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Note

Taste Characteristics of Various Amino Acid Derivatives

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Summary Amino acids contribute to the taste of foods. Previous studies on the taste of amino acids focused mainly on α-amino acids, and therefore, the taste characteristics of amino acid derivatives remain unclear. In the present study, we targeted 6 different amino acid derivatives, β-alanine, citrulline, creatine, γ-aminobutyric acid, taurine, and ornithine, and evaluated their taste characteristics in a human sensory study. All tested amino acid derivatives showed multiple taste qualities; no derivatives had only a single taste quality. However, their taste intensities were relatively weak even at high concentrations. Given that the interactions between amino acid derivatives and nucleotide result in taste enhancements, we investigated the effect of inosine 5′-monophosphate (IMP) on the taste characteristics and found that the taste intensity of ornithine increased in the presence of IMP. This finding will be useful for understanding the role of amino acid derivatives as taste substances in daily foods.

Key Words amino acid derivatives, taste, sensory evaluation, taste enhancement, ornithine

Amino acids are mainly present as components of protein in foods. In particular, free-form amino acids contribute to the flavor of food. Generally, food proteins are composed of α-amino acids, and previous studies have reported the taste characteristics of α-amino acids. Interestingly, their taste is rich in variety (*1*); glutamic acid and aspartic acid have an umami taste, glycine and alanine are mainly sweet, while branched-chain amino acids, such as valine and leucine, are mainly bitter.

Various amino acid derivatives are present in food. However, previous studies analyzing the taste characteristics of amino acids have focused primarily on α-amino acids (*1–4*). Therefore, the taste characteristics of amino acid derivatives remain unclear. Some of these amino acids exert beneficial effects on human health. Therefore, understanding the taste characteristics of these amino acids can be useful in promoting their use in food products.

In the present study, we selected 6 different amino acid derivatives, β-alanine, citrulline, creatine, γ-aminobutyric acid (GABA), taurine, and ornithine, and evaluated their taste characteristics in a human sensory study. One well-known characteristic of amino acids is that their synergistic interaction with nucleotides, such as inosine 5′-monophosphate (IMP) and guanosine 5′-monophosphate, enhances their flavor profile (*5*). Therefore, we also investigated whether the taste of these derivatives is enhanced by IMP.

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Materials and Methods

Reagents. β-Alanine, L-citrulline, creatine monohydrate, GABA, taurine, ornithine monohydrochloride, and monosodium glutamate (MSG) were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). IMP was obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan).

Sensory evaluation. A total of 74 female subjects (mean age, 22.0±0.3 y), who attended the Kyoto Women's University, were recruited in the study. All the subjects were non-smokers and had good physical health. All psychophysical tests were performed according to the protocol approved by the Kyoto Women's University Ethics Committee (Approval Number: 2020-19).

Taste intensity was assessed using a visual analog scale (VAS). The subjects rated the taste intensity by marking the appropriate position on a 100 mm VAS. The right and left ends of the scale represented "strong" and "weak" tastes, respectively.

The subjects described the taste qualities in terms of no taste, sweetness, sourness, saltiness, bitterness, umami, astringency, pungency, and other tastes.

In a paired-difference test, one sample set consisted of two cups: one cup containing an amino acid derivative and another cup containing the first solution mixed with 0.5 mM IMP. The subjects were provided with a sample set and asked to select a cup that had a strong taste intensity.

For each taste evaluation, the subjects were asked to rinse their mouth thoroughly with distilled water, sip the stimulus solution, swirl it around their mouth for several seconds to taste, and then spit it out. Tasting of each sample was followed by rinsing with distilled water.

