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Behavioral Comparisons of the Tastes of L-Alanine and Monosodium Glutamate in Rats

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Abstract

Recent research has implicated T1R1/T1R3 as the primary taste receptor in mammals for detecting L-amino acids, including L-monosodium glutamate (MSG) and L-alanine. Previous behavioral studies with rodents found only minimal evidence that these two substances share perceptual qualities, but those studies did not control for the taste of sodium associated with MSG. This study used several behavioral methods to compare the perceptual qualities of MSG and L-alanine in rats, using amiloride (a sodium channel blocker) to reduce the sodium component of MSG taste. Detection thresholds of L-alanine in rats ranged between 0.4 and 2.5 mM, with or without amiloride added, which are similar to threshold estimates for MSG. Conditioned taste aversion (CTA) found that rats showed strong cross-generalization of CTA between MSG and L-alanine when mixed with amiloride, indicating the two substances have similar perceptual qualities. Discrimination methods showed that rats easily discriminated between L-alanine and MSG unless the cue function of sodium was reduced. The discrimination became significantly more difficult at concentrations <100 mM when amiloride was added to all stimuli and became even more difficult when NaCl was also added to L-alanine solutions to match the sodium concentrations of MSG. These results indicate that, perceptually, MSG and L-alanine have quite similar taste qualities and support the hypothesis that these two L-amino acids activate a common taste receptor. The differences in perceptual qualities also suggest separate afferent processing of one or both substances may also be involved.

Key words: conditioned taste aversion, discrimination, L-alanine, MSG, threshold

Introduction

L-monosodium glutamate (MSG) is a non-essential amino acid found in high-protein foods such as meats, fish, cheese and vegetables. Its detection as a taste sensation signals the presence of protein in the diet and when added in small amounts to food, it can increase the palatability of food and thus can influence dietary intake (Bellisle, 1999). The taste of MSG is often considered prototypical of substances with an 'umami' taste, which is considered a fifth primary taste distinct from sweet, salty, sour and bitter (Yamaguchi, 1967; Lindemann *et al.*, 2002). A second defining property of umami substances is their ability to interact synergistically with 5′-ribonucleotide monophosphates, specifically inosine monophosphate (IMP) and guanosine monophosphate (GMP) to potentiate the perceived intensity of the substance (Sato *et al.*, 1970; Rifkin and Bartoshuk, 1980; Delay *et al.*, 2000; Kawai *et al.*, 2002). Even though MSG is known for its umami taste, when amiloride, an epithelial sodium channel antagonist (Heck *et al.*, 1984), is added to MSG to reduce the Na^+ taste, rats can confuse its taste with substances such as sucrose that humans perceive as sweet (Yamamoto *et al.*, 1991; Chaudhari *et al.*, 1996; Stapleton *et al.*, 1999; Heyer *et al.*, 2003). These findings have led to the suggestion that MSG elicits a characteristic sweet sensation (Yamamoto *et al.*, 1991; Sugimoto *et al.*, 2001; Stapleton *et al.*, 2002; Heyer *et al.*, 2003).

Interestingly, another non-essential amino acid, L-alanine, appears to share many of the characteristics of MSG. L-alanine is found in high concentrations in many of the same foods as MSG, although in humans L-alanine elicits a sweet sensation (Schiffman *et al.*, 1981). MSG and L-alanine are both preferred by rodents (Iwasaki *et al.*, 1985; Delay *et al.*, 2000) and a conditioned taste aversion (CTA) to sucrose generalizes to L-alanine in mice (Kasahara *et al.*, 1987; Harada *et al.*, 1997). The linkage between MSG and L-alanine as taste stimuli has been strengthened by recent descriptions of T1R G-protein coupled taste receptors (Nelson *et al.*, 2002; Damak *et al.*, 2003; Zhao *et al.*, 2003). The T1R family of receptors form heterodimers that appear to be selective for certain taste stimuli. The T1R2/T1R3 receptor appears to be selective for substances that elicit a

sweet sensation in human such as natural sugars, artificial sweeteners and D-amino acids and in molecular expression studies are unaffected by the presence of IMP (Nelson *et al.*, 2002; Zhao *et al.*, 2003). On the other hand, the T1R1/T1R3 receptor is selective for L-amino acids, including L-glutamate and L-alanine, and its response to both amino acids is potentiated by the addition of IMP (Nelson *et al.*, 2002; Zhao *et al.*, 2003). These findings support the hypothesis that the T1R1/T1R3 receptor is responsible for detection of L-amino acids. If both L-amino acids activate the same taste receptors, then hypothetically they should have very similar if not identical taste characteristics, especially when the cue function of the sodium ion of MSG is minimized.

