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

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Abstract

Generalization of a conditioned taste aversion (CTA) is based on similarities in taste qualities shared by the aversive substance and another taste substance. CTA experiments with rats have found that an aversion to a variety of sweet stimuli will cross-generalize with monosodium glutamate (MSG) when amiloride, a sodium channel blocker, is added to all solutions to reduce the taste of sodium. These findings suggest that the glutamate anion elicits a sweet taste sensation in rats. CTA experiments, however, generally do not indicate whether two substances have different taste qualities. In this study, discrimination methods in which rats focused on perceptual differences were used to determine if they could distinguish between the tastes of MSG and four sweet substances. As expected, rats readily discriminated between two natural sugars (sucrose, glucose) and two artificial sweeteners (saccharin, SC45647). Rats also easily discriminated between MSG and glucose, saccharin and, to a lesser extent, SC45647 when the taste of the sodium ion of MSG was reduced by the addition of amiloride to all solutions, or the addition of amiloride to all solutions and NaCl to each sweet stimulus to match the concentration of Na^+ in the MSG solutions. In contrast, reducing the cue function of the Na^+ ion significantly decreased their ability to discriminate between sucrose and MSG. These results suggest that the sweet qualities of glutamate taste is not as dominate a component of glutamate taste as CTA experiments suggest and these qualities are most closely related to the taste qualities of sucrose. The findings of this study, in conjunction with other research, suggest that sweet and umami afferent signaling may converge through a taste receptor with a high affinity for glutamate and sucrose or a downstream transduction mechanism. These data also suggest that rats do not necessarily perceive the tastes of these sweet stimuli as similar and that these sweet stimuli are detected by multiple sweet receptors.

Key words: glucose, MSG, saccharin, SC45647, SC45647 threshold, sucrose

Introduction

Glutamate is a naturally occurring amino acid that is found in many protein-rich foods such as meats, fish, cheese and some vegetables. The taste of glutamate is believed to have a unique quality called 'umami' that is distinct from the other primary tastes of sweet, sour, salty and bitter (Yamaguchi, 1967). Monosodium glutamate (MSG) is considered to be the prototypical umami substance and has long been incorporated into Asian cuisine to enhance flavor (Maga, 1983). The ability of an organism to detect glutamate is important because its taste signals the presence of dietary protein and it can increase the palatability of food, thereby increasing food intake (Bellisle, 1999). Even though glutamate is an important food additive and a substance naturally present in many foods, the peripheral mechanisms responsible for the taste sensation of MSG are not yet well understood.

Although humans perceive the taste of MSG as umami, under certain conditions rats perceive the taste of MSG as similar to that of sucrose. If a taste aversion is conditioned (CTA) to MSG mixed with amiloride (an Na^+ channel blocker that reduces the Na^+ component of MSG taste), this CTA will also cause rats to avoid sucrose, suggesting that MSG and sucrose share perceptual qualities (Yamamoto *et al.*, 1991; Chaudhari *et al.*, 1996; Stapleton *et al.*, 1999; Heyer *et al.*, 2003). These findings have piqued the interest of researchers and spawned a surge of research activity in umami taste transduction. Behavioral and molecular evidence indicate that, in rats, glutamate activates a novel taste variant of a G-protein coupled class III metabotropic glutamate receptor (mGluR4). Chaudhari *et al.* (2000) cloned a taste-mGluR4 receptor that is identical to the brain mGluR4 except the n-terminus of the taste-mGluR4

receptor is truncated and has a lower affinity for glutamate. Behavioral studies have also supported the role of this receptor in umami taste (Chaudhari *et al.*, 1996; Delay *et al.*, 2000, 2004). 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 (Nelson *et al.*, 2002; Damak *et al.*, 2003; Zhao *et al.*, 2003). Behavioral and molecular studies have shown that this T1R1 + T1R3 heterodimer is responsive to MSG and other substances that elicit an umami taste and appears to be able to detect certain other L-amino acids as well. In contrast, stimuli that elicit a sweet sensation in humans activate a different heterodimeric receptor (T1R2 + T1R3) that is not activated by umami substances (Nelson *et al.*, 2002; Zhao *et al.*, 2003). In short, a combination of behavioral and molecular data support the notion that MSG and sweet stimuli activate different afferent pathways.

