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Olfactory Perception of 6 Amino Acids by Human Subjects

Matthias Laska

IFM Biology, Linköping University, SE-581 83 Linköping, Sweden

Correspondence to be sent to: Matthias Laska, IFM Biology, Linköping University, SE-581 83 Linköping, Sweden. e-mail: malas@ifm.liu.se

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Abstract

The olfactory properties of 6 amino acids were assessed in 20 human subjects using psychophysical tests of detectability, discriminability, and chemesthesis. Mean olfactory detection thresholds were found to be 10 μM for D-methionine, 80 μM for L-methionine, 200 μM for L-cysteine, 220 μM for D-cysteine, 75 mM for D-proline, and 100 mM for L-proline. When presented at clearly detectable and intensity-matched concentrations, the subjects readily discriminated between the odors of the L-forms of cysteine, methionine, and proline, whereas they failed to distinguish between the L- and D-forms of a given amino acid. The subjects also failed in localizing the side of monorhinal stimulation with all 6 amino acids when presented at the same concentrations as in the discrimination tasks. These results suggest that amino acids may contribute to the flavor of food not only as taste stimuli but also as olfactory stimuli perceived via ortho- or retronasal smelling. In contrast, it is unlikely that amino acids contribute to flavor perception via chemesthesis. Given that the odors of 4 of the 6 amino acids tested here were detected at concentrations lower than their corresponding taste detection thresholds, this may have important implications for the widespread use of amino acids as food additives as well as for the evaluation of off-flavors caused by amino acids.

Key words: amino acids, chemesthesis, olfactory discriminability, olfactory detection thresholds

Introduction

It is well established that amino acids evoke specific taste sensations (Schiffman et al. 1981) and thus contribute to the flavor of food (Kirimura et al. 1969). Surprisingly little, in contrast, is known about the olfactory properties of amino acids as perceived by humans and their potential contribution to the flavor of food via ortho- or retronasal smelling. This is all the more surprising given that amino acids are present in free form in a wide variety of foods (Maarse 1991) and have been shown to act as ligands for fish olfactory receptors with high affinity and specificity (Caprio and Byrd 1984; Bruch and Rulli 1988; Nikonov and Caprio 2007a). Furthermore, amino acids are known to play a crucial role as olfactory cues in a variety of nonhuman species (Valentincic and Caprio 1994; Hubbard et al. 2003; Ferrer and Zimmer 2007). In a series of landmark studies, Caprio and coworkers demonstrated that fishes are able to perceive amino acids not only via the gustatory system but also via the olfactory system and that the latter is more sensitive than the former for this group of stimuli (Caprio 1977, 1978; Caprio and Byrd 1984; Nikonov and Caprio 2001, 2007a, 2007b). Electrophysiological evidence suggests that certain molecular properties of amino acids such as chirality, functional groups, and side chain polarity are encoded by the fish olfactory system and form the basis for olfactory discrimination of amino acids (Nikonov and Caprio 2007b). Recent genetic studies have shown that the olfactory receptors interacting with amino acids are not confined to fish but can be found in all classes of vertebrates (Niimura and Nei 2006). Thus, it is likely that humans, too, are able to smell amino acids. This supposition is supported by studies that reported human subjects capable of discriminating the odor of certain amino acids from solvent (Dietz and Traud 1978; Naim et al. 1997).

In order to gain information about the olfactory properties of amino acids as perceived by humans, it was the aim of the present study to determine olfactory detection thresholds and to assess the olfactory discriminability and chemesthetic properties of 6 amino acids in a group of human subjects. Using the L- and D-forms of cysteine, methionine, and proline allowed me to additionally assess the impact of chirality and other molecular structural features on olfactory perception of these odorants. Comparing the olfactory detection thresholds determined here with human taste detection thresholds reported in earlier studies allowed me to assess the potential contribution of the odors of the amino acids under investigation to the flavor of food via ortho- or retronasal smelling.

Experiment 1: olfactory detection thresholds of 6 amino acids

Materials and methods

Subjects

Twenty healthy, unpaid volunteers, 10 males and 10 females between 21 and 35 years of age, participated. The average age of the males was 27.6 ± 6.3 years and that of the females was 27.7 ± 4.1 years. None of the subjects had any history of olfactory dysfunction or suffered from an acute upper respiratory tract infection. All subjects were informed as to the aims of the study and provided written consent. The study was performed in accordance with the declaration of Helsinki/Hong Kong.