The experiments reported in this study examined several characteristics of MSG and L-alanine tastes. First, threshold experiments were conducted to determine the degree of sensitivity rats have for detecting L-alanine. Secondly, two CTA experiments were conducted to determine if MSG and L-alanine, mixed with amiloride to reduce Na^+ taste, had comparable taste qualities. Thirdly, since the CTA results suggested that these substances appear to elicit quite similar taste qualities, discrimination experiments were conducted to determine if rats could distinguish between the tastes of these substances. Collectively, these studies indicate that MSG and L-alanine have quite similar perceptual qualities and provide insights into transduction mechanisms for these two substances and for amino acids in general.

Threshold experiment

The detection threshold for L-alanine was determined for rats in the first experiment. This information is important for establishing minimum behaviorally relevant concentrations of L-alanine, with or without amiloride present, and for selecting concentrations for subsequent experiments. It is also an important feature of L-alanine taste perception that can be compared with MSG.

Materials and methods

Subjects

Six male albino rats (Harlan Sprague–Dawley) aged between 90 and 100 days old at the beginning of training and weighing between 300 and 425 g served as subjects. The rats were housed individually in a colony kept on a 12 h light/dark schedule where lights were turned on at 7:30 a.m. The rats were put on a 21 h water deprivation schedule beginning at least 1 week prior to the start of the training session. Food was provided *ad libitum*.

Apparatus

Threshold and discrimination experiments were conducted in computer controlled Knosys Ltd gustometers (Brosvic and Slotnick, 1986) housed in individual bench top stations. Each test apparatus consisted of a Plexiglas operant chamber measuring 25.4 × 15.9 × 20.6 cm. The rat could access a lick spout through a circular opening (2.2 cm diameter) in one wall, centered 11.4 cm from the floor of the chamber. The lick spout was positioned 3 mm behind the opening. A fan was mounted in the ceiling of the chamber to reduce olfactory cues by forcing air out of the chamber through the opening of the spout. Taste solutions were stored in ten 10 ml unpressurized syringe barrels positioned at least 15 cm above the lick spout. Fluid output from the syringe barrels 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 lick 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 0.6 s. Licks were counted when the animal's tongue made contact with the lick spout. Contact with the spout completed a 64 nA contact current through a stainless steel plate on the floor of the chamber. An incandescent light inside the station provided 30 ± 5 lx illumination during a test session. To reduce potential auditory cues, an independent solenoid was activated simultaneously with the solenoid delivering the stimulus. In addition, masking noise was generated by the ceiling fan and by a Radio Shack Sleep Machine (SPL A scale: 75 ± 5 dB).

Procedure

In the threshold experiment, six rats learned to distinguish between S+ (deionized water) and S– (L-alanine) tastants. The start of each trial consisted of a presentation of 50 µl of a taste stimulus followed by a 2 s decision period where the rat had to either continue licking if the stimulus was the S+ or to discontinue licking if the stimulus was an S–. The rat received a 75 µl water reinforcer if it correctly detected the stimulus as an S+ whereas it received a mild shock if it continued to lick when the stimulus was the S–. Shock was titrated for each rat to just above threshold to discourage licking but not strong enough to cause the rat to stop licking all together. The rats were initially trained to identify 10, 25, 50, 100, 150 and 200 mM of L-alanine (Sigma, St Louis, MO) and water until performance was consistently over 80% correct for all solutions. Thereafter, each day 25, 50 and 100 mM L-alanine were presented along with four randomly selected from the following concentrations of L-alanine: 0.05, 0.1, 0.5, 1, 2.5 or 5.0 mM. The three high concentrations were presented each day to help maintain stimulus control with the S– and continued responding even when the rats could not detect all of the S– stimuli. This was repeated until each concentration was tested 10 times in each of four sessions. Each session was either 160 trials or 1 h depending on which occurred first. The order of stimulus presentation was randomized according to a Latin square design and a different order was used each day. In addition, each solution was stored in a different syringe barrel each day. Since amiloride would be used in subsequent experiments, the threshold testing procedures were replicated with 30 µM

amiloride added to all solutions to determine if it would have any effect on thresholds for L-alanine.

