Chem. Senses 24: 449–457, 1999

The Taste of Monosodium Glutamate (MSG), L-Aspartic Acid, and *N*-Methyl-D-aspartate (NMDA) in Rats: Are NMDA Receptors Involved in MSG Taste?

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Abstract

Monosodium glutamate (MSG) is believed to elicit a unique taste perception known as umami. We have used conditioned taste aversion assays in rats to compare taste responses elicited by the glutamate receptor agonists MSG, L-aspartic acid (L-Asp), and *N*-methyl-D-aspartate (NMDA), and to determine if these compounds share a common taste quality. This information could shed new light upon the receptor mechanisms of glutamate taste transduction. Taste aversions to either MSG, L-Asp or NMDA were produced by injecting rats with LiCl after they had ingested one of these stimuli. Subsequently, rats were tested to determine whether they would ingest any of the above compounds. The results clearly show that a conditioned aversion to MSG generalized to L-Asp in a dose-dependent manner. Conversely, rats conditioned to avoid L-Asp also avoided MSG. Conditioned aversions to MSG or L-Asp generalized to sucrose when amiloride was included in all solutions. Importantly, aversions to MSG or L-Asp did not generalize to NMDA, NaCl or KCl, and aversions to NMDA did not generalize to MSG, L-Asp, sucrose or KCl. These data indicate that rats perceive MSG and L-Asp as similar tastes, whereas NMDA, NaCl and KCl elicit other tastes. The results do not support a dominant role for the NMDA subtype of glutamate receptors in taste transduction for MSG (i.e. umami) in rats.

Introduction

Free amino acids are present in many foods and are significant gustatory stimuli. In humans, amino acids elicit complex tastes, including sweetness, saltiness, sourness and bitterness (Schiffman *et al.*, 1981). Glutamate is also an important amino acid in many foods and is normally present in the form of the monosodium salt, monosodium glutamate (MSG). MSG is believed to elicit a taste that is distinct from sweet, sour, salty or bitter and is known as umami (Kawamura and Kare, 1986), a Japanese term roughly translated as 'good taste'. It is likely that, at the level of sensory receptor cells, signal transduction for amino acid taste responses involves membrane receptors on the apical, chemosensitive tips of taste bud cells. Researchers have searched intensely for amino acid receptors in taste buds, and the receptors for MSG taste in particular. Several approaches have been taken to investigate putative receptors for MSG in taste buds, including receptor binding assays (Torii and Cagan, 1980; Cagan, 1986), animal behavioral studies (Ninomiya and Funakoshi, 1989a; Yamamoto *et al.*, 1991; Chaudhari *et al.*, 1996), electrophysiological recordings (Ninomiya and Funakoshi, 1989b; Adachi and Aoyama, 1991; Brand *et al.*, 1991; Faurion, 1991; Hellekant and Ninomiya, 1991; Kumazawa *et al.*, 1991; Nishijo *et al.*, 1991; Ninomiya *et al.*, 1992; Plata-Salaman *et al.*, 1992; Rolls *et al.*, 1996; Bigiani *et al.*, 1997; Hellekant *et al.*, 1997), Ca^{2+}-imaging (Hayashi *et al.*, 1996) and molecular biological experiments (Chaudhari *et al.*, 1996). One approach has been to search for ligands other than MSG that mimic taste responses to glutamate and that might, collectively, define a pharmacological profile for the putative receptor(s) responsible for this unique taste. This strategy was applied in a study where taste-evoked activity in the chorda tympani nerve in hamsters was recorded in response to stimulating the tongue with glutamate receptor agonists, including L-aspartic acid (L-Asp) (Faurion, 1991). Prior to that report, it was known from human studies that L-Asp somewhat resembles the taste of MSG (Maga, 1983), and it was believed that L-Asp activated ionotropic glutamate receptors of the *N*-methyl-D-aspartate (NMDA) subtype. Faurion (Faurion, 1991) concluded that MSG taste was transduced by more than one type of glutamate receptor, including NMDA-like and non-NMDA-like sites. Electrophysiological recordings from lipid bilayer membranes in which membranes from mouse lingual tissue had been incorpor-

ated also suggested that NMDA receptors were present in taste buds (Brand *et al.*, 1991). More recently, however, studies at the molecular biological level showed that a class III metabotropic glutamate receptor, mGluR4, is expressed in rat taste buds and not in surrounding non-taste cells, and may transduce MSG taste (Chaudhari *et al.*, 1996). Furthermore, rat behavioral experiments carried out in this same study showed that agonists for mGluR4 mimicked MSG taste, supporting the interpretation that mGluR4 is involved in taste transduction for MSG. Human psychophysical studies have also implicated a class III metabotropic glutamate receptor(s) in taste transduction for glutamate (Kurihara and Kashiwayanagi, 1998). This combination of molecular biological, animal behavioral and human psychophysical studies provides strong support for the hypothesis that a metabotropic glutamate receptor, and in particular mGluR4, functions as a taste receptor in gus- tatory sensory organs.

The fact remains, however, that the NMDA receptor agonist L-Asp elicits a taste that is described as umami and elicits impulses in the chorda tympani nerve of experimental animals (Faurion, 1991). This conundrum of an NMDA receptor agonist mimicking what is postulated to be a metabotropic receptor mechanism might be explained by what is now recognized as the rather widespread actions of L-Asp on glutamate receptor subtypes, including metabotropic glutamate receptors (Littman and Robinson, 1994; Littman *et al.*, 1995). In an attempt to determine if ionotropic glutamate receptors of the NMDA subtype play a role in taste transduction for MSG and to lay the foundation for future molecular and cellular studies, we re-examined taste responses in rats to solutions of MSG, NMDA and L-Asp. Specifically, we used conditioned taste aversion (CTA) assays to determine whether L-Asp and NMDA mimic the taste of MSG in rats. Our findings question whether NMDA receptors play a principal role in MSG taste in rats.

