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Original

Effects of straight alkyl chain, extra hydroxylated alkyl chain and branched chain amino acids on gastric emptying evaluated using a non-invasive breath test in conscious rats

Masayuki Uchida, Orie Kobayashi, Kaori Iwasawa and Kimiko Shimizu

Food Science Research Laboratories, Research and Development Division, Meiji Co., Ltd., Kanagawa, Japan

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Abstract

Aim: Some amino acids been known to influence gastric emptying. Thus we have evaluated the effects of straight alkyl chain, extra hydroxylated alkyl chain and branched chain amino acids on gastric emptying. Materials and Methods: Gastric emptying was evaluated in rats after feeding with Racol (nutrient formulae) containing [1-^{13}C] acetic acid. Using a breath test, the content of $^{13}CO_2$ in their expired air was measured by infrared analyzers. Rats were orally administered with test amino acids, while control rats were administered orally with distilled water. Results: The expired $^{13}CO_2$ content in the expired air increased with time, peaked after about 30 min and decreased thereafter. Among the amino acids having an alkyl chain, L-serine, L-alanine and L-glycine, significantly decreased the $^{13}CO_2$ content and Cmax, and delayed Tmax, suggesting inhibition and delay of gastric emptying. AUC_{120min} values of L-alanine and L-glycine also decreased significantly. L-Threonine significantly decreased $^{13}CO_2$ content and delayed Tmax, but had no influence on Cmax and AUC_{120min} values, suggesting a delay of gastric emptying. L-Isoleucine and L-leucine and L-valine significantly decreased $^{13}CO_2$ content, suggesting inhibition of the gastric emptying, but Cmax, Tmax and AUC_{120min} values were not significantly affected. Conclusion: The results show that the amino acids used in the present study had different effects on gastric emptying. Moreover, it was found that inhibition and delay of gastric emptying were clearly classifiable by analyzing the change in $^{13}CO_2$ content of the expired air and the Cmax, Tmax and AUC_{120min} values.

Key words: Amino acid, Breath test in rats, Gastric emptying

Corresponding author: Masayuki Uchida, Food Science Research Laboratories, Research and Development Division, Meiji Co., Ltd. 540 Naruda, Odawara, Kanagawa 250-0862 Japan
Phone: +81-465-37-3664 Fax: +81-465-37-3638 e-mail: masayuki.uchida@maiji.com

Introduction

The ingredients and energy density of meals have been known to influence gastric emptying. Bell and Webber (1) reported that the amino acid tryptophan delayed gastric emptying in rats. Carney et al. (2) also found that a sense of fullness was greater after L-tryptophan than after ingestion of D-tryptophan. We also found that L-tryptophan delayed gastric emptying as evaluated by breath test using ^{13}C-acetic acid (3). In addition, Jordi et al. (4) reported that L-arginine, L-lysine and L-glutamic acid inhibit food intake via the area postrema or vagal afferents. On the contrary, the enhancement of gastric motility was reported for monosodium glutamate (5). However, the effects of other amino acids on gastric emptying have not been clarified.

Among existing techniques for evaluating gastric motility, radio scintigraphy is generally accepted as the gold standard for measuring gastric emptying in humans (6). The breath test using ^{13}C-octanoic acid has been frequently applied in the clinical diagnosis of gastric emptying disorder since it was reported first by Ghoos et al. (7) in 1993. In basic research, we recently developed a simple and non-invasive breath test system for monitoring gastric emptying in conscious rats using ^{13}C-acetic acid (8). Moreover, we have already reported the reliability of this method by comparing the traditional phenol red method and the present breath test, and that the latter method is useful in the assessment of the effect of drugs and gut function pharmacologically (9).

Thus, in this study we aimed to evaluate the effects of amino acids having straight alkyl chain and extra hydroxylated alkyl chain, and also branched chain amino acids on gastric emptying using the breath test in conscious rats following administration of ^{13}C-acetic acid.

