test diets. The 8 and 16% protein diets (as net protein; protein
content = \( N \times 6.25 \)) contained 9.4 and 18.8% of casein material (ALACID, New
Zealand Dairy Board, Wellington, N.Z.), or 9.6 and 19.2% SPI material (Fujipro
R, Fuji Oil Co., Osaka, Japan), respectively.

\( p \)-Aminobenzoic acid was purchased from Wako Pure Chemical Industry
(Osaka, Japan). [Carboxy-\(^{14}\)C] \( p \)-aminobenzoic acid (1.8 GBq/mmol, ICN Bio-
chemicals Inc., Irvine, California) was diluted by Non-labeled PABA to 370 kBq/1 g PABA.

_Anhmals._ Male Sprague-Dawley rats (Japan SLC Inc., Hamamatsu, Japan)
were housed in individual cages in a temperature-controlled room at 22°C
throughout the experiments.

_Portal absorption of PABA (Experiment 1):_ After a 3-day feeding of the stock
diet, portal cannula was implanted on twenty rats weighing 230–250 g under
sodium pentobarbital anesthesia (40 mg/kg body weight, Nembutal, Abbott, North
Chicago, IL.). The cannula (polyethylene tube, sp 28; ID 0.4 mm, OD 0.8 mm,
Table 1. Composition of diets (%).

|                | Stock diet | 8% protein diets | 16% protein diets |
|----------------|------------|------------------|-------------------|
| Casein¹        | 25.0       | 9.4              | —                 |
| SPI¹           | —          | —                | 18.8              |
| Sucrose        | 63.1       | 78.5 78.3        | 69.1 68.7         |
| Corn oil²      | 5.0        | 5.0 5.0          | 5.0 5.0           |
| Mineral mixture³ | 4.0        | 4.0 4.0          | 4.0 4.0           |
| Vitamin mixture⁴ | 1.0        | 1.0 1.0          | 1.0 1.0           |
| Vitamin E granule⁵ | 0.1        | 0.1 0.1          | 0.1 0.1           |
| Choline chloride | 2.0        | 2.0 2.0          | 2.0 2.0           |

¹ Nitrogen contents of casein and SPI materials are 13.7 and 13.4%, respectively, as evaluated by the semimicro Kjeldahl method. ² Retinyl palmitate (6,000 IU/kg diet) and ergocalciferol (800 IU/kg diet) were added to the corn oil. ³ The mineral mixture is identical to the mineral mixture (MM2) described by Ebihara et al. (9). ⁴ The vitamin mixture was prepared in accordance with the AIN-76 mixture (10) except that menadione and L-ascorbic acid were added to give a 1 mg/kg (11) and 50 mg/kg (12) diet, respectively. ⁵ Vitamin E (Yuvela, Eisai Co., Tokyo) supplied 200 mg all-rac-α-tocopheryl acetate in one kg diet. ⁶ One percent of p-aminobenzoic acid added to these diets.

Natsume Seisakusho, Tokyo was directly inserted into the portal vein. The details were described previously (13). After a 3-day recovery period with the stock diet and a 24-h fast, rats, whose portal blood could be drawn out through the catheter, were divided into two groups and were given 2 g of the test diets containing 1% PABA (PABA: 146 μmol/2 g test diet). Almost all given diets were consumed. Portal blood was collected at specified intervals (shown in Figs. 1 and 2) for 4 h after feeding. Forty microliters of blood was sampled at a time.

**Gastrointestinal movement of PABA (Experiment 2):** After a 5–7 day feeding of the stock diet and a 24-h fast, twelve rats weighing 240–260 g were divided into two groups and fed 2 g of the test diets containing 1% radio-labeled PABA (7.4 kBq/146 μmol PABA/2 g test diet). The rats were killed by decapitation at 20 and 60 min after feeding. Almost all given diets were consumed within 20 min. The stomach, small intestine, and cecum were removed with their contents. The small intestine was divided into 5 parts of equal length without loss of the content.

**Analyses.** Plasma PABA concentrations were measured by HPLC as phenyl thiocarbamyl (PTC) derivatives with phenyl isothiocyanate (Tokyo Kasei Kogyo, Tokyo) (14, 15). To determine the remaining PABA, the stomach, cecum, and segments of small intestine (those with contents were freeze-dried), were added to a mixture of acetonitrile : ethanol : water (2:1:2) and homogenized by the Polytron homogenizer (KINEMATICA, Amlehnhalde, Switzerland). After a centrifugation, the radioactivity of the supernatant was determined in 15 ml of toluene-methylcellosolve scintillator by a liquid scintillation system (LSC-700, Aloka, Tokyo). The amount of PABA emptied from the stomach was estimated by subtracting the amount of remaining PABA in the stomach 20 or 60 min after...
feeding from the amount of PABA fed.

