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An Analysis of 5′-Inosine and 5′-Guanosine Monophosphate Taste in Rats

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Abstract

Inosine monophosphate (IMP) and guanosine monophosphate (GMP) elicit an umami taste in humans and synergistically increase the intensity of the umami taste of monosodium glutamate (MSG). Conditioned taste aversion (CTA) studies in rodents indicate that these nucleotides and MSG elicit quite similar tastes, but recent physiological evidence suggests that these nucleotides and MSG may not activate the same population of taste receptors and therefore may not elicit identical taste qualities. This study reports the findings of several behavioral experiments with rats that compared the taste properties of IMP and GMP with each other and with those of MSG. Well-trained rats were able to detect both nucleotides at nanomolar concentrations, but they did not respond to either nucleotide in two-bottle preference tests or brief-access CTA tests at concentrations less than 0.5 mM. Discrimination experiments found that the tastes of these nucleotides could not be discriminated from each other, but both could be discriminated from MSG, even when the taste of Na^+ was controlled. Overall, these experiments indicate the taste properties of the two 5′-ribonucleotides are quite similar to each other, and even though they may elicit an umami sensation, these sensations are not identical to the taste of MSG.

Key words: amiloride, behavior, GMP, IMP, monosodium glutamate, taste discrimination, taste preferences, taste thresholds

Introduction

The chemical sense of taste is important for locating food sources, maintaining nutritional equilibrium, and avoiding harmful or toxic substances. Many substances produce taste sensations, but there are only a few taste primaries. Each primary taste perception (sweet, sour, salt, and bitter) is typically associated with a different category of food. Sweet, for example, is associated with carbohydrates, and sucrose is generally accepted as the prototypical sweet substance. Another important taste, ''umami'' (roughly translated as ''good taste'' or ''savory''), is considered a primary taste by Asian cultures, but Western science has been somewhat slower accepting umami as a primary. Nevertheless, umami taste signals the presence of dietary protein and is associated with foods rich in protein such as meats, fish, vegetables, and cheeses (Yamaguchi 1967; Maga 1983). It can also increase the palatability of food and, thus, food intake. Bellisle (1999) and others have argued that knowledge about umami taste is extremely valuable as a tool to help improve food intake of people who often do not eat well and thus have dietary deficiencies (e.g., diabetic, elderly). The prototypical umami taste stimulus is generally thought to be monosodium glutamate (MSG). Umami is also a taste quality of 2 5′-ribonucleotide monophosphates, 5′-inosine monophosphate (IMP), and 5′-guanosine monophosphate (GMP) and some other L-amino acids. However, even though these 3 compounds are thought to elicit the same taste, the comparability of the tastes qualities of these substances has rarely been tested.

There are 2 defining characteristics of umami taste (Yamaguchi 1967; Maga 1983; Ninomiya 2003). The first is its unique taste quality and the second is a synergistic interaction between an umami taste substance such as MSG with IMP or GMP. Taste synergy is observed when the intensity of the response to a mixture of taste stimuli is greater than the sum of the responses to the individual components (Rifkin and Bartoshuck 1980). These nucleotides can potentiate responses to MSG in taste receptor cells (Li et al. 2002; Nelson et al. 2002; Lin et al. 2003), the chorda tympani nerve (Adachi and Aoyama 1991; Nakamura and Norgren 1993; Sako and Yamamoto 1999; Kurihara and Kashiwayanagi 2000), the nucleus of the solitary tract (Pritchard and Scott 1982), and the cortex (Hellekant and Ninomiya 1991; Scott