In spite of apparent differences in afferent mechanisms activated by MSG and sweet stimuli, strong cross-generalization of CTA between MSG mixed with amiloride and the natural sugars sucrose and glucose (and, to a lesser extent, maltose) and the artificial sweeteners saccharin and SC45647 has been reported for rats (Heyer *et al.*, 2003). These data suggest that the taste of glutamate mimics the taste qualities of sucrose and other natural sugars and artificial sweeteners. In addition, Stapleton *et al.* (2002) found that rats had difficulty discriminating between sucrose and MSG when the cue function of the Na^+ ion was reduced by either adding amiloride to test solutions or by matching the Na^+ content of sucrose to that of MSG. Collectively, these findings suggest that to rats glutamate has a strong, maybe even dominating 'sweet' component and raise the question of whether rats can discriminate between any of these sweet substances and MSG. It is possible that rats may not be able to differentiate between substances identified as 'sweet' and 'umami' by humans. To address this issue, behavioral discrimination experiments were conducted with rats to determine the degree to which rats could discriminate between the tastes of MSG and several natural sugars (sucrose and glucose) and artificial sweeteners (sodium saccharin and SC45647) that previously were reported to show strong bi-directional generalization of CTA. If glutamate primarily elicits a sweet taste in rats, then they should have difficulty discriminating between MSG and these sweet substances, especially when the role of Na^+ taste is reduced.

Material and methods

Subjects

Twenty-nine male albino Sprague–Dawley rats were obtained from Harlan Sprague–Dawley (Indianapolis, IN). At the beginning of the experiment all animals were 90–120 days old, weighed between 300 and 450 g and were housed individually. Two weeks prior to testing, the rats were placed on a 21 h water deprivation schedule that was maintained throughout the experiment. Purina Lab chow was available *ad libitum*. The colony lighting was regulated according to a 12 h light/dark cycle with the lights turned on at 7:00 a.m. All testing took place during the light portion of the cycle and each rat was tested at the same time each day.

Apparatus

Each test station contained a computer controlled Knosys Ltd gustometer (Brosvic and Slotnick, 1986) housed in individual benchtop stations. Each test apparatus consisted of a Plexiglas operant chamber (25.4 × 15.9 × 20.6 cm) with a small circular opening (2.2 cm diameter) in one wall, centered 11.4 cm from the floor of the chamber, that permitted access to a drinking spout positioned 3 mm behind the portal. A fan, mounted in the ceiling of the chamber, forced air out of the chamber through the opening for the spout to reduce olfactory cues. Each taste solution was stored in one of ten 10 ml unpressurized syringe barrels that were located at least 15 cm above the drinking spout. The flow of solution from each syringe barrel was regulated by solenoids, located at least 20 cm from the chamber. All syringe barrels were connected to capillary tubing through which each solution flowed 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 55 µl aliquot delivered over 0.6 s. Licks were counted when the animal's tongue made contact with the drinking spout and completed a 64 nA contact current through a stainless steel plate on the floor of the chamber. All testing was conducted under 30 ± 5 lx illumination from a white incandescent bulb inside the station. To reduce auditory cues, an independent solenoid was activated simultaneously with the solenoid delivering the stimulus. In addition, a Radio Shack Sleep Machine generated masking noise (SPL A scale: 75 ± 5 dB) throughout the test period.

Procedures

Training procedures

Training and discrimination methods were similar to those used in previous experiments (Stapleton *et al.*, 2002; Delay *et al.*, 2004). Six rats were randomly assigned to four test groups. Each group was trained to discriminate between one of four sweet taste substances—sucrose, glucose, sodium saccharin (Sigma, St Louis, MO) and SC45647 (Nofre *et al.*, 1990)—and MSG (Sigma). Initially, half of the rats in each group were randomly assigned to discriminate MSG from deionized water and the other half of the animals were assigned to discriminate easily detectable concentrations of the sweet substance from deionized water. To initiate a trial, the rats licked on a variable ratio 20 schedule that resulted in a 35 µl water rinse. Three seconds later, the rat could begin a second variable ratio 20 schedule which, when completed, resulted in the delivery of the 55 µl stimulus aliquot. Once

the stimulus was delivered, the rat had 2 s (decision interval) to determine if the stimulus was an S+ or an S–. After the delivery of an S+ solution, the rat had to lick the spout during the last 0.4 s of the decision interval to receive a 70 μl water reinforcer (i.e. correct detection of the S+). Upon delivery of an S– solution, 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 correctly respond to the S– taste stimulus, a weak shock (30–35 mV) 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 briefly stopped licking when the shock was applied. Shock was always presented to the lick spout for 2 s following the end of the decision interval of S– trials. However, 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 session was completed after the animal completed 160 trials or an hour had elapsed, whichever came first. During each training session, seven of the stimulus barrels contained different concentrations of the taste stimulus (S–) and three contained deionized water (S+). An equal number of S+ and S– trials were presented within each session and the order of the S+/S– presentations followed a random sequence established with a Latin square design. A different concentration sequence was tested each day and each concentration was stored in a different syringe barrel each day to minimize the possibility that the rat could identify a taste stimulus using the location of the stimulus delivery in the spout. When the detection rate of a concentration was 80% in three consecutive sessions, the range of concentrations was decreased in the next session until the test concentrations were reached. Following the end of a session, the rat was returned to its home cage and 15 min later the rat received an additional hour of access to water.