Results and discussion

Detection threshold was defined as the concentration that can be detected 50% of the time. The detection threshold measured for L-alanine in this study ranged between 0.4 and 2.5 mM (geometric mean = 1.12 mM) for these six animals (see Figure 1). Adding amiloride to L-alanine did not alter their threshold ($P > 0.15$). These are very close to the threshold estimated by Pritchard and Scott (1982) from two-bottle preference studies and are also nearly equivalent to estimates of detection thresholds (1–4 mM) for MSG in rats (Stapleton *et al.*, 2002). These results suggest that taste receptor affinities for MSG and L-alanine are comparable and could even be the same receptor.

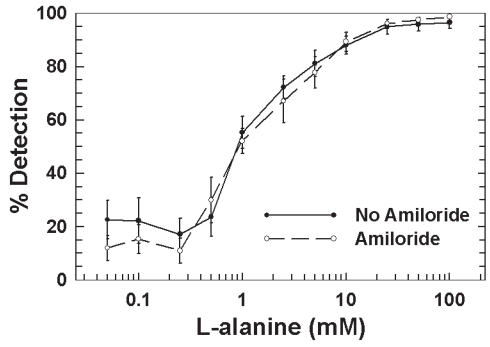

Figure 1 Mean (± SEM) percentage correct detections of each concentration of L-alanine when rats were tested without amiloride or with 30 μM amiloride in all solutions. The presence of amiloride did not affect threshold estimates. The geometric mean threshold for L-alanine across both amiloride conditions was 1.12 mM.

CTA experiments

Two experiments were conducted using CTA procedures. CTA experiments are excellent methods for identifying substances that elicit similar taste qualities (Spector and Grill, 1988; Spector, 2003). When a subject has learned an aversion to a taste substance, it will avoid ingesting that substance and any other substance that is perceived as processing taste qualities similar to the first substance. Moreover, the degree of avoidance of the second substance is proportional to the degree of similarity between the second substance and the first substance. The first CTA experiment was conducted to establish a concentration–response gradient for L-alanine to select stimulus concentrations for the second experiment. The second experiment was designed to examine cross-generalization of CTA between MSG and L-alanine.

Materials and methods

Subjects

All subjects were male Sprague–Dawley rats aged between 90 and 120 days old at the beginning of training and weighing between 300 and 430 g. Sixteen rats were used in the concentration–response experiment and 32 rats were used in the generalization experiment. They were housed individually in the same colony and under the same conditions as described for the threshold experiment. The rats were put on a 21 h water deprivation schedule beginning at least 2 weeks prior to the start of the experiment. When the experiment started, the rats were given 45 min access to water, beginning 30 min after the end of each session.

CTA apparatus

All CTA procedures were conducted in Davis MS80 (Dilog Instruments, Tallahassee, FL) lickometer systems. This system consisted of an eight-tube mobile tray unit for stimulus presentations, an operant chamber, a control unit and an IBM-compatible computer. The operant chamber was 15 cm wide, 30 cm long and 20 cm high. The stainless steel front wall had an oval-shaped opening 3.2 cm wide and 4.0 cm high to give the rat access to a drinking spout when a shutter uncovered the opening. Taste stimuli were stored in 50 ml centrifuge tubes with drinking spouts. The computer controlled the shutter and the positioning of each centrifuge tube with its drinking spout. During a trial the tip of the drinking spout was centered 3 mm behind the oval opening. When the rat made contact with the spout, a circuit with a current of 64 nA was completed through the stainless steel floor and a lick was counted. To reduce potential olfactory cues, a fan moved air into the back of the chamber and out through the opening around the drinking spout. Background lighting was ~15 lx illumination and masking noise (Radio Shack Sleep machine) was 70 ± 5 dB (re: 20 $\mu N/m^2$) inside the operant chamber.