Experiment 1

Although MSG has been studied extensively in CTA experiments, less is known about the taste perception in rats of L-Asp and NMDA. In particular, little is known about what concentrations of these compounds rats will ingest and recognize as salient stimuli. Thus, the first experiment was designed to determine appropriate concentrations of MSG, L-Asp and NMDA to use in CTA assays. Experiment 1 establishes a dose–response function for each taste stimulus that allowed us to select appropriate concentrations in subsequent experiments.

Materials and methods

Subjects

Male albino Sprague–Dawley rats (n = 36) were obtained from Harland Sprague–Dawley (Indianapolis, IN). At the beginning of the experiment these animals were 70–90 days old, weighed ~300–350 g and were housed individually with Purina Lab chow available *ad libitum*. Beginning 7 days before the experiment, naive rats were placed on a 21.5 h water deprivation schedule that was maintained throughout the experiment. The colony lighting was regulated according to a 12 h light/dark schedule with the lights turned on at 7.30 a.m. All rats were tested during the light portion of the cycle.

Apparatus

All conditioning and testing took place in a computer-controlled Davis MS80 Lickometer system (DiLog Instruments, Tallahassee, FL) with an enclosed Plexiglas operant chamber. An oval-shaped opening covered by a computer-operated shutter was located in the wall at one end of the chamber. Taste solutions were placed in 50 ml centrifuge tubes fitted with stainless steel spouts and mounted on an eight-bottle moveable platform behind the oval-shaped opening. Rats had access to a taste stimulus when the shutter was opened. During a stimulus presentation, the tip of the stimulus delivery spout was positioned 3 mm behind the center of the opening. When the rat licked from the metal spout, a 64 nA contact current was counted and the rate of licking was recorded. To minimize potential olfactory cues, air flowed into the operant chamber from a tube mounted on the far wall of the chamber and out of the chamber through the opening where the delivery spout was located. Masking noise (75 ± 5 dB; Radio Shack Sleep Machine) was present during all sessions. A white incandescent light provided 30 ± 5 lx illumination inside the operant chamber.

Procedures

Behavioral training and testing sessions were carried out in the Davis Lickometer for 7 days consecutively, as follows. During the first 3 days of the experiment, rats were trained to drink water in the Davis Lickometer. Each 32-trial session lasted ~15–20 min. Rats were given up to 60 s to begin a trial before the shutter was closed and the next delivery spout was positioned for a new trial. The rat initiated a trial by making contact with the delivery spout. Each trial lasted 10 s and was followed by a 5 s intertrial interval. Half an hour after the end of the session, each rat was given access to a water bottle for an additional 1.5 h.

The animals were randomly divided into six groups (six rats per group). Two groups were assigned to each of three taste stimuli for conditioning: 100 mM MSG, 100 mM L-ASP or 50 mM NMDA. The concentrations of each conditioned stimulus (CS) were selected based on prior studies (Chaudhari *et al.*, 1996) and because they produced similar reliability and strength of conditioning during pilot studies. On the fourth day, rats were presented with their respective CS. During the conditioning session, the CS was presented at least 14 times. At least one water rinse trial separated each stimulus presentation. In this and all subsequent

conditioning and testing sessions for this study, all solutions, including water presentations, contained amiloride (30–50 μM). Amiloride at these concentrations does not elicit responses and was included to reduce potentially confounding taste responses elicited by Na^+ (Heck *et al.*, 1984; Chaudhari *et al.*, 1996). Immediately after the conditioning session, one group of rats (experimental) received i.p. injections of 0.3 M LiCl (127 mg/kg, 1 ml/100 g body wt) to induce gastric distress. This procedure produces a conditioned aversion to the taste stimulus (Nachmann and Ashe, 1973; Spector and Grill, 1988; Chaudhari *et al.*, 1996). The other group (control) received i.p. injections of 0.15 M NaCl (1 ml/100 g body wt). Trials on days 5 and 6 served as water-only sessions in the Davis Lickometer to allow the animal to recover from any ill effects of the conditioning procedure.

On the seventh day, a series of seven taste stimuli and water was presented to each animal to test whether a taste aversion to the CS had been established, and if so, to which concentrations. Animals conditioned to avoid MSG or L-ASP were presented with 0.15, 1.5, 15, 50 and 150 mM of their respective CS (i.e. MSG or L-ASP respectively). Animals conditioned to avoid NMDA were tested with 0.05, 0.5, 5 and 25 mM NMDA and 150 mM MSG. In addition, all animals were tested with 100 mM sucrose, 20 mM KCl and deionized water. As during conditioning, every taste stimulus, including water, contained amiloride. KCl was employed as a negative control to ascertain whether or not rats were simply avoiding any unknown taste stimulus after conditioning ('dirty water effect') (Spector and Grill, 1988). Prior CTA data (unpublished) indicated that 20 mM KCl is at or slightly above the threshold under the conditions of our experiments and thus is detectable by rats. All experimental solutions were presented twice in the testing session. The order of stimulus presentations to each rat was randomized using a Latin square design. Solutions of NMDA and L-ASP were adjusted to pH 6.5–7.0 with NaOH. Procedures for presenting these stimuli were the same as those used during conditioning.

Data analyses

The lick rates of each rat during the test session were converted to percent scores to normalize the drinking data for statistical analysis. The mean lick rate for each test stimulus was divided by the mean lick rate for all water presentations in that session, then multiplied by 100. Data for each CS were subjected to a two-way analysis of variance (ANOVA) for mixed designs, then further analyzed by simple effects tests where appropriate. Where the results of simple effects tests are reported, the degrees of freedom reported for the denominator represent f' (Howell, 1997), a conservative estimate of degrees of freedom that corrects for potential heterogeneous sources of error in mixed designs.

