Umami Taste Enhancement of MSG/NaCl Mixtures by Subthreshold L-α-Aromatic Amino Acids

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ABSTRACT: L-Phenylalanine (L-Phe) and L-tyrosine (L-Tyr) are L-α-aromatic amino acids that have recently been discovered to be important components of the savory fractions of soy sauce in addition to L-glutamate. Their effects are evaluated on the umami or savory taste of monosodium L-glutamate (MSG), with or without sodium chloride (NaCl). Because L-Phe at subthreshold concentration (1.0 mM) significantly enhances an umami taste of a MSG/NaCl mixture ($P = 0.000$), combinations of 4 subthreshold concentrations (0, 0.5, 1.5, and 5.0 mM) of L-Phe with a weakly suprathreshold MSG (4.0 mM) and NaCl (80 mM) mixture were then rated for salty and umami intensities relative to those of standard solutions. L-Phe was found to significantly enhance the umami tastes of the MSG/NaCl mixtures when it was added in a concentration range of 0.5 to 5.0 mM ($P = 0.000$). However, neither the umami taste of MSG alone nor the salty taste of NaCl alone was intensified. In a further experiment, L-Tyr at the 3 subthreshold concentrations (0, 0.5, and 1.5 mM) studied was shown to have the same activity as L-Phe. The phenomenon of umami or savory enhancement by subthreshold aromatic amino acids in the soy sauce system has been established.

Keywords: aromatic amino acid, umami taste, salty taste, savory taste, umami enhancement

Introduction

L-α-Aromatic amino acids, such as L-phenylalanine (L-Phe), L-tyrosine (L-Tyr), and L-tryptophan, are well known for their bitter taste above certain threshold concentrations (Kirimura and others 1969; Solms 1969; Nishimura and Kato 1988; Keast and others 2003; Soldo and others 2003). The threshold concentration for L-Phe is 900 mg/L or 5.45 mM according to Kirimura and others (1969). Work on the taste of bitter compounds has so far focused mainly on their threshold or suprathreshold values and on their interactions with suprathreshold salty tastants (Tamura and others 1989; Kemp and Beauchamp 1994; Breslin and Beauchamp 1995; Breslin 1996; Keast and others 2004; Lawless and others 2004). Perception of their bitterness was generally suppressed by salty tastants, especially by sodium salts, whereas salty tastes are not affected by bitterness (Keast and Breslin 2002). Moreover, Keast and others (2004) reported that several sodium salts that taste umami also suppressed the bitterness of aromatic amino acid. Thus far, interactions of the aromatic amino acids, especially at their subthreshold concentrations, with a mixture of salty and umami tastants, have not yet been studied in depth.

Enhancement by L-α-amino acids at concentrations near their threshold or weakly suprathreshold value of the taste of umami tastants, mainly MSG and certain nucleotides, that is, inosine monophosphate (IMP) and guanosine monophosphate (GMP), has been reported by some researchers (Tanaka and others 1969a, 1969b; Yokotsuka and others 1969; Kawai and others 2002). Tanaka and others (1969a) found that although most L-α-amino acids near their threshold values did not enhance the umami taste of MSG or IMP/GMP alone, they did enhance the umami taste of a mixture of MSG and IMP/GMP in the presence of NaCl (171 mM). In this study, L-α-aromatic amino acids did not show any umami-enhancing effect at their concentrations studied (3 to 6 mM). Furthermore, Kawai and others (2002) reported that various L-α-amino acids at their weakly suprathreshold concentrations could exhibit umami enhancement of IMP alone. These were found to contain acidic and neutral amino acid groups. They also revealed in their study that aromatic amino acids did not exhibit any umami-enhancing effect at weak suprathreshold concentrations (10 to 25 mM).

NaCl itself has a salty taste at its threshold concentration of 10.0 mM at pH 7.0 (Soldo and others 2003), whereas MSG has an umami taste and a threshold concentration of 1.56 mM at pH 6.0 (Spanier and others 1997) or 1.5 mM at pH 5.0/7.0 (Soldo and others 2003). It is known that the physiological mechanisms of perception of salty and umami tastes are different. Umami taste is perceived via a separate umami-binding site, and the mechanisms were similar to those for sweet and bitter tastes (Fuke and Ueda 1996; Lindemann 2001; Heyer and others 2003; Zhang and others 2003), whereas salty tastes (Na^+ ions) are detected through sodium channels in the oral space (Fuke and Ueda 1996; Lindemann 2001).