Materials and Methods

The following animal studies were performed in accordance with the *Guiding Principles for the Care and Use of Laboratory Animals* approved by Meiji Co., Ltd.

Animals

Male Sprague-Dawley rats weighing about 200 g were purchased from SLC (Shizuoka, Japan) and housed for 1 week prior to the commencement of the experiments under a constant temperature of 21 ± 2 degree centigrade, humidity of 55 ± 15% and a 12-hour light/dark cycle. The rats were fasted in mesh cages for 18 h before each experiment in order to prevent coprophagy, but were allowed free access to drinking water during this period.

Amino acids treatment

After fasting, 1 g/kg of amino acid dissolved or suspended in distilled water for injection was administered orally in a volume of 5 ml/kg. In the control rats, distilled water alone was administered instead of amino acid. The breath test was performed 30 min after amino acid treatment. Time schedule of the experiment was shown in Fig. 1.

Breath test system

The breath test was performed according to the method reported by us as shown in Fig. 2 (8). Dessicators were selected as the animal chambers, because they were easy to set up and relatively inexpensive. Each animal chamber was connected to an aspiration pump (Masterflex L/S, Cole-Palmer Inst. Co., USA). A desiccator with a volume of 2,000 ml was employed so that the rats could move freely within the chamber and the

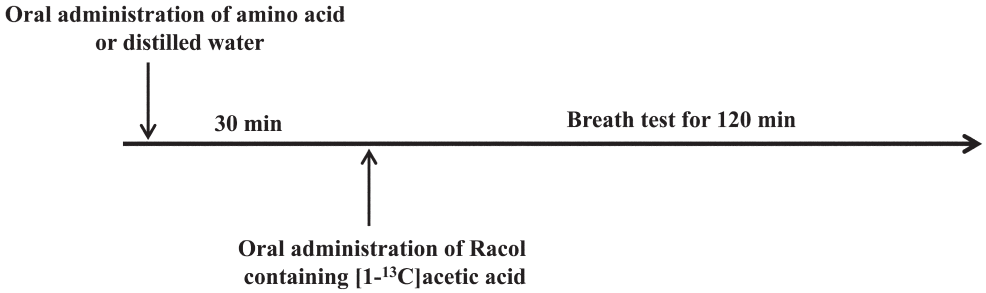

Fig. 1. Time schedule of the present study
Thirty min after oral administration of amino acid or distilled water, Racol containing [1-^{13}C]acetic acid was administered orally and the breath test was performed.

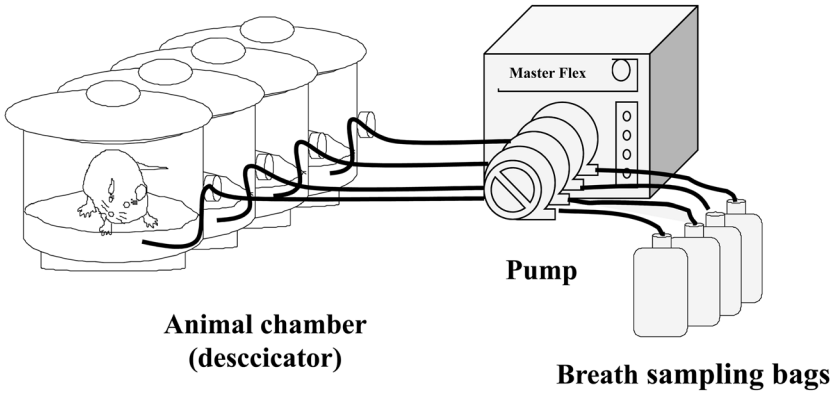

Fig. 2. Schematic illustration of the system used for the present study
This system comprised a desiccator that was used as an animal chamber, a pump and a breath-sampling bag. Aspirating the expired air caused fresh air to automatically flow into the desiccator to replace it through a hole in the side of the chamber. The expired $^{13}CO_2$ was collected in the breath bag and measured with an infrared spectrometer (POCone).

expired air could be collected effectively in the breath sampling bag (Otsuka Pharmaceutical Co. Ltd., Tokyo, Japan). Aspirating the expired air caused fresh air to be automatically drawn into the desiccator through a hole in the side of the dessicator (Fig. 2). The air in the chamber was continuously aspirated during the experimental period. Aspirated air was discharged to the exterior of this breath test system except for the period collecting expired air in the breath-sampling bag. POCone infrared spectrometer (Otsuka Electronics, Co., Ltd., Japan) was chosen as they allowed $^{13}CO_2$ to be measured simply and effectively.