Results

MSG as the CS

Simple effects tests showed that rats injected i.p. with LiCl after ingesting 100 mM MSG (experimental animals) significantly suppressed their rate of licking to all concentrations of MSG when compared with control animals (rats injected i.p. with NaCl) [$F(1,78) = 5.41$, 5.60 ($P < 0.025$), 12.62, 31.75 and 50.50 ($P < 0.001$) for 0.15, 1,5, 15, 50 and 150 mM MSG respectively]. Licking rates decreased in a dose-dependent manner to increasing concentrations of MSG (Figure 1). Interestingly, rats conditioned to avoid MSG also showed significant suppression to 100 mM sucrose [$F(1,78) = 47.49$, $P < 0.001$].

L-Asp as the CS

Similarly, rats conditioned to avoid L-Asp subsequently showed significant concentration-dependent suppression in drinking for all concentrations of L-Asp except 0.15 mM [all $F(1,30) = 9.12$, $P < 0.01$ or greater]. These rats also suppressed their drinking behavior significantly when presented with 100 mM sucrose solution [$F(1,30) = 19.18$, $P < 0.001$].

NMDA as the CS

Rats conditioned to avoid NMDA had a significantly lower rate of licking than control rats when presented with 5 and 25 mM NMDA [$F(1,53) = 10.83$, $P < 0.005$; $F(1,53) = 31.64$, $P < 0.001$ respectively] but not at lower concentrations (0.05 and 0.5 mM; Figure 1). However, the ingestion of the other stimuli, including sucrose, was not significantly affected by NMDA conditioning procedures (also see experiment 4 and Table 1).

To summarize, results of the first experiment showed that rats readily acquire a concentration-dependent conditioned aversion to MSG, L-Asp and NMDA in the presence of amiloride. These data also suggest that equimolar MSG and L-Asp have similar taste intensities. As previously reported (Yamamoto *et al.*, 1991; Chaudhari *et al.*, 1996), an aversion to MSG generalized to sucrose. A conditioned aversion to L-Asp also generalized to sucrose. These results also show that rats easily detect NMDA at concentrations of 5 mM and greater, and, importantly, a conditioned aversion to NMDA did not generalize to sucrose.

Experiment 2

Experiment 2 was designed to examine the key question of whether a conditioned aversion to MSG would generalize to L-Asp and, conversely, whether a conditioned aversion to L-Asp would generalize to MSG. That is, does the taste of L-Asp mimic that of MSG and vice versa? To answer this question, rats were presented with their respective CS as well as the other amino acid during the test session.

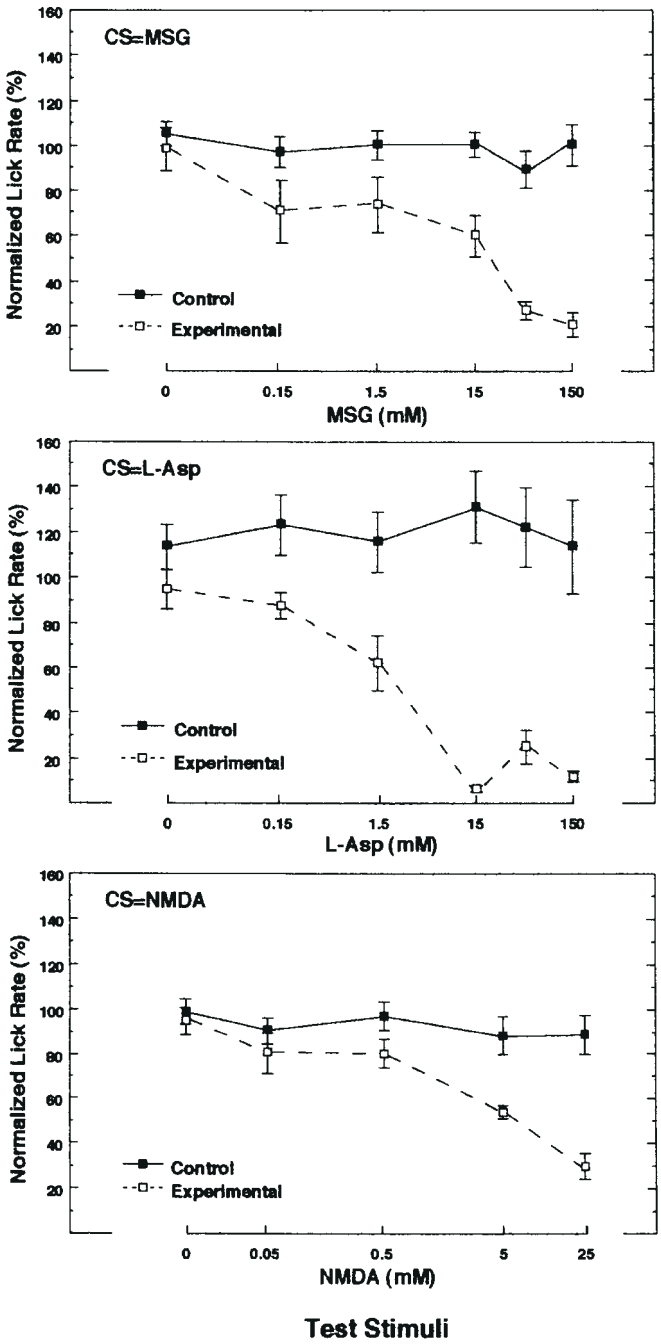

Figure 1 Rats can be conditioned to avoid the taste of MSG, L-Asp or NMDA. Each point represents the normalized lick rate (mean ± SEM) of control rats (filled squares) or experimental rats (open squares) when presented with increasing concentrations of MSG **(top)**, L-Asp **(middle)** and NMDA **(bottom)**. The CS was 100 mM MSG (top), 100 mM L-Asp (middle) or 50 mM NMDA (bottom). To normalize lick rates, the mean lick rate for each test stimulus was divided by the mean lick rate for all water presentations in that session, then multiplying by 100. The ordinate shows the normalized lick rates and the abscissa shows the concentrations of the test solutions. In this figure as well as all subsequent figures, amiloride was present in all solutions to reduce Na^+ taste as a possible confounding parameter, as described in the text.