Before the discrimination experiments with SC45647 were conducted, five additional rats were trained with the above procedures with concentrations as low as 0.001 mM to establish the detection threshold, defined as the concentration detected 50% of the time. Each concentration was tested in at least three sessions. A second set of tests was conducted with 30 μM amiloride added to all solutions. The range of detection thresholds for SC45647 for these rats was between 0.0012–0.0045 mM without amiloride and 0.0022–0.0047 mM with amiloride.

Discrimination procedures

Once the rats reached a stable rate of performance with water as the S+ and the test concentrations of one of the taste substances as the S–, discrimination procedures were initiated by changing the S+ condition from water to the opposite taste substance. For example, of the six rats assigned to the sucrose/MSG discrimination, three were tested with sucrose as the S+ and MSG as the S– and three were tested with MSG as the S+ and sucrose as the S–.

During each test session, 5 of 10 stimulus tubes contained different concentrations of the S+ and 5 contained different concentrations of the S–. The stimulus tubes and the stimulus presentation order were randomized each day with a Latin square.

To minimize the possibility that stimulus intensity rather than stimulus quality could serve as a discriminative cue, five concentrations of each substance were tested each day. The concentrations of each substance used in the discrimination experiments were based on a combination of detection threshold values, pilot data and previous CTA studies in which strong cross-generalization between the sweet substance and MSG was reported (Stapleton et al., 1999; Heyer et al., 2003). The test concentrations of MSG were 10, 25, 50, 100 and 150 mM in all experiments. The range of glucose concentrations was 100, 200, 300, 400 and 500 mM. Because of apparently steeper psychophysical functions, the stimulus concentrations of sodium saccharin and SC45647 were divided into high and low ranges. For saccharin, the low range of concentrations included 0.5, 0.625, 0.75, 1.0 and 1.25 mM and the high range included 1.25, 1.5, 2.0, 2.5 and 3.0 mM. For SC45647, the low range included 0.005, 0.0075, 0.01, 0.015 and 0.02 mM and the high range included 0.02, 0.025, 0.03, 0.035 and 0.04 mM. The pH of all stimuli was between 6.75–7.0.

Because the Na^+ ion of MSG could serve as a cue to differentiate MSG from the other substances, these experiments were conducted under three separate conditions to control for sodium taste: (1) no amiloride, (2) amiloride (30 μM) in all solutions and (3) amiloride (30 μM) in all solutions and NaCl added to the sweet solutions. The last condition was conducted because amiloride, although tasteless at 30 μM (Markison and Spector, 1995), does not fully block Na^+ taste at the higher concentrations (Geran and Spector, 2000). Thus, to further minimize the cue function of sodium taste, NaCl was added to each solution of the sweet substance to match the Na^+ content of each concentration of MSG. When testing glucose, for example, 10 mM of NaCl was added to the 100 mM concentration of glucose, 25 mM of NaCl was added to the 200 mM concentration of glucose and so on.

Discrimination training began with at least 14 days of the no amiloride condition to ensure stable performance. The rats were then run an additional 4 days for data collection. This was followed by 6 days of training with the amiloride condition and then 4 days of testing. Another 6 days of training followed and then 4 days of testing with the amiloride and NaCl matching condition. Finally, the amiloride condition was repeated for an additional 6 days to control for experience and the data from the last 4 days were averaged with the first set of scores. After completion of each sodium cue condition, an additional test session was conducted to determine if any of the rats were able to discriminate between stimulus tubes on the basis of spout location or some equipment generated cue. All experimental

parameters were maintained during this session except each tube was filled with water and randomly assigned as an S+ or S−. For the rats in the saccharin and SC45647 experiments, discrimination began with the low range of concentrations and then the procedures were repeated for the high range of concentrations.

Results

Even though the substances treated as the S+ and S− were counterbalanced in each experiment, the data were examined to see if there were any differences in performance related to the specific substance being tested as the S+ and S−. No significant differences were detected for this factor and thus to simplify the analyses, scores for the two substances were pooled for the S+ and S− conditions in each experiment. The primary analysis of the data for each experiment examined the number of correct detections for each stimulus. This within subject analysis of variance examined the effects of sodium cue condition (3) and concentrations matched by ordinal ranking (5) on correct detections.

Sucrose versus MSG

In general, the discrimination between sucrose and MSG was easy for these rats but, as seen in Figure 1, it became much more difficult when the cue function of Na+ was reduced. The analysis of these data indicate that detection rates decreased systematically as the concentrations of the two substances decreased [$F_{(4,20)} = 58.91$, $P < 0.001$]. This ANOVA also revealed a significant main effect for the sodium cue condition [$F_{(2,10)} = 11.44$, $P < 0.005$]. Further analysis of this variable using simple effects tests and

Bonferroni *t*-tests (Howell, 1997) indicated that detection rates were significantly higher in the no amiloride condition than in either the amiloride or the amiloride plus NaCl conditions and that these differences were seen primarily at 10, 25 and 50 mM (all Ps ≤ 0.05). Detection rates in the no amiloride condition were between 86 and 98%, whereas detection rates for the other two sodium cue conditions were between 72 and 94%. These results indicate that the discrimination between sucrose and MSG is rather easy for these rats, but when the cue function of Na+ is reduced, this discrimination is much more difficult.