Soy sauce has an intense savory taste due to its balanced intensities of salty and umami tastes (Kuramitsu 1998). It usually contains 15% to 20% NaCl and 1% to 2% MSG (Fukuchi and others 1969; Fukushima 1981; Flegel 1988; Kaneko and others 1994; Kuramitsu 1998; Lioe and others 2004). Besides MSG, our previous study of major compounds in savory fractions of Indonesian soy sauce (Lioe and others 2004) found that aromatic amino acids, that is, L-Phe and L-Tyr, were also responsible for the intense umami taste of the fractions, even though their concentrations in the soy sauce are below their threshold values. This finding led to the present study of the umami enhancement activity of subthreshold L-Phe and L-Tyr in simple MSG/NaCl mixtures, applying the concentrations found in the soy sauce fractions previously studied.

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Materials and Methods

Subjects

Nine selected and trained subjects (6 women and 3 men, age 22 to 33 years) were volunteers in the assessment of L-Phe activity, and 10 subjects (6 women, 4 men) in the assessments of L-Tyr activity. All were Japanese students and employees at the Dept. of Bioscience and Biotechnology of the Univ. of the Ryukyus in Japan.

They were selected by a triangle test done in duplicate and a ranking test, following the procedures described by Carpenter and others (2000). In a triangle test, 5 sets of 3 NaCl solutions and 5 sets of 3 MSG solutions were examined by each subject for salty and umami taste attributes, respectively. Two of the 3 NaCl solutions were of the same concentration, that is, 86 mM or 137 mM, whereas 2 MSG solutions were of 1.2 mM or 3.6 mM. All solutions were coded by random three-figure numbers, and tasted from left to right in each set. Interval time between tastings of the 2 attributes was 15 min. Subjects who were able to correctly determine the odd samples of 7 sets from the total 10 sets were selected. In a ranking test, a set of 5 NaCl solutions of 14, 34, 86, 137, and 171 mM for salty attribute and a set of 5 MSG solutions of 0.6, 1.8, 3.6, 5.9, and 9.5 mM for umami attribute were judged by the subjects. The MSG or NaCl solutions in each set were coded by random three-figure numbers, and presented in a balanced order. Interval time between tastings of the 2 set solutions was 10 min. Subjects who were able to rank all of 5 MSG solutions and 5 NaCl solutions correctly were selected.

Ten subjects, from 21 subjects recruited and screened, who passed both triangle and ranking tests were then selected for sensory analysis of samples. The selected subjects were trained in applying the analysis procedures described below, so as to familiarize them with using a linear line with standard scores, describing the taste intensities of standard solutions (see below), and judging the taste intensities of a sample.

Taste stimuli

Stimulus for the basic umami taste is MSG, and that for the basic salty taste is NaCl. These were obtained from Wako Pure Chemical Industries Ltd. (Osaka, Japan). The L-α-aromatic amino acids used in this study, L-Phe and L-Tyr, were also obtained from the same company.

Taste intensity analysis

Taste intensity analysis was used to rate the salty and umami intensities of sample mixtures based on the intensity scores of standards (Table 1). Throughout the analyses, Milli-Q water (Milli-Q SP Reagent water system, Millipore Corp., Bedford, Mass., U.S.A.) was used to prepare each sample and standard solution, and also for oral rinsing. It is noted that MSG was used as the only umami standard at concentration range of 3.0 to 14.8 mM, because it could exhibit predominantly umami or savory taste quality with very low, almost undetected, salty taste at the concentrations below than 20 mM (Kuramitsu 1998; Keast and others 2004).

Subjects were instructed to taste a set of standard solutions prior to tasting a taste attribute of a sample. They were asked to take about 1 mL of a solution into their mouths and experience it for 5 s. Once a set of standard solutions had been tasted, subjects were asked to rate the taste intensity of a sample.

