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Glutamate Taste: Discrimination between the Tastes of Glutamate Agonists and Monosodium Glutamate in Rats

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Abstract

Taste aversion studies have demonstrated that rats conditioned to avoid monosodium glutamate (MSG) with amiloride added to reduce the intensity of the sodium component of MSG taste, generalize this aversion to aspartic acid and to L-AP4, but not to ionotropic glutamate receptor agonists. That is, MSG, L-AP4 and aspartate have similar tastes to rats. However, conditioned taste aversion methods are unable to show to what extent the tastes of two substances are different. If two substances activate the same afferent processes (e.g. taste receptors), they are likely to produce the same tastes, but if they activate different afferent processes, the subject may detect differences between the tastes of the substances. In this study, rats were tested to determine if they could discriminate between the tastes of these agonists and MSG. We also established the detection thresholds for NMDA, aspartic acid and L-AP4, with and without amiloride (a sodium channel antagonist). Taste threshold values were 1–4 mM for NMDA and aspartic acid and 0.5–2.5 μM for L-AP4. None were affected by 30 μM amiloride. Rats could readily distinguish between the tastes of MSG and NMDA but they had difficulty discriminating between the tastes of aspartic acid and MSG. Rats could also easily distinguish between 10–100 mM MSG and 0.01–5 mM L-AP4. However, in two separate experiments error rates increased significantly when L-AP4 concentrations were between 10–100 mM, indicating that the tastes of L-AP4 and MSG were similar at these concentrations.

Key words: amiloride, aspartic acid, L-AP4, monosodium glutamate, NMDA, taste discrimination, taste thresholds

Introduction

The taste of glutamate, an amino acid found in many protein-rich food items such as meats, fish, cheese and some vegetables, signals dietary protein. Glutamate is thought to possess a unique taste quality called 'umami' that is distinct from sweet, sour, salty and bitter (Yamaguchi, 1967). Monosodium glutamate (MSG) is the prototypical umami substance and has long been used in Asian cuisine to enhance flavor (Maga, 1983). Despite its importance as a food additive and as a substance present naturally in many foods, relatively little is known about the peripheral mechanisms responsible for the taste of MSG.

In recent years there has been a surge of interest in umami taste transduction. Earlier work suggested an *N*-methyl-D-aspartate (NMDA) or an NMDA-like ionotropic glutamate receptor may be responsible for detection of glutamate taste (Brand *et al.*, 1991; Faurion, 1991), but more recent reports have implicated G-protein coupled receptors in umami taste transduction (Bigiani *et al.*, 1997; Lin and Kinnamon, 1999; Stapleton *et al.*, 1999; Nakashima *et al.*, 2001; Damak *et al.*, 2003; Zhao *et al.*, 2003). A taste-specific type III metabotropic glutamate receptor (taste-specific mGluR4) responds to MSG at concentrations similar to those found to be effective in behavioral assays (Chaudhari *et al.*, 1996, 2000). Another taste specific G-protein coupled receptor, a heterodimer formed from the combination of T1R1 and T1R3 subunits, has also been implicated in umami taste. This receptor is responsive to MSG and other substances that elicit an umami taste and may be more broadly tuned to detect certain other amino acids (Li *et al.*, 2002; Nelson *et al.*, 2002; Damak *et al.*, 2003; Zhao *et al.*, 2003).

The results of several behavioral studies also favor metabotropic receptor involvement in glutamate taste. In rats (Chaudhari *et al.*, 1996; Stapleton *et al.*, 1999) and mice (Nakashima *et al.*, 2001), a conditioned taste aversion (CTA) to MSG generalizes to L-2-amino-4-phosphonobutyrate (L-AP4), a potent agonist for mGluR4 receptors,

but does not generalize to agonists for ionotropic glutamate receptors. These results suggest that the taste of L-AP4 mimics the taste of MSG, at least to some degree, and that these substances activate the same taste receptors or other afferent signaling processes within the gustatory system. However, these experiments are unable to indicate the degree to which the tastes of two stimuli are different, a condition that would occur if different afferent mechanisms are involved in the coding of taste sensations of two substances. Perceptual differences are more easily detected with stimulus discrimination methods because the subject associates a different response and response consequence with each stimulus. The present experiments used discrimination procedures to ask how well rats can distinguish between the tastes of MSG and the glutamate agonists NMDA, L-AP4 and another amino acid that elicits an umami taste, aspartic acid (ASP).

Material and methods

Subjects

The subjects for these experiments were 39 male albino Sprague–Dawley rats, obtained from Harlan Sprague–Dawley (Indianapolis, IN). They were ~90 days of age and weighed between 250 and 300 g at the beginning of the experiment. The subjects were housed individually in separate cages in the colony with Purina Lab chow available *ad libitum*. One week prior to testing the rats were placed on a 20 h water deprivation schedule that was maintained for the duration of the study. Colony lighting was set on a 12 h light–dark cycle with the lights turned on at 7 a.m. Each rat was tested at the same time each day between 9 a.m. and 12:30 p.m.