Materials and methods

Subjects

The naive animals (n = 28) for this experiment were as described and were housed in the same manner as the rats in experiment 1. They were also subjected to the same deprivation procedures as rats in experiment 1. Rats were randomly assigned to one of four groups (seven rats per group) prior to the start of the experiment.

Apparatus and procedure

The Davis Lickometer used in experiment 1 was also used in experiment 2, and the procedures used were identical but with the following modifications. After training, one group of rats was injected i.p. with NaCl and another group was injected with LiCl following exposure to 100 mM MSG as described in experiment 1. The other two groups were likewise injected after exposure to 100 mM L-Asp.

On the test day, the rats were presented with 10 and 100 mM solutions of their respective CS, and 10, 25 and 100 mM solutions of the other stimulus. All rats were also presented with 100 mM sucrose, 20 mM KCl and deionized water. As previously stated, all solutions contained amiloride and were presented at a pH between 6.5–7.0.

Results

Lick rates were normalized as before and examined initially with a three-way ANOVA for mixed designs treating the CS (2) and unconditioned stimuli (2) as between-subject variables, with taste stimuli (8) as a within-subject variable. This ANOVA revealed significant effects of unconditioned stimuli [$F(1,24) = 95.17$, $P < 0.001$], taste stimuli [$F(7,168) = 15.22$, $P < 0.001$] and interactions between these two variables [$F(7,168) = 22.76$, $P < 0.001$]. No significant main or interactive effect for the CS variable was found (all $F < 2.00$). The data were then partitioned by CS condition and analysed further with two-way ANOVAs and simple effects tests. A summary of the data can be found in Table 1.

L-*Asp as the CS*

As observed in experiment 1, animals conditioned to avoid L-Asp in experiment 2 showed significant suppression of drinking when presented with 10 or 100 mM L-Asp. More importantly, these same experimental rats significantly reduced their rate of licking when they encountered 25 and 100 mM MSG, and 100 mM sucrose [all $F(1,56) \geq 28.68$, $P < 0.001$; Figure 2].

MSG as the CS

Similar results were obtained for animals conditioned to avoid MSG. The experimental rats exhibited a significant aversion to 10 and 100 mM MSG [$F(1,67) = 11.22$ and 37.69 respectively, $P < 0.001$]. In addition, experimental rats avoided all concentrations of L-Asp in a dose-dependent manner (Figure 2) and 100 mM sucrose as well [$F(1,67) \geq 8.83$, $P < 0.005$)].

In summary, the results of experiment 2 indicate that rats

Table 1 Summary of means (± SEM) for MSG, L-Asp and NMDA taste aversion in experiments 2–4

Test stimulus[a]	Conditioned stimulus					
	100 mM MSG		100 mM L-Asp		25 mM NMDA	
	Control[b]	Experimental[c]	Control	Experimental	Control	Experimental
MSG						
0 mM	101.3 (4.4)	103.1 (7.0)				
10 mM	112.3 (11.6)	71.3 (11.8)**	101.3 (9.1)	81.0 (16.5)		
25 mM			122.0 (4.3)	53.6 (10.5)***		
100 mM	100.7 (10.4)	25.5 (4.8)***	105.3 (8.1)	29.3 (7.2)***	109.5 (4.3)	99.0 (10.3)
L-Asp						
0 mM			109.8 (8.1)	112.6 (6.8)		
10 mM	95.1 (12.2)	58.8 (8.8)**	120.1 (5.3)	56.6 (13.5)***		
25 mM	108.0 (8.8)	49.0 (13.1)***				
100 mM	112.4 (6.7)	13.8 (2.7)***	128.3 (6.5)	19.2 (7.6)***	95.3 (6.8)	98.4 (7.1)
Sucrose, 100 mM	117.6 (4.9)	42.3 (6.8)***	119.4 (13.9)	23.8 (5.4)***	95.0 (6.8)	99.2 (6.9)
KCl, 20 mM	106.0 (7.5)	96.3 (8.6)	111.0 (6.6)	89.0 (4.3)	104.9 (6.5)	109.3 (5.9)
NaCl						
50 mM	108.2 (2.8)	83.9 (7.4)*	110.2 (10.7)	88.8 (7.5)		
100 mM	102.8 (4.7)	90.5 (5.0)	102.1 (10.0)	84.1 (3.1)		
NMDA						
10 mM	94.2 (7.9)	100.4 (4.3)	96.3 (14.3)	72.1 (11.2)	104.7 (3.5)	77.0 (7.5)**
25 mM	85.2 (8.0)	94.1 (7.3)	79.7 (6.5)	92.1 (3.7)	87.6 (7.2)	32.0 (2.3)***

*$P < 0.01$; **$P < 0.005$; ***$P < 0.001$.

[a]Where test conditions were replicated in experiments 2–4, only the data for the first stimulus presentation are listed.

[b]Control: each animal was conditioned with an injection of NaCl.

[c]Experimental: each animal was conditioned with an injection of LiCl.

conditioned to avoid MSG show a strong, dose-dependent aversion to L-Asp. Conversely, rats conditioned to avoid L-Asp show an equally strong dose-dependent aversion to MSG. The findings suggest that, in rats, L-Asp mimics the taste of MSG and vice versa. Furthermore, similar to findings from experiment 1, a conditioned aversion to either amino acid generalizes to sucrose.