Procedures

CTA procedures were conducted over a 7 day period. During the first 3 days, the rats were trained to drink de-ionized water from the lick spouts. Each session consisted of 32 trials. A trial began when the shutter opened. Once the rat made contact with the lick spout, the number of licks emitted during the next 10 s was counted and then the shutter was closed. If the animal did not lick for 60 s, the trial was ended, the door to the spout was closed and a new trial began after a 5 s inter-trial interval. On day 4 (conditioning day) rats were presented with the conditioned stimulus (CS) in 16 of the 32 trials. Half of the subjects were randomly selected to receive an injection of LiCl (IP, 127 mg/kg, 1 ml/100 g body wt) as an unconditioned stimulus (US) to induce gastric distress and thus a conditioned aversion to the taste CS (Nachman and Ashe, 1973; Spector and Grill, 1988). The rats assigned to the control group received injections of 0.15 M NaCl (i.p., 1 ml/100 g body wt) as the US. The rats were presented only de-ionized water for the next two sessions and CTA testing was conducted on the seventh day. Amiloride (30 μM) was mixed in all solutions,

including water, during conditioning and test days in both experiments.

In the first experiment, designed to establish a concentration-response gradient, 125 mM L-alanine was presented as the CS on the fourth day. Eight of the rats then received LiCl injections and the other eight received NaCl injections. On the seventh day, CTA testing was conducted with seven concentrations (0.1, 1.0, 5.0, 10, 15, 50 and 100 mM) of the CS, each presented twice during a single session. These were selected as a result of the threshold experiments to include concentrations that ranged from undetectable to easily detected. The order of stimulus presentations to each rat was randomized using a Latin square. Stimulus presentations were separated by one to three water trials. Suppression was measured as a percentage of licks for a stimulus relative to licks for water. Thus, lick rates <100% indicate avoidance of a stimulus relative to water, and lick rates by LiCl-conditioned rats that are less than those seen for NaCl-conditioned rats indicate CTA.

In the second experiment, designed to test for cross-generalization between MSG and L-alanine, the CS for 16 of the animals was 100 mM L-MSG (Sigma, St Louis, MO) while the CS for the other 16 rats was 100 mM L-alanine (both mixed with amiloride). Half of the rats in each CS group received LiCl injections and the other half received NaCl injections. During generalization testing, rats in each CS group were presented 10 and 100 mM of their respective CS to assess the strength of conditioning and 10, 25 and 100 mM of the opposite amino acid to assess the degree of stimulus generalization. The test concentrations were selected from the L-alanine concentration–response gradients established in the first CTA experiment and from a similar experiment with MSG (Stapleton *et al.*, 1999). Likewise, CS concentrations were selected because they are approximately equivalent in their capacity to suppress lick rates. Rats were also presented with 25 mM NMDA to determine if they were avoiding all detectable taste stimuli (neophobia). In addition, 100 mM sucrose was presented to determine if the CTA generalized to a prototypical substance that elicits a sweet taste. At these concentrations, both NMDA and sucrose elicit behavioral characteristics for conditioning and testing comparable to those elicited by 100 mM MSG and 100 mM L-alanine (Chaudhari *et al.*, 1996; Stapleton *et al.*, 1999; Heyer *et al.*, 2003). All solutions were mixed with 30 μM amiloride. Each stimulus was presented twice during testing and was separated from other stimuli by one to three water (de-ionized water) trials. The order of stimulus presentation was randomized for each rat using a latin square.

Results and discussion

The data from both experiments were first normalized by dividing the mean lick rate for each stimulus by the mean lick rates for the water trials and then multiplying by 100. For the 0 mM concentration, all series of two or more consecutive water trials were identified and two trials were randomly selected from these series (the first trial of each series was excluded). The average of these lick rates were then normalized in the same manner as the rest of the stimuli.