Apparatus

Computer-controlled gustometers (Knosys Ltd, www.knosysknosys.com; Brosvic and Slotnick, 1986), located in individual bench top stations, were used for testing. Each gustometer consisted of a Plexiglas operant chamber (25.4 × 15.9 × 20.6 cm high) with a fan mounted in the ceiling to draw fresh air into the chamber and force air out of the chamber. Each subject had access to a lick spout located 3 mm behind a small circular opening (2.2 cm diameter) in one wall, centered 11.4 cm from the floor of the chamber. Taste solutions and water for reinforcement were stored in 10 ml unpressurized syringe barrels. The bottoms of the barrels were at least 15 cm above the drinking spout. Solenoids controlled the flow of solution from each barrel through capillary tubing to individual 24 gauge stainless steel tubes within the drinking spout. The tips of these tubes were recessed 2 mm from the end of the spout. Each taste stimulus was presented as a 50 µl aliquot delivered over 0.5 s. A lick of the spout completed a 64 nA contact current through a stainless steel plate on the floor of the chamber and was counted by the computer. All testing was conducted under 30 ± 5 lx illumination from a white incandescent bulb inside the station in which the operant equipment was housed. Masking noise (SPL A scale: 75 ± 5 dB; Radio Shack Sleep Machine) was also present during all testing.

Procedures

Detection threshold experiments

Threshold and discrimination methods are similar to those used in previous experiments (Stapleton *et al.*, 2002). The purpose of the first experiments was to establish detection thresholds for NMDA, ASP and L-AP4. These experiments also tested whether the addition of amiloride would affect detection thresholds for these stimuli.

Five animals were tested with ASP (Sigma, St Louis, MO), six were tested with NMDA (Sigma) and six were tested with L-AP4 (Tocris, Bristol, UK). The concentrations of NMDA and ASP tested were 0.01, 0.1, 1, 2.5, 5, 10, 25, 50 and 100 mM, in deionized water. Concentrations of L-AP4 ranged from 0.00001 to 5.0 mM in half-log increments. The pH of all stimuli was adjusted to 6.75–7.0. Detection thresholds for each substance were first determined without amiloride, then with 30 µM amiloride in each solution. Rats were first trained to discriminate the five highest concentrations of the substance from deionized water until they reached 80% detection rate for each concentration in two consecutive sessions. The rats licked on a variable ratio 20 schedule which resulted in a 35 µl water 'rinse'. Three seconds later the rat could begin a second variable 20 schedule which, when completed, initiated the delivery of the 50 µl taste stimulus. Once the stimulus was delivered, the rat had 2 s (decision interval) to determine if the stimulus was an S+ or an S–. After an S+ solution was delivered, the rat had to lick the spout during the last 0.4 s of the decision interval to receive 70 µl water reinforcement (i.e. correct detection of the S+). After the S– substance was delivered, a correct detection was registered if the rat did not lick during the last 0.4 s of the decision interval. If the animal failed to identify the S– taste stimulus, a weak shock was delivered through the lick spout to the animal's tongue. The shock intensity was adjusted for each animal by increasing the intensity until the rat stopped licking briefly when shock was applied. Shock was always presented to the lick spout for 2 s following the end of the decision interval of each S– trial. The animal only experienced shock if it licked the spout during the shock presentation. A 10 s intertrial interval occurred before the start of the next variable ratio 20. A test session ended after the animal completed 160 trials or an hour had elapsed, whichever occurred first.

During each test session, seven of the stimulus barrels contained different concentrations of the taste stimulus (S–) and four contained deionized water (S+). An equal number of S+ and S– trials were presented within each session and the order of S+/S– presentations followed a random counterbalanced sequence. Different concentrations and

sequences were tested each day and each concentration was stored in a different barrel each day to minimize the possibility that a rat could identify a taste stimulus on the basis of the location of stimulus delivery within the spout. After the subject had been tested, it was returned to its home cage where, after another hour, it received an additional 2 h of access to water. All rats received at least 10 days training on the discrimination task before data collection began. At the end of each experiment, an additional test session was conducted to determine if any of the rats were able to discriminate between stimulus tubes on the basis of location or some equipment generated cue. All experimental parameters were maintained except each tube was filled with water and then randomly assigned as either S+ or S–.

Discrimination experiments

ASP versus MSG. This experiment repeated the discrimination procedures described above to determine if rats could differentiate between two closely related amino acids with an umami taste, ASP and MSG. The animals were tested in the same apparatus and under the same general protocol for the discrimination paradigm as stated in the threshold experiments with the following exceptions. Six rats were tested on the discrimination, of which three were randomly assigned to be tested with ASP as the S+ and MSG as the S– and the other three were assigned to the opposite stimulus conditions. The concentrations of ASP and MSG tested were 10, 25, 50, 100, 150, 200 and 300 mM. During each test session, 5 of 10 stimulus barrels contained different concentrations of the S– and five contained matched concentrations of the S+. Different concentrations and orders were tested each day and the order of stimulus presentations within a session was randomized with a Latin square design. Each rat received 15 days training before data collection began and an additional 20 days were used to collect the discrimination data for analysis. These procedures were then replicated with 30 μM amiloride added to all solutions. This was followed by water only sessions to test for extraneous cues.

NMDA versus MSG. Six rats were tested to determine how well they could discriminate between the tastes of NMDA and MSG. Four concentrations (10, 25, 50 and 100 mM) of NMDA and of MSG were tested each day to minimize intensity as a cue. For three of the rats, NMDA was the S+ stimulus and MSG was the S–. The consequence and thus the role of each solution were reversed for the other three rats. The order of presentation was determined with a Latin square and a different sequence was used each day. Every rat received 12 days training on the discrimination task before data collection began and all data were collected within the next 10 sessions. All other testing procedures were identical with those established during threshold experiments, including a water-only test the day after the last session. Once data were collected without amiloride, the experiment was repeated except with 30 μM amiloride added to all solutions, including the water used for reinforcement.