Each sample solution was coded using random three-figure numbers. All standard and sample solutions tasted were in the pH range of 5.7 to 7.1, centered around neutral, which is known to give an optimal umami taste intensity (Wang and others 1996; Soldo and others 2003). Solutions were prepared simply by diluting the tastants in Milli-Q water at room temperature, other than solutions containing L-Tyr. In that case, L-Tyr was diluted 1st using hot Milli-Q water (at 80 to 90 °C) before putting another tastant in the mixtures. All solutions were served at room temperature.

Table 1 — Standard solutions used for taste intensity analysis[a]

Taste attribute	Tastant	Score	Concentration (mM)
Salty	Sodium chloride	50	42.8
		80	85.6
		150	213.9
Umami	Monosodium L-glutamate	50	3
		80	5.9
		150	11.8
		200	14.8

[a]Milli-Q water was used to prepare the solutions.

Influence of subthreshold L-Phe on the MSG/NaCl mixture

To test whether there is an influence of L-Phe at its subthreshold concentration on a weakly suprathreshold MSG/NaCl mixture that mimics the phenomenon found in the previous study (Lioe and others 2004), solutions containing MSG (4.0 mM, about 3 times the MSG threshold value) alone and a mixture of MSG (4.0 mM) and NaCl (80 mM, about 8 times the NaCl threshold value) were rated in the absence or presence of L-Phe (1.0 mM) according to the procedures described above. This analysis was conducted in duplicate. In each replicate, 9 subjects rated both the umami and salty taste intensities of 4 samples.

Activity of L-Phe on differing subthreshold levels

To study the enhancement activity of L-Phe, we performed 3 separate analyses. Each analysis was done in duplicate, using the same procedures as described above. In the 1st analysis, L-Phe at concentrations of 0, 0.5, 1.5, and 5.0 mM was added to a mixture of MSG (4.0 mM) and NaCl (80 mM). In the 2nd analysis, L-Phe at the 4 subthreshold levels was added to MSG solution (4.0 mM) alone. Only the umami quality was rated in this analysis, so that only a set of umami standards was served. In the 3rd analysis, L-Phe at the 4 concentrations was mixed with NaCl solution (80 mM) alone, and the salty taste intensity of each mixture was then rated. In each analysis, 4 samples were rated by 9 subjects per replicate.

Influence of subthreshold L-Tyr on the MSG/NaCl mixture

Solutions containing MSG (4.0 mM) alone and a mixture of MSG (4.0 mM) and NaCl (80 mM) were rated in the presence or absence of L-Tyr (1.0 mM) using the same standard solutions and procedures as in the analysis for L-Phe above. This analysis was performed in duplicate, with 4 samples rated by 10 subjects per replicate.

Activity of L-Tyr on different subthreshold levels

As with L-Phe, L-Tyr was used at 3 subthreshold levels (0, 0.5, 1.5 mM) and mixed with MSG (4.0 mM) and/or NaCl (80mM) to set up 3 analyses, each examined in duplicate by applying the procedures described above. In each analysis, 3 samples were rated by 10 subjects per replicate.

Data analysis

In each taste analysis described above, the umami or salty scores of each mixture rated by the same subject were averaged across replicates, so that there are 9 or 10 averaged scores for each attribute in each mixture tasted. These data were then analyzed by a one-way repeated measures analysis of variance (ANOVA) for each taste analysis, using the SPSS program version 13.0 (SPSS Inc. 2003, Chicago, Ill., U.S.A.), to examine whether there was a significant effect among the mean scores of sample mixtures. The mean scores reported were arithmetic means obtained from the 9 or 10 averaged scores. All statistical analysis results revealed below are significant at *P* value less than 0.05 unless stated.

Results and Discussion

Influence of subthreshold L-Phe on the MSG/NaCl mixture

MSG as an umami tastant at a weakly suprathreshold concentration of 4.0 mM, about 3 times the MSG threshold value (Soldo and others 2003), has an umami taste intensity rated with a score of 57, and a very weak, only just detectable, salty taste (Figure 1). In the presence of L-Phe (1.0 mM), the umami taste of MSG (4.0 mM) was slightly enhanced to a score of 65 (Figure 1).

