Gustatory Responses to Amino Acids in the Chorda Tympani Nerve of C3H Mice

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Abstract

Integrated neural responses to various amino acids were recorded from the chorda tympani (facial) nerve in C3H mice. The basic amino acids hydrochlorides L-Arg-HCl and L-Lys-HCl evoked large magnitude integrated taste responses, similar to that for NaCl, and had estimated electrophysiological thresholds of 0.0001 M. No significant difference was indicated between the response magnitudes for the L- and D-forms of the basic amino acid hydrochlorides; however, responses to the basic amino acid hydrochlorides cross-adapted with NaCl. Responses to neutral L-amino acids (Ser, Ala, Gly), which taste sweet to humans, showed higher thresholds (>0.0003 M), similar to that for sucrose, and did not cross-adapt with basic amino acid hydrochlorides or with NaCl. Responses to the neutral amino acids L-Ser and L-Ala were larger than those to their D-amino acid enantiomers. The acidic amino acids L-Asp and L-Glu showed concentration–response functions different from that for HCl. Both acidic amino acids were more stimulatory than HCl at the same pH, although the responses to them were cross-adapted by HCl, indicating a pH effect. A comparison of the stimulatory effectiveness among amino acid derivatives and analogues suggested that the α-amino group is essential for the stimulatory effectiveness of neutral amino acids.

Introduction

Amino acids are often contained in foods in relatively high amounts and are important as chemical cues for feeding behavior in animals. An analysis of taste activity recorded from single geniculate ganglion cells revealed units highly responsive to amino acids in the cat, dog and rat but not the goat (Boudreau et al., 1985). The amino acid units in the dog and rat were responsive to a broad range of amino acids and were also excited by sugars. Electrophysiological thresholds for amino acids estimated from rat chorda tympani recordings varied from 0.0001 to 0.08 M. The lower thresholds were for amino acids with a basic side chain or HCl radical (Cys-HCl) and the higher thresholds were for those amino acids reported by humans as being sweet (Pritchard and Scott, 1982a). Our previous recordings from the chorda tympani of the C3H mouse showed that HCl salts of amino acids with basic side chains and with neutral pH produced robust responses with much lower thresholds (~0.00003 M) than those for neutral amino acids (Harada et al., 1982). Essentially similar results were reported in the ddy mouse (Iwasaki et al., 1985).

In the present experiments, neural response properties of the chorda tympani nerve to various L- and D-amino acids, their analogues and derivatives, including those with acetylated amino groups, were examined in C3H mice. An analysis of the structural contribution of amino acids that have such a large difference in stimulatory effectiveness (i.e. between HCl salts and free base amino acids) has not previously been reported.

Materials and methods

Animals

Twenty-five male and five female mice (C3H) weighing between 22 and 45 g (mean ± SD 30.1 ± 7.3 g) were studied. Each mouse was anesthetized deeply with Nembutal® (Abbott Lab., 50 mg/ml sodium pentobarbital). The anesthetic solution was diluted to one-fifth with mammalian Ringer's solution and injected i.p. at 0.5 ml/100 g body wt. The surgical level of anesthesia was maintained by supplemental doses (0.2 ml/100 g) of diluted Nembutal®.

Surgical procedures

The head of the animal was fixed in a non-traumatic head holder made of Plexiglas and the trachea was cannulated with a polyethylene tubing. An incision was made ventrally along the angle of the left mandible and the CT was dissected from the point of entry into the tympanic bulla down to its branching point from the lingual nerve.

Electrophysiological recording

The exposed CT was placed on a 100 μm Ag–AgCl hook electrode and an indifferent electrode was placed on the inner wall of the wound, both electrodes being soaked in liquid paraffin. The animal was grounded by a 0.5 mm Ag wire attached to the wounded margin. Neural activity from the whole nerve was led to a high-impedance probe (JB-101J; Nihon Kohden) and an A/C amplifier (ABV-11; Nihon Kohden). Amplified signals were monitored on an oscilloscope and an audio monitor, and recorded on a PCM data recorder (RD-111T; TEAC) for later analysis. Responses of the whole nerve were integrated (RC = 0.3 s, EI-600G; Nihon Kohden) and recorded on a chart recorder (WI-641G; Nihon Kohden) at a speed of 1 mm/s.

Stimulation

Taste stimuli were applied to a distilled water (DW) rinse (1 ml/s) through polyethylene tubing (2 mm i.d.) placed adjacent to the anterior portion of the tongue. The rinse constantly flowed over the tongue and was switched to the stimulus solution (SS) for 10 s by a three-way electromagnetic valve controlled by a microcomputer system (PC9801RX; NEC). For the cross-adaptation experiments, DW was switched to the adapting solution (AS), which was applied for 10 s prior to the application of the SS for 10 s. Test stimuli were dissolved in the AS so that the concentration of the adapting substance did not change throughout adaptation and stimulation. Chemical solutions were made with reagent grade chemicals (Sigma Chemicals and Nacalai Tesque, Inc.) in DW. Concentrations of the basic taste stimuli tested were sequential 1/2 log step increases in concentration of sucrose (0.001–1 M), NaCl (0.00001–1 M), HCl and quinine hydrochloride (Q-HCl; 0.00001–0.01 M). A total of nine basic and neutral L-amino acids (Arg-HCl, Lys-HCl, Ala, Met, Gln, Asn, Ser, Val and Cys; 0.00003–0.1 M), two acidic L-amino acids (Asp and Glu; 0.00001–0.01) and their NaCl salts (0.00003–0.01 M) were tested. Test L-amino acids and their analogues and derivatives are listed in Table 1 and 3. Three D-amino acids (Arg-HCl, Ala and Ser) at 0.1 M were also tested. Amino acids, analogues and derivatives were dissolved in DW. With the exceptions of 0.1 M L-Asn (pH 4.0) and the acidic amino acids L-Asp and L-Glu, the pH of each solution was not adjusted since the pH ranged between 5.2 and 7.4, which had little effect. Solutions were prepared weekly and stored at 5°C. Stimuli and rinse solutions were presented to the tongue at 20 ± 1°C.