L-AP4 versus MSG. Two discrimination experiments were conducted to determine whether rats could distinguish between MSG and L-AP4, an mGluR4 agonist. Because the detection thresholds for L-AP4 were considerably lower than those for MSG, the procedures of the first experiment were modified to accommodate potential effects of stimulus intensity on discrimination. Three rats were trained with L-AP4 as the S– and MSG as the S+ and three more rats were trained with the opposite conditions. Concentrations of MSG were maintained throughout the experiment at 10, 25, 50 and 100 mM. Rats were initially trained for 18 days with 0.01, 0.025, 0.05 and 0.1 mM L-AP4. These concentrations were chosen because the lowest concentration for each substance was approximately one log unit above threshold and each range spanned one log unit. All solutions were mixed with 30 μM amiloride to reduce the cue-function of the higher concentrations of Na^+ in MSG. Data were collected for 3 days after training was completed. Since amiloride could not completely eliminate the taste of Na^+ at the higher concentrations of MSG, these procedures were repeated for an additional 5 days with NaCl added to L-AP4 at concentrations matching Na^+ in MSG. For example, 10 mM NaCl was added to 0.01 L-AP4, 25 mM NaCl was added to 0.025 mM L-AP4 and so on. As before, all solutions were mixed with 30 μM amiloride. Because the rats readily discriminated between L-AP4 and MSG under all of these conditions, the rats were then tested for three days with L-AP4 incremented by one log unit to 0.1, 0.25, 0.5 and 1.0 mM and then for 3 days with matched concentrations of NaCl added to L-AP4. This cycle was repeated with 1.0, 2.5, 5.0 and 10.0 mM L-AP4. Finally, these rats were tested with 10, 25, 50 and 100 mM L-AP4. Since in the latter series the concentrations of Na^+ were similar for L-AP4 and MSG, rats were initially tested without amiloride and then with 30 μM amiloride mixed in all solutions. After the last test session, two additional sessions were conducted with water only to ensure that the rats were not responding to non-taste cues.

The results of the first experiment indicated that these rats easily discriminated between MSG and L-AP4 except at the highest concentrations of L-AP4, suggesting that, between 10 and 100 mM, L-AP4 tastes quite similar to MSG. However, it could be argued that the rats did not receive adequate training with L-AP4 at the 10–100 mM concentrations, especially since the absence of amiloride was a new stimulus condition for these rats. Therefore a second discrimination experiment was conducted with four naïve rats to compare the tastes of L-AP4 and MSG when both substances were 10–100 mM. Training and testing procedures were similar to the first experiment, except that two of the rats were trained with MSG as the S– and two were trained with L-AP4 as the S–. One rat assigned to each S–

condition was trained with 30 μM amiloride in all solutions, while the others were trained without. All rats received 18 days of training followed by 4 days of data collection. They were then switched to the opposite amiloride condition and trained an additional 5 days before data were collected for 4 days. Finally, control sessions with water solutions were conducted to test for nongustatory cues.

Data analyses

For the threshold and discrimination experiments, the percent correct detection was calculated for each stimulus concentration during a test session and then averaged across sessions. Initial analyses of the data for each experiment did not reveal group differences related to the specific agonist serving as S+ or S–. Therefore, the data for all rats in each experiment were combined and organized to compare detection of the S+ stimulus with that of the S– (stimulus valence variable). Data for the NMDA and the ASP experiments were then subjected to a three-way within-subject ANOVA examining stimulus valence, concentration and amiloride factors. Simple effects tests and Newman–Keuls *post hoc* tests were applied as appropriate. Similar procedures were used for each experiment with L-AP4, except that the scores for the lowest concentration of L-AP4 were matched to those of 10 mM MSG, the second lowest concentrations were matched with 25 mM MSG and so on. None of the data collected during control experiments in which only water was presented suggested any evidence of non-gustatory cues (all Fs < 1.0) and, thus, are not reported below.

The difficulty of each discrimination was also assessed by examining mean percentage errors with ANOVA procedures and *post hoc* tests. Error scores were calculated for matched concentrations of each substance by the following formula:

$$\frac{[(100 - \%\ \text{detection of S+}) + (100 - \%\ \text{detection of S–})]}{2}$$

This measure eliminates any specific role for stimulus valence, an imbalance between the motivational effects of water reinforcement or shock, or any bias in response strategy based on stimulus valence a rat may have adopted during the course of the discrimination. However, since this type of measure is the equivalent of a two-choice discrimination (i.e. each response is either correct or incorrect), a mean error rate that approaches zero indicates an easy discrimination, whereas an error rate that approaches 50% indicates performance that is near chance levels.

Results

Detection thresholds

Detection thresholds, defined as the concentration detected in 50% of the trials, were between 1 and 4 mM for NMDA and ASP (geometric means = 2.67 and 2.36 mM, respectively), and between 0.0005 and 0.0025 mM (geometric mean = 0.0017 mM) for L-AP4. Threshold data for each substance were analyzed using a two-way repeated measures analysis of variance examining the effects of 30 μM amiloride and NMDA, ASP, or L-AP4 concentration. While the concentration factor was significant in each analysis [$F(9,36)$ = 184.5 or higher, $P < 0.001$], amiloride did not have a significant effect on any of these threshold functions. These values compare with detection thresholds between 1 and 4 mM for MSG in rats (Stapleton *et al.*, 2002).