When MSG (4.0 mM) was combined with NaCl (80 mM), the umami taste intensity increased to a score of 82 with the salty taste rated to 78 (Figure 1). In the presence of L-Phe (1.0 mM), the umami taste intensity of the mixture was significantly increased up to a score of 115 [$F(3,24) = 48.894$, $P = 0.000$], whereas the salty taste intensity was unaffected (Figure 1). Consequently, there was an enhancement by subthreshold L-Phe of the umami taste of the MSG/NaCl mixture. This result confirmed the phenomenon that was newly found in the previous study (Lioe and others 2004) and led to further analyses using the same MSG/NaCl mixture.

Enhancement activity of subthreshold L-Phe

To evaluate the enhancement by L-Phe of the umami taste of the MSG/NaCl mixture, L-Phe was added at 4 subthreshold levels (0, 0.5, 1.5, and 5.0 mM) to MSG (4.0 mM) and NaCl (80 mM), either alone or in the mixture.

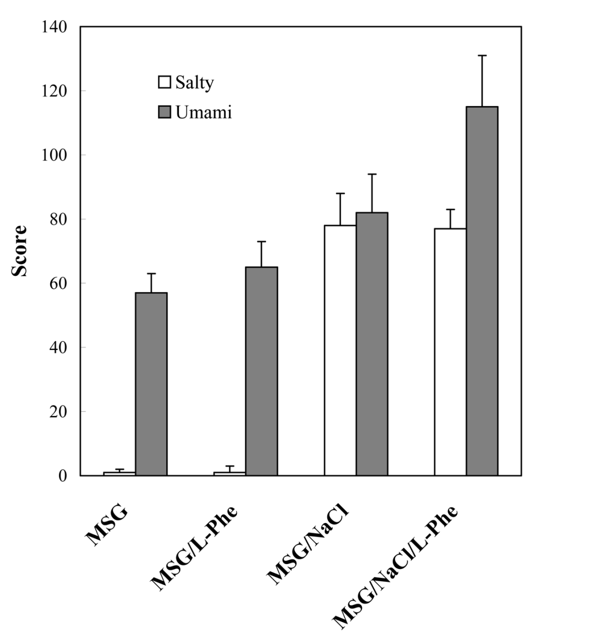

Figure 1—Effect of L-Phe (1.0 mM) on the tastes of MSG (4.0 mM) alone and the mixture of MSG (4.0 mM) and NaCl (80 mM). Each score was the arithmetic mean across subjects and replicates. The error bars show the standard deviations of the means.

Figure 2 shows that L-Phe significantly enhanced the umami taste of the MSG/NaCl mixture when it was added at subthreshold concentrations of 0.5, 1.5, and 5.0 mM to the mixture [$F(3,24) = 11.470$, $P = 0.000$], the umami score was increased from 124 to 156, 166, and 140, respectively. It can be seen that the effect of L-Phe was increased as concentration increased up to 1.5 mM, and then decreased at concentration 5.0 mM. In contrast, the salty taste of the mixture was not affected by the addition of L-Phe [$F(3,24) = 0.585$, $P = 0.631$]. The overall mean of salty scores of the mixtures containing L-Phe, MSG and NaCl was 76, and the pooled standard deviation was 6. The subthreshold concentration of L-Phe (1.5 mM) that led to the most intense umami taste was nearly the same as that found in soy sauce fractions tasted in the previous study (Lioe and others 2004).

To know whether L-Phe affected the individual perceived taste of MSG or NaCl in the MSG/NaCl mixture, the taste analyses of the mixtures containing L-Phe at the 4 subthreshold concentrations and MSG (4.0 mM) alone or NaCl (80 mM) alone were performed. The results revealed that neither the umami taste of MSG alone [$F(3,24) = 1.195$, $P = 0.333$] nor the salty taste of NaCl alone [$F(3,24) = 1.247$, $P = 0.315$] was significantly affected by L-Phe. The overall mean of umami scores of the mixtures containing L-Phe and MSG was 63, and the pooled standard deviation was 12; whereas, the overall mean of salty scores of the mixtures containing L-Phe and NaCl was 75, and the pooled standard deviation was 5. This suggests that umami enhancement activity of the subthreshold tastant in the MSG/NaCl mixture is not determined by the taste of MSG or NaCl alone.