et al. 1993; de Araujo et al., 2003). In humans, both ribonucleotides potentiate the perceived intensity of MSG and other L-amino acids (Rifkin and Bartoshuck 1980; Schiffman et al. 1994; Kawai et al. 2002). Similar evidence of synergy in the perception of umami substances has been observed in rats and mice (Bachmanov et al. 2000, 2001; Delay et al. 2000; Ruiz et al. 2003; Zhao et al. 2003). The possibility that synergy occurs in the natural environment seems likely because IMP is often found in the same foods as MSG or L-aspartate (Yamaguchi and Ninomiya 2000; Ninomiya 2003). IMP and GMP also elicit taste responses independently. For example, humans perceive the taste of IMP and GMP as umami and similar to MSG (Yamaguchi 1967; Rifkin and Bartoshuk 1980; Maga 1983). Rats and mice show strong generalization of conditioned taste aversion (CTA) between MSG and either IMP or GMP, indicating that both ribonucleotides elicit a taste similar or identical to MSG in these species (Ninomiya and Funakoshi 1989; Yamamoto et al. 1991). Collectively, these studies suggest that MSG, IMP, and GMP may possess quite similar or even identical umami qualities.

The apparent similarities between afferent signaling of MSG and these 2 nucleotides have influenced the nature of research on umami taste. Curiously, though, relatively little is known about the specific taste characteristics of IMP and GMP or whether they elicit similar or identical taste qualities. For example, selection of concentrations of IMP or GMP for synergy experiments is often arbitrary, ranging from 0.01 to 10 mM, and is often assumed to be below the subject's ability to detect or recognize the taste of the nucleotide or, if detectable, its taste qualities are identical to MSG. For nonbehavioral taste studies, it is generally difficult to know the behavioral relevance of a selected concentration. Moreover, because both nucleotides are disodium salts, they are often tested with amiloride, an epithelial sodium channel antagonist that reduces afferent signals associated with Na^+, but again little is know of the impact of amiloride on the perception of either nucleotide. In view of their apparent umami properties and their critical role for studying the synergistic qualities of umami stimuli, there is a significant need for systematic behavioral profiles of these substances. We are reporting the results of several experiments with rats intended to profile the taste characteristics of IMP and GMP (without amiloride or mixed with amiloride) and to compare their taste qualities with each other and with MSG. Specifically, we tested detection thresholds of IMP and GMP to determine what concentrations are detectable to rats and to compare sensitivity for each substance. We also conducted 2-bottle preference and CTA experiments to compare concentration-preference functions of IMP and GMP in rats. Finally, we conducted discrimination experiments to determine if rats could distinguish between the tastes of IMP and GMP and between MSG and either IMP or GMP. In general, we found that rats perceive the tastes of IMP and GMP as nearly identical to each other but not to MSG.

Experiment 1: detection thresholds

To our knowledge, no one has ever established the detection or absolute threshold for either IMP or GMP in the rat. This experiment measured and compared the absolute thresholds of the 2 nucleotides. In addition, detection thresholds were measured in the presence of amiloride to determine if it altered the threshold of either nucleotide.

Materials and methods

Subjects

The subjects for these experiments were 10 male albino Sprague–Dawley rats obtained from Harlan Sprague-Dawley (Indianapolis, IN). They were approximately 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 21-h water deprivation schedule. Colony lighting was set on a 12-h light:dark cycle with the lights turned on at 7 AM. Each rat was tested at the same time each day between 9 AM and 12:30 PM.

Apparatus

Computer-controlled gustometers (Brosvic and Slotnick 1986; Knosys Ltd. [Lutz, FL], www.knosysknosys.com), located in individual bench top stations, were used for threshold and all discrimination testing. Each gustometer consisted of a Plexiglas operant chamber ($25.4 \times 15.9 \times 20.6$ cm high) with a fan mounted in the ceiling to draw fresh air into the chamber and force air out of the chamber. A small circular opening (2.2 cm diameter) was centered in one wall 11.5 cm above the floor of the chamber. Each subject had access to a lick spout located 3 mm behind the opening. 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 60 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 and 75 ± 5 dB masking noise generated by a Radio Shack Sleep Machine. As an additional mask of the sound of the individual solenoids, an independent solenoid was mounted directly to the chamber above the lick spout and was activated at the same time as the solenoid delivering the stimulus.