The results of the first experiment were analyzed with a 2 (US) × 8 (taste stimuli) analysis of variance (ANOVA) for mixed designs. The main effects for US [$F(1,15) = 49.03$, $P < 0.001$] and taste stimuli [$F(7,105) = 21.32$, $P < 0.001$] were significant as was the interaction [$F(7,105) = 13.87$, $P < 0.001$]. Since the interaction was significant, simple effects tests (Howell, 1997) were used to determine that 5 mM was the lowest concentration at which LiCl rats showed significantly lower lick rates than the NaCl rats ($P < 0.005$). These results (see Figure 2) show that rats begin to avoid L-alanine at concentrations just above detection thresholds and exhibit a concentration-avoidance gradient for L-alanine that is similar to that seen for MSG using the same protocol (Stapleton *et al.*, 1999).

In the generalization experiment, the 2 × 8 ANOVA applied to the normalized data of each CS group indicated a significant interaction [MSG, $F(7,98) = 18.91$, $P < 0.001$; L-alanine, $F(7,98) = 21.31$, $P < 0.001$]. The simple effects tests comparing the groups conditioned with MSG (see Figure 3, lower panel) found that the LiCl group licked significantly less of the 10 mM ($P < 0.05$) and 100 mM MSG ($P < 0.001$) than the NaCl group, indicating that the LiCl animals acquired the aversion. More importantly, however, the LiCl rats also showed a concentration dependent generalization of the aversion of L-alanine (all Ps < 0.001). The LiCl rats also significantly decreased their lick rates of sucrose ($P < 0.001$) but their lick rates for NMDA did not differ from NaCl rats (see Table 1). The simple effects tests of the data for the groups conditioned to avoid L-alanine revealed

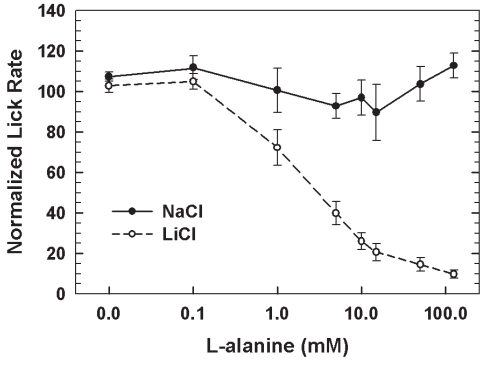

Figure 2 Mean (± SEM) normalized lick rates for several concentrations of L-alanine mixed with amiloride after being conditioned with either NaCl (filled circles) or LiCl (open circles) to induce a taste aversion. The CS concentration was 125 mM. To normalize lick rates, the mean lick rate for each test stimulus was divided by the mean lick rate for all water presentations in the session, then multiplied by 100. The ordinate shows the normalized lick rates and the abscissa shows the concentrations of the test solutions. LiCl-conditioned rats licked significantly less L-alanine than NaCl-conditioned rats at 5 mM and higher concentrations.