Discrimination experiments

ASP versus MSG

Rats had a great deal of difficulty discriminating between the tastes of ASP and MSG and the addition of 30 μM amiloride significantly worsened detection rates (Fig. 1). The three-way ANOVA revealed significant main effects for stimulus valence [$F(1,5) = 9.20$, $P < 0.05$], concentration [$F(6,30) = 9.92$, $P < 0.001$] and amiloride [$F(1,5) = 10.60$, $P < 0.025$]. The concentration variable also interacted significantly with stimulus valence [$F(6,30) = 18.30$, $P < 0.001$] and with amiloride [$F(6,30) = 2.49$, $P < 0.05$]. To break down these interactions, the data for each amiloride condition were analyzed with a two-way ANOVA. Without amiloride, detection of the S+ remained relatively high, while S– improved over the range of concentrations [$F(6,30) = 13.54$, $P < 0.001$]. Simple effects tests ($P < 0.01$) showed that the S+ was detected significantly more often than the S– at concentrations between 10 and 100 mM, but both were detected equally well between 150 and 300 mM. With amiloride, the interaction between stimulus valence and concentration was also significant [$F(6,30) = 6.18$, $P < 0.001$], but simple effects tests indicated that the S+ was more accurately detected than the S– only at 10 and 25 mM ($P < 0.01$).

The presence of amiloride also increased error rates [$F(1,5) = 10.61$, $P < 0.025$], but the effect of amiloride depended upon the concentrations of the solutions [$F(6,30) = 2.50$, $P < 0.05$; Fig. 2]. Simple effects tests revealed that mean error rates were high and approaching chance levels (35–46%) for all concentrations when amiloride was present. Comparably

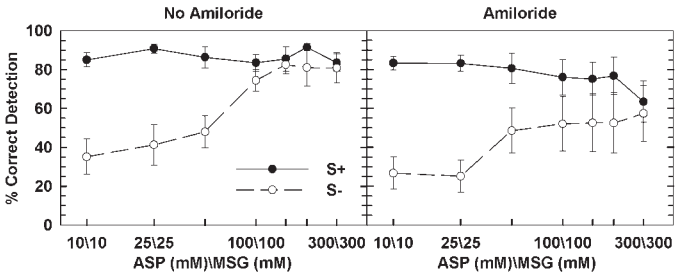

Figure 1 Mean (SEM) percent detection rates for S+ (solid line) and S– (dashed line) stimuli when rats were tested with ASP and MSG. The concentrations of both substances were 10, 25, 50, 100, 150, 200 and 300 mM. Rats had difficulty discriminating between the tastes of these two amino acids at concentrations <100 mM, with (right panel) or without 30 μM amiloride (left panel). Their discrimination performance improved significantly at the higher concentrations of the two substances.

high error rates were seen at concentrations <100 mM when amiloride (30 μM) was absent from all solutions, but significantly fewer errors ($P < 0.05$) were observed at concentrations between 100 and 300 mM.

NMDA versus MSG

Rats were able to discriminate between NMDA and MSG at 79% or higher in the presence or absence of amiloride (Fig. 3). The three-way ANOVA applied to the detection data showed a significant interaction between stimulus valence and concentration [$F(3,15) = 7.92$, $P < 0.005$]. Simple effects tests showed that the detection of the S+ was significantly greater than the detection of the S– at 10 and 25 mM [$F(1,5) = 31.37$ and 45.17, respectively, $P < 0.001$]. Briefly, rats accurately identified the S+ significantly more than the S– at 10 mM (mean ± SEM = 95.8 ± 0.8 and 79.4 ± 1.9, respectively) and also at 25 mM (mean ± SEM = 92.5 ± 1.5 and 85.5 ± 1.1, respectively). The analysis that compared the mean error rates in the presence and absence of amiloride and across concentrations, shown in Figure 4, did not detect any significant differences between any of these conditions nor across concentrations. Average error rates were <15% at all concentrations, indicating that rats made relatively few errors on this discrimination.

L-AP4 versus MSG

The detection and error data for each concentration range of L-AP4 in the first experiment were analyzed separately and selected comparisons across concentration ranges were made. Discrimination between L-AP4 and MSG was relatively easy at the lowest range of concentrations of L-AP4 (0.01–0.1 mM). Even so, the S+ stimuli (mean ± SEM = 94.2 ± 1.1%) were detected significantly more often than the S– [mean ± SEM = 77.3 ± 3.1%; $F(1,5) = 10.71$, $P < 0.025$]. Between 1.0 and 10 mM L-AP4, the rats detected S+ and the S– stimuli at similar rates of at least 80% and generally much higher. Moreover, detection rates over all three ranges were unaffected by the addition of NaCl to L-AP4 to match the concentrations of Na^+ of MSG (all Ps > 0.10). Figure 5 illustrates the discrimination performance when the concentrations of L-AP4 were between 1 and 10 mM. Error rates were similar across these three ranges of L-AP4 and were also unaffected by the addition of NaCl (all Ps > 0.10; Figure 6).