That fact led to the expectation that the perceptions of umami and salty tastes have distinct mechanisms, which are also described by Fuke and Ueda (1996) and Lindemann (2001). However, when both umami and salty stimuli are mixed, they can potentially interact to give a more intense umami taste perception, as described above, and this interaction is then found to be intensified by L-Phe at its subthreshold concentrations. In this condition, L-Phe may interact directly with the 2 taste mechanisms, because the activity of subthreshold L-Phe was significantly emerged only in the MSG/NaCl mixture. This possible effect gives an insight that a subthreshold tastant can affect the intensity of the other taste attribute. Moreover, the phe-

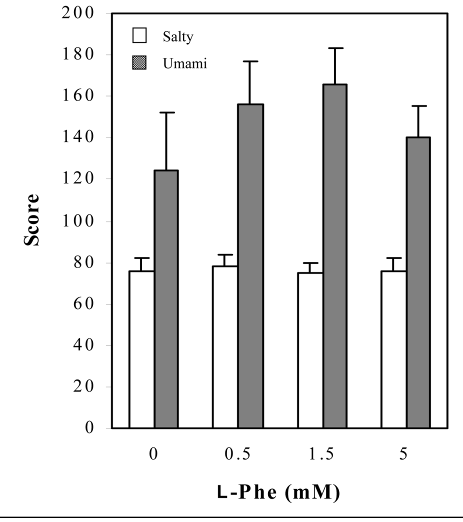

Figure 2—Effect of L-Phe at different subthreshold levels on the tastes of the mixture of MSG (4.0 mM) and NaCl (80 mM). Each score was the arithmetic mean across subjects and replicates. The error bars show the standard deviations of the means.

nomenon of subthreshold umami enhancement is interesting. The same phenomenon was also found in another study reported by Yamaguchi (1967). She found that IMP at a subthreshold range of 0.005% to 0.010% w/v (its threshold value is 0.025% w/v) significantly enhanced the umami taste of 2.4 to 2.7 mM of MSG.

Influence of subthreshold L-Tyr on the MSG/NaCl mixture

Similar results were obtained for L-Phe, although L-Tyr slightly lowered the umami taste of MSG (4.0 mM) alone (Figure 3). Figure 3 showed that when MSG (4.0 mM) and NaCl (80 mM) were mixed, the umami intensity of MSG (4.0 mM) was increased and this umami intensity was significantly intensified by the addition of L-Tyr [$F(3,27) = 44.331$, $P = 0.000$], as with L-Phe. Umami enhancement activity by subthreshold L-Tyr on the MSG/NaCl mixture was therefore present.

Enhancement activity of subthreshold L-Tyr

To evaluate the enhancement caused by subthreshold L-Tyr of the umami taste of the MSG/NaCl mixture, L-Tyr at 3 subthreshold levels (0, 0.5, and 1.5 mM) was added to MSG (4.0 mM) and/or NaCl (80 mM). No 5.0-mM L-Tyr concentration was used because of its limiting dilution capacity in water. Figure 4 shows that significant umami enhancement occurs when L-Tyr at concentrations 0.5 and 1.5 mM is added to the MSG/NaCl mixture [$F(2,18) = 47.433$, $P = 0.000$], even though the salty tastes of mixtures containing L-Tyr, MSG, and NaCl were slightly lowered [$F(2,18) = 5.445$, $P = 0.014$]. In contrast, addition of L-Tyr to MSG alone [$F(2,18) = 1.194$, $P = 0.326$] or to NaCl alone [$F(2,18) = 0.956$, $P = 0.403$] gave no significant taste enhancement. The overall mean of umami scores of the mixtures containing L-Tyr and MSG was 63, and the pooled standard deviation was 9; whereas, the overall mean of salty scores of the mixtures containing L-Tyr and NaCl was 77, and the pooled standard deviation was 4. In these analyses, it is obvious that L-Tyr may also interact directly with the umami and salty taste mechanisms, because the activity of subthreshold L-Tyr was also significantly emerged only in the MSG/NaCl mixture. To summarize the results, subthreshold tastants can affect the intensity of the other taste attribute.