Odorants

A set of 6 odorants comprising the L- and D-forms of cysteine, methionine, and proline was used. For each stimulus, a geometric dilution series using demineralized water as the solvent was prepared, starting at a concentration of 500 mM and progressing by a factor of 5. Stem dilutions were designated step 1, and subsequent dilutions step 2, 3, and so forth. Fresh dilutions were prepared every other day following the initial preparations. All substances were of the highest available purity (>99.5% with the 3 L-amino acids and >99.0% with the 3 D-amino acids) and were obtained from Sigma-Aldrich. Gas phase concentrations for the headspace above the diluted odorants were calculated using published vapor pressure data (Dykyi et al. 2001) and corresponding formulas (Weast 1987).

Figure 1 shows the molecular structure of the odorants.

Test procedure

A 40 mL aliquot of each odorant was presented in a 250 mL high-density polyethylene (HDPE) squeeze bottle equipped with a flip-up spout. Bottles containing the pure diluent served as blanks. Subjects were instructed as to the manner of sampling and at the start of the first session were allowed time to familiarize themselves with the bottles and the sampling technique. Care was taken that the spout was only a short distance (1–2 cm) from the nasal septum during sampling of an odorant in order to allow the stimulus to enter both nostrils.

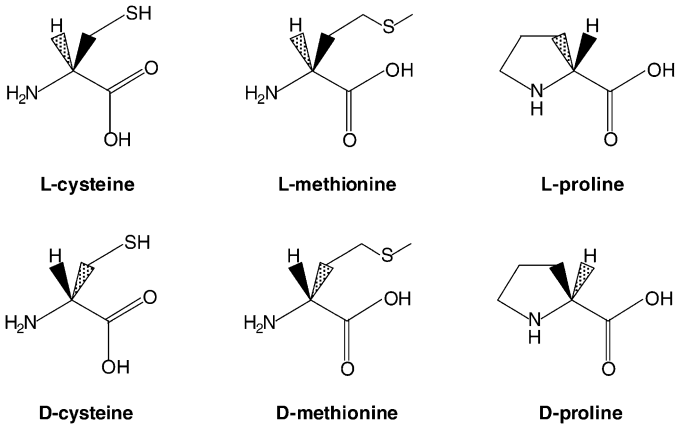

Figure 1 Molecular structures of the 6 amino acids.

Olfactory detection thresholds were determined using a 3-alternative forced-choice procedure in which the subjects were presented with 3 randomly arranged bottles, 2 of which contained pure diluent and the third the stimulus (Laska and Hudson 1991; Laska and Teubner 1999; Laska 2004). In order to minimize adaptation effects, testing followed an ascending staircase procedure. Each bottle could be sampled twice per trial with an interstimulus interval of at least 5 s. Sampling duration was restricted to 1 s per presentation in order to minimize adaptation effects. Subjects were required to decide whether there was no difference between the bottles or identify one as containing the stimulus. In the case of "no difference," testing proceeded to the next dilution step (with a higher concentration of the odorant), otherwise the bottles were rearranged and the subject allowed to sample a second time. If both choices were correct, this was provisionally recorded as the threshold dilution. However, if these had been preceded by one correct and one incorrect choice, the previous dilution (with a lower concentration of the odorant) was again tested, and if both choices were then correct this was taken as threshold. In this way, olfactory detection thresholds were determined for each subject. Testing was repeated in 2 more sessions as previous studies using the same method have shown that a subject's performance may increase (i.e., detection thresholds may decrease) with repeated testing and may reach a plateau by the third session (Laska and Hudson 1991; Laska and Teubner 1999; Laska 2004). The sessions took about 60 min each and were performed 1–3 days apart. Care was taken to systematically vary the order in which the 6 odorants were presented across sessions.

Data analysis

Comparisons of group performance across sessions were made using the Friedman 2-way analysis of variance (ANOVA). When ANOVA detected differences between sessions, this was then followed by pairwise Wilcoxon signed-rank tests for related samples to evaluate which sessions were responsible. Comparisons of group performance between odorants were made using the Wilcoxon signed-rank test for related samples. Possible differences in sensitivity between male and female subjects were assessed using the Mann–Whitney *U*-test for independent samples. Data are reported as means ± standard deviations (SDs).