In contrast, discrimination performance was much poorer when the concentrations of L-AP4 matched those of MSG. An ANOVA comparing detection rates under the two amiloride conditions found that amiloride significantly worsened detection accuracy [$F(1,5) = 12.81$, $P < 0.025$; Fig. 7] compared to the no-amiloride condition. A significant interaction between concentration and amiloride [$F(3,15) = 3.98$, $P < 0.05$] was evaluated by analyzing the data for the amiloride and no-amiloride conditions with separate ANOVAs. Without amiloride, the S+ was detected significantly more frequently than the S– [$F(1,5) = 8.91$, $P < 0.05$]. With amiloride added to all solutions, there was a significant interactive effect between valence and concentration on detection rates [$F(3,15) = 5.74$, $P < 0.01$]. Simple effects tests

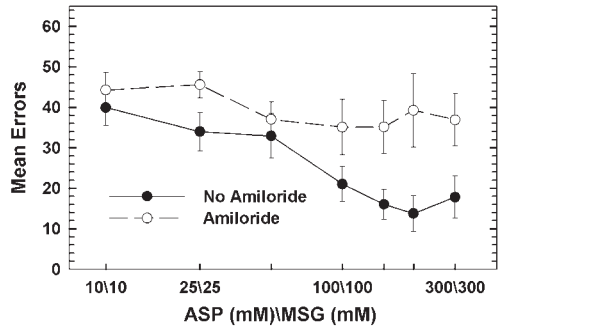

Figure 2 Mean (SEM) error rates when rats were tested with ASP and MSG. The concentrations of both substances were 10, 25, 50, 100, 150, 200 and 300 mM. Mean error rates were high at all concentrations when amiloride was not present in either solution (solid line). Mean error rates were high at concentrations <100 mM when amiloride (30 μM) was added to all solutions (dashed line), but fewer errors were made at the higher concentrations of the amino acids.

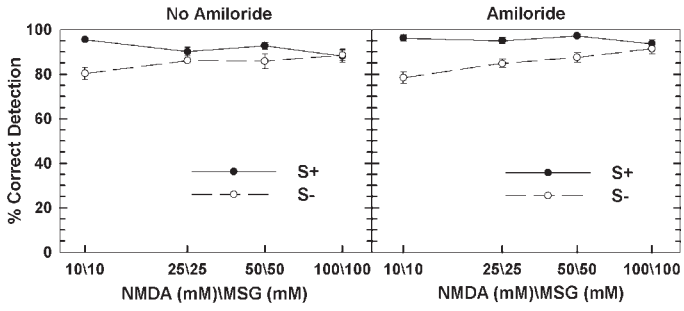

Figure 3 Mean (SEM percent correct detection of S+ (solid line) and S– stimuli (dashed line) when rats were tested with NMDA and MSG. Concentrations of NMDA and MSG were 10, 25, 50 and 100 mM. Rats easily discriminated between the tastes of these two substances, with (right panel) or without (left panel) 30 μM amiloride added to all solutions.

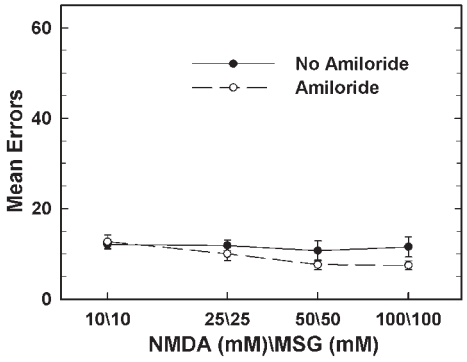

Figure 4 Mean (SEM) error rates when rats were tested with NMDA and MSG. Concentrations of NMDA and MSG were 10, 25, 50 and 100 mM. Error rates were low for all concentrations of these two substances, with (dashed line) and without (solid line) 30 μM amiloride added to all solutions.

($P < 0.05$) verified that rats detected the S+ significantly more accurately than the S– when stimulus concentrations were 10 and 25 mM, but significantly less accurately than the S– when stimulus concentrations were 50 and 100 mM when amiloride was present.

In general, errors made during the first L-AP4/MSG discrimination were low when the concentrations of L-AP4 were <10 mM and increased significantly between 10 and 100 mM. An analysis of errors made when amiloride (but no NaCl) was present in all solutions revealed that error rates were not equivalent across the concentration ranges [$F(3,15) = 14.34$, $P < 0.005$; Figures 6 and 8]. Games and Howell (1976) *post hoc* tests indicated that rats made significantly more errors at 10–100 mM than at any of the other concentration ranges of L-AP4 (all Ps < 0.01). At the highest concentrations of L-AP4 (10–100 mM) adding amiloride to reduce the sodium taste significantly increased the number of errors compared to the non-amiloride condition [$F(1,5) = 12.81$, $P < 0.025$; Fig. 8].