Data analysis

The magnitude of the phasic portion of the integrated response to 0.1 M NaCl was used as a standard. The standard was applied prior to and after each concentration series and between every three or four tests of other stimuli.
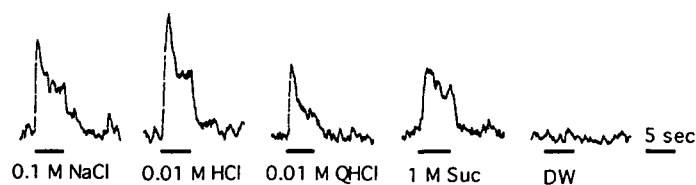

Figure 1   Integrated responses of the CT in a mouse to four taste stimuli representing the four basic taste and DW. Stimuli were applied for 5 s.

The peak height of the initial phasic portion of the integrated response was used as a measure of the response magnitude to each stimulus. For each concentration series the responses were compared with a two-way analysis of variance (stimulation versus concentration). Differences in the responses to the 0.1 M solution were analyzed using the two-tailed t-test.

Results

Responses to the four basic taste stimuli

All four stimuli resulted in robust phasic and tonic responses (Figure 1). A small 'off' response occurred to 1 M sucrose. Mean chorda tympani thresholds to NaCl, HCl and Q-HCl were estimated at 0.00003 M (Figure 2). The magnitude of the responses to the three chemicals increased monotonically with an increase in concentration up to the maximum concentration tested. The threshold for sucrose was 0.01 M. Responses to sucrose increased with increases in concentration above 0.1 M more steeply than those to NaCl, reaching 95.8 ± 18.0% ($n = 5$) relative to that for 0.1 M NaCl (Figure 2).

Responses to basic amino acids

Basic L-amino acids (Arg-HCl and Lys-HCl) evoked robust taste responses that increased with an increase in concentration, reaching at 0.1 M 102.9 ± 8.0% ($n = 5$) and 89.6 ± 6.7% ($n = 5$), respectively, of the response to the standard (Figures 5 and 7, and Table 1). CT thresholds to L-Arg-HCl and L-Lys-HCl were estimated at 0.00003 M, whereas the threshold to L-His free base was 0.00001 M; the response to 0.1 M L-His reached 69.3 ± 6.3% ($n = 4$) of the standard. The concentration–response relations for L-Arg-HCl and L-Lys-HCl were similar to that for NaCl (Figure 3); however, that for L-His free base was significantly different (ANOVA, $P < 0.0001$). The response to L-His-HCl (92.1 ± 6.5%, $n = 6$, pH 3.8) was significantly larger than that to the free base ($P < 0.0002$) L-His, and significantly smaller than that to L-Arg-HCl ($P = 0.0004$) and Lys-HCl ($P = 0.0021$). The pH of the 0.1 M L-Arg-HCl and L-Lys-HCl solutions were both 5.2 (Table 1), and HCl at the same pH was not stimulatory (Figure 8B).

Responses to neutral amino acids

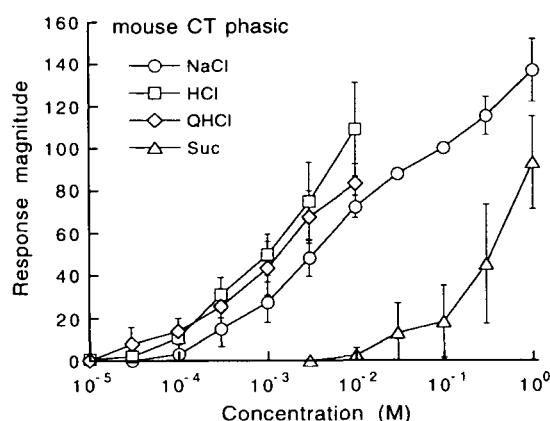Gustatory thresholds to the seven neutral amino acids

Figure 2 Mean concentration–response functions to NaCl, HCl, Q-HCl and sucrose (Suc). Phasic responses are expressed as percentages to the magnitude of phasic responses to 0.1 M NaCl. Error bars show the SD of the mean. Means were calculated from five preparations.
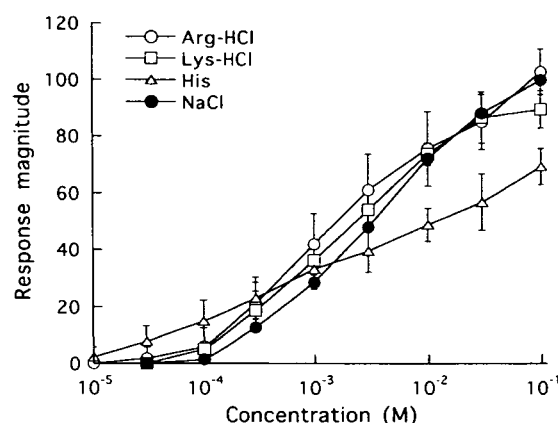

Figure 3 Mean concentration–response functions to three basic amino acids—Arg-HCl, Lys-HCl and His free base—and NaCl. Phasic responses are expressed as percentages to the magnitude of phasic responses to 0.1 M NaCl. Error bars show the SD of the mean. Means were calculated from five preparations.