Test meal and breath test for evaluating gastric emptying

Racol nutrient formula containing [1-^{13}C]acetic acid (16 mg/kg) was used as test meal and administered orally in a volume of 2.5 ml/kg 30 min following the amino acid treatment (Fig. 1). The rats were placed in the chamber immediately after the oral administration of the test meal. While the air in the chamber was continuously aspirated, expired air was collected at 5-min intervals for the first 70 min, with further samples taken at 90 and 120 min. The aspiration rate was set at 150 ml/min. At each sample point, the expired air was collected into a breath sampling bag for 1.5 min. A gaseous mixture of 5%CO_2 and 95% O_2 was used as the reference gas. The $^{13}CO_2$ levels were measured by placing the breath sampling bags into the inlet port of the infrared analyzer. The measured values were presented as $\Delta^{13}CO_2$ (‰). The maximum concentration (C_{max}; ‰), the time taken to

reach the maximum concentration (T_{max}; min) and the area under the curve (AUC_{120min}; ‰·min) were calculated using the measured $\Delta^{13}CO_2$ values.

In this study, as used in the previous reports (6) C_{max}, T_{max} and AUC_{120min}values were used as pharmacokinetic parameters in addition to the change in expired $^{13}CO_2$.

Agents

Amino acids and [1-^{13}C]acetic acid were purchased from Wako Pure Chemical (Tokyo, Japan) and Cambridge Isotope Laboratories Inc. (MA, USA), respectively. Racol and distilled water for injection were obtained from Otsuka Pharmaceutical Co., Ltd. (Tokyo) and Otsuka Pharmaceutical Factory, Inc. (Tokushima), respectively

Data analysis

All results are presented as the mean ± standard deviation (S.D.) or standard error (S.E.) Statistical analysis was performed by Dunnett's multiple comparison test and $P<0.05$ was considered to be significant.

Results

The changes in expired $^{13}CO_2$ air from both control rats and amino acid-treated rats are shown in Figs. 3 and 4. The effects of amino acids on the pharmacokinetic parameters are given in Table 1.

In the control group, the expired $^{13}CO_2$ air increased with time and peaked at about 30 min before decreasing (Fig. 3 and 4). Cmax, Tmax and AUC_{120min} values were 386.6 ± 47.3 ‰, 28.8 ± 4.8 min and 25,123 ± 1,823 ‰·min, respectively (Table 1).

Judging from the values of Tmax, Cmax and AUC_{120min} in the control group, there were no amino acids that enhanced gastric emptying under the present experimental conditions (Table 1).

The effects of L-glycine, L-serine, L-alanine and L-threonine are show in Fig. 3. L-Serine significantly delayed gastric emptying, because the Tmax and Cmax values were significantly delayed and decreased, respectively, as compared with control, but the AUC_{120min} value was almost the same as in the control (Table 1). L-Glycine and L-alanine significantly delayed and inhibited gastric emptying, because Cmax and AUC_{120min} were significantly decreased and Tmax was also significantly delayed as compared with the control (Table 1). L-Threonine significantly delayed gastric emptying, because Tmax was significantly delayed, while Cmax and AUC_{120min} values were almost the same as control (Table 1).

The effects of branched chain amino acids were show in Fig. 4. L-Isoleucine and L-leucine markedly inhibited gastric emptying, although Tmax, Cmax and AUC_{120min} values were not markedly different from those of the control group (Table 1). L-Valine significantly delayed gastric emptying judging from the changes of the expired $^{13}CO_2$ (Fig. 4), although Tmax, Cmax and AUC_{120min} values were almost the same as in the control group (Table 1).