Experiment 3

This experiment tested whether rats perceive MSG, L-Asp and NMDA as having similar taste. Would a conditioned aversion to MSG or to L-Asp generalize to NMDA? This information would provide insights into the question of which receptor subtypes transduce the taste of glutamate in taste bud cells.

Materials and methods

Subjects

The naive rats ($n = 28$) in this experiment were of the same description, housed in the same manner and subjected to the same deprivation schedule as rats in experiments 1 and 2. Each rat was randomly assigned to one of four groups (seven rats per group) prior to the start of the experiment.

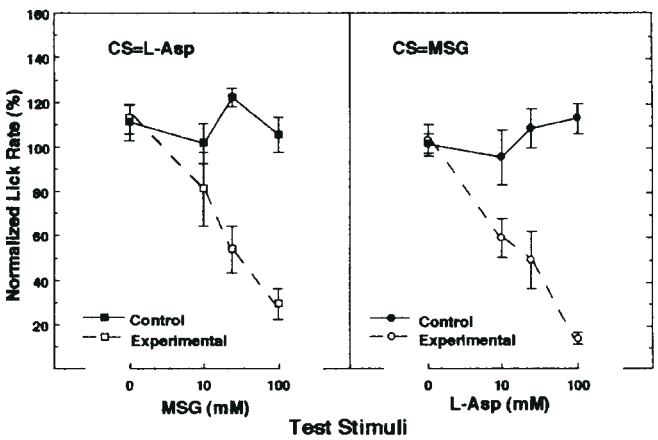

Figure 2 Rats conditioned to avoid the taste of L-Asp also avoid MSG; conversely, a conditioned aversion to MSG generalizes to L-Asp. The CS was 100 mM L-Asp **(left)** and 100 mM MSG **(right)**. Data are presented in the same format as in Figure 1.

Apparatus and procedure

The apparatus, CTA conditioning and test procedures used in this experiment were identical to those described for experiment 2 except for the solutions presented on the test day. The solutions (all containing amiloride) used on the

test day were: 100 mM MSG, 100 mM L-Asp, 50 and 100 mM NaCl, 10 and 25 mM NMDA, 20 mM KCl and deionized water. In all cases, conditioned aversions for each animal were verified on the test day by presenting the CS and measuring a significantly decreased lick rate.

Results

The normalized scores for each rat were subjected to the same type of analyses used in experiment 2. These data are summarized in Table 1. The three-way ANOVA for mixed designs revealed significant main effects due to the unconditioned stimuli [$F(1,24) = 45.09$, $P < 0.001$], to the various taste stimuli [$F(7,168) = 16.91$, $P < 0.001$] and to the interaction between these two variables [$F(7,168) = 16.91$, $P < 0.001$]. These data were then partitioned to examine the lick rates for each CS.

No significant differences between rats conditioned to avoid MSG and L-Asp were detected (all $F < 1.2$). As seen in Figures 3 and 4, animals conditioned to avoid L-Asp suppressed their drinking when presented with L-Asp or MSG ($P < 0.001$) but not when presented with NMDA, NaCl or KCl. Similarly, rats conditioned to avoid MSG suppressed their drinking when presented with L-Asp or MSG ($P < 0.001$) but not the other taste stimuli, including NMDA. Rats conditioned to avoid MSG drank less of the 50 mM NaCl solution than control rats ($P < 0.01$) but there was no significant difference in lick rates when rats were presented with 100 mM NaCl.

In summary, the results from experiment 3 show that animals conditioned to avoid MSG also find L-Asp aversive but they do not alter their drinking behavior to NMDA, NaCl (at the higher concentration) or KCl. Likewise, rats with a conditioned aversion to L-Asp avoid MSG but not NMDA, NaCl or KCl. These results indicate that the taste sensations of MSG and L-Asp have common properties but that rats did not identify similar salient taste sensations in the other taste stimuli tested in this experiment.

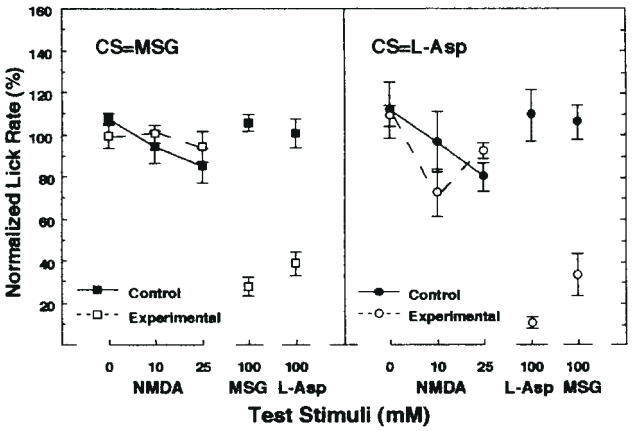

Figure 3 Conditioned taste aversions to MSG or to L-Asp do not generalize to NMDA. The CS was 100 mM MSG **(left)** and 100 mM L-Asp **(right)**. Data are presented as in Figure 1.