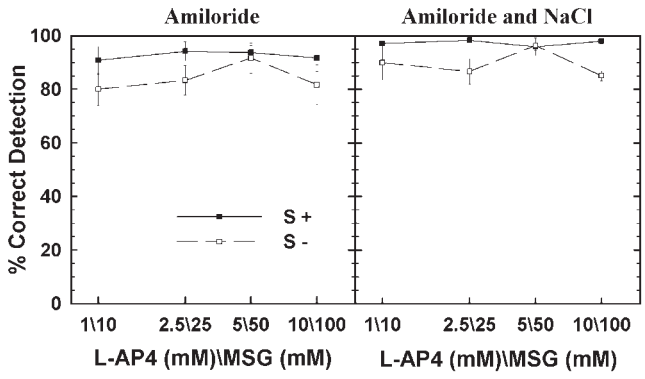

Figure 5 Mean (SEM) percentage correct detection for S+ (solid lines) and S– stimuli (dashed lines) when rats were tested with L-AP4 and MSG in the first experiment (see text for details). Concentrations of L-AP4 were 1, 2.5, 5 and 10 mM. Concentrations of MSG were 10, 25, 50 and 100 mM. Amiloride (30 μM) was present in all solutions. Rats easily discriminated between the tastes of these two substances, whether with NaCl added to L-AP4 to match the concentrations of Na+ in MSG (right panel) to minimize the cue function of sodium, or without NaCl added (left panel).

Similar analyses were applied to the detection and error rate data obtained during the second experiment in which the rats received extensive training with 10–100 mM L-AP4 and MSG. With amiloride in all solutions, these rats showed significantly better detection rates as the concentrations of the substances increased [$F(3,15) = 4.51$, $P < 0.025$], but detection rates of S+ and S– stimuli were comparable at each concentration. Without amiloride in the solutions, these rats discriminated between S+ and S– stimuli significantly better at the higher concentrations than the lower concentrations [$F(3,15) = 11.23$, $P < 0.001$; Fig. 9]. More insights were revealed by the analysis of errors comparing the effects of concentration and amiloride conditions. Like the first experiment, average errors for matched concentrations were high, ranging from 22 to 45% (Fig. 10). In contrast to the first experiment, the rats in the second experiment made significantly fewer errors when amiloride was present in the solutions than when it was not present [$F(1,5) = 8.27$, $P < 0.05$] and detection rates were significantly better at the higher concentrations than at the lower concentrations [$F(3,15) = 5.68$, $P < 0.01$]. Simple effects tests indicated that these rats made significantly fewer errors at 25 ($P < 0.05$) and 50 mM ($P < 0.005$) when amiloride was present. Nevertheless, despite the differences related to experience, the overall finding remained that at L-AP4 concentrations ≥10 mM, even well-trained rats had difficulty distinguishing the taste of L-AP4 from MSG.

To summarize, rats had a great deal of difficulty discriminating between the tastes of ASP and MSG and adding 30 μM amiloride made this discrimination more difficult. Rats readily discriminated between the tastes of NMDA and MSG, with or without amiloride added to the solutions. In general, rats were able to discriminate between the tastes elicited by L-AP4 and MSG unless the concentrations of the two substances were the same. In the second

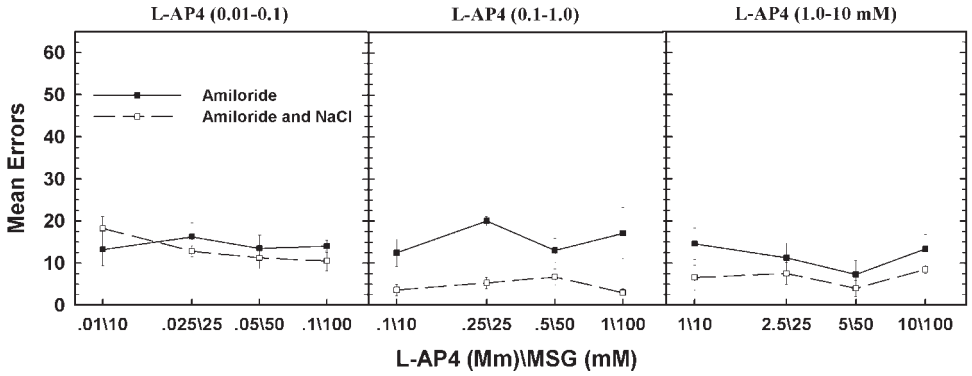

Figure 6 Mean (SEM) error rates when rats were tested with MSG (10–100 mM) and L-AP4 at concentration ranges of 0.01–0.1 mM (left panel), 0.1–1.0 mM (center panel) and 1.0–10 mM (right panel). Amiloride (30 μM) was present in all solutions. Rats easily discriminated between the tastes of these two substances, whether with NaCl added to L-AP4 to match the concentrations of Na+ in MSG (dashed lines) to minimize the cue function of sodium, or without NaCl added (solid lines).

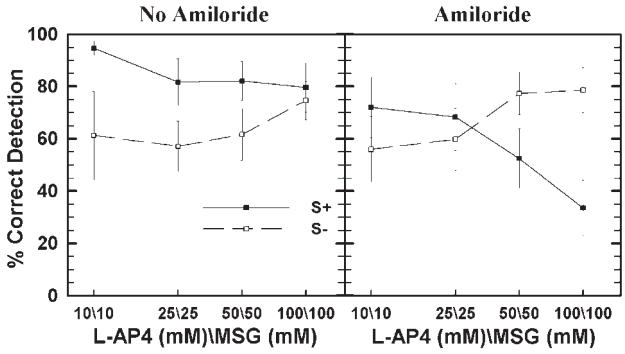

Figure 7 Mean (SEM) percentage detection rates for S+ (solid line) and S– (dashed line) stimuli when rats were tested in the first experiment with L-AP4 and MSG. Concentrations of both substances were 10, 25, 50 and 100 mM. Rats had difficulty discriminating between the tastes of these two substances at these concentrations without amiloride (left panel). Their discrimination performance worsened when amiloride (30 μM) was present in all solutions (right panel).