Discussion

The terms 'inhibition of gastric emptying' and 'delay of gastric emptying' are frequently confused. Strictly speaking, inhibition of gastric emptying results in residual ingested material in the stomach even though most of the ingested material was eliminated from the stomach, while a delay of gastric emptying results in the ingested material being almost eliminated but the eliminated peak time is longer than normal.

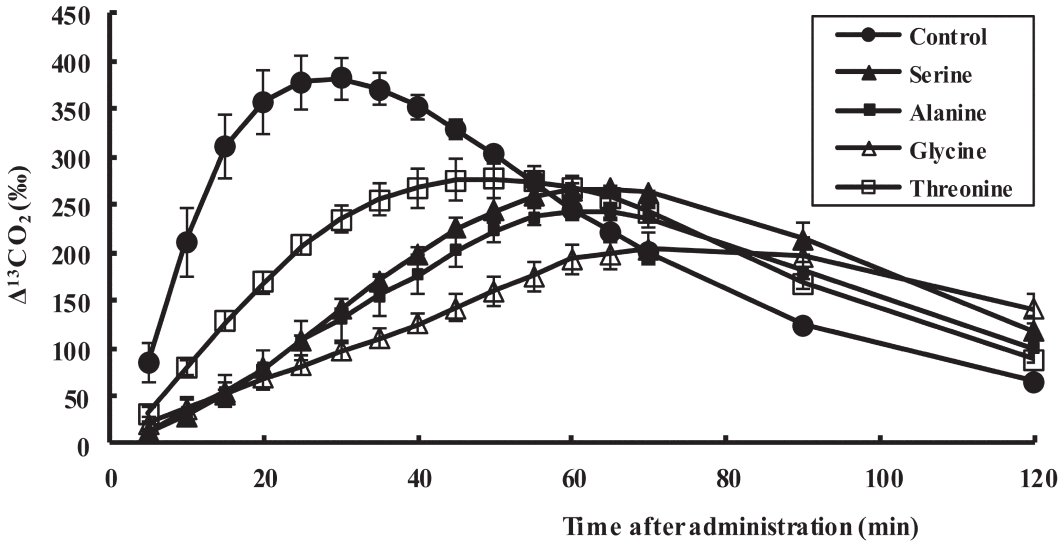

Fig. 3. Effects of amino acids having a straight alkyl chain and an extra hydroxylated alkyl chain on the time course of expired $\Delta^{13}CO_2$ in rats administered with Racol containing [1-^{13}C]acetic acid.
Values represent the mean ± standard error of the mean (SEM) (n = 3 or 4).

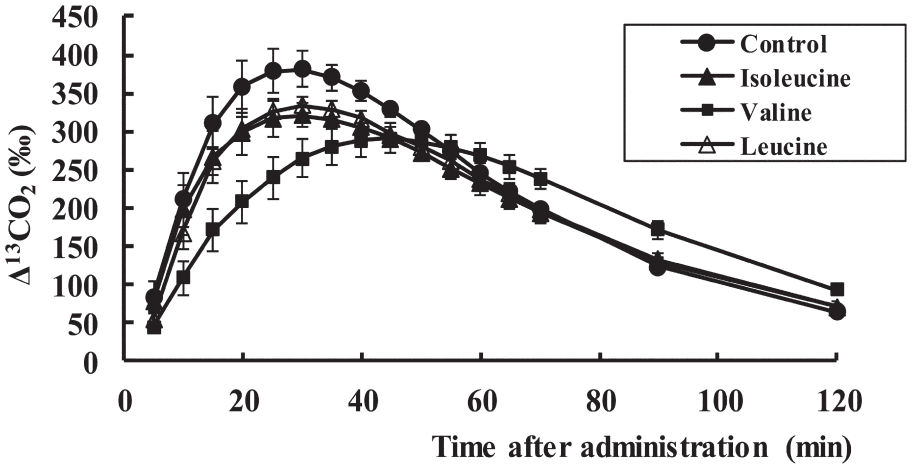

Fig. 4. Effects of branched amino acids on the time course of expired $\Delta^{13}CO_2$ in rats administered Racol containing [1-^{13}C]acetic acid.
Values represent the mean ± standard error of the mean (SEM) (n = 3 or 4).