The presence of L-Tyr in soy sauce fractions, as observed previously, was 1.0 mM (Lioe and others 2004), which is in the range of the effect. This fact follows that umami enhancement by subthreshold L-Tyr as well as subthreshold L-Phe cannot be ruled out in the generating of umami or savory taste of soy sauce. This newly identified umami-enhancing effect provides a new insight into umami or savory taste perception in sensory science. Furthermore, because MSG can also interact synergistically with IMP/GMP (Yamaguchi 1967; Yamaguchi and others 1971; Maga 1983; Kawai and others 2002), investigations on the taste enhancement of the umami tastant mixtures using single or binary mixtures of subthreshold aromatic amino acids in the absence or presence of NaCl will become the future study.

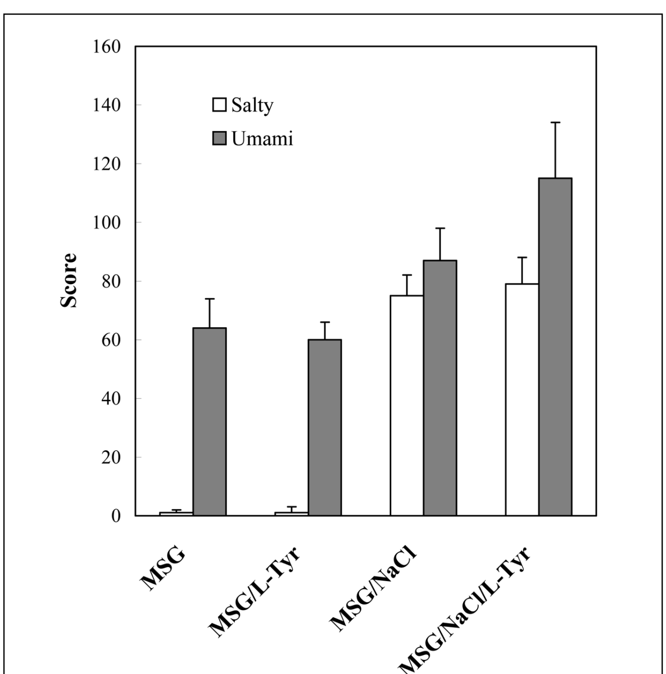

Figure 3—Effect of L-Tyr (1.0 mM) on the tastes of MSG (4.0 mM) alone and the mixture of MSG (4.0 mM) and NaCl (80 mM). Each score was the arithmetic mean across subjects and replicates. The error bars show the standard deviation of the means.

Conclusions

Both aromatic amino acids, that is, L-phenylalanine and L-tyrosine, at their subthreshold concentrations showed significant umami-enhancing effect on the umami taste of MSG/NaCl mixtures. This is a novel phenomenon for the so-called bitter amino acids.

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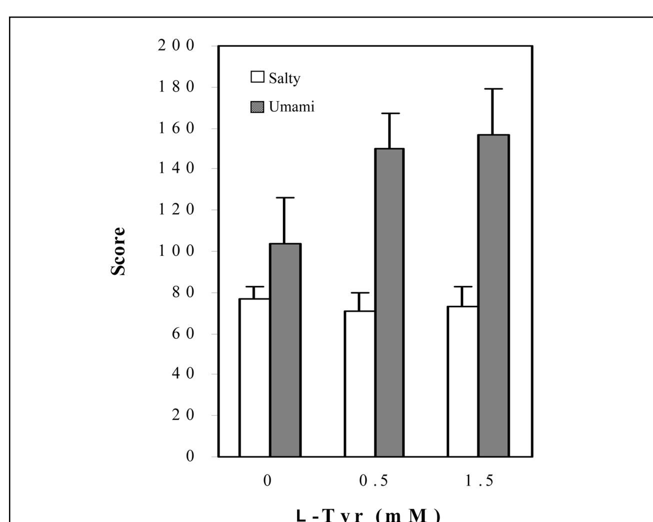

Figure 4—Effect of L-Tyr at different subthreshold levels on the tastes of the mixture of MSG (4.0 mM) and NaCl (80 mM). Each score was the arithmetic mean across subjects and replicates. The error bars show the standard deviation of the means.

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