Glucose versus MSG

Rats easily discriminated between glucose and MSG, regardless of the sodium cue condition (Figure 2). Mean detection rates ranged between 93.1 and 98.7%. The ANOVA detected a significant effect of concentration on detection rates [$F_{(4,20)} = 9.63$, $P < 0.001$] which was due to a small but significantly lower detection rate for the 100 mM glucose/10 mM MSG pair of concentrations than for the other concentrations. The analysis did not find any effect related to the sodium cue condition.

Saccharin versus MSG

The rats also found the saccharin/MSG discrimination task easy to perform at all concentrations. The analysis of the data for the low range of saccharin concentrations (0.5–1.25 mM) revealed a significant effect related to concentrations [$F_{(4,20)} = 14.03$, $P < 0.001$] and to the interaction between the sodium cue condition and concentration [$F_{(4,20)} = 2.23$, $P < 0.05$]. Simple effects and Bonferroni *t*-tests indicated that detection rates at the lowest concentrations of MSG and saccharin were significantly higher in the no amiloride
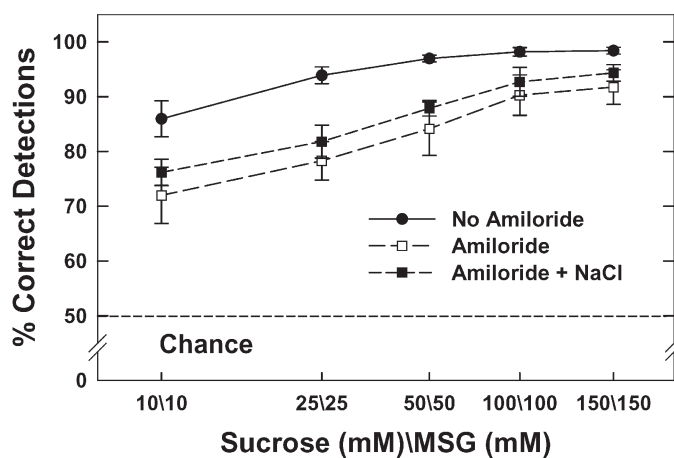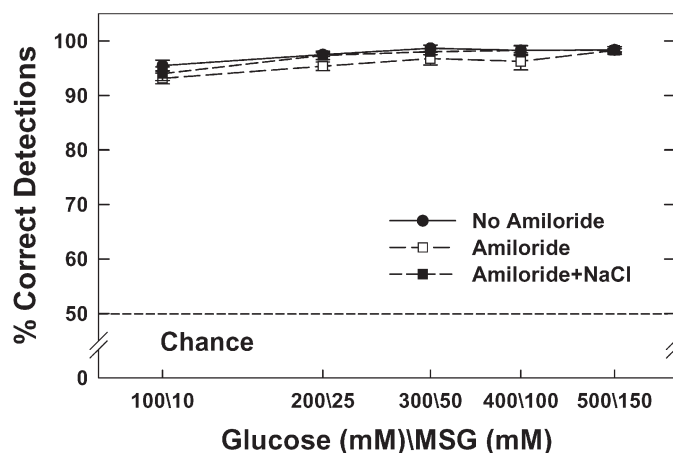

Figure 1 Mean (SEM) percentage correct detection when rats were tested with sucrose and MSG. The concentrations of both substances were 10, 25, 50, 100 and 150 mM. Detection rates were high at all concentrations when amiloride was not present in either solution (filled circle). Rats had difficulty discriminating between the tastes of these two substances at concentrations <100 mM when amiloride (30 μM) was added to all solutions (open square) or when amiloride was added to all solutions and NaCl was added to sucrose to match the concentrations of Na+ in MSG (filled square) to minimize the cue function of sodium.

Figure 2 Mean (SEM) percentage correct detection when rats were tested with glucose and MSG. Test concentrations were 10, 25, 50, 100 and 150 mM for MSG and 100, 200, 300, 400 and 500 mM for glucose. Rats easily discriminated between the tastes of these two substances. Detection rates of all concentrations were high in all three sodium cue conditions.

condition than in either of the other two sodium cue conditions ($P < 0.01$). The sodium cue conditions did not affect performance at any of the higher concentrations (Figure 3).