In our improved method of investigating gastric emptying, the inhibition of gastric emptying and the delay of gastric emptying are clearly distinguished. The reason is as follows: the T_{max} value indicates the time of the maximum concentration of expired $^{13}CO_2$ air and shows the peak of gastric emptying, while a decrease in the AUC_{120min} value shows the decrease of total materials eliminated from the stomach within 120 min. Therefore, we evaluated the effect of amino acid on the gastric emptying in view of the difference between delay and inhibition of gastric emptying, although the evaluation time is 120 min.

In our preliminary experiments, it was found that L-tyrosine and L-phenylalanine had no influence at all on the gastric emptying evaluated by the present breath test using [1-^{13}C] acetic acid, suggesting all amino acids do not inhibit or delay gastric emptying, although the dosage used in the present experiments (1 g/kg) is comparatively high.

Table 1. Effects of amino acids on the pharmacokinetic parameters of the expired $\Delta^{13}CO_2$ from rats treated with [1-^{13}C]acetic acid

Treatment	Cmax (‰)	Tmax (min)	AUC_{120min} (‰·min)
Control	386.6 ± 47.3	28.8 ± 4.8	25,123 ± 1,823
Amino acids having a straight alkyl chain and an extra hydroxylated alkyl chain			
L-Glycine	205.4 ± 28.7*	76.7 ± 11.5*	16,890 ± 2,270*
L-Alanine	247.4 ± 14.7*	60.0 ± 5.8*	18,462 ± 1,428*
L-Serine	270.5 ± 8.6*	65.0 ± 5.0*	20,673 ± 871
L-Threonine	278.7 ± 34.3	50.0 ± 5.0*	22,164 ± 1,153
Branched chain amino acids			
L-Leucine	334.5 ± 24.3	28.8 ± 2.5	23,819 ± 1,136
L-Isoleucine	330.3 ± 30.3	31.3 ± 9.5	23,555 ± 369
L-Valine	294.7 ± 29.9	46.7 ± 7.6	23,616 ± 1,433

Values represent the mean ± standard deviation of used rats (3 or 4).
*, **: Significant difference from the control (P<0.05, 0.01).

It has been reported that the vago-vagal reflex is a part of the mechanism involved in the delay and inhibition of gastric emptying (10). In addition, the duodenal brake has been shown to be involved in gastric emptying (11). Indeed, the stomach is supplied with a large number of vagal afferents with receptors in the muscle and the mucosa (12). Cholecystokinin, a hormone released from endocrine cells of the upper small intestine in response to amino acids and fatty acids in chyme, inhibits gastric emptying mediated by cholecystokinin-induced activation of an inhibitory vago-vagal reflex involving vasoactive intestinal peptide-induced relaxation of the gastric fundus. Therefore, changes in this gastrointestinal hormone may explain the mechanism of the inhibition or delay of gastric emptying observed in the present study.

In the stomach, postganglionic parasympathetic neurons form two distinct pathways. The first is an excitatory cholinergic pathway that increases gastric tone, motility and secretion via activation of muscarinic cholinergic receptors, while the second is an inhibitory non-adrenergic, non-cholinergic (NANC) pathway that inhibits gastric functions via release of nitric oxide or vasoactive intestinal polypeptide. Therefore, the gastric functions may be inhibited either by activation of the NANC pathway or by inhibition of the tonic cholinergic pathway (13). In the present study, all amino acids inhibited or delayed gastric emptying. These findings may suggest that amino acids inhibit gastric emptying through the vago-vagal reflex and the cholecystokinin pathway. In our preliminary experiments, L-tyrosine and L-phenylalanine did not influence gastric emptying**,** even though the same 1 g/kg amino acid (4 kcal) was administered in the same way. However, the involvement of the vago-vagal reflex and cholecystokinin pathway can not be absolutely excluded on the present study results. To clarify the difference, the effect of varying the hormone level on gastric emptying need to be examined. In addition, amino acid receptors may be involved in this mechanism. Further studies research is needed to clarify these questions.