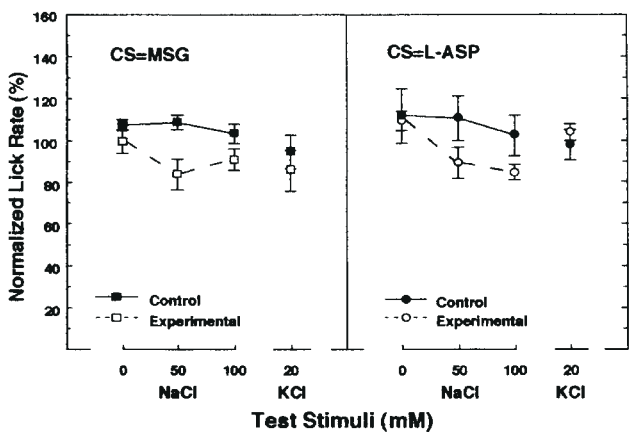

Figure 4 Conditioned taste aversions to MSG or to L-Asp do not generalize to NaCl, ruling out possible complications from Na^+ taste in this study. As in all other figures, amiloride was present to reduce Na^+ taste. 20 mM KCl was presented to test for a 'dirty water effect'; see text. The CS was 100 mM MSG **(left)** and 100 mM L-Asp **(right)**. Data are presented as in Figure 1.

Experiment 4

The results of experiment 3 indicated that in rats an aversion to MSG or L-Asp did not generalize to NMDA. However, one could argue that NMDA, a selective ligand for only one subclass of glutamate receptors, might elicit fewer salient taste components than MSG, a ligand that activates all classes of glutamate receptors. That is, some aspects of the taste of NMDA might generalize to MSG or L-Asp even though the converse did not occur (experiment 3). Experiment 4 tested whether a conditioned aversion to NMDA generalized to MSG or L-Asp.

Materials and methods

Subjects

Naive rats ($n = 14$) in this experiment were of the same description, housed in the same manner and subjected to the same deprivation schedule as rats in the previous experiments. Each rat was randomly assigned to one of two groups (seven rats per group) prior to the start of the experiment.

Apparatus and procedure

As before, the apparatus, CTA conditioning and test procedures used in this experiment were identical to those described for the previous experiments except for the solutions presented on the conditioning and the test days. Rats were conditioned with 25 mM NMDA. The solutions used on the test day were 10 and 25 mM NMDA, 100 mM L-Asp, 100 mM MSG, 100 mM sucrose, 20 mM KCl and deionized water, all in the presence of amiloride. To determine if the presence of amiloride might affect generalization of the

taste aversion to MSG, 100 mM MSG without amiloride was also tested. All solutions were presented at a pH of 6.5–7.0.

Results

The normalized scores for each rat were subjected to the same type of analyses used in previous experiments. The ANOVA revealed significant differences between groups [$F(1,12) = 8.46$, $P < 0.025$], between taste stimuli [$F(7,84) = 16.01$, $P < 0.001$], and in the interaction between groups and taste stimuli [$F(7,84) = 6.65$, $P < 0.025$; Table 1]. The simple effects tests showed that the only significant difference between the control and the experimental rats was that experimental rats suppressed licking in a dose-dependent fashion when presented with NMDA (Figure 5). The aversion did not generalize to other test stimuli. Thus, there was no evidence that the rats perceived the taste of MSG, L-Asp or any of the other taste stimuli as similar to that of NMDA.

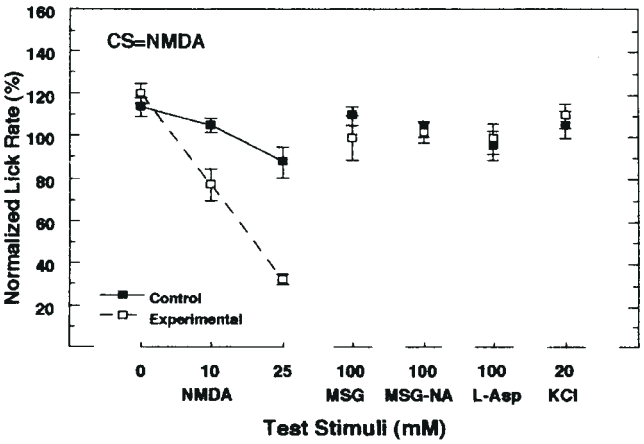

Figure 5 Rats conditioned to avoid NMDA do not generalize this aversion to MSG, L-Asp or KCl. The CS was 25 mM NMDA. Solutions contained amiloride except for the points labeled MSG-NA, which represent tests of 100 mM MSG without amiloride. Although a strong aversion to NMDA was established, this conditioned taste aversion did not generalize to other taste stimuli tested. Data are presented as in Figure 1.

Discussion

The findings clearly show that L-Asp closely mimics the taste of MSG when both stimuli are presented at similar concentrations in rats. Conditioning a taste aversion to one taste stimulus produces a dose-dependent aversion to the other and vice versa. Conditioning an aversion to either amino acid generalized to sucrose when amiloride was added to all solutions. These findings confirm and extend earlier reports that L-Asp elicits glutamate taste (i.e. umami) (Maga, 1983). The present findings, considered with previous reports, define a group of chemical compounds (L-glutamate, L-Asp and L-AP4) that appear to elicit similar tastes and thus might be used to study transduction mechanisms at the cellular and molecular levels.

Furthermore, the results question whether glutamate receptors of the NMDA subtype are directly or solely involved in glutamate taste transduction. In this study rats with aversions to either MSG or L-Asp did not generalize their aversion to NMDA. Conversely, rats with an aversion to NMDA did not generalize the aversion to MSG, L-Asp or sucrose. It seems unlikely that the lack of generalization between NMDA and the other two amino acids could be explained by differences in perceived stimulus intensities rather than stimulus qualities among the test solutions. First, rats showed similar and relatively strong aversions to the higher concentrations of each of the respective CSs. Second, generalization of aversions from MSG to L-Asp, and vice versa, was clearly observed for several different concentrations, but not for NMDA at any of the concentrations tested in these experiments. Instead, these data suggest that at least in part the rats were responding to qualitative features of each test stimulus. The lack of generalization between NMDA and the other two amino acids, even at the higher concentrations, suggests that certain aspects of the taste of NMDA are qualitatively different from the taste of MSG or L-Asp.