Statistics. Statistical analyses were performed by one-way, two-way, and three-way ANOVA. The results are presented in the figure legends. Significant differences between the mean of casein- and SPI-fed groups and the correlation coefficient were determined by Student t-test, and among the different times or different sites of small intestine were determined by Least Significant Difference.

RESULTS

Changes in the portal concentrations of PABA after the feeding of 8 and 16% protein diets are shown in Figs. 1 and 2, respectively. After feeding, the concentrations of PABA increased rapidly, and after 10 min the concentration of PABA of all groups reached the same level. The levels of PABA for the initial 80 min were significantly higher than those at 200 and 240 min in both SPI groups. In contrast, in the 8 and 16% casein groups, significantly higher concentrations compared to the values at 200 and 240 min continued for the initial 30 min and for the initial 60 min, respectively. The increased levels of portal PABA, which were significantly higher than those at 200 and 240 min after feeding, continued longer in the SPI group than in the casein group. The portal concentrations at 200 and 240 min were regarded as the values after the termination of PABA absorption from the intestine.

The results of the gastric movement of PABA are shown in Figs. 3, 4, 5, and 6. A significantly larger amount of PABA was emptied from the stomach for the initial 60 min after the feeding of an 8% casein diet than after the feeding of an 8% SPI diet (Fig. 3). The same tendency was obtained at the 16% protein level without significant difference (Fig. 4). The gastric emptying rate slowed after 20

Fig. 1. Changes in the portal concentrations of p-aminobenzoic acid (PABA) after the feeding of an 8% casein diet (CAS) or an 8% soybean protein diet (SPI) containing 1% PABA. Two g of each test diet were given to a rat starved for 24 h. Each value is the mean for four (casein group) and five (SPI group) rats. Vertical bars represent SEM. From results of ANOVA, 'Diet' did not influence, and 'Time' influenced significantly ($p<0.01$). Asterisks represent the values significantly higher than the values at 200 and 240 min after feedings among the same group ($p<0.05$).
Fig. 2. Changes in the portal concentrations of p-aminobenzoic acid (PABA) after the feeding of a 16% casein diet (CAS) or a 16% soybean protein diet (SPI) containing 1% PABA. Two g of each test diet were given to a rat starved for 24 h. Each value is the mean for seven (casein group) and eight (SPI group) rats. Vertical bars represent SEM. From results of two-way ANOVA, 'Diet' did not influence, and 'Time' influenced significantly (p<0.01). Asterisks represent the values significantly higher than the values at 200 and 240 min after feedings among the same group (p<0.05).

Fig. 3. The amount of PABA emptied from the stomach for the initial 20 or 60 min after the feeding of an 8% casein or an 8% SPI diet containing 1% PABA. Two g of each test diet (PABA: 7.4 kBq of [14C]-PABA/146 μmol) were given to a rat starved for 24 h. Each value is the mean for six rats. Vertical bars represent SEM. In the points with no bar, SEMs were too small to record. The asterisk shows the significant difference between the casein- and SPI-fed group at each point (p<0.05).

min in all the groups (Figs. 3 and 4).

In the first segment of the small intestine, which is part of the duodenum and proximal jejunum, a larger amount of PABA was retained at 20 min after the feeding of an 8% casein diet than after the feeding of an 8% SPI diet (Fig. 5A). The contents of PABA in the other parts of the small intestine were very low at 20 min after feeding. At 60 min (Fig. 5B), the PABA contents were increased in the 4th and 5th segments (ileum) of both the 8% casein- and 8% SPI-fed groups. The contents of PABA in the intestinal lumen of the rats fed the 16% protein diets were
Fig. 4. The amount of PABA emptied from the stomach for the initial 20 or 60 min after the feeding of a 16% casein or a 16% SPI diet containing 1% PABA. Two g of each test diet (PABA: 7.4 kBq of [14C]-PABA/146 μmol) were given to a rat starved for 24 h. Each value is the mean for six rats. Vertical bars represent SEM. In the points with no bar, SEMs were too small to record.

Fig. 5. The amount of p-aminobenzoic acid (PABA) in the content of the small intestinal segment and cecum 20 min (A) or 60 min (B) after the feeding of an 8% casein or an 8% SPI diet containing 1% PABA. Two g of each test diet (PABA: 7.4 kBq of [14C]-PABA/146 μmol) were given to a rat starved for 24 h. Each value is the mean for six rats except 20 min after SPI diet (five rats). Vertical bars represent SEM. In the points with no bar, SEMs were too small to record. From the results of ANOVA, 'Diet' and 'Site' at 20 or 60 min influenced significantly ($p < 0.05$). The asterisk shows the significant difference between the casein- and SPI-fed group at each point ($p < 0.05$). At 20 min, site 1 was significantly higher than the other sites after the feeding of both the casein and SPI diets. At 60 min, site 4 was significantly higher than site 2 after the feeding of the casein diet and higher than sites 2 and 3 after the feeding of the SPI diet.