While acetylcholine is the principal neurotransmitter released on activation of the vago-vagal reflex, from both vagal efferent terminals and preganglionic vagal fibers that excite enteric neurons, they also release glutamine (14). Glutamic acid is an excitatory amino acid, and γ-aminobutyric acid (GABA) is an inhibitory amino acid. GABA is synthesized by glutamate decarboxylase from glutamic acid or glutamine. Therefore, glutamine may inhibit or enhance the gastric emptying under different experimental conditions. GABA is located throughout the gastrointestinal tract and has been localized in enteric nerves as well as in endocrine-like cells. These findings implicate that GABA is both a neurotransmitter and an endocrine mediator in the

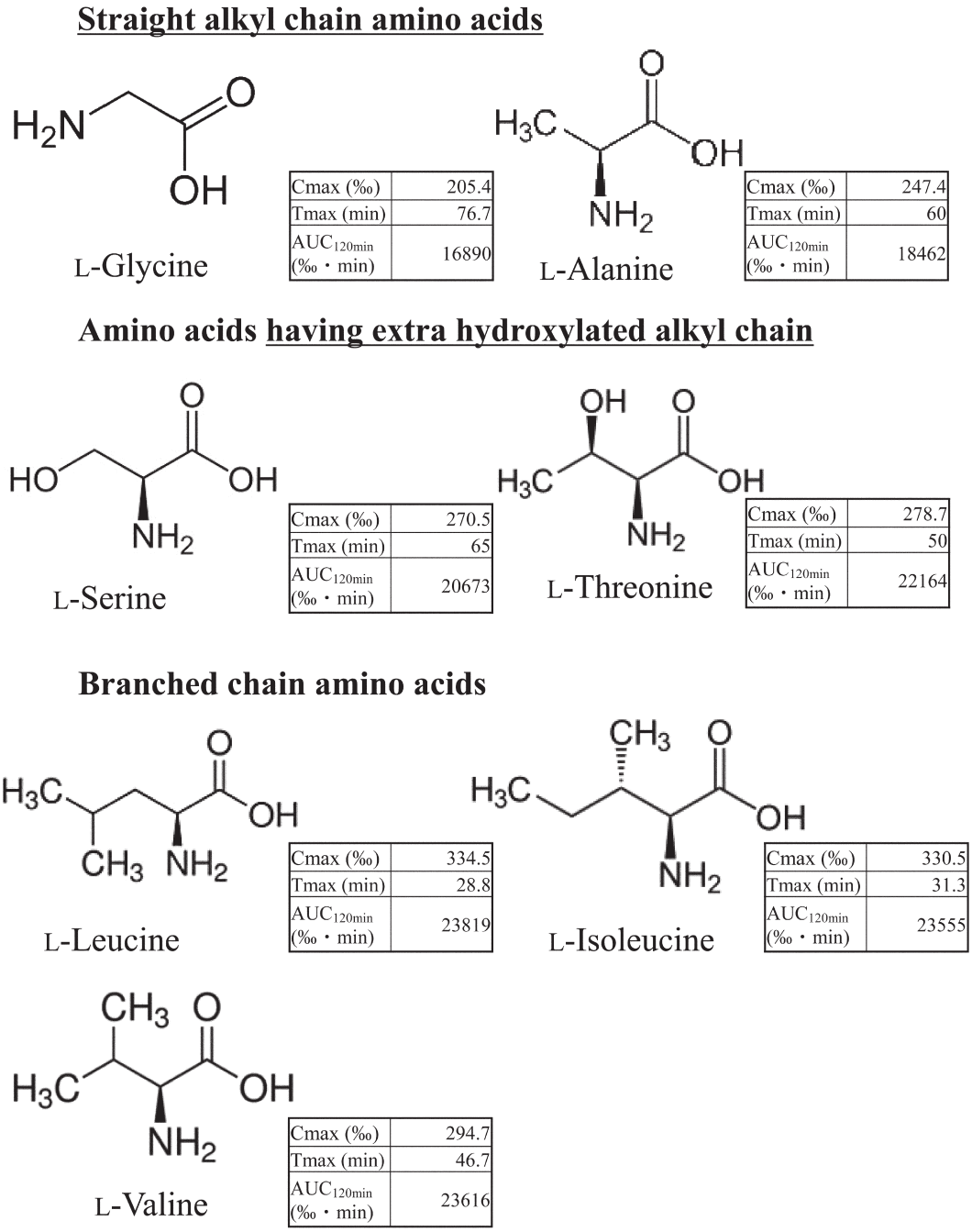

Fig. 5. Chemical structures of the amino acids used in the present study and their pharmacokinetic parameters evaluated by the breath test using Racol containing [1-^{13}C]acetic acid.

gastrointestinal tract. Steinert et al. (15) reported that glutamine had no effect on antral, duodenal or pyloric pressures, or plasma cholecystokinin in healthy men, suggesting no effect on gastric emptying. On the contrary, baclofen, a GABA agonist, accelerated gastric emptying of solids but delayed emptying of liquid in ^{13}C-breath test in mice (16). These findings show that glutamine has differential effects on proximal and distal stomach emptying.

Akao and Kobashi (17) reported that L-glycine lowered the gastric emptying rate, resulting in the suppression of ethanol absorption from the gastrointestinal tract. This finding is in accord with our present study. Barker et al. (18) also reported that the slowing of gastric emptying via duodenal osmoreceptors was about 10%

greater for L-glycine than it was for glucose. Also in this study, L-glycine significantly inhibited and delayed gastric emptying.

There have been no reports concerning gastric emptying by L-serine and L-threonine. But in the present study, we have shown for the first time that L-serine and L-threonine significantly delayed and inhibited gastric emptying under the present experimental conditions.