The analyses of the correct detection data for the high range of saccharin concentrations (1.25–3.0 mM) revealed only a significant effect of concentration [$F(4,20) = 21.36$, $P < 0.001$]. Although detection rates (Mean = $91.8 \pm 0.7\%$) were significantly lower at the lowest concentrations of saccharin (1.25 mM) and MSG (10 mM) than at the other concentrations, this effect was small (Figure 3). No effect related to the sodium condition was found. Thus, these rats easily distinguished between saccharin and MSG at all concentrations of the solutions tested.

SC45647 versus MSG

Importantly, a paired comparison t-test indicated that the threshold estimates for SC45647 were unaffected by amiloride ($P > 0.10$) (without amiloride, mean threshold estimate = 0.0037 mM; with amiloride, mean threshold estimate = 0.0041 mM). In the discrimination experiments, the rats generally found this discrimination task easy to perform. The mean correct detections ranged between 81.8 and 98.8% for the low range of concentrations of SC45647 (0.005–0.02 mM) and MSG (left panel, Figure 4). Although these detection rates indicate that this was a relatively easy discrimination for rats, the ANOVA procedures revealed significant effects for the sodium cue condition [$F(2,10) = 14.80$, $P < 0.005$], concentration [$F(4,20) = 16.12$, $P < 0.001$] and their interaction [$F(8,40) = 3.74$, $P < 0.001$]. Simple effects tests indicated significant differences between sodium cue conditions at the three lowest concentrations of MSG and SC45647 [all $Fs(2,10) \geq 6.43$, $P < 0.001$]. Bonferroni t comparisons of the two lowest stimulus concentrations showed that detection rates were significantly lower when amiloride or amiloride and NaCl were added to neutralize the cue function of the Na^+ ion ($P < 0.01$). At 50 mM MSG and 0.01 mM SC45647, detection rates in the amiloride condition were significantly lower than in the other two conditions ($P < 0.01$). Thus, even though these are relatively high detection rates, reducing the importance of the sodium ion for detecting MSG produced a small but significant decrease in discrimination accuracy at the lowest concentrations of each substance.

Detection rates of the higher range of SC45647 and MSG were very high, ranging from 88.9 to 98.4% accuracy, indicating this discrimination was also very easy for rats to perform (right panel, Figure 4). The ANOVA analysis comparing discrimination performance of the higher range of SC45647 and MSG uncovered a significant increase in detection rates related to increases in stimulus concentrations [$F(4,20) = 10.34$, $P < 0.001$]. Significant effects for the sodium cue condition [$F(2,10) = 7.28$, $P < 0.025$] and the interaction between concentration and sodium cue condition [$F(8,40) = 4.38$, $P < 0.001$] were found. Simple effects and Bonferroni t-tests indicated that at the lowest concentration of SC45647 and MSG, detection in the amiloride and the amiloride plus NaCl conditions were slightly but significantly lower than the no amiloride condition ($P < 0.05$).