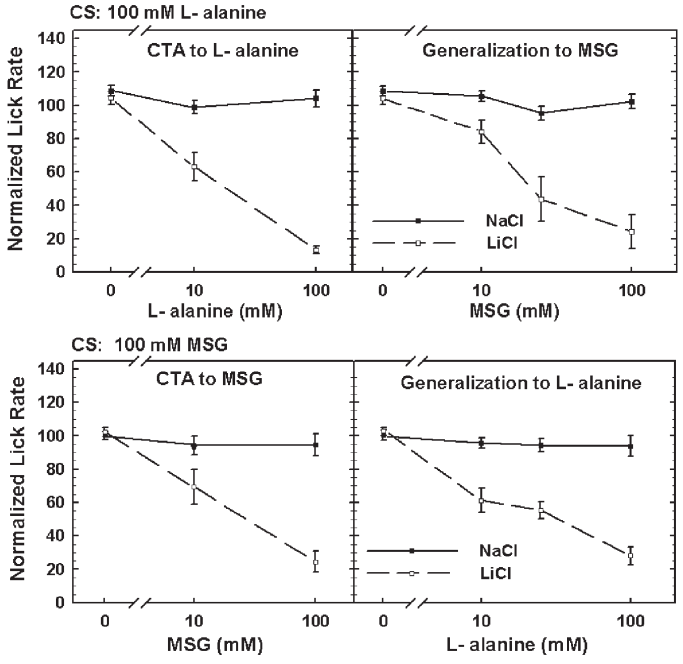

Figure 3 Rats conditioned to avoid 100 mM L-alanine (top, left panel) also avoided MSG during generalization testing (top, right panel). Conversely, rats conditioned to avoid 100 mM MSG (lower, left panel), generalized the avoidance to L-alanine (lower, right panel). Data are presented in the same format as in Figure 2.

nearly identical findings (see Figure 3, upper panel). Compared to the NaCl rats, LiCl rats licked significantly less of: (1) 10 mM ($P < 0.005$) and 100 mM ($P < 0.001$) L-alanine; (2) 10 mM ($P < 0.025$), 25 mM ($P < 0.005$) and 100 mM ($P < 0.001$) MSG; and (3) 100 mM sucrose ($P < 0.001$). The two groups did not differ in their ingestion of 25 mM NMDA (see Table 1).

CTA methods are very good at identifying substances that have similar taste qualities because the animal generalizes the learned aversion (stimulus generalization) to the degree that two substances taste alike. In the second CTA experiment, the magnitude of conditioning toward each amino acid CS and the amount of generalization to the opposite amino acid were similar for both CSs at iso-molar concentrations. These results indicate that both amino acids elicit highly similar taste sensations in the rat. By themselves, these data strongly support the idea that both amino acids share afferent signaling process, probably beginning with a common taste receptor in the rat.

Discrimination experiment

The results of the threshold and the CTA experiments collectively suggest that in rats the taste sensations elicited by MSG and L-alanine are quite similar. However, two taste substances may share many taste qualities and still have taste qualities that distinguish one substance from another (e.g. Heyer *et al.*, 2003, 2004). Stimulus discrimination methods are much better behavioral methods for determining whether or not two substances elicit different taste qualities. These methods are typically designed to motivate the subject to attend to differences in stimulus characteristics instead of similarities (Spector, 2003). If the taste sensations elicited by MSG and L-alanine are nearly identical, then rats will have difficulty discriminating between their tastes, but if the two amino acids also have other dissimilar taste qualities, then the discrimination will be relatively easy for the rats.

Table 1 Mean lick rates of control stimuli after conditioning to either L-alanine or L-MSG

CS	US	Sucrose	NMDA
L-Alanine	NaCl	107.32	100.09
	LiCl	18.15*	103.48
L-MSG	NaCl	92.47	96.12
	LiCl	23.99*	93.18

*$P < 0.001$.

Materials and methods

Subjects and apparatus

Subjects were six naïve male albino rats of the same description and maintained in the same manner as described for the threshold experiment. The apparatus was also the same as that used in the threshold experiment.

Procedures

The discrimination trials followed the same procedures as the threshold work described above. Three of the rats were trained with MSG as the S– and three were trained with L-alanine as the S–. All six rats were trained to >80% accuracy on the discrimination with deionized water as the S+ and their respective S–. Once the animals met criteria for five consecutive days, the S+ was changed from water to the opposite amino acid. During each session five matched concentrations of both the S+ and S– were tested: 10, 25, 50, 100 and 150 mM. The stimuli were presented in three amiloride conditions designed to assess the cue function of the Na^+ ion in the discrimination. In the first condition, all solutions were presented with 30 μM amiloride including the reinforcement water, the second condition presented the same concentrations but without amiloride. Finally, in the third condition 30 μM amiloride was added to all of the stimuli. Also, NaCl was added to the L-alanine only and was calculated to match the concentration of sodium present in the same concentration of MSG. Testing under each condition lasted for 10 days. After each amiloride condition, the rats were tested one day with only water presented as both the S– and the S+ to ensure that the animals were not detecting other cues to make the discrimination.