Table 1 Relative stimulatory effectiveness, pH and pI of 20 L-amino acids tested at 0.1 M except tryptophan, which was tested at 0.05 M

Amino acids	Abbr.	Mean ± SD	n	pH	pI
L-Arginine-HCl	L-Arg-HCl	98.4 ± 10.4	8	5.2	
L-Lysine-HCl	L-Lys-HCl	88.4 ± 8.6	8	5.2	
L-Histidine(free)	L-His	69.9 ± 2.5	4	7.4	7.47
L-Cysteine(free)	L-Cys	83.3 ± 8.4	6	5.2	5.07
L-Serine	L-Ser	78.0 ± 12.2	8	5.5	5.68
L-Alanine	L-Ala	77.9 ± 9.8	8	5.6	6.00
L-Glutamine	L-Gln	75.1 ± 12.9	7	5.4	5.65
Glycine	Gly	64.8 ± 12.6	7	5.8	5.97
L-Methionine	L-Met	61.8 ± 15.0	9	6.1	5.74
L-Threonine	L-Thr	59.7 ± 11.3	6	5.8	5.64
L-Asparagine	L-Asn	59.2 ± 12.1	5	4.0	5.41
L-Valine	L-Val	37.4 ± 8.0	7	5.9	5.96
L-Leucine	L-Leu	29.6 ± 5.0	8	5.7	5.98
L-Phenylalanine	L-Phe	16.5 ± 7.8	5	5.6	5.48
L-Isoleucine	L-Ile	16.5 ± 2.8	5	5.7	5.94
L-Hydroxyproline	L-Hyp	14.3 ± 7.8	5	5.3	5.74
L-Proline	L-Pro	12.7 ± 3.5	6	5.6	6.30
L-Tryptophan	L-Trp	5.7 ± 7.4	6	5.4	5.89

tested ranged between 0.0003 M for L-Ala and 0.001 M for L-Val (Figure 4). Responses to these amino acids increased monotonically with an increase in concentration. A group of neutral L-amino acids (Ser, Ala, Gln, Gly, Thr and Asn) at 0.1 M evoked taste responses ranging between 59.7 and 83.8% of the standard, while other neutral L-amino acids with larger side chains (Val, Leu, Phe, Ile, Hyp, Pro and Trp) at 0.1 M evoked relatively smaller responses, ranging from 5.7 to 37.4% of the standard. The measured pH of aqueous solutions of these neutral amino acids ranged between 4.0 and 6.1 (Table 1). The taste response to L-Cys-HCl (133.1 ± 7.7%, n = 6) was significantly larger (P < 0.000001) than that to the other amino acids tested.

Responses to L- and D- amino acids

There was no significant difference between the responses to L- and D-Arg-HCl, while the response to D-Ala was significantly smaller than that to L-Ala (P = 0.0003); a similar effect as observed for D- and L-Ala occurred for L- and D- Ser (D L, P = 0.000003) (Figures 5 and 6).

Cross-adaptation

Adaptation to 0.05 M NaCl or L-Arg-HCl completely eliminated the phasic responses to 0.05 M L-Lys-HCl, while the response to L-Ser was not depressed (Figure 7). In contrast, adaptation to 0.05 M L-Ala did not depress the response to 0.05 M L-Arg-HCl, while the response to 0.05 M
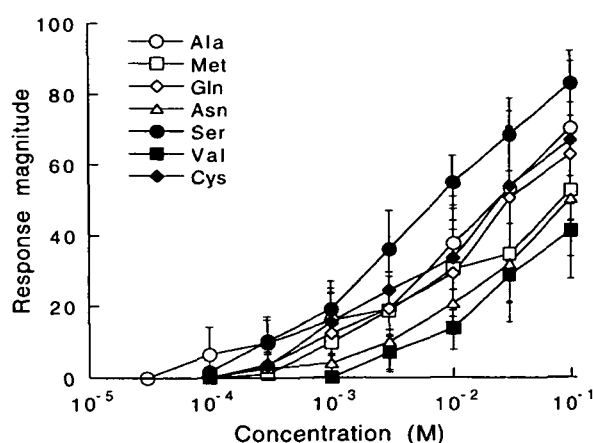
Figure 4 Mean concentration–response functions to seven neutral amino acids. Phasic responses are expressed as percentages to the magnitude of phasic responses to 0.1 M NaCl. Error bars show the SD of the mean. Means were calculated from five preparations.
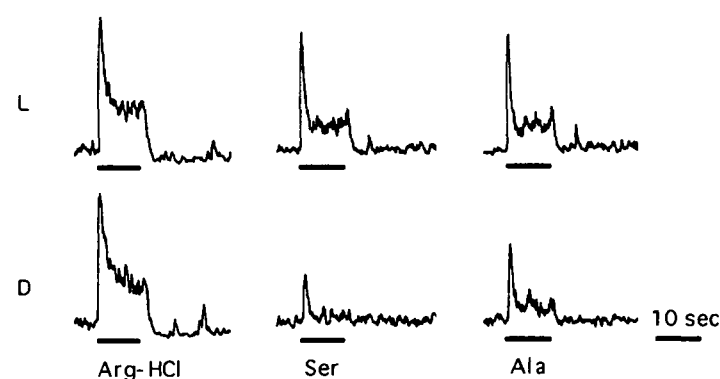

Figure 6 Comparison of mean phasic responses to D- and L-forms of Arg-HCl, Ser and Ala in the CT of a mouse. Error bars show the SD of the mean. Asterisks indicate statistically significant difference between the two forms (two-tailed t-test; $P < 0.0005$).