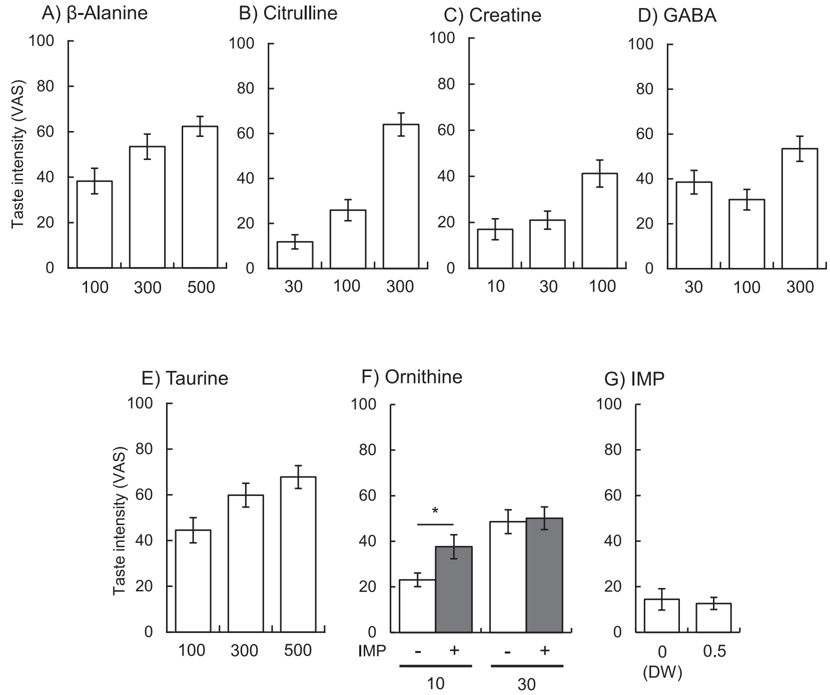

Fig. 1. Evaluation of taste intensities of various amino acid derivatives using a visual analog scale (VAS). Change in taste intensities of (A) *β*-alanine, (B) citrulline, (C) creatine, (D) *γ*-aminobutyric acid (GABA), (E) taurine, (F) ornithine and ornithine with 0.5 mM inosine 5′-monophosphate (IMP), (G) distilled water (DW) and 0.5 mM IMP alone. The numbers on the *x*-axis indicate the concentration in mM; *$p<0.05$, $n=20$.