*J. Nutr. Sci. Vitaminol.*
Fig. 6. The amount of p-aminobenzoic acid (PABA) in the content of the small intestinal segment and cecum 20 min (A) or 60 min (B) after the feeding of a 16% casein or a 16% SPI diet containing 1% PABA. Two g of each test diet (PABA: 7.4 kBq of [14C]-PABA/146 μmol) were given to a rat starved for 24 h. Each value is the mean for six rats. Vertical bars represent SEM. In the points with no bar, SEMs were too small to record. From the results of ANOVA, 'Diet' and 'Site' at 20 or 60 min influenced significantly (p<0.05). At 20 min, site 1 was significantly higher than the other sites after feeding of both the casein and SPI diets. At 60 min, sites 4 and 5 were significantly higher than sites 2 and 3 after the feeding of the casein diet and higher than sites 1-3 after the feeding of the SPI diet.

Fig. 7. The relationships between the amount of PABA emptied from the stomach for the initial 20 min and the logarithmic values of the amount of PABA in the first segment of the small intestine 20 min after feeding both 8% protein diets and 16% protein diets are shown in Fig. 7. There was a high correlation in the rats fed 16% protein diets, but no correlation

similar to that found in the rats fed the 8% protein diets, both at 20 min (Fig. 6A) and 60 min (Fig. 6B). In the cecum, the radioactivity of PABA was not detectable in any group.

The relationships between the amount of PABA emptied from the stomach and logarithmic values of the content of PABA in the first part of the small intestine 20 min after feeding both 8% protein diets and 16% protein diets are shown in Fig. 7. There was a high correlation in the rats fed 16% protein diets, but no correlation
in those fed 8% protein diets.

DISCUSSION

The absorption of PABA in the test diets is determined by several factors, probably mainly gastric emptying and small intestinal transit. Our observations revealed that the changes in the portal absorption of PABA in casein-fed groups were similar to those in SPI-fed groups, on the whole. Therefore, the time course of the gastric emptying of the casein- and SPI-fed groups at both the 8 and 16% protein levels may progress in almost the same manner. But there are several discrepancies between gastric emptying and the absorption of PABA.

We observed the slowing of gastric emptying after 20 min in all groups. In contrast, the PABA concentrations in the portal blood are maintained at the increased level for the initial 80 min after the feeding of both the SPI diets and for the initial 30–60 min after the feeding of both the casein diets (Figs. 1 and 2). Also, we observed that the amount of PABA emptied from the stomach for the first 60 min was larger in the 8% casein group than in the 8% SPI group (Fig. 3). In contrast, the increased level of portal PABA concentration continued longer in the SPI group than in the casein group. These rather small discrepancies between gastric emptying and portal absorption may be due to the different transit speed and the different distribution of PABA along the small intestine depending on the transit.

We observed that PABA was more widely distributed at 60 min than at 20 min after the feeding of the diets (Figs. 5 and 6). The results reveal that PABA contacts the absorptive site more widely at 60 min than at 20 min after feeding, which may cause the enhancement of PABA absorption at 60 min.

We also observed, in a separate study, that the chyme transit in the small intestine after the feeding of an 8% SPI diet was faster than after the feeding of an 8% casein diet using a nonabsorbable substance by direct duodenum challenge through a chronic duodenal catheter.* In the present study, a lesser amount of PABA was retained in the first segment of the small intestine of the SPI group than in the casein group, which supports the faster transit of chyme after the feeding of the SPI diet. The mechanism of the enhancement of small intestinal transit rate by SPI is unknown.

The relationship between small intestinal transit and absorption has not been consistent, namely, faster transit do not causes the higher absorption rate (16–18). The present study concludes, first, that the absorptive rate of PABA is affected not only by gastric emptying but also by small intestinal transit, and, second, that faster transit and wide distribution of PABA, depending on transit, enhance the PABA absorptive rate.

* Different effects of casein and soybean protein on gastric emptying of protein and small intestinal transit after spontaneous feeding of diets in rats.

J. Nutr. Sci. Vitaminol.
Read et al. reported that small intestinal transit was independent of gastric emptying in man (19). Figure 7 demonstrates a strong correlation between gastric emptying and PABA content in the first segment of the small intestine in rats fed the 16% protein diets but not in those fed the 8% protein diets. These results show that in the 16% protein diet the PABA contents of the first segment depend on the rate of PABA emptying from the stomach. In contrast, the PABA content of the first segment in the 8% protein groups may influence small intestinal transit rather than gastric emptying of PABA.

The accumulation of PABA in the first segment of the small intestine suggests that small intestinal transit is affected by a rate-limiting site in the duodenum or the proximal jejunum.

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