Figure 5 Integrated responses L- and D-forms of Arg-HCl, Ser and Ala. Stimuli were applied for 10 s.

L-Cys was greatly depressed (Figure 7). NaCl and the basic L-amino acids (Arg-HCl and Lys-HCl) showed self-adaptation, but did not cross-adapt the responses to L-His or to the neutral L-amino acids (Cys, Ser, Ala and Glu) (Table 2). The neutral amino acids, which also showed self-adaptation, did not cross-adapt the responses to NaCl or to the basic amino acids (Table 2).

Responses to amino acids analogues and derivatives

The magnitudes of the gustatory responses to neutral amino acids were affected by the size of the carbon chain. Among five amino acids tested (Table 3A), a significant negative correlation ($r = -0.882$, $P = 0.0499$) was found between molecular weight and relative response magnitudes. Similarly, the response to Ile having a longer side chain at the α-hydrogen was significantly larger ($P = 0.0002$) than that to L-Val (Table 3C). The response to Thr, possessing an OH-group, was significantly larger ($P = 0.0016$) than that to L-Val. As for the position of an amino group in the
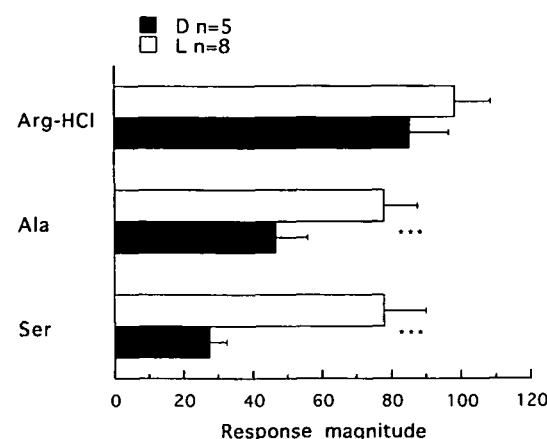
molecule, the response to β-Ala was significantly larger ($P = 0.0124$) than that to β-Ala, but there was no significant difference between the responses to α-aminobutyric acid and GABA (Table 3B). The response to N-acetyl-L-Ala was significantly larger than that to L-Ala, while no significant difference was observed between the response to N-acetyl-L-Cys and L-Cys (Table 3D). The structure of the side chain in simple neutral amino acids (L-Ala, L-Ser and L-Cys) had little effect on stimulatory effectiveness (Table 3E).

Responses to acidic amino acids

Approximate thresholds for L-Asp and L-Glu were 0.00001 and 0.00003 M respectively. Responses to both acidic amino acids increased monotonically with increases in concentration (Figure 8A). The concentration–response function for L-Asp was significantly different from those for L-Glu and HCl (ANOVA, $P = 0.0223$ and 0.0267 respectively), and the response function for L-Glu was significantly different from that for HCl (ANOVA, $P = 0.0009$). The pH versus response function showed that the responses to L-Asp and Glu were larger than that to HCl (Figure 8B). Adaptation to 0.005 M HCl depressed the taste responses to L-Asp and L-Glu to 15 and 33% respectively (Figure 10). The gustatory threshold to L-Asp-Na was 0.00003 M and that to L-Glu-Na was 0.0001 M, the same as for NaCl (Figure 9). Responses to both acidic amino acid NaCl salts increased monotonically with increases in concentration. The concentration–response function for each NaCl salt was significantly different from that for NaCl (ANOVA, L-Asp-Na, $P = 0.0018$; L-Glu-Na, $P = 0.0034$). Compared at 0.1 M, the response to L-Glu-Na was smaller than that to both L-Asp-Na ($P = 0.0011$) and L-Glu-Na ($P = 0.0063$). Adaptation to 0.05 M NaCl did not completely depress the phasic responses to L-Asp-Na (44%) and L-Glu-Na (33%) (Figure 10).
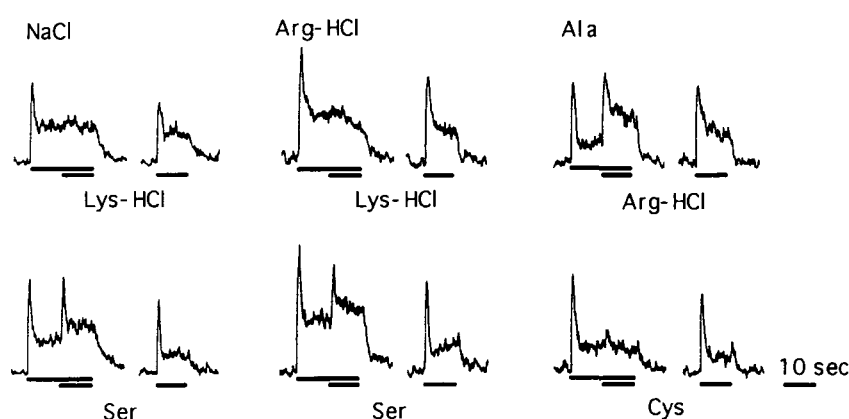
Figure 7 Integrated responses of the CT in a mouse in a cross-adaptation experiment with basic and neutral L-amino acids, and NaCl. Each stimulus was at 0.05 M applied for 10 s. The adapting solution was applied for 10 s prior to the stimulation before switching to the stimulus solution for 10 s. Since the stimulus substance was solved in the adapting solution, concentration of the adapting substance did not change throughout the adaptation and stimulation.