Results

Figure 2 shows the mean detection thresholds of 20 subjects for each of the 6 odorants tested across 3 sessions. With all 6 odorants, threshold values were quite stable and did not

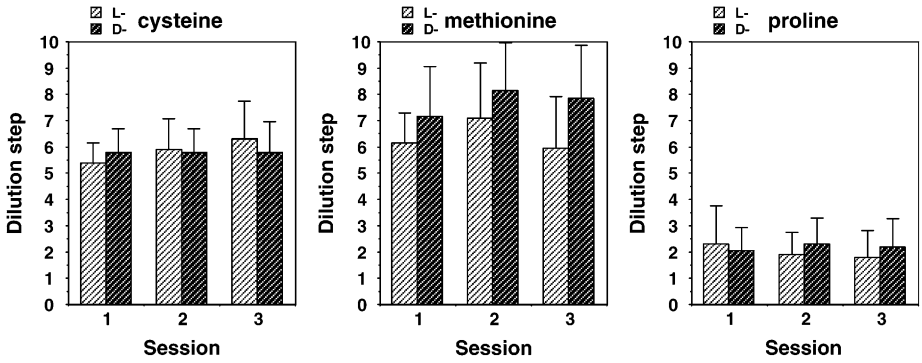

Figure 2 Olfactory detection thresholds for the 6 amino acids. Means and SDs (n = 20 subjects) for each of the 3 test sessions are given.

differ significantly across sessions (Friedman, $P > 0.05$ for all 6 odorants) and thus no significant learning or training effects at the group level were found. Therefore, a grand average across the 3 sessions was calculated for each odorant: L-cysteine was detected at dilution step 5.87 ± 1.20 and D-cysteine at dilution step 5.80 ± 0.97, corresponding to mean detection threshold concentrations of 200 and 220 μM, respectively. L-Methionine was detected at dilution step 6.40 ± 1.82 and D-methionine at dilution step 7.72 ± 1.92, corresponding to mean detection threshold concentrations of 80 and 10 μM, respectively. L-Proline was detected at dilution step 2.00 ± 1.13 and D-proline at dilution step 2.18 ± 0.97, corresponding to mean detection threshold concentrations of 100 and 75 mM, respectively.

Detectability of L- and D-cysteine did not differ significantly from each other in any of the 3 sessions and also when the grand averages across sessions were compared (Wilcoxon, $P > 0.05$). The same is true for L- and D-proline (Wilcoxon, $P > 0.05$). In contrast, detectability of L- and D-methionine differed significantly in 2 of the 3 sessions (Wilcoxon, $P < 0.05$ in sessions 1 and 3, respectively). Accordingly, a comparison between the grand averages for L- and D-methionine yielded a statistically significant difference (Wilcoxon, $P < 0.001$) with threshold values for the D-form being lower (indicating a higher sensitivity of the group of subjects for this odorant) than those for the L-form.

L- and D-proline were both detected at significantly higher concentrations than any of the 4 other odorants (Wilcoxon, $P < 0.01$), and D-methionine, but not L-methionine, was detected at significantly lower concentrations than L- and D-cysteine (Wilcoxon, $P < 0.01$).

Interindividual variability with a given odorant was comparatively low as can be inferred from the SDs in Figure 2 which ranged from 0.75 dilution steps (i.e., a factor of 3.3) for L-cysteine in session 1 to 2.10 dilution steps (i.e., a factor of 30) for L-methionine in session 2.

No significant differences in sensitivity between males and females were found with any of the 6 odorants (Mann–Whitney, $P > 0.05$).

Table 1 summarizes the mean olfactory threshold dilutions of the human subjects for the 6 amino acids and shows various measures of corresponding gas phase concentrations (Weast 1987) allowing readers to easily compare the data obtained in the present study with those reported by other authors using one of these convertible measures. With L- and D-cysteine as well as with L- and D-methionine, threshold dilutions correspond to gas phase concentrations <1 ppb. With L- and D-proline threshold dilutions correspond to gas phase concentrations <1 ppm.