Procedures

General procedures. Threshold and discrimination methods were similar to those used in previous experiments (Stapleton et al. 2002; Delay et al. 2004). These methods are designed to induce differential responding to each of 2 taste stimuli by pairing one stimulus (S+ stimulus) with water reinforcement and pairing the second taste stimulus (S− stimulus) with shock. To initiate a trial, the rat had to lick the spout an average of 20 times to receive a 35-μl water "rinse." After another 3 s delay, the rat again licked an average of 20 more times to receive the 50-μl taste stimulus. Stimulus delivery was followed by a 2-s "decision period." Responding during the last 0.4 s of the decision period determined the response outcome. A "detection" occurred if 1) the S+ (e.g., water) was the stimulus and the rat licked during the last 0.4 s of the decision period, then the rat received a 70-μl water reinforcer or 2) the S− was the taste stimulus (e.g., IMP) and the rat did not lick, then the rat avoided shock applied to the spout after the decision period. Shock was always presented to the lick spout for 2 s following the end of the decision interval of each S− trial. Shock intensity (28–33 VDC; Brosvic and Slotnick 1986) was titrated for each rat to just above threshold to induce avoidance but not to stop all licking. The animal only experienced shock if it licked the spout during the shock presentation. Opposite responses during the last 0.4 s in the decision period were errors. A 10-s intertrial interval began after the response consequence. Each session ended after 1 h or 160 trials, whichever came first.

Threshold procedures. Five rats were tested with IMP, and 5 other rats were tested with GMP. During each test session, 7 of the stimulus barrels contained different concentrations of the taste stimulus (S−) and 4 contained deionized (Millipore filtered) 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. The rats were first trained with relatively high concentrations of IMP or GMP (1, 2.5, 5, 10, and 15 mM) until they consistently detected each substance >80% of the time. Concentrations were gradually lowered by half-log increments to 0.00005 mM. To maintain stimulus control, 4 relatively easy to detect concentrations (0.1, 0.5, 1.0, and 2.5 mM) were presented in every session. Two additional concentrations, randomly selected from the concentration range below 0.1 mM, were also tested each day. Within session sequences of stimulus presentations were based on latin square procedures and different sequences were tested each day. 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. The pH of all stimuli was adjusted to 6.75–7.0, and fresh solutions were used each day. After testing, the subject was returned to its home cage. One hour later, the rat received an additional hour of access to water.

Because amiloride was going to be used in the discrimination experiments and is often used in umami research, the same procedure was repeated with 30 μM amiloride added to all solutions to determine whether this substance has an effect on threshold. Rats do not detect amiloride at this concentration (Markison and Spector 1995). To ensure optimum estimations of thresholds, training and testing of each nucleotide continued for 48 consecutive days without amiloride and an additional 30 consecutive days with amiloride. Data for a session were included only if the detection rate of water (S+) trials was >80% during the session. This criterion prevented the inclusion of data from sessions in which the rat might have adopted a strategy dominated by avoidance responding and inappropriately inflating avoidance of concentrations at or below thresholds. Finally, every 8–10 days, water-control sessions were run to determine if the rats were using nongustatory cues to detect the stimuli. During these sessions, all tubes were filled with water and randomly assigned the role of S+ and S−.

Results and discussion

Thresholds were defined as the stimulus concentration detectable in 50% of the trials. The geometric mean threshold was 0.004 mM for IMP and 0.0025 mM for GMP (Figure 1). An analysis of variance (ANOVA) indicated that the thresholds for IMP and GMP did not differ and the presence of amiloride did not significantly alter detection thresholds for either IMP or GMP (all F values < 1.0). Mean (standard error of mean) false alarm rates (incorrectly responding to a water stimulus as if it was a nucleotide) were 5.3% (± 0.8) for IMP and 10.4% (±1.3) for GMP. Spector (2003) has argued that in animal psychophysics, a more accurate measure of thresholds is the midpoint between minimum