A correlation between the chemical structure of the amino acid and gastric emptying was shown in Fig. 5. Amino acids having a straight alkyl chain, such as L-glycine and L-serine, had almost the same AUC_{120min} values. When the lateral chain of an amino acid was short, Tmax was delayed, and Cmax was decreased (L-glycine vs. L-serine). With the amino acids that had an extra hydroxylated alkyl chain, the Tmax was delayed when a lateral chain of an amino acid became short (L-serine vs. L-threonine). However, AUC_{120min} values were almost the same between L-serine and L-threonine.

Among the essential amino acids, Stephen et al. (19) reported that only L-tryptophan significantly slowed emptying at a concentration above 4 mM. However, in the present study, the essential amino acids L-leucine and L-isoleucine markedly inhibited gastric emptying and L-valine also significantly inhibited and delayed gastric emptying. This difference may have been caused by experimental conditions. Branched chain amino acids showed almost the same AUC_{120min} and Cmax values. When a lateral chain of an amino acid was short, Tmax was delayed (Fig. 5). This was also observed in amino acids having a straight alkyl chain or an extra hydroxylated alkyl chain (Fig. 5).

Recently, Kusano et al. (20) reported a significant correspondence between postprandial dyspepsia syndromes and accelerated gastric emptying in the early postprandial period. Therefore, amino acids that inhibit and/or delay gastric emptying would be of interest in relation to postprandial dyspepsia.

In conclusion, it was found that amino acids having a straight alkyl chain and an extra hydroxylated alkyl chain, and branched chain amino acids influence gastric emptying**.** This may be correlated to the structure of the amino acid. In addition, delay and inhibition of gastric emptying is clearly distinguishable by using the breath test following administration of [1-^{13}C] acetic acid in rats.

Conflict of interest

The authors declare that they have no conflict of interest.

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