Table 1 Mean olfactory detection threshold values in human subjects (n = 20) for 6 amino acids expressed in various measures of gas phase concentrations

	Liquid concentration			Gas phase concentration		
	mM	Molec./cm^3 air	ppm	Log ppm	Mol/L	Log Mol/L
L-Cysteine	0.2	2.4×10^{10}	0.00089	−3.05	4.0×10^{-11}	−10.40
D-Cysteine	0.22	2.6×10^{10}	0.00096	−3.01	4.3×10^{-11}	−10.36
L-Methionine	0.08	7.9×10^{9}	0.00029	−3.53	1.3×10^{-11}	−10.88
D-Methionine	0.01	9.8×10^{8}	0.000036	−4.44	1.6×10^{-12}	−11.79
L-Proline	100	1.8×10^{13}	0.67	−0.18	3.0×10^{-8}	−7.52
D-Proline	75	1.4×10^{13}	0.52	−0.29	2.3×10^{-8}	−7.63

Experiment 2: olfactory discrimination of 6 amino acids

Materials and methods

Subjects

Twenty healthy, unpaid volunteers, 10 males and 10 females between 20 and 27 years of age, participated. The average age of the males was 23.1 ± 2.5 years and that of the females was 23.0 ± 1.0 years. Ten of the subjects (5 males and 5 females) had also participated in Experiment 1. None of the subjects had any history of olfactory dysfunction or suffered from an acute upper respiratory tract infection. All subjects were informed as to the aims of the study and provided

written consent. The study was performed in accordance with the declaration of Helsinki/Hong Kong.

Odorants

The same 6 amino acids as in Experiment 1 were used. L- and D-cysteine as well as L- and D-methionine were presented at a concentration of 100 mM, whereas L- and D-proline were presented at a concentration of 500 mM. The rationale for using these concentrations was that they are clearly above detection threshold and provide stimuli of approximately equal subjective intensity.

Test procedure

A 40 mL aliquot of each odorant was presented in a 250 mL HDPE squeeze bottle equipped with a flip-up spout. Subjects were instructed as to the manner of sampling and at the start of the first session were allowed time to familiarize themselves with the bottles and the sampling technique. Care was taken that the nosepiece was only a short distance (1–2 cm) from the nasal septum during sampling of an odorant in order to allow the stimulus to enter both nostrils.

In a 3-alternative forced-choice procedure 20 subjects were asked to compare 3 bottles and to identify the one containing the odd stimulus (Laska and Teubner 1999; Laska 2004). Each bottle could be sampled twice with an interstimulus interval of at least 5 s. Sampling duration was restricted to 1 s per presentation in order to minimize adaptation effects. The sequence of presenting the stimulus pairs was systematically varied between sessions and individual subjects while taking care that the presentation of a given odorant as odd or even stimulus was balanced within and between sessions. In order to control for possible cross-adaptation effects, the order in which the stimuli of a given triad were sampled was systematically varied between sessions. Approximately 30 s were allowed between trials and no feedback regarding the correctness of the subjects' choice was given.

The following 6 stimulus pairs were tested for discriminability: L-cysteine versus D-cysteine, L-methionine versus D-methionine, L-proline versus D-proline, L-cysteine versus L-methionine, L-cysteine versus L-proline, and L-methionine versus L-proline. The first 3 stimulus pairs assessed the subjects' ability to discriminate between the 2 enantiomeric forms of a given amino acid and the last 3 stimulus pairs assessed the subjects' ability to distinguish between the L-forms of the amino acids under investigation.

The 6 stimulus pairs were presented 5 times per session, and testing was repeated in one more session 1–3 days after the first one, enabling 10 judgments per stimulus pair and subject to be collected.

Data analysis

The criterion for an individual subject to be regarded as capable of discriminating a given odor pair was set at 7 or more of 10 decisions correct (2-tailed binomial test, $P < 0.05$). Accordingly, the criterion for the group of subjects to be regarded as capable of discriminating a given odor pair was set at 12 or more of 20 subjects performing significantly above chance (2-tailed binomial test, $P < 0.05$).

Comparisons of group performance across tasks were made using the Friedman 2-way ANOVA. When ANOVA detected differences between tasks, this was then followed by pairwise Wilcoxon signed-rank tests for related samples to evaluate which tasks were responsible. Possible differences in discrimination performance between male and female subjects and between subjects that had or had not participated in Experiment 1, respectively, were assessed using the Mann–Whitney *U*-test for independent samples. Data are reported as means ± SDs.