Table 2 Cross-adaptation effects among NaCl, and basic and neutral L-amino acids at 0.05 M

Adaptation	Test stimulus											
	NaCl	Arg-HCl	Lys-HCl	His	Cys	Ser	Ala	Gln	Gly	Thr	Asn	Met
NaCl		+	+	+	− −	− −	− −	−	−	−	−	−
Arg-HCl	±		+	+	±	−	±	±	±	±	−	±
Lys-HCl	+	+		+	−	− −	− −	±	−	−	−	− −
His	−	−	±		±	±	−	− −	−	±	±	−
Cys		−	− −	− −		+	+	+	+	+	±	+
Ser	− −	− −	− −	− −	+		+	+	+		+	+
Ala	− −	− −	−	−	+	+		+	+	+	+	+
Gln	− −	− −	−	− −		+	+		+	+	+	+

A phasic response magnitude of testing stimuli during an adaptation was measured and the percentage to the phasic response without adaptation was calculated. The ratios were scored by 20 steps: − − (80–100), − (60–79), + (40–59), + (20–39), + + (0–19).

Discussion

Chemical stimulation applied to the anterior tongue of the C3H mouse produced robust integrated neural responses in the CT. Among the four basic taste stimuli, NaCl was the most stimulatory and produced the most evident phasic and tonic responses. The estimated electrophysiological threshold of the CT to NaCl was 0.0001 M, which is similar to that observed in the ddy mouse (Iwasaki and Sato, 1984), rat (Pfaffman et al., 1967; Harada et al., 1997) and hamster (Harada and Smith, 1992). The low thresholds and large taste response magnitudes to basic amino acid HCl salts in the present experiments were also observed in the ddy mouse (Iwasaki et al., 1985) and rats (Pritchard and Scott, 1982a). Such high sensitivities to these compounds in the present report cannot be ascribed to the acidity of the solutions, as the pH for both the L-Arg-HCl and the L-Lys-HCl taste solution tested was 5.2, a pH which does not produce taste activity in the chorda tympani in C3H mice when tested with HCl. Although the stimulatory effectiveness of the basic amino acids free base (pH

10.2–11.0) was small, the response increased dramatically upon lowering the pH down to 5.2 by titration with various acids (Harada et al., 1983).

Cross-adaptation effects between L-His free base and NaCl were rather small despite the observation that basic amino acid hydrochlorides cross-adapted well to NaCl. Also the rat geniculate ganglion did not respond to three free base 0.05 M L-His and L-Lys (Boudreau et al., 1983), while single fiber analysis in the rat CT indicated that L-Arg-HCl showed a similarity to the response to NaCl (Pritchard and Scott, 1982b). These results suggest that basic amino acids may have different stimulatory mechanisms prominent at different pHs. The finding that there was no significant difference between the responses to L- and D-basic amino acid hydrochlorides also suggest that the effectiveness of basic amino acid hydrochlorides may be related to the extent of their ionic charge rather than to their three-dimensional structure. The high stimulatory effectiveness of the basic amino acid hydrochloride may therefore be attributed to an

Table 3 Relationships between structure and stimulatory effectiveness among amino acids and analogues at 0.1 M and relative response magnitudes to 0.1 M NaCl