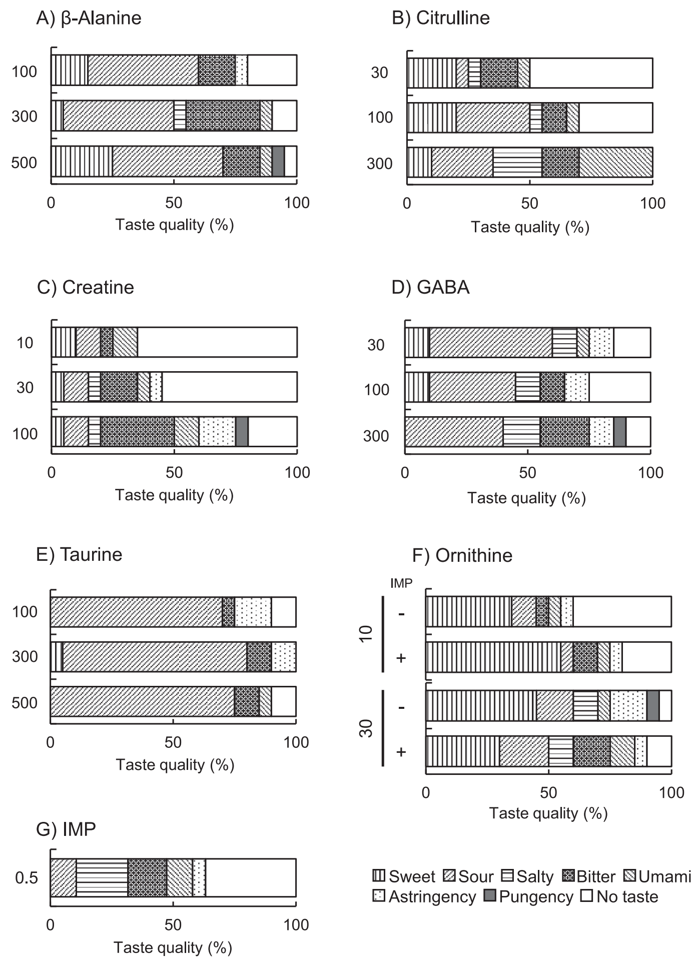

Fig. 2. Taste qualities of various amino acid derivatives. Change in taste qualities of (A) *β*-alanine, (B) citrulline, (C) creatine, (D) *γ*-aminobutyric acid (GABA), (E) taurine, (F) ornithine and ornithine with 0.5 mM inosine 5′-monophosphate (IMP), and (G) IMP alone. The numbers on the *y*-axis indicate the concentration in mM; $n=20$.

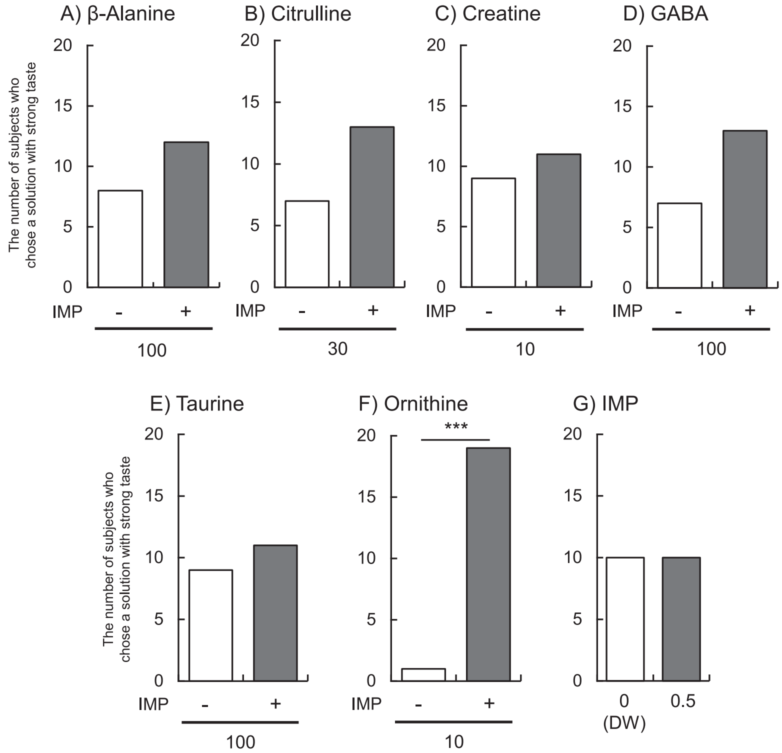

Fig. 3. Comparison of the taste intensity of amino acid derivatives with and without 5′-monophosphate (IMP) using a paired-difference test. Comparison of the taste intensities of (A) β-alanine, (B) citrulline, (C) creatine, (D) γ-aminobutyric acid (GABA), (E) taurine, and (F) ornithine with and without 0.5 mM IMP. (G) Comparison of the taste intensity between distilled water (DW) and 0.5 mM IMP. The numbers on the x-axis indicate the concentration in mM; $^{***}p<0.001$. $n=20$.

We requested the subjects not to ingest anything except water 1 h before the test. All the solutions (30 mL) were poured into plastic cups and were given at room temperature. The tastant solutions were (in mM): 100, 300, and 500 β-alanine, 30, 100, and 300 citrulline, 10, 30, and 100 creatine, 30, 100, and 300 GABA, 100, 300, and 500 taurine, 10 and 30 ornithine, 1–30 MSG, and 0.5 IMP. Additionally, 0.5 IMP mixtures with 100 β-alanine, 30 citrulline, 10 creatine, 100 GABA, 100 taurine, 10 and 30 ornithine and/or 1 mM MSG were also prepared. All tastatnt solutions were prepared using distilled water.