L-Asp originally was believed to be an agonist for the NMDA subtype of glutamate receptor (Olverman *et al.*, 1984, 1988) and L-Asp taste was considered as evidence for NMDA-like receptor sites being activated in umami taste (Faurion, 1991). However, these findings were reported before metabotropic glutamate receptors were cloned and characterized (Masu *et al.*, 1991; Nakanishi, 1994). It is now recognized that L-Asp activates several glutamate receptor subtypes, including metabotropic glutamate receptors (Littman and Robinson, 1994; Littman *et al.*, 1995). In light of this newer information and the results of this study, L-Asp taste responses can no longer be ascribed solely to NMDA receptor mechanisms.

An enigmatic finding in this study is that some aspect of sucrose taste mimics the taste of MSG when amiloride is added to reduce Na^+ responses. Others have reported this anomalous observation (Yamamoto *et al.*, 1991; Chaudhari *et al.*, 1996). Moreover, sucrose also mimics the taste of L-Asp in the presence of amiloride, further stressing the taste similarities of MSG and L-Asp. Investigators have obtained electrophysiological activity from single chorda tympani (CT) fibers (Sato *et al.*, 1970; Ninomiya and Funakoshi, 1989b; Hellekant and Ninomiya, 1991), from neurons in the nucleus of the solitary tract (NST) (Adachi and Aoyama, 1991) and from neurons in the parabrachial nucleus (PBN) in rats (Nishijo *et al.*, 1991) that may correspond to these behavioral data. Namely, CT fibers, and NTS and PBN neurons excited by stimulating the tongue with sucrose often are also activated by lingual stimulation with MSG. These experiments, taken together with the present findings, reveal that glutamate taste and sucrose

taste interact at some point during signal processing. It is presently unknown whether these interactions occur at a membrane receptor level, between cells in the taste bud or at the level of synaptic excitation of primary afferent axons.

It should be emphasized that the findings in this report do not rule out the participation of NMDA receptors in peripheral mechanisms of gustation. The present results in fact indicate that rats do taste NMDA (cf. experiments 1 and 4), but that activation of peripheral NMDA receptors is, on its own, unable to explain MSG taste in rats *per se*. Interestingly, in humans, too, NMDA fails to elicit a taste comparable to that of glutamate (Kurihara and Kashiwayanagi, 1998). Nonetheless, when applied as a taste stimulus, NMDA clearly elicits excitatory responses in chorda tympani axons of hamsters (Faurion, 1991); in lipid bilayers in which membranes from taste papillae of mice have been incorporated (Brand *et al.*, 1991); and in individual taste cells of mice and rats (Hayashi *et al.*, 1996; Lin and Kinnamon, 1998). Whether these responses are due to activation of NMDA subtypes of glutamate receptors remains to be established and how the animals perceive the taste of NMDA is not known. It is conceivable that MSG taste requires co-activation of metabotropic glutamate receptors and NMDA receptors (if they exist in taste buds). Metabotropic and NMDA glutamate receptors are activated by MSG and by L-Asp. However, if co-activation of ionotropic and metabotropic glutamate receptors is required for glutamate taste, it is difficult to explain taste responses of L-AP4. L-AP4, a ligand for class III metabotropic glutamate receptors, mimics glutamate taste in rats (Chaudhari *et al.*, 1996) and humans (Kurihara and Kashiwayanagi, 1998). In brief, our experiments indicate that stimulation of NMDA receptors is not, on its own, sufficient to explain glutamate taste transduction and may not in fact be related to MSG taste. Further experiments are needed to test if NMDA contributes in some manner to glutamate taste, or whether NMDA receptors are necessary for the taste transduction of glutamate.

Acknowledgements

This research was supported by NIH/NIDCD grant 1 RO1 DC03013 to S.D.R. Brief abstracts of these findings have appeared elsewhere (Stapleton *et al.*, 1998a, 1998b).

References

Adachi, A. and **Aoyama, M.** (1991) *Neuronal responses of the nucleus tractus solitarius to oral stimulation with umami substances*. Physiol. Behav., 49, 935–941.

Bigiani, A., Delay, R.J., Chaudhari, N., Kinnamon, S.C. and **Roper, S.D**. (1997) *Responses to glutamate in rat taste cells*. J. Neurophysiol., 11, 3048–3059.

Brand, J.G., Teeter, J.H., Kumazawa, T., Huque, T. and **Bayley, D.L.** (1991) *Transduction mechanisms for the taste of amino acids*. Physiol. Behav., 49, 899–904.

Cagan, R.H. (1986) *Allosteric regulation of glutamate taste receptor function*. In Kawamura, Y. and Kare, M.R. (eds), Umami: a Basic Taste. Marcel Dekker, New York, pp. 144–172.

Chaudhari, N., Yang, H., Lamp, C., Delay, E., Cartford, C., Than, T. and **Roper, S.** (1996) *The taste of monosodium glutamate: membrane receptors in taste buds*. J. Neurosci., 16, 3817–26.

Faurion, A. (1991) *Are umami taste receptor sites structurally related to glutamate CNS receptor sites?* Physiol. Behav., 49, 905–912.

Hayashi, Y., Zviman, M.M., Brand, J.G., Teeter, J.H. and **Restrepo, D.** (1996) *Measurement of membrane potential and* $[Ca^{2+}]_i$ *in cell ensembles: application to the study of glutamate taste in mice*. Biophys. J., 71, 1057–1070.

Heck, G.L., Mierson, S. and **DeSimone, J.A.** (1984) *Salt taste transduction occurs through an amiloride-sensitive sodium transport pathway*. Science, 223, 403–405.

Hellekant, G. and **Ninomiya, Y.** (1991) *On the taste of umami in chimpanzee*. Physiol. Behav., 49, 927–34.