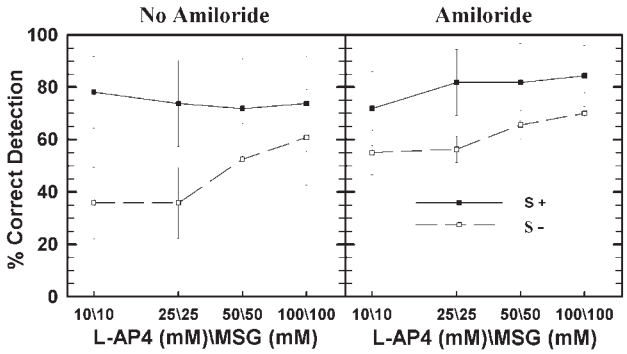

Figure 9 Mean (SEM) percentage detection rates for S+ (solid line) and S– (dashed line) stimuli when rats were tested in the second experiment with L-AP4 and MSG. Rats received extensive training with both substances at 10, 25, 50 and 100 mM. Rats had difficulty discriminating between the tastes of these two substances at these concentrations without amiloride (left panel). Their discrimination performance improved significantly when amiloride (30 μM) was present in all solutions (right panel).

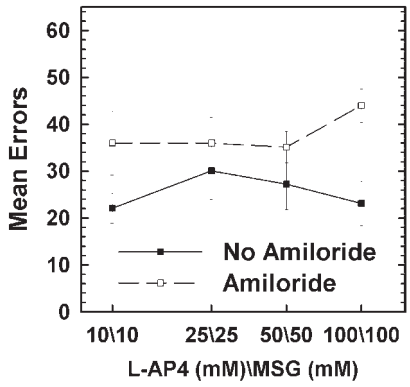

Figure 8 Mean (SEM) error rates when rats were tested in the first experiment with L-AP4 and MSG, both at concentrations of 10, 25, 50 and 100 mM. Mean error rates were high for all concentrations of these two substances without amiloride (solid line). Mean error rates were significantly higher when 30 μM amiloride was added to all solutions (dashed line).

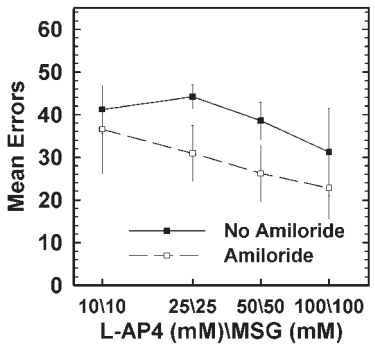

Figure 10 Mean (SEM) error rates when rats were tested in the second experiment with L-AP4 and MSG. Rats received extensive training with both substances at 10, 25, 50 and 100 mM. Mean error rates were high for all concentrations of these two substances without amiloride (solid line). Mean error rates were significantly lower when 30 μM amiloride was added to all solutions (dashed line).

experiment in which the rats received extensive training with matched concentrations of L-AP4 and MSG, accurate discrimination between L-AP4 and MSG remained difficult, but it was significantly better when amiloride was present in the solutions.

Discussion

This study measured the detection thresholds for NMDA, L-AP4 and ASP in rats and tested the ability of rats to discriminate between these taste stimuli under a variety of conditions. The findings verify and extend previous results from conditioned taste aversion studies indicating that there are marked differences between the tastes of NMDA and MSG but there are similarities between the tastes of L-AP4 and MSG and between ASP and MSG.

Detection thresholds, defined as the concentration detected in 50% of the trials, ranged between 1 and 4 mM for NMDA and ASP and between 0.5–2.5 μM for L-AP4. Like detection thresholds (1–4 mM) of MSG (Stapleton *et al.*, 2002), these thresholds were unaffected by 30 μM amiloride. Amiloride raises the detection threshold for Na^+ (as NaCl) from 5 to 40–50 mM in rats (Geran and Spector, 2000). Thus amiloride would be expected to reduce the contribution of Na^+ to the taste of the sodium salts of glutamate, NMDA, ASP and L-AP4 in these experiments. It also means that the taste elicited by the glutamate anion is more salient in the presence of amiloride and presumably plays a more important role in the discrimination experiments. Parenthetically, rats apparently are unable to taste amiloride at concentrations up to 100 μM (Markison and Spector, 1995). Our experiments were conducted well below that concentration.

ASP and MSG were very difficult for rats to identify correctly, especially when amiloride was present, although at the highest concentrations (150–300 mM) detection rates improved. Previous CTA studies had shown that an aver-

sion to either substance generalized strongly to the opposite substance (Stapleton *et al.*, 1999). Taken together, these data indicate that, perceptually, both substances share many common characteristics in rats and that both substances may activate many of the same afferent mechanisms such as the taste-mGluR4 receptor or some other taste receptor. Recent publications have shown that MSG and ASP activate an amino acid receptor that also appears to elicit umami taste (Li *et al.*, 2002; Nelson *et al.*, 2002; Zhao *et al.*, 2003).