Statistical analysis. Data are expressed as mean± standard error of the mean. Data obtained from the paired-difference test was analyzed by the test for proportion. The number of subjects who chose a solution with the strong taste intensity was determined. For a sample size of 20 (19), significance was demonstrated at the 5%, 1%, and 0.1% significance levels when the number of subjects who correctly chose the solution with a strong taste intensity was greater than 15 (14), 16 (15), and 18 (17), respectively (one-sided testing). Data obtained from the VAS were analyzed by a Wilcoxon signed-rank test. The Wilcoxon signed-rank test was conducted using the GraphPad Prism 6 software (GraphPad Software, San Diego, CA, USA). The differences were considered to be significant at $p<0.05$.

Results

Taste intensity and quality of amino acid derivatives

The changes in taste intensities and taste qualities for each amino acid derivative are shown in Fig. 1 and Fig. 2, respectively. The taste intensities of amino acid derivatives increased in a concentration-dependent manner, however, high concentrations were required to show taste intensity with moderate range (Fig. 1). On the other hand, they induced different taste qualities (Fig. 2). β-Alanine induced mainly sourness, but some subjects also reported tasting bitterness and sweetness (Fig. 2A). Citrulline tasted sweet at low concentrations, while sourness, saltiness, and umami were reported by subjects at high concentration (Fig. 2B). For creatine, the percentage of subjects who reported tasting bitterness increased in a concentration-dependent manner (Fig. 2C). The tastes of GABA and taurine were predominantly sour (Fig. 2D and E). Although the percentage of subjects who tasted sweetness in the 10 mM ornithine was high, the percentage of subjects who tasted sourness, saltiness, and astringency increased at 30 mM ornithine (Fig. 2F).

Evaluation of taste-enhancement between amino acid derivatives and IMP

Initially, in order to confirm whether the subjects can correctly evaluate taste enhancement by IMP, we tested the enhancement effect between MSG and IMP (Fig. S1, Supplemental Online Material). The taste intensity of MSG increased in a concentration-dependent manner

(Fig. S1A) and several subjects tasted umami at 3 mM MSG and higher (Fig. S1B). By adding 0.5 mM IMP to 1 mM MSG, the taste intensity was significantly increased ($p<0.001$; Fig. S1A). The taste intensity was comparable with that of 30 mM MSG alone. In addition, the percentage of subjects who experienced umami increased drastically (Fig. S1B). When comparing the taste intensities between 1 mM MSG and 1 mM MSG+ 0.5 mM IMP, all the subjects responded that the mixture had a stronger taste than MSG alone (Fig. S1C). Thus, we confirmed that the subjects who participated in this study can evaluate the taste enhancement effect by IMP.

We compared the taste intensity of each amino acid derivative and its IMP mixture by the paired-difference test (Fig. 3). No significant increase in taste intensity was observed when IMP was added to *β*-alanine, citrulline, creatine, GABA, and taurine (Fig. 3A–E). On the other hand, with the addition of IMP to ornithine, a significant number of subjects reported that the IMP-containing solution had a stronger taste than ornithine alone solution ($p<0.001$; Fig. 3F).

Subsequently, we performed quantitative analysis on the taste enhancement of ornithine by the addition of IMP. When 0.5 mM IMP was added to 10 mM and 30 mM ornithine solutions, a significant increase in taste intensity was observed in the 10 mM ornithine solution ($p<0.05$; Fig. 1F). Addition of 0.5 mM IMP to 10 mM ornithine increased the percentage of subjects who reported tasting sweetness (Fig. 2F). However, no apparent enhancement in taste intensity and quality was observed when IMP was added to 30 mM ornithine solution (Figs. 1F and 2F). We also assessed the intensity and quality of taste of the 0.5 mM IMP alone. The intensity of the taste of 0.5 mM IMP was similar to that of DW (Fig. 1G). The subjects were unable to distinguish the intensity of taste between 0.5 mM IMP and water (Fig. 3G). Moreover, the majority of the subjects stated that 0.5 mM IMP had no taste (Fig. 2G). These results indicated that there was no obvious intensity or quality to the taste of 0.5 mM IMP. Taken together, these results suggest that the taste of ornithine may be enhanced by the addition of IMP.