Compound	Structure	Mean ± SD	n	Mol. wt
(A) Number of carbon atoms				
Gly	$CH_2(NH_2)COOH$	64.8 ± 12.6	7	75.1
Ala	$CH_3CH(NH_2)COOH$	77.9 ± 9.8	8	89.1
α-Aminobutyric acid	$CH_3CH_2CH(NH_2)COOH$	49.4 ± 18.7	6	103.1
Norvaline	$CH_3(CH_2)_2CH(NH_2)COOH$	29.4 ± 8.9	2	117.2
Norleucine	$CH_3(CH_2)_3CH(NH_2)COOH$	28.4 ± 16.1	5	131.2
(B) Position of the amino group				
α-Ala	$CH_3CH(NH_2)COOH$	77.9 ± 9.8	8	89.1
β-Ala	$CH_3(NH_2)CHCOOH$	54.9 ± 11.8	10	89.1
α-Aminobutyric acid	$CH_3CH_2CH(NH_2)COOH$	49.5 ± 18.7	6	103.1
γ-Aminobutyric acid	$H_2NCH_2CH_2CH_2COOH$	41.8 ± 12.7	6	103.1
(C) Substitution at the β-hydrogen				
Val	CH_3 \ CH_3 / $CHCH(NH_2)COOH$	37.4 ± 8.0	7	117.2
Thr	CH_3 \ OH / $CHCH(NH_2)COOH$	59.7 ± 11.3	6	119.1
Ile	CH_3CH_2 \ CH_3 / $CHCH(NH_2)COOH$	16.5 ± 2.8	5	131.2
(D) Acetylation at the α-amino group				
Ala	$CH_3CH(NH_2)COOH$	77.9 ± 9.8	8	89.1
N-Acetyl-L-Ala	$CH_3CH(NHCOCH_3)COOH$	94.4 ± 19.7	6	131.1
Cys	$HSCH_2CH(NH_2)COOH$	83.8 ± 8.4	6	121.2
N-Acetyl-L-Cys	$HSCH_2CH(NHCOCH_3)COOH$	74.7 ± 11.2	2	163.2
(E) Terminal group				
Ala	$CH_3CH(NH_2)COOH$	77.9 ± 9.8	8	89.1
Ser	$HOCH_2CH(NH_2)COOH$	78.0 ± 12.2	8	105.1
Cys	$HSCH_2CH(NH_2)COOH$	83.8 ± 8.4	6	121.2
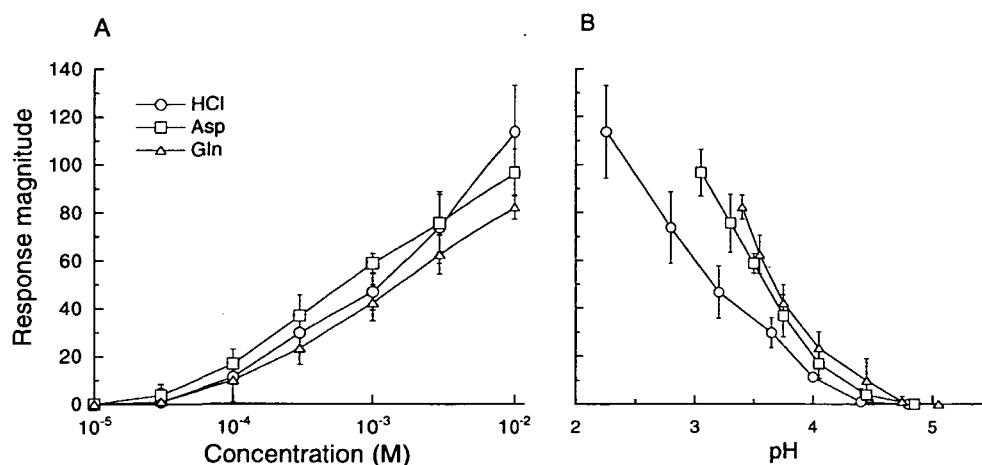
Figure 8 **(A)** Mean concentration–response functions to two acidic amino acids and HCl. **(B)** Replot of (A) by the pH–response function. Phasic responses are expressed as percentages to the magnitude of phasic responses to 0.1 M NaCl. Error bars show SD of the mean. Means were calculated from five preparations.
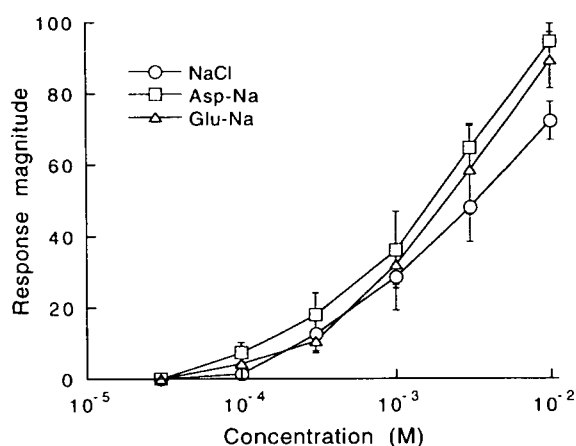
Figure 9   Mean concentration–response functions to NaCl and two Na salts of acidic amino acids. Phasic responses are expressed as percentages to the magnitude of phasic responses to 0.1 M NaCl. Error bars show the SD of the mean. Means were calculated from five preparations.
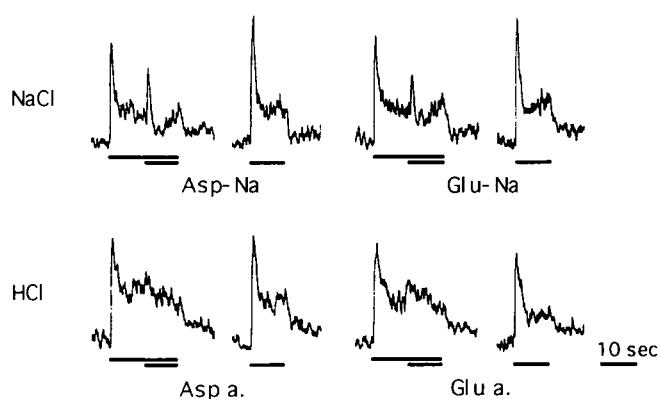

Figure 10   Integrated responses in an adaptation effects of 0.1 M NaCl to 0.1 M L-Asp-Na and L-Glu-Na, and 0.01 M HCl to 0.01 M Asp and L-Glu. The adapting solution applied for 10 s prior to the stimulation before switching to the stimulus solution for 10 s. Since the stimulus substance was dissolved in the adapting solution, the concentration of the adapting substance did not change throughout the adaptation and stimulation.

increase in the ratio of molecules possessing a charged amino group (Tierney and Atema, 1988).

In contrast to the highly stimulatory effectiveness in neural responses, a two-bottle preference test showed that L-Lys-HCl solution was not discriminated from DW by either the ddy mouse (Iwasaki et al., 1985) or the rat (Pritchard and Scott, 1982a) until the amino acid concentration was 0.1 M. Similarly, basic amino acids at pH 6.0 are tasteless in man (Solms et al., 1965). On the other hand, behavioral experiments in mice employing a conditioned taste aversion paradigm (Harada et al., 1987) revealed that the taste of a basic amino acid hydrochloride generalized to other basic amino acid hydrochloride and Q-HCl solutions but not to NaCl. The previous behavioral experiments also revealed that the taste of Q-HCl generalized to basic amino

acid hydrochlorides but not to NaCl. Further, taste aversion conditioning to NaCl resulted in the rejection of only NaCl itself. These behavioral data suggest that the gustatory neural responses in mice to the basic amino acid hydrochlorides contain a taste component similar to that for Q-HCl, despite the rather small neural response, that produces a quite different perceived taste from that for NaCl. This may be supported by the results from psychophysical experiments that show the bitter taste component for 0.1 M L-Arg was rather higher than the other taste components in humans (Stone, 1967).