Hellekant, G., Danilova, V. and **Ninomiya, Y.** (1997) *Primate sense of taste: behavioral and single chorda tympani and glossopharyngeal nerve fiber recordings in the rhesus monkey,* Macaca mulatta. J. Neurophysiol., 77, 978–993.

Howell, D.C. (1997) Statistical Methods for Psychology, 4th edn. Wadsworth Publishing Company, New York.

Kawamura, Y. and **Kare, M.R.** (1986) Umami: a Basic Taste. Marcel Dekker, New York.

Kumazawa, T., Nakamura, M. and **Kurihara, K.** (1991) *Canine taste nerve responses to umami substances*. Physiol. Behav., 49, 875–881.

Kurihara, K. and **Kashiwayanagi, M.** (1998) *Introductory remarks on umami taste*. Ann. N.Y. Acad. Sci., 855, 393–397.

Lin, W. and **Kinnamon, S.C.** (1998) *Responses to monosodium glutamate and guanosine 5′-monophosphate in rat fungiform taste cells*. Ann. N.Y. Acad. Sci., 855, 407–411.

Littman, L. and **Robinson, M.B.** (1994) *The effects of L-glutamate and* trans*-(+)-1-amino-1,3-cyclopentanedicarboxylate on phosphoinositide hydrolysis can be pharmacologically differentiated*. J. Neurochem., 63, 1291–1302.

Littman, L., Chase, L.A., Renzi, M., Garlin, A.B., Koerner, J.F., Johnson, R.L. and **Robinson, M.B.** (1995) *Effects of quisqualic acid analogs on metabotropic glutamate receptors coupled to phosphoinositide hydrolysis in rat hippocampus*. Neuropharmacology, 34, 829–841.

Maga, J.A. (1983) *Flavor potentiators*. Crit. Rev. Food. Sci. Nutr., 18, 231–312.

Masu, M., Tanabe, Y., Tsuchida, K., Shigemoto, R. and **Nakanishi, S.** (1991) *Sequence and expression of a metabotropic glutamate receptor*. Nature, 349, 760–765.

Nachman, M. and **Ashe, J.H.** (1973) *Learned taste aversions in rats as a function of dosage, concentration, and route of administration of LiCl*. Physiol. Behav., 10, 73–78.

Nakanishi, S. (1994) *Metabotropic glutamate receptors: synaptic transmission, modulation, and plasticity*. Neuron, 13, 1031–7.

Ninomiya, Y. and **Funakoshi, M.** (1989a) *Behavioural discrimination between glutamate and the four basic taste substances in mice*. Comp. Biochem. Physiol. A, 92, 365–370.

Ninomiya, Y. and **Funakoshi, M.** (1989b) *Peripheral neural basis for behavioural discrimination between glutamate and the four basic taste substances in mice*. Comp. Biochem. Physiol. A, 92, 371–376.

Ninomiya, Y., Kurenuma, S., Nomura, T., Uebayashi, H. and **Kawamura, H.** (1992) *Taste synergism between monosodium glutamate and 5′-ribonucleotide in mice*. Comp. Biochem. Physiol. A, 101, 97–102.

Nishijo, H., Ono, T. and **Norgren, R.** (1991) *Parabrachial gustatory neural responses to monosodium glutamate ingested by awake rats*. Physiol. Behav., 49, 965–971.

Olverman, H.J., Jones, A.W. and **Watkins, J.C.** (1984) *L-Glutamate has higher affinity than other amino acids for [^{3}H]-D-AP5 binding sites in rat brain membranes*. Nature, 307, 460–462.

Olverman, H.J., Jones, A.W., Mewett, K.N. and **Watkins, J.C.** (1988) *Structure/activity relations of* N*-methyl-D-aspartate receptor ligands as studied by their inhibition of [^{3}H]D-2-amino-5-phosphonopentanoic acid binding in rat brain membranes*. Neuroscience, 26, 17–31.

Plata-Salaman, C.R., Scott, T.R. and **Smith-Swintosky, V.L.** (1992) *Gustatory neural coding in the monkey cortex: L-amino acids*. J. Neurophysiol., 67, 1552–1561.

Rolls, E.T., Critchley, H.D., Wakeman, E.A. and **Mason, R.** (1996) *Responses of neurons in the primate taste cortex to the glutamate ion and to inosine 5′-monophosphate*. Physiol. Behav., 59, 991–1000.

Sato, M., Yamashita, S. and **Ogawa, H.** (1970) *Potentiation of gustatory response to monosodium glutamate in rat chorda tympani fibers by addition of 5′-ribonucleotides*. Jpn. J. Physiol., 20, 444–464.

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.

Spector, A.C. and **Grill, H.J.** (1988) *Differences in the taste quality of maltose and sucrose in rats: issues involving the generalization of conditioned taste aversions*. Chem. Senses, 13, 95–113.

Stapleton, J.R., Roper, S.D. and **Delay, E.R.** (1998a) *Comparison of MSG and L-aspartic acid using conditioned taste aversion in rats*. Chem. Senses, 23, 643.

Stapleton, J.R., Roper, S.D. and **Delay, E.R.** (1998b) *Generalization of conditioned taste aversion between monosodium glutamate and L-aspartic acid*. Soc. Neurosci. Abstr., 24, 1887.

Torii, K. and **Cagan, R.H.** (1980) *Biochemical studies of taste sensation. IX. Enhancement of L-[^{3}H]glutamate binding to bovine taste papillae by 5′-ribonucleotides*. Biochim. Biophys. Acta, 627, 313–323.

Yamamoto, T., Matsuo, R., Fujimoto, Y., Fukunaga I., Miyasaka, A. and **Imoto, T.** (1991) *Electrophysiological and behavioral studies on the taste of umami substances in the rat*. Physiol. Behav., 49, 919–925.

Accepted May 4, 1999