Results

Figure 3 summarizes the mean performance of 20 subjects in discriminating between the 6 odor pairs. As a group, the human subjects performed significantly above chance in the 3 tasks involving the L-forms of the different amino acids (2-tailed binomial test, $P < 0.01$), whereas they failed to do so with the 3 tasks involving the L- and D-forms of a given amino acid (2-tailed binomial test, $P > 0.05$). Accordingly, only one subject of 20 failed to significantly discriminate between L-cysteine and L-methionine (and none of the 20 subjects failed with L-cysteine vs. L-proline and with L-methionine vs. L-proline), whereas only 2 of 20 subjects succeeded in discriminating between L- and D-methionine (and none of the 20 subjects succeeded with L-cysteine vs. D-cysteine and with L-proline vs. D-proline).

Although interindividual variability was comparatively high, particularly in tasks that were not significantly

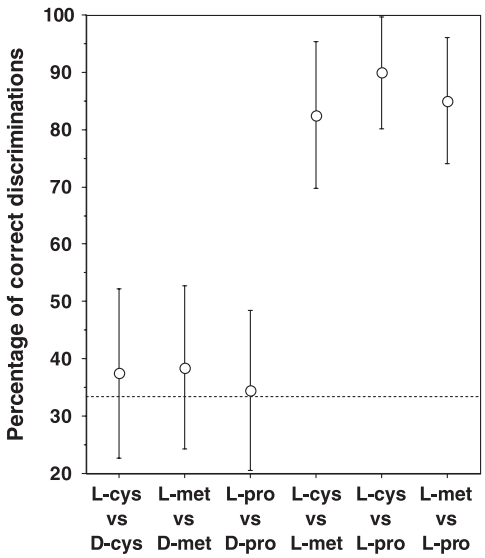

Figure 3 Performance of 20 subjects in discriminating between 6 pairs of amino acids. Each data point represents the percentage (means ± SD) of correct choices from 10 decisions per odor pair and subject.

discriminated at the group level (see SDs in Figure 3), ANOVA detected significant differences in the group's performance between tasks (Friedman, $P < 0.001$) and subsequent pairwise tests revealed that the 3 odor pairs involving the L- and D-forms of a given amino acid were significantly more difficult to discriminate compared with the 3 odor pairs involving the L-forms of the different amino acids (Wilcoxon, $P < 0.01$). Discrimination scores within these 2 groups of odor pairs did not differ significantly from each other (Wilcoxon, $P > 0.05$).

No significant differences in discrimination performance between males and females were found with any of the 6 odor pairs (Mann–Whitney, $P > 0.05$). Similarly, the 10 subjects that had participated in Experiment 1 did not differ significantly in their discrimination performance with any of the 6 odor pairs from the 10 subjects that had not (Mann–Whitney, $P > 0.05$).

A number of subjects spontaneously commented on the odor quality of the amino acids. L- and D-cysteine were most often described as "sulfur" or "rotten eggs"; L- and D-methionine were labeled as "moldy," "old potatoes," and "rotten dairy products"; and L- and D-proline were described to smell of "semen," "sperm," and "chlorine."

Experiment 3: chemesthetic potency of 6 amino acids

Materials and methods

Subjects

Twenty healthy, unpaid volunteers, 10 males and 10 females between 21 and 25 years of age, participated. The average age of the males was 22.5 ± 2.7 years and that of the females was 23.4 ± 1.9 years. Ten of the subjects (5 males and 5 females) had also participated in Experiment 1. None of the subjects had any history of olfactory dysfunction or suffered from an acute upper respiratory tract infection. All subjects were informed as to the aims of the study and provided written consent. The study was performed in accordance with the declaration of Helsinki/Hong Kong.

Odorants

The same 6 amino acids as in Experiments 1 and 2 were used. They were presented at the same concentrations as in Experiment 2.

Test procedure

Using a custom-made squeezer, air from two 250 mL HDPE squeeze bottles was applied to the right and the left nostril of a subject. One bottle contained 40 mL of an odorant, whereas the other bottle contained 40 mL of the odorless solvent. Both bottles were equipped with a flip-up spout which for testing was fitted with a handmade Teflon nosepiece. Care was taken that the nosepieces were in direct contact with the nostrils during sampling in order to ensure that each stimulus entered one nostril only. Presentation of an odorant was synchronized with a subject's inhalation, and the squeezer was calibrated to deliver 20 mL of air to each nostril.