In addition, a second within subject analysis of variance was used to examine the data of each experiment for stimulus valence (S+/S–) and concentration (5) within each sodium cue condition to detect potential effects related to stimulus valence such as shifts in response strategy or motivational factors. No effect of valence was detected for SC45647, but a significant effect of valence was detected for both ranges of saccharin and glucose. Simple effects tests showed that these were due to significantly lower detection rates of the S– than the S+ only at the lowest concentration of each substance [all $Fs(1,5) \geq 9.90$, $P < 0.05$]. In the sucrose/MSG discrimination experiments, the 10 and 25 mM S+ in all three sodium cue conditions and the 50 mM S+ in the amiloride and amiloride plus NaCl conditions was detected significantly more often than the corresponding S– [all $Fs(1,5) \geq 8.94$, $P < 0.05$; Figure 5]. In general, the rats in
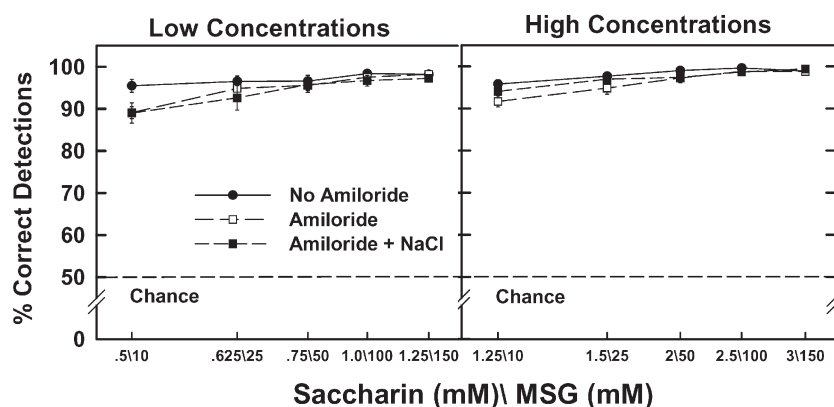
Figure 3 Mean (SEM) percentage correct detection in two experiments in which rats were tested with saccharin and MSG. Two concentration ranges of saccharin (left panel, 0.5, 0.625, 0.75, 1.0 and 1.25 mM; right panel, 1.25, 1.5, 2, 2.5 and 3 mM) were tested. MSG concentrations were 10, 25, 50, 100 and 150 mM in both experiments. Rats easily discriminated between the tastes of these two substances in all three sodium cue conditions.
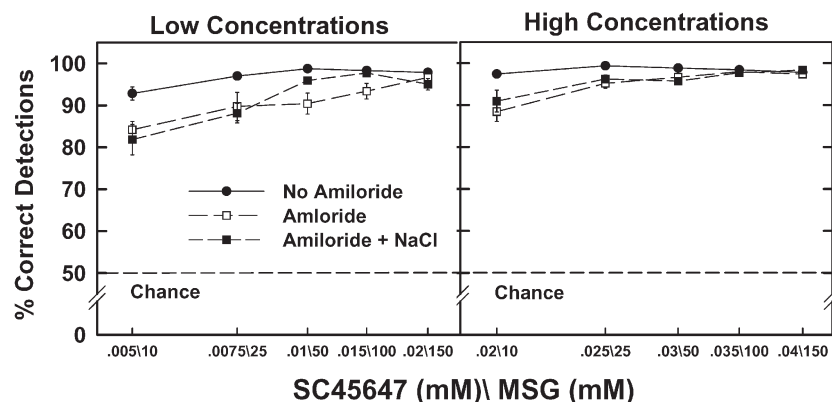
Figure 4 Mean (SEM) percentage correct detection of the artificial sweetener SC45647 and MSG in two experiments. Two concentration ranges of SC45647 (left panel, 0.005, 0.0075, 0.01, 0.015 and 0.02 mM; right panel, 0.02, 0.025, 0.03, 0.035 and 0.04 mM) were tested. MSG concentrations were 10, 25, 50, 100 and 150 mM in both experiments. Rats easily discriminated between MSG and the higher concentrations of SC45647. The discrimination was made moderately but significantly more difficult with the lowest concentrations of SC45647 when the sodium taste of MSG was reduced by amiloride and amiloride + NaCl.
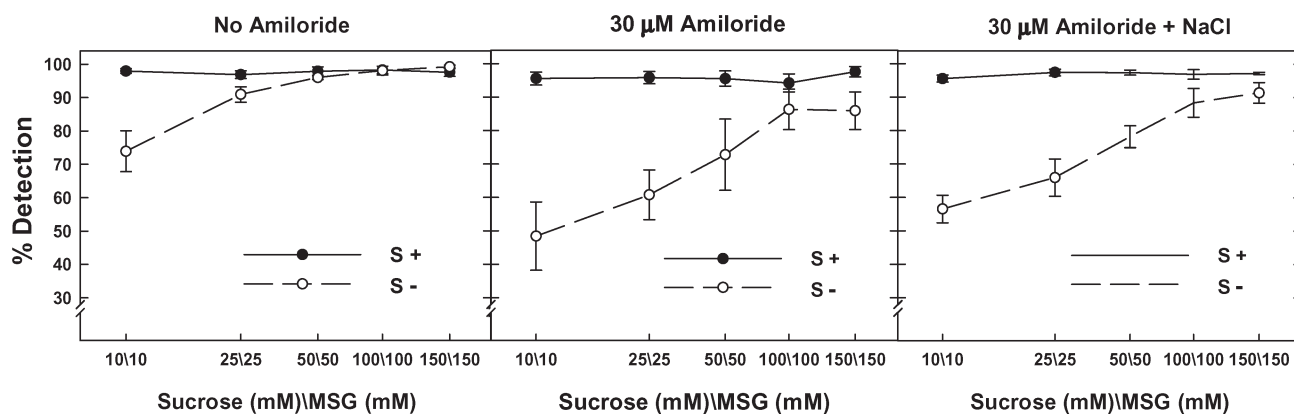

Figure 5 Mean (SEM) percentage detection of S+ (filled circles) and S− (open circles) stimuli when rats were tested with sucrose and MSG. The concentrations of both tastants were 10, 25, 50, 100 and 150 mM. Discrimination was easy when solutions were only sucrose and MSG (left panel). However, all rats had difficulty discriminating between the tastes of these two substances when the cue function of the Na+ was reduced by amiloride (central panel) or amiloride + NaCl (right panel). This difficulty manifested itself primarily by reducing the accuracy of detecting the S− stimuli, especially at the lower concentrations.

all of these experiments tended to miss the S− more than the S+ as the difficulty of the discrimination increased either because the concentration decreased or because of the change in sodium cue effect.