The CT may play a more important role in the discrimination of the taste of sucrose in mice than it does in the rat and hamster. The magnitude of the integrated CT taste response to 1 M sucrose in the C3H mouse (present report) and in the ddy mouse (Iwasaki and Sato, 1984) was 80% of that to 0.1 M NaCl, whereas in the CT of the rat (Pfaffman et al., 1967; Harada et al., 1997) and hamster (Harada and Smith, 1992) the response to 1 M sucrose was <50% of the response to 0.1 M NaCl. However, the GSP nerve that innervates palatal taste buds in the rat (Nejad, 1986; Harada et al., 1997) and hamster (Harada and Smith, 1992) responds to sucrose significantly greater than does the CT. In support of these electrophysiological findings, behavioral experiments in the rat and hamster confirmed that the GSP is important for the discrimination of sucrose taste (Krimm et al., 1987; Harada, 1992).

Thresholds for neutral L-amino acids were >0.0003 M, one log unit higher than those for basic L-amino acid HCl salts. With the exception of L-Cys, the more stimulatory L-amino acids (Ser, Ala, Glu and Gly) either produce a sweet taste sensation or are tasteless to humans (Solms et al., 1965; Solms, 1969) and are preferred by mice (Iwasaki et al., 1985; Kasahara et al., 1987) and rats (Pritchard and Scott, 1982a). Individual neutral L-amino acids cross-adapt taste responses to other neutral amino acids but had little effect on taste responses to either basic L-amino acid HCl salts or to NaCl. Similarly, results of conditioned taste aversion studies in mice suggested that neutral amino acids which produce a sweet taste in humans produce a sensation similar to that for sucrose (Harada et al., 1987). The high gustatory responsiveness to these neutral amino acids in C3H mice (present study) and in ddy mice (Iwasaki et al., 1985) may therefore be related to a pleasant (i.e. sweet) taste. According to a molecular theory on the structural feature required for sweet substances (Kier, 1972), sweet amino acid molecules may have a third structural feature relative to the A-H/B feature (Shallenberger and Acree, 1967). Taste responses in the present experiments to 0.1 M D-Ala and D-Ser were significantly smaller than to their L-forms, indicating that the taste response resulting in a sweet sensation is affected by the three-dimensional structure of the stimulus.

It has commonly been observed that as the carbon chain length increases in a homologous series, an initially sweet amino acid stimulus gradually becomes either bitter or

tasteless; carbon chain branching can also have a similar effect (Schiffman *et al.*, 1981; Venanzi, 1984). Similarly, a significant negative correlation ($r = -0.882$, $P = 0.0499$) was found in the present experiment between molecular weight and the taste response magnitude for the five L-amino acids and analogues tested. The low-molecular-weight neutral amino acids are relatively potent taste stimuli in mice in this experiment and others (Iwasaki *et al.*, 1985), and are perceived preferable (Iwasaki *et al.*, 1985) or as having a taste similar to sucrose (Harada *et al.*, 1987). The loss of neural responsiveness with increasing molecular weight of these amino acid may related to the concomitant decrease in perceived sweetness.

L-Val, which has one fewer methyl-group in the side chain than L-Ileu, was more stimulatory than L-Ileu. Although L-Thr was a more potent electrophysiological stimulus than L-Val, this effect may have been influenced by the OH-group present in Thr. Similarly, the OH-group in L-Ser and the SH-group in L-Cys may be important for producing the same response magnitude as observed for the lower-molecular-weight L-Ala.

The position of the amino group in an amino acid has little effect on the magnitude of the integrated taste response except for the response to α- and β-Ala, where the response to α-Ala was significantly greater than that to β-Ala. Although substitution at the α-amino group resulted in the significant decline of neural responses to L-Ala and L-Cys in fish (Marui *et al.*, 1983), acetylation of the α-amino group of L-Ala and L-Cys showed little effect on the responses to these L-amino acids in the mouse CT. These results suggest that the taste effectiveness of the amino group in neutral amino acids may not depend on the charge of the amino group, which is fundamentally different in basic amino acids.

Although one of the acidic amino acids, L-Glu, has been extensively studied as a component in the 'umami' taste in humans (Yoshii *et al.*, 1982), their basic character of stimulatory effectiveness as acidic organic compounds has yet to be examined thoroughly. The taste response magnitudes in the rat CT for acids at the same pH decrease in the order: acetic > formic > lactic > oxalic > HCl (Beidler, 1967). This relationship also holds for the order of intensity for sour taste in humans (Kurihara and Beidler, 1969). The concentration–response function for L-Asp and L-Glu were significantly different from that for HCl. Comparison of the pH–response function for their acidic HCl salts revealed that these compounds were more stimulatory than HCl at the same pH. Responses to the Na salts of these two acidic amino acids were significantly larger than that to NaCl. Adaptation to NaCl did not completely suppress the response to L-Asp-Na or to L-Glu-Na, suggesting a difference in the receptor mechanism for Na$^+$ and L-Asp or L-Glu. Acidic amino acids have a prominent sour taste, but salty and bitter sensations were also perceived in a psychophysical experiment in humans (Stone, 1967). Those non-acidic taste

components in the integrated responses to acidic amino acids may relate to the unsuppressed responses for L-Asp-Na or L-Glu-Na by the adaptation to NaCl.