In a forced-choice test procedure, 20 subjects were asked to identify the side of stimulation with an odorant (Laska and Teubner 1999; Laska 2004). The sequence of presenting the stimuli was systematically varied between sessions and individual subjects while taking care that the presentation of a given odorant to the left or the right nostril was balanced within and between sessions. Approximately 30 s were allowed between trials and no feedback regarding the correctness of the subjects' choice was given. The 6 stimuli were presented 5 times per session, and testing was repeated in 3 more sessions, each 1–3 days apart, enabling 20 judgments per stimulus and subject to be collected.

Data analysis

The criterion for an individual subject to be regarded as capable of localizing the side of monorhinal stimulation with a given stimulus was set at 14 or more of 20 decisions correct (2-tailed binomial test, $P < 0.05$). Accordingly, the criterion for the group of subjects to be regarded as capable of localizing a given stimulus was set at 14 or more of 20 subjects performing significantly above chance (2-tailed binomial test, $P < 0.05$).

Comparisons of group performance across sessions were made using the Friedman 2-way ANOVA, and comparisons of group performance between tasks involving the L- and the D-form of a given amino were made using the Wilcoxon signed-rank test for related samples. Possible differences in lateralization performance between male and female subjects and between subjects that had or had not participated in Experiment 1, respectively, were assessed using the Mann–Whitney *U*-test for independent samples. Data are reported as means ± SDs.

Results

Figure 4 summarizes the mean performance of 20 subjects in localizing the side of monorhinal stimulation with the L- and D-forms of cysteine, methionine, and proline, respectively, when presented at the same concentrations as in Experiment 2.

As a group, the human subjects failed to perform significantly above chance in all 6 tasks, with between 16 (with L-methionine) and 19 (with L-proline) of 20 individuals not reaching the criterion of at least 14 of 20 decisions correct.

Pairwise comparisons of performance between the L- and the D-form of a given amino acid revealed that the enantiomers of cysteine, methionine, and proline, respectively, did not differ significantly in their chemesthetic potency at the concentrations tested (Wilcoxon, $P > 0.10$).

Interindividual variability was comparatively low (see SDs in Figure 4), and individual scores averaged across the 6 tasks

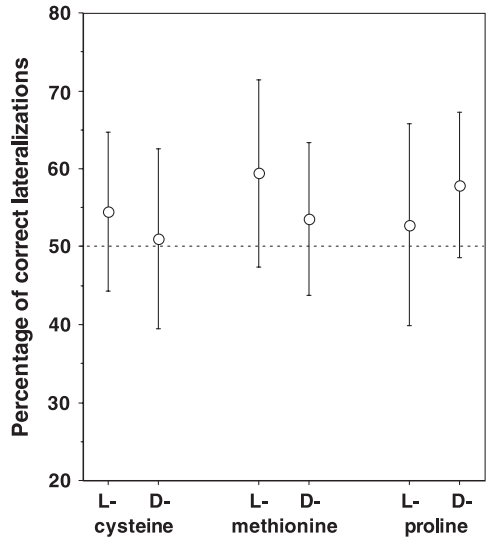

Figure 4 Performance of 20 subjects in correctly localizing the side of monorhinal stimulation. Each data point represents the percentage (means ± SD) of correct choices from 20 decisions per odor and subject.

ranged from 42% correct for the poorest performing subject to 68% correct for the best performing subject. Only one subject succeeded in reaching the criterion of 70% correct (corresponding to a 5% level of statistical significance) with 2 of the 6 stimuli.

Lateralization performance of the 20 subjects across the 4 test sessions was quite stable and did not differ significantly between sessions (Friedman, $P > 0.05$), and thus no significant learning or training effects at the group level were found.

No significant differences in lateralization performance between males and females were found with any of the 6 stimuli (Mann–Whitney, $P > 0.05$). Similarly, the 10 subjects that had participated in Experiment 1 did not differ significantly in their lateralization performance from the 10 subjects that had not (Mann–Whitney, $P > 0.05$).

Discussion

The results of this study demonstrate that human subjects are able to detect the odors of the L- and D-forms of cysteine and methionine at μM concentrations, and those of proline at mM concentrations. Furthermore, they show that humans are able to discriminate between the odors of the L-forms of these 3 amino acids, whereas they fail to distinguish between the L- and D-forms of a given amino acid. They also fail to correctly localize the side of monorhinal stimulation with any of the 6 stimuli.