Discussion

Previous experiments with rodents using CTA methods showed strong bi-directional generalization of aversion between each of these sweet substances and MSG mixed with amiloride (Yamamoto *et al.*, 1991; Chaudhari *et al.*, 1996; Stapleton *et al.*, 1999; Nakashima *et al.*, 2001; Heyer *et al.*, 2003). Generalization within a CTA experiment is based on the degree of similarity in taste qualities shared by each stimulus and the results of these experiments suggest that glutamate elicits a sweet taste with qualities shared by a range of artificial sweeteners and natural sugars. However, the findings of the discrimination experiments reported here

suggest that may not be the case. As reported by Stapleton *et al.* (2002), rats found it difficult to distinguish between the tastes of sucrose and MSG when the sodium taste associated with glutamate was reduced or minimized by amiloride and by NaCl matching, especially at concentrations <100 mM. In contrast, the tastes of saccharin and glucose were easily discriminated from MSG by these rats, regardless of the sodium cue condition. Moreover, their ability to discriminate between the artificial sweetener SC45647 and MSG was only slightly, although significantly, diminished when the sodium taste of MSG was reduced. Thus, even though CTA experiments indicate that glutamate elicits taste qualities that mimic qualities elicited by several sweet substances, the taste of glutamate is most similar but not identical to the taste sensation elicited by sucrose.

One might ask whether the ease with which rats distinguished glucose, saccharin and SC45647 from MSG was due

to differences in apparent intensity when the psychophysical functions of the two substances are dissimilar or if the thresholds are quite different. If a wide range of concentrations of one substance is tested while holding constant the concentrations of the second substance, then the perceived intensities of both substances should overlap at some point and the rat will then be forced to use only qualitative taste features to make the discrimination. The concentrations of the sweet substances tested in these experiments were the same as those eliciting strong stimulus generalization in CTA experiments with MSG (Heyer *et al.*, 2003). For example, in this study glucose was tested at concentrations between 100 and 500 mM. Heyer *et al.* (2003) found that cross-generalization of CTA between 100 mM MSG (with amiloride) and glucose was apparent at 50 mM glucose and increased at higher concentrations of glucose. Similarly, the concentrations selected for the low concentration range of saccharin readily showed cross-generalization of CTA with 100 mM MSG. When the rats had little difficulty discriminating the tastes of these substances and MSG, a higher concentration range was tested to ensure a sufficient portion of the psychophysical function of each substance was compared to MSG. Saccharin was not tested at concentrations >3 mM to avoid the emergence of bitter taste qualities that occur at higher concentrations of saccharin (Dess, 1993). The moderate difficulty in discrimination that was seen for the lower range of SC45647 when the Na^+ taste of MSG was reduced may be at least partially related to the proximity of detection thresholds for SC45647 and MSG. Still, these rats readily discriminated between SC45647 and MSG in all sodium conditions at the higher concentrations where CTA readily cross-generalizes (Heyer *et al.*, 2003). Although it is possible that intensity was the basis for the high degree of accuracy in these discrimination experiments (except sucrose), the wide range of concentrations tested for each sweet substance makes this unlikely. Finally, one must at least consider whether non-taste cues, such as equipment-generated cues, might play some role in these experiments. This seems unlikely since water-only test days did not reveal any systematic bias in responding. Also, several steps were taken to minimize potential odor cues. First, stimulus volumes were small and rats were required to emit a response within a short time after stimulus delivery to minimize the possibility that retro-oral odor cues might facilitate discrimination. In addition, fresh solutions were used daily and the delivery system (e.g. lick spout) was designed to minimize odor cues (Brosvic and Slotnick, 1986). Although these procedures may not have completely eliminated all non-taste cues, the saliency and reliability of any residual non-taste cues should have been much less than those of the taste stimuli serving as discriminative stimuli in these experiments.

Although there is evidence that umami and sweet signals may travel different afferent pathways (cf. Sako *et al.*, 2000; Nelson *et al.*, 2002), it seems unlikely that these pathways are completely separate. Several investigators have suggested that glutamate and sweet afferent pathways may interact or converge at some point (Yamamoto *et al.*, 1991, 2001; Chaudhari and Kinnamon, 2001; Sugimoto *et al.*, 2001; Heyer *et al.*, 2003). For example, studies of candidate taste receptors have identified two heterodimeric G-protein coupled receptors, T1R2/T1R3 for detecting sweet stimuli and T1R1/T1R3 for detecting umami stimuli and other L-amino acids (Li *et al.*, 2002; Nelson *et al.*, 2002; Damak *et al.*, 2003; Zhao *et al.*, 2003). Zhao *et al.* (2003) reported that genetically eliminating the T1R1 and T1R3 subunits in mice abolished preferences for umami and artificial sweeteners and most of the response to natural sugars. Damak *et al.* (2003) independently developed a T1R3 knockout mouse and similarly found a loss of response for artificial sweeteners, but only a moderate loss of response for umami substances and natural sugars. The findings reported in the present study suggest there may be another sweet receptor that also responds to glutamate. One possibility is another candidate G-protein coupled glutamate receptor, a taste-mGluR4 receptor, that mimics the cellular effects and umami taste perception of MSG (Chaudhari *et al.*, 1996, 2000). Within taste receptor cells, sucrose increases cAMP while artificial sweeteners increase IP_3, downstream second messengers also affected by MSG stimulation (Lindemann, 1996; Chaudhari *et al.*, 2000; Sugimoto *et al.*, 2001; Abaffy *et al.*, 2003). Convergence of these signals within the same cell could account for the difficulty in the sucrose-MSG discrimination.