Discussion

Previously, we have reported that the taste characteristics of L-theanine (5-*N*-ethylglutamine), an amino acid derivative present in green tea, has a complicated taste profiles, which include umami and sweet, and demonstrated the taste enhancement of L-theanine by IMP (*6*). Additionally, we confirmed that this enhancement occurred via the umami receptor T1R1/T1R3 (*7*). Furthermore, we found that the L-theanine has a taste improving effect, as the addition of L-theanine suppressed sourness in citric acid solution (*8*). This characterization of the taste of specific food components helps in promoting their usage in food products.

In the present study, we focused on 6 different amino acid derivatives, *β*-alanine, citrulline, creatine, GABA, taurine, and ornithine, and evaluated their taste features. All these derivatives are commonly found in our diets. The characteristics of the amino acid derivatives tested in the present study are described below. *β*-Alanine combines with histidine to form carnosine. This amino acid derivative is found in animal sources, such as poultry and meat. *β*-Alanine is the rate-limiting precursor of carnosine formation, and therefore, *β*-alanine supplementation has ergogenic effects on exercise performance, increasing muscle carnosine (*9*, *10*). Citrulline is a ubiquitous amino acid in mammals. Watermelon is the principal source of citrulline in the diet (*11*). Citrulline exerts beneficial effects on the cardiovascular system by enhancing nitric oxide production (*12*). It is known that several proteins contain citrulline as a result of a posttranslational modification (*13*). Creatine is a compound derived from three amino acids, glycine, arginine, and methionine. Creatine is naturally found in meat. Creatine is converted to phosphocreatine and is then used to regenerate the primary source of energy adenosine triphosphate in muscle cells. Creatine supplementation has been demonstrated to have performance-enhancing properties during various types of physical activity (*14*). GABA can be found in a few food sources such as spinach, potato, kale, and broccoli (*15*). GABA is an amino acid that serves as the primary inhibitory neurotransmitter in the central nervous system (*16*). It has been reported that oral intake of GABA reduces stress and has a relaxing effect (*17*, *18*). Taurine is a sulfur-containing *β*-amino acid present in high concentrations in various tissues, including skeletal muscle, liver, blood, and brain. The main taurine sources are meat, dairy, and seafood (*19*). Taurine is involved in various physiologic processes including antioxidant processes, energy metabolism, stabilization of the plasma membrane, inflammation, osmoregulation, regulation of ion channels, and Ca^{2+} handling by the sarcoplasmic reticulum (*20*). It has been reported that taurine supplementation can improve metabolic syndrome, and exercise-induced fatigue and recovery (*21*, *22*). Ornithine is found in animals as a free amino acid and is a vital component of the urea cycle in the liver. Ornithine is found in fish and cheese, but also in freshwater clams, which contain considerably more ornithine than other foods (*23*). The effectiveness of ornithine supplementation on exercise performance has been reported (*24*, *25*).