Olfactory sensitivity for amino acids

To the best of my knowledge, only 2 studies so far reported human olfactory detection thresholds for amino acids. Dietz and Traud (1978) reported human subjects to detect the odors of L-tyrosine and DL-phenylalanine at concentrations >60 μM and >55 μM, respectively, and Naim et al. (1997) found the human olfactory detection threshold for L-cysteine, one of the amino acids used in the present study, to be 1.8 mM. Although the former study employed a rather coarse method and the latter used orange juice as the solvent they are both in line with the present finding that humans are capable of detecting the odor of amino acids at concentrations in the mM and, in some cases, even the μM range.

A comparison of the olfactory detection thresholds determined here with those obtained with amino acids in other species suggests that humans are not generally less sensitive to these odors than fishes. This is remarkable given that amino acids are known to be important food-associated odor stimuli in aquatic animals. Valentincic and Caprio (1994) found that channel catfish show behavioral responses to the odor of L-proline at a concentration of 0.1 mM and thus at a considerably lower concentration than humans. However, the same species responded to the odors of L-alanine and L-arginine at 10 μM and thus to the same concentration as humans did with D-methionine. Unfortunately, olfactory detection thresholds for cysteine and methionine were not determined in fish. However, such across-species comparisons of olfactory detection thresholds should take into account that different methods may lead to widely differing results (Hastings 2003).

Although the amino acids used here were of the highest available purity (>99.5% with the 3 L-amino acids and >99.0% with the 3 D-amino acids), and fresh dilutions were prepared every other day and I cannot exclude the possibility that impurities or degradation products might have affected their detectability and discriminability. However, there was no indication that olfactory detection thresholds or discrimination scores determined on the day the dilutions were prepared and on the following day differed. Similarly, there was no indication that the chemesthetic properties of the amino acids changed over the 2 days that a given set of prepared dilutions was used.

Comparison between olfactory and gustatory sensitivity for amino acids

A comparison of the olfactory detection thresholds determined here with those of human taste detection thresholds reported in earlier studies (Schiffman et al. 1981; Haefeli and Glaser 1990) shows that the human olfactory system is more sensitive than the gustatory system with 4 of the 6 amino acids (Table 2). This suggests that at least the L- and D- forms of cysteine and methionine may contribute to the flavor of food via ortho- or retronasal olfaction at concentrations that are not detected by the sense of taste.

Given the rather negative verbal labels that the human subjects assigned to the odors of the amino acids tested here, this finding may have important implications for the widespread use of amino acids as food additives (Burdock 2005). Cysteine and cysteine-S conjugates have also been found to play an important role in the olfactory perception of fruits and

Table 2 Comparison of human olfactory detection thresholds and taste detection thresholds for 6 amino acids

Stimulus	ODT[a]	TDT[b]	TDT[c]
L-Cysteine	0.20	0.63	1.98
D-Cysteine	0.22	0.85	1.46
L-Methionine	0.08	3.72	12.70
D-Methionine	0.01	5.01	6.50
L-Proline	100	15.1	16.10
D-Proline	75	60.4	60.4

All values are given in mM. ODT, olfactory detection threshold; TDT, taste detection threshold.
[a]Present study.
[b]Schiffman et al. (1981).
[c]Haefeli and Glaser (1990).

vegetables (Starkenmann, Le Calvé, et al. 2008) and in food flavors (Starkenmann, Troccaz, and Howell 2008). Low olfactory detection thresholds for sulfur-containing odorants such as thiols are interpreted as an evolutionary adaptation to the perception of putrefaction processes, that is: the microbial degradation of proteins and thus may help to avoid ingestion of spoiled food (Laska et al. 2007). The present finding that humans are capable of detecting the odors of the L- and D-forms of cysteine and methionine, 2 sulfur-containing amino acids, at μM concentrations is in line with this idea.

Olfactory discriminability of amino acids

The finding that human subjects had little difficulty in discriminating between the odors of the L-forms of cysteine, methionine, and proline supports the notion that a variety of olfactory receptors belonging to the phylogenetic class I which are typical for aquatic animals and which have been shown to interact with amino acids are functional in humans (Sanz et al. 2005). However, the failure to discriminate between the odors of the L- and D-forms of a given amino acid suggests that humans might lack enantioselective receptors for these odorants. Previous studies on the ability of humans to discriminate between the odors of enantiomers other than amino acids found a clear substance-specificity ranging from readily discriminable to completely indiscriminable optical antipodes (Laska and Teubner 1999; Laska 2004). Future studies using the L- and D-forms of other amino acids are needed to clarify whether the present finding may represent a more general phenomenon pertaining to the olfactory discriminability of amino acids.