Rats readily discriminated between the tastes of NMDA and MSG with or without amiloride. Accurate discrimination between two taste substances is based upon the identification of salient features that are not shared by the two stimuli; that is, the greater the differences in taste qualities, the easier the discrimination between the substances becomes. It is possible that nongustatory cues such as equipment or odor cues might be responsible for these results, but this seems unlikely. Equipment related cues were not detected when only water was presented to the rats during the control experiments. To minimize odor cues, fresh solutions were used each day and a fan was used to push air out of the chamber, including through the opening in which the stimulus delivery tube was located. Moreover, the stimulus aliquot was delivered while the rat licked the tube, minimizing the possibility that the rat could analyze the stimulus as an odor rather than as a taste. Daily non-systematic observations throughout all experiments failed to detect rats approaching the stimulus tube with its nose rather than its tongue once it learned to lick the tube. In addition, the poor performance on the ASP/MSG discrimination and the L-AP4/MSG discrimination at L-AP4 concentrations ≥10 mM, but not at lower concentrations of L-AP4, argue against the notion that the rats used olfactory cues during these discrimination experiments. Individually, none of these factors guarantee that discrimination performance was based only on taste cues but, collectively, they suggest that the discrimination was much more likely to be based on the more salient gustatory cues rather than olfactory cues.

It is generally assumed that if peripheral gustatory transduction mechanisms (e.g. taste receptors) or the afferent pathways are identical for two chemical compounds, there should be little or no perceptual differences between their tastes. However, if taste transduction or neuronal activity for two taste stimuli differs (e.g. the stimuli activate different membrane receptors, different subpopulations of taste cells, or different afferent fibers or neurons), the perceived tastes may also differ. Early molecular studies suggested that NMDA-like receptors were responsible for the umami taste elicited by glutamate (Brand *et al.*, 1991; Faurion, 1991). However, more recent behavioral data using CTA procedures have not supported this position. These studies did not find any generalization of taste aversion between MSG and NMDA in either rats or mice (Chaudhari *et al.*, 1996; Nakashima *et al.*, 2001; Stapleton *et al.*, 1999), suggesting that these substances elicit different taste qualities. The high rate of accuracy in discrimination performance seen in this study adds further support to this position. Taken together, these behavioral data suggest that NMDA and MSG activate, at least in part, different afferent mechanisms and that by itself NMDA may not elicit an umami taste, although it may if combined with other substances (cf. Nakashima *et al.*, 2001).

However, a different story emerges when one considers L-AP4. Molecular evidence has shown that candidate umami taste receptors are stimulated by L-AP4 and MSG at taste relevant concentrations (Chaudhari *et al.*, 2000; Li *et al.*, 2002; Nelson *et al.*, 2002; Zhao *et al.*, 2003) and CTA experiments have shown that L-AP4 and MSG share taste qualities (Chaudhari *et al.*, 1996; Nakashima *et al.*, 2001). The present discrimination task posed different problems, however. Our experiments were modified to take into account the much lower behavioral detection threshold for L-AP4 compared to the threshold for MSG. Surprisingly, these rats rather easily discriminated between MSG and the lower concentrations of L-AP4. Even though behavioral experiments show CTA generalization between these two substances at these concentrations (Chaudhari *et al.*, 1996; Nakashima *et al.*, 2001), generally rats could distinguish between the tastes of the two substances when concentrations of L-AP4 were between 0.01 and 5 mM and concentrations of MSG were between 10 and 100 mM, whether or not the cue function of the sodium ion of MSG was minimized. Yet, when the concentrations of L-AP4 were ≥10 mM, equivalent to the concentrations of MSG, rats found the tastes of L-AP4 and MSG difficult to differentiate. Interestingly, amiloride improved discrimination performance of the rats with extensive training at high concentrations of L-AP4. This suggests that when the taste of the sodium ion is reduced, more differences in taste qualities between L-AP4 and MSG emerge. It is possible that the rats discriminated between L-AP4 at the lower concentrations and MSG on the basis of differences in intensity. That is, perceived intensities of L-AP4 at concentrations under 10 mM were less than the intensities elicited by MSG solutions. However, this explanation is weakened by the fact that CTA generalization occurs at these concentrations and by the fact that the lowest concentration of MSG in these experiments is also near recognition thresholds in rats (Stapleton *et al.*, 1999). Another possibility, implicated by nerve recording data (Sako *et al.*, 2003), is that low concentrations of L-AP4 may be activating a different set of taste receptors than MSG and, therefore, may elicit a taste sensation that is distinguishable from the taste of MSG. However, when the concentration of L-AP4 is above ~0.5 mM, it may activate the same subset of taste receptors as MSG and thus elicit similar gustatory sensations. In short, it is possible that L-AP4 elicits two or more tastes, depending upon the concentration of L-AP4. Regardless, L-AP4 and MSG elicit similar, although not identical, tastes when the concentra-

tions of both substances are 10 mM or greater, an effect that may result from activation of related or common afferent signal pathways within the gustatory system.

In summary, detection thresholds for NMDA, ASP and MSG are similar whereas the detection threshold for L-AP4 is much lower. None of these thresholds were affected by amiloride. Rats accurately discriminated between NMDA and MSG, but had difficulty discriminating between ASP and MSG at concentrations as high as 300 mM. Rats also easily discriminated between low concentrations of L-AP4 and MSG, but had difficulty discriminating between the tastes of equimolar concentrations of L-AP4 and MSG. These results suggest that L-AP4, ASP and MSG may share signal transduction mechanisms or overlapping afferent signal pathways.

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