We investigated concentration-dependent changes in taste qualities and taste intensities of amino acid derivatives. *α*-Amino acids induce multiple taste qualities rather than a single taste qualities (*3*, *4*). Similarly, all amino acid derivatives tested showed multiple taste qualities. The taste intensities of each amino acid derivative increased in a concentration-dependent manner. The taste intensities of *β*-alanine and taurine were approximately 40 at 100 mM and increased to approximately 60 at 500 mM. However, some subjects reported that 500 mM *β*-alanine and/or taurine had no taste. The taste intensity of citrulline was weak at 30 mM and 100 mM, and all subjects were able to taste the citrulline at 300 mM. Regarding creatine, some subjects stated that there was no taste even at 100 mM; however, because 100 mM of creatine is close to a saturated concentration, we could not evaluate greater concentrations therein. The taste intensity of GABA was approximately 50 even at 300 mM. Among the amino acid derivatives tested, ornithine showed the strongest taste intensity at a lower concentration. However, even at 30 mM solution, the taste intensity was approximately 50. Thus, even at the highest concentration of each derivative tested, the solution exhibited moderate taste intensity. Although it is generally thought that each derivative contributes to the taste of foods to differing degrees, their role as taste substances therein might be weaker than expected.

Some subjects felt sourness to β-alanine, citrulline, GABA, and taurine solutions. Among the α-amino acids, it has been reported that proline, serine, and threonine, in addition to acidic amino acids such as aspartic acid and glutamic acid, induce sourness (*3*). Except for acidic amino acids, each of these compounds has one carboxyl group (or sulfo group in taurine). H^+ is important for the generation of sourness, while anion which is a counter ion of H^+ has been reported to also affect sourness (*26*). Therefore, the anionic part may affect their sourness.

Although individual α-amino acids do not themselves have a strong taste, the latter is intensified in the presence of other compounds, such as nucleotides (*5*, *27*, *28*). Among the various taste interactions, the most prominent and well-investigated phenomenon is the considerable umami enhancement of MSG by IMP (*27*, *28*). It has been elucidated that this enhancement is mediated by the umami receptor T1R1/T1R3 (*29*). Therefore, we determined whether amino acid derivatives also enhanced taste after IMP was added. As it was easier to evaluate the enhancement effect when the taste intensity of the test sample was low, the enhancement effect was examined when IMP was added to amino acid derivatives at the lower concentration tested in this study. First, we evaluated the enhancement of the umami of MSG by IMP. We confirmed that the subjects who participated in this study were able to detect the umami enhancing effect. Among the amino acid derivatives tested, we observed an enhancement between 10 mM ornithine and IMP. In the paired-difference test, a significant number of subjects reported that the IMP-containing ornithine solution had a stronger taste than the solution containing ornithine alone. Furthermore, in the quantitative analysis using the VAS, we confirmed the enhancement effect of adding IMP to ornithine. However, we did not observe an enhancement between 30 mM ornithine and IMP in VAS analysis. For MSG, IMP is enhanced over a wide range of MSG concentrations (*27*). Thus, enhancement by ornithine is thought to be weaker than that by MSG. The threshold for IMP is 0.025 g/dL (approximately 0.7 mM) (*27*). Thus, 0.5 mM IMP used in this study was a concentration lower than the threshold. In fact, the taste intensity of 0.5 mM IMP showed a similar value as that of water, and the subjects could not distinguish between IMP and DW. Therefore, the enhancement effect of ornithine is not thought to be caused by the addition of IMP to ornithine. Regarding taste quality, although MSG enhanced umami with the addition of IMP, ornithine increased the number of subjects who tasted sweetness after its addition. This is similar to the results of a previous study that reported sweetness was perceived as the most enhanced taste quality of D-alanine because of its interaction with IMP (5). In the future, we plan to investigate the molecular mechanism by which IMP enhances sweetness.

In the present study, we analyzed the taste characteristics of 6 amino acid derivatives and found that each amino acid derivative has a complex taste profile. Among the amino acid derivatives tested, we found that only ornithine has a taste enhancement interaction with IMP. We believe that this finding will be useful for understanding the role of amino acid derivatives as taste substances in daily foods.

Authorship

Research conception and design: MN; experiments: RT, RS, and YM; statistical analysis of the data: RT and MN; interpretation of the data: RT and MN; writing of the manuscript: MN.

Disclosure of state of COI

The authors have declared that no competing interests exist.

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Supporting information

Supplemental online material is available on J-STAGE.

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