Nerve recording studies also point to convergence of umami and sweet signaling. For instance, Yamamoto *et al.* (2001) found that mixtures of MSG and IMP and of L-AP4 (a potent mGluR4 agonist) and IMP elicited activity in chorda tympani nerve recordings that are suppressed by gurmarin, a sweet inhibitor. Moreover, mixtures of L-AP4 and several sweet substances produced a synergism that was not seen with mixtures of MSG and sweet substances. Curiously, Sako *et al.* (2003) detected synergy between L-AP4 and sweet substances (e.g. sucrose, glucose) that was blocked by gurmarin in fibers that respond only weakly to sucrose. These data, along with the data reported here, strengthen the possibility that the taste-mGluR4 receptor may have played a role in these discrimination experiments. Gustatory neurons within the nucleus of the solitary tract, like fibers of the afferent nerves, respond better to selected taste stimuli than to others. Some of the NTS neurons that respond best when the rat's tongue is bathed with sucrose also respond to MSG and show synergy to mixtures of MSG and IMP (Adachi and Aoyama, 1991; Nakamura and Norgren, 1993). Thus, while afferent signaling of umami stimuli may travel in pathways separate from sweet, molecular and nerve recording data, along with CTA data, suggest that there is at least some convergence of afferent signals for sweet and umami taste early in the afferent system. The discrimination data reported in this study also support this

hypothesis. Although these experiments do not directly address the manner of convergence, the difficulty these rats had discriminating between sucrose and MSG and the ease with which these rats discriminated between all other sweet substances and MSG, suggest that the interaction between sweet and umami may be through a receptor with a high affinity for glutamate and sucrose, or a point further downstream such as a shared transduction process within taste receptor cells, or possibly cellular interactions within a taste bud. Further study with other glutamate agonists, L-amino acids and sweet stimuli may help elucidate the nature of this convergence.

Although this study was designed to explore the sweet qualities of MSG, the results may provide some insights into sweet transduction as well. Recent behavioral and electrophysiological studies with knockout mouse studies support the hypothesis that the T1R2/T1R3 receptor may be the primary receptor for many artificial sweeteners and an important but almost certainly not the only receptor for natural sugars (Li *et al.*, 2002; Damak *et al.*, 2003; Zhao *et al.*, 2003). In this study the taste of sucrose was not easily discriminated from MSG by rats yet glucose, in sharp contrast, was quite readily discriminated from MSG. These results suggest that rats do not perceive the tastes of these four sweet substances as identical and that at least two different sweet receptors may be involved in the detection of natural sugars by rats. Additionally, a previous CTA study found that while sucrose and glucose showed strong stimulus generalization with MSG, maltose showed only weak cross-generalization to MSG (Heyer *et al.*, 2003) or sucrose (Spector and Grill, 1988; Heyer *et al.*, 2003). These results indicate that rats perceive maltose as only weakly similar to either MSG or sucrose at the concentrations used in these studies and suggests that maltose may also activate receptors different from those that respond to sucrose or MSG. The apparent differences in taste qualities of these natural sugars relative to MSG, when considered together with numerous other studies, suggest that rats have at least one sweet receptor that can detect some artificial sweeteners and multiple receptors capable of detecting carbohydrates (e.g. Lawless and Stevens, 1983; Dubois, 1997; Ninomiya *et al.*, 1999). One possibility, suggested by Sclafani and others (Nissenbaum and Sclafani, 1987; Sclafani *et al.*, 1987; Sako *et al.*, 1994), is that in addition to a receptor for sucrose, there are 'polysaccharide' receptors. Another possibility, suggested by the differential effects of gurmarin on synergistic responses induced by glutamate agonists and either IMP or natural sugars in the chorda tympani nerve, is that there may be 'gurmarin-sensitive' and 'gurmarin-insensitive' sweet receptors (Ninomiya *et al.*, 1999; Sako and Yamamoto, 1999; Sako *et al.*, 2003). Although the results of the present study do not elucidate the number or the specific nature of sweet receptors in the rat, they clearly support the notion that there are multiple receptors for detecting sweet stimuli.

In summary, the behavioral data obtained in this study suggest that sweet and umami afferent signaling may converge through a common taste receptor with a high affinity for glutamate and sucrose, a downstream transduction mechanism, or cell–cell interactions. These data also support the hypothesis that artificial sweeteners and natural sugars are detected by multiple sweet receptors.

Acknowledgements

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