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References

Beidler, L.M. (1967). *Anion influences on taste receptor response.* In Hayashi, T. (ed.), Olfaction and Taste II. Pergamon Press, Oxford, pp. 509–534.

Boudreau, J.C., Hoang, N.K., Oravec, J. and **Do, L.T.** (1983) *Rat neurophysiological taste responses to salt solutions.* Chem. Senses, 8, 131–150.

Boudreau, J.C., Sivakumar, L., Do, L.T., White, T.D., Oravec, J. and **Hoang, N.K.** (1985) *Neurophysiology of geniculate ganglion (facial nerve) taste systems: species comparisons.* Chem. Senses, 10, 89–127.

Harada, S. (1992) *Effects of transection of the greater superficial petrosal and the chorda tympani nerves on conditioned taste aversion to sucrose in the hamster.* Jpn. J. Oral Biol., 34, 690–700.

Harada, S. and **Smith, D.V.** (1992) *Gustatory sensitivities of the hamster's soft palate.* Chem. Senses, 17, 37–51.

Harada, S., Marui, T. and **Kasahara, Y.** (1982) *Amino acids as taste stimuli in the mouse.* In Proceedings of the 16th Japanese Symposium on Taste and Smell, Tokyo, pp. 123–126.

Harada, S., Marui, T. and **Kasahara, Y.** (1983) *Gustatory stimulatory effectiveness of basic amino acids in rats and mouse.* Proceedings of the 17th Japanese Symposium on Taste and Smell, Kagoshima, pp. 61–64.

Harada, S., Marui, T. and **Kasahara, Y.** (1987). *Neural and behavioral responses to amino acids in mice and rats.* In Roper, S.D. and Atema, J. (eds), Olfaction and Taste IX. New York Academy of Science, New York, pp. 345–346.

Harada, S., Yamamoto, T., Yamaguchi, K. and **Kasahara, Y.** (1997) *Different characteristics of gustatory responses between the greater superficial petrosal and chorda tympani nerves in the rat.* Chem. Senses, 22, 133–140.

Iwasaki, K., Kasahara, T. and **Sato, M.** (1985) *Gustatory effectiveness of amino acids in mice: behavioral and neurophysiological studies.* Physiol. Behav., 34, 531–542.

Iwasaki, K. and **Sato, M.** (1984) *Neural and behavioral responses to taste stimuli in the mouse.* Physiol. Behav., 32, 803–807.

Kasahara, T., Iwasaki, K. and **Sato, M.** (1987) *Taste effectiveness of some D- and L-amino acids in mice.* Physiol. Behav., 39, 619–624.

Kier, L.B. (1972) *A molecular theory of sweet taste.* J. Pharm. Sci., 61, 1394–1397.

Krimm, R.F., Nejad, M.S., Smith, J.C., Miller, I.J. and **Beidler, L.M.** (1987) *The effect of bilateral sectioning of the chorda tympani and the greater superficial petrosal nerves on the sweet taste in the rat.* Physiol. Behav., 41, 495–501.

Kurihara, K. and **Beidler, L.M.** (1969) *Mechanism of the action of taste-modifying protein.* Nature, 222, 1176–1179.

Marui, T., Harada, S. and **Kasahara, Y.** (1983) *Gustatory specificity for amino acids in the facial taste system of the carp,* Cyprinus carpio L. J. Comp. Physiol., 153, 299–308.

Nejad, M.S. (1986) *The neural activities of the greater superficial petrosal*

nerve of the rat in response to chemical stimulation of the palate. Chem. Senses, 11, 283–293.

Pfaffman, C., Fisher, G.L. and **Frank, M.K.** (1967). The sensory and behavioral factors in taste responses. In Hayashi, T. (ed.), Olfaction and Taste II. Pergamon Press, Oxford, pp. 361–381.

Pritchard, T.C. and **Scott, T.R.** (1982a) Amino acids as taste stimuli. I. Neural and behavioral attributes. Brain Res., 253, 81–92.

Pritchard, T.C. and **Scott, T.R.** (1982b) Amino acids as taste stimuli. II. Quality coding. Brain Res., 253, 93–104.

Schiffman, S.S., Sennewald, K. and **Gagnon, J.** (1981) Comparison of taste qualities and thresholds of D- and L-amino acids. Physiol. Behav., 27, 51–59.

Shallenberger, R.S. and **Acree, T.E.** (1967) Molecular theory of sweet taste. Nature, 216, 480–482.

Solms, J. (1969) The taste of amino acids, peptides, and proteins. J. Agric. Food Chem., 17, 686–688.

Solms, J., Vuataz, L. and **Egli, R.H.** (1965) The taste of L- and D-amino acids. Experientia, 21, 692–694.

Stone, H. (1967). Gustatory response to L-amino acids in man. In Hayashi, T. (ed.), Olfaction and Taste II. Pergamon Press, Oxford, pp. 289–306.

Tierney, A.J. and **Atema, J.** (1988) Amino acid chemoreception: effects of pH on receptors and stimuli. J. Chem. Ecol., 14, 135–141.

Venanzi, T.J. (1984) Hydrophobicity parameters and the bitter taste of L-amino acids. J. Theor. Biol., 111, 447–450.

Yoshii, K., Yoshii, C., Kobatake, Y. and **Kurihara, K.** (1982) High sensitivity of Xenopus gustatory receptors to amino acids and bitter substances. Am. J. Physiol., 243, R42–R48.