Results and discussion

Prior to analysis, the percentage of correctly detected S+ and S– stimuli for each concentration in each amiloride condition was determined for each subject. This procedure simplifies each response to either a correct discrimination or an error. As such, chance performance is a 50% rate of discrimination. These data were then subjected to a 3 (amiloride) × 5 (concentration) within-subject ANOVA, which indicated significant effects related to amiloride conditions [$F(2,10) = 22.54$, $P < 0.001$], concentration [$F(4,20) = 46.71$, $P < 0.001$] and the amiloride by concentration interaction [$F(8,40) = 19.74$, $P < 0.001$]. To examine the interaction, or in other words, the effects of the different amiloride conditions on discrimination performance, simple effects tests and Bonferroni multiple-comparison procedures were applied to the data for each concentration (Howell, 1997). The simple effects tests indicated that the amiloride condition significantly altered discrimination rates at all concentrations except 100 mM [all Fs(2,10) = or >11.53, $P < 0.01$]. Bonferroni comparisons ($P < 0.05$) found that discrimination rates were significantly different under all three amiloride conditions at 10 and 25 mM (see Figure 4). At these two concentrations, rats easily discriminated between MSG and L-alanine when the taste of Na^+ associated with MSG was unaltered. The addition of amiloride significantly decreased accuracy compared to the no amiloride condition and adding NaCl to L-alanine along with amiloride to all solutions significantly decreased accuracy compared to the other amiloride conditions, approaching chance performance at the two lowest concentrations. At 50 mM, discrimination rates were significantly lower in the two amiloride added conditions than in the no amiloride condition. Discrimination between L-alanine and MSG was most accurate at 100 and 150 mM, but at 150 mM discrimination rates in the amiloride plus NaCl condition were significantly poorer than in the other two conditions. Analysis of the data for the control tests did not reveal any systematic response that might be attributable to an extraneous cue, although olfactory cues cannot be ruled out completely. These findings indicated that when the taste of the Na^+ ion is reduced, the tastes of glutamate and L-alanine are difficult for rats to differentiate, even in a behavioral paradigm designed to force rats to attend to stimulus differences rather than similarities. This difficulty is concentration dependent, however. These data show that the rats found the two substances easier to differentiate as the concentration increased, suggesting that at the higher concentrations one or both substances begin to elicit salient taste qualities not shared by the opposite amino acid.

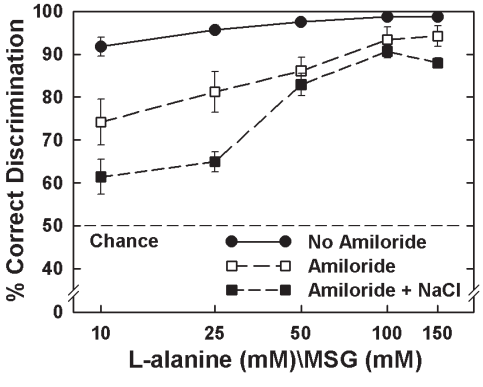

Figure 4 Mean (± SEM) percentage correct discriminations when rats were tested with L-alanine and MSG. The concentrations of both substances were 10, 25, 50, 100 and 150 mM. Discrimination rates were high at all concentrations when amiloride was not added to any solution (filled circles). Rats had difficulty discriminating between the tastes of these two substances at concentrations below 100 mM when amiloride (30 μM) was added to all solutions (open squares). Performance dropped to near chance levels at the lower concentrations when amiloride was added to all solutions and NaCl was added to L-alanine to match the concentrations of Na^+ in MSG (filled squares) to minimize the cue function of sodium.

General discussion

Behavioral studies have shown that MSG has taste qualities similar to those elicited by the mGluR4 agonist, L-AP4, but not with ionotropic glutamate agonists such as NMDA, kainite or AMPA (Chaudhari *et al.*, 1996; Stapleton *et al.*, 1999; Nakashima *et al.*, 2001). Of the amino acids, the umami taste of MSG is often thought to be most closely related to another polar amino acid, L-aspartic acid. Both CTA and discrimination data have verified these similarities (Yamamoto *et al.*, 1991; Stapleton *et al.*, 1999; Delay *et al.*, 2004). The results of the present study show that when the sodium component of MSG is reduced, L-alanine also elicits taste qualities quite similar to L-glutamate in rats, especially at lower concentrations.