Not surprisingly, catfish have been shown to readily discriminate between the odors of almost all the proteinogenic L-amino acids and also between the L- and D-forms of cysteine and alanine (Valentincic et al. 2000). Unfortunately, methionine and proline were not tested with regard to chiral discrimination. Similarly, lobsters have been reported to have olfactory receptors that are enantioselective for amino acids (Michel et al. 1993). Thus, the odors of the enantiomers of amino acids are discriminable for some aquatic animals. Interestingly, the human gustatory system appears to be able to distinguish between the enantiomers of amino acids. This notion is supported by the fact that the taste qualities of most of the L- and D-forms of the 20 proteinogenic amino acids have been described with clearly different verbal labels (Schiffman et al. 1981). This suggests human taste receptors responding to amino acids to be enantioselective.

Chemesthetic properties of amino acids

The finding that human subjects failed to correctly localize the side of stimulation with any of the 6 amino acids tested here suggests that the nasal trigeminal system did not contribute to the discrimination of the L-forms of cysteine, methionine, and proline. Given that the odorants were presented at fairly high concentrations that are only rarely found in natural foods it seems unlikely that oral and/or nasal chemesthesis plays a role in flavor perception of these amino acids. However, when used as food additives, amino acids may be present at concentrations higher than the ones employed in the present study and thus the possibility of a trigeminal contribution of amino acids to flavor perception cannot be generally excluded. Here, too, future studies are needed to elucidate whether the present finding may represent a more general phenomenon with regard to the chemesthetic properties of amino acids.

Odor structure-activity relationships

The odors of the sulfur-containing amino acids L- and D-cysteine and L- and D-methionine, respectively, were detected at significantly lower concentrations than the odors of L- and D-proline, which are both lacking sulfur. Electrophysiological recordings from the olfactory mucosa in the catfish (Caprio 1977), the zebrafish (Michel and Lubomudrov 1995), and the hammerhead shark (Tricas et al. 2009) showed that the stimulatory effectiveness of L-cysteine and L-methionine to be much higher compared with L-proline, an interesting parallel to the results of the present study. Similarly, the same order of sensitivity has been reported for the corresponding human taste detection thresholds (see Table 2). However, whether the observed difference in detectability is due to the presence or absence of sulfur in the stimulus molecules or due to the fact that the amino group is secondary in proline, whereas it is primary in cysteine and methionine remains to be answered. The human olfactory detection thresholds for both the L- and the D-form of methionine were lower than those of the L- and D-form of cysteine suggesting that the type of sulfur-containing functional group, a thioether group in the case of methionine and a thiol group in the case of cysteine, may affect the interaction with olfactory receptors and thus detectability. Electrophysiological studies on the properties of fish olfactory

receptors responding to amino acids have found that polarity of the side chain may also affect the ligands' binding affinity (Luu et al. 2004; Nikonov and Caprio 2007b). As all amino acids employed in the present study have a nonpolar side chain, no conclusions as to the possible impact of this molecular feature on detectability or discriminability can be drawn.

Chirality had a substance-specific effect on detectability: whereas the olfactory detection thresholds for L- and the D-forms of cysteine and proline, respectively, did not differ significantly from each other, D-methionine was detected at significantly lower concentrations compared with L-methionine. This is in line with findings from enantiomeric odor pairs other than amino acids where some optical antipodes were found to differ in detectability, whereas others were not (Laska and Teubner 1999; Laska 2004). Furthermore, chirality was not a molecular property allowing for olfactory discrimination of the 2 optical antipodes of a given amino acid.

Taken together, the results of the present study suggest that amino acids may contribute to the flavor of food not only as taste stimuli but also as olfactory stimuli perceived via ortho- or retronasal smelling. Given that the odors of some of the amino acids tested here were detected at concentrations lower than their corresponding taste detection thresholds, this may have important implications for the widespread use of amino acids as food additives as well as for the evaluation of off-flavors caused by amino acids.

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