Learned taste aversions are helpful in determining if two substances have similar taste qualities because once the aversion is learned for one substance, the subject will also avoid other substances with similar tastes. Moreover, the greater the similarities between the conditioned stimulus and the test stimulus, the more the subject will avoid the test stimulus. The degree of aversion and the similarity of cross-directional generalization between L-alanine and MSG in the presence of amiloride indicate that these substances have quite similar taste qualities. The CTA findings in this study are in contrast to those reported by Ninomiya and Funakoshi (1989). They did not find generalization of CTA between MSG and several L-amino acids in mice, including L-alanine, but their study did not control for the Na^+ component of MSG. As a result, the taste of Na^+ may have masked the perceptual similarities between MSG and the other amino acids. Interestingly, in this study the CTA to each substance also generalized to 100 mM sucrose, replicating previous findings (Kasahara *et al.*, 1987; Yamamoto *et al.*, 1991; Harada *et al.*, 1997; Heyer *et al.*, 2003) and indicating that to rats both substances also have salient taste qualities that mimic those of sucrose. Taken as a whole, the similarities in the profiles of CTA generalization of MSG and L-alanine suggest that they may have quite similar taste characteristics. However,

one of the limitations of CTA methods is that subjects may show generalization of an aversion between two substances and yet one or both substances may possess substantial qualities that are distinctive or absent from the qualities of the other substance (Spector and Grill, 1988; Frank *et al.*, 2003). To test this possibility, discrimination methods requiring subjects to attend to perceptual differences rather than similarities can be used. The discrimination experiments in this study indicated that rats readily discriminate between L-alanine and MSG under normal circumstances. However, when the taste of sodium associated with MSG is reduced, the differences between the perceptual qualities of the two amino acids were nearly eliminated at the lower concentrations almost to the same extent as seen when rats tried to discriminate between MSG and L-aspartic acid (Delay *et al.*, 2004). As with aspartic acid, the discrimination between MSG and L-alanine was easier at the higher concentrations. This latter effect may be due to at least two possible factors. First, amiloride raises the detection threshold of sodium to ~45 mM in the rat (Geran and Spector, 2000). As a result, the taste of sodium probably became increasingly apparent at the highest concentrations. Secondly, it may be that perceptual qualities unique to each substance became sufficiently salient at the higher concentrations to enable the rats to more accurately discriminate between the two substances. Still, when the results of the CTA are combined with the discrimination experiments, it appears that L-alanine and the glutamate anion of MSG have quite similar, although not identical taste qualities.

The strong resemblance between the taste characteristics of glutamate and L-alanine implies that the two substances may share afferent pathways, maybe beginning with a common taste receptor such as the T1R1/T1R3 heterodimer. Cellular expression experiments have shown the T1R1/T1R3 responds to L-glutamate, L-alanine and other L-amino acids, especially when IMP is present (Nelson *et al.*, 2002). Zhao *et al.* (2003) reported that removal of the T1R3 portion of the receptor in knockout mice eliminated behavioral and nerve responses to MSG, L-alanine and other L-amino acids. Thus the closely related perceptual characteristics of MSG and L-alanine may be the result of both amino acids having similar affinities for activating this taste receptor. While it is tempting to attribute all of the taste qualities of L-glutamate and L-alanine to the T1R1/T1R3 receptor, it must be noted that Damak *et al.* (2003) independently developed a T1R3 knockout mouse that exhibited only a reduced response to MSG. These investigators suggested that another taste receptor may also be activated by glutamate. Quite similar tastes can be elicited by different receptor mechanisms and other glutamate receptors such as the taste-mGluR4 (Chaudhari *et al.*, 2000), or possibly NMDA receptors (Faurion, 1991) have not been tested with L-alanine. If those receptors are selective for or have a greater affinity for glutamate than for other amino acids, then glutamate may elicit afferent signals, and thus perceptual qualities, not associated with other L-amino acids. The improved discrimination performance at the highest concentrations and the generalization of CTA between the two amino acids and sucrose may support this hypothesis but further study is needed to test these possibilities.

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