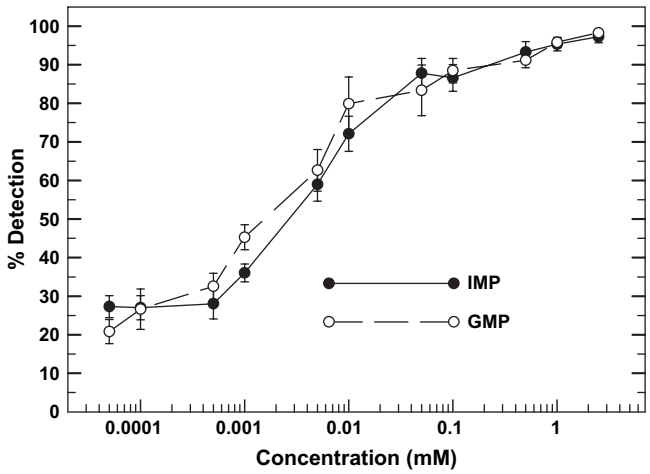

Figure 1 Mean detection (±standard error of mean) of each concentration of IMP (solid line, filled circle) and GMP (dashed line, open circle) in the detection threshold experiments are shown. Detection thresholds, defined as the concentration detected 50% of the time, were in the nanomolar range for both nucleotides.

and maximum asymptotic values of the concentration–response function. For comparison, threshold values were also estimated using Spector's recommendations. These thresholds (geometric means) were 0.005 mM for IMP and 0.003 mM for GMP. Neither did the ANOVA detect a difference between IMP and GMP nor did it detect any effect of amiloride.

Surprisingly, the detection thresholds for IMP and GMP are considerably lower than the behavioral thresholds of rats for most substances thought to have an umami taste quality (Pritchard and Scott 1982; Stapleton et al. 2002; Delay et al. 2004; Taylor-Burds et al. 2004). For example, detection thresholds for MSG and L-aspartate have been established around 1–4 mM in rats (Stapleton et al. 2002; Delay et al. 2004), but detection thresholds for IMP and GMP are much lower in the present experiments. An important question is whether animals are affected by nongustatory cues, but much effort was taken to exclude these possibilities (e.g., masking solenoid, fan, fresh solutions). Mean detection rates during the water-control sessions were between 41% and 57% and did not reveal any evidence that the rats were using nontaste cues. Thus, although the rats might have been able to use nontaste cues, there was no evidence that they did.

Experiment 2: 2-bottle preference tests

Studies evaluating the hedonic qualities of either IMP or GMP have generally found that rats and mice increasingly prefer IMP or GMP up to 10 mM or higher (Bachmanov et al. 2000; Delay et al. 2000). Even though it is generally assumed that IMP and GMP elicit comparable, if not identical, tastes, to our knowledge, no one has directly compared the hedonic value of IMP and GMP in rats. Moreover, few have actually examined the potential effects of amiloride on the preference for either IMP or GMP. Because both nucleotides are disodium salts, amiloride might influence the preference for these substances by diminishing the taste of the Na^+. Two-bottle preference tests are often used to assess the hedonic properties of a taste substance (Spector 2003), in part because of its simplicity and in part because it is easy to conduct because it does not require special equipment. In spite of the potential for confounding by postingestive effects, the 24-h 2-bottle preference test has frequently been the method of choice for studying the hedonic properties of umami and other stimuli. Consequently, we chose to use 2-bottle preference tests to compare the preference of rats for IMP and GMP, with and without amiloride.

Materials and methods

Subjects

Thirty-two male albino rats (Harlan Sprague-Dawley) served as subjects. They were over 90 days of age and 300 g at the beginning of the experiment. They were housed individually in the home colony with food available ad libitum. The colony was maintained on a 12-h light:dark cycle with lights on at 7:30 AM.

Procedures

All rats were presented with taste stimuli in 50 ml graduated cylinders with stoppers equipped with lick spouts. Each day, rats were presented with 2 cylinders, one containing the assigned taste stimulus and the other containing the vehicle solution. The locations of the tastants were counterbalanced across subjects each day. After 24 h, the amount consumed was measured and the cylinders were cleaned, refilled with fresh solutions, and returned to the cages but with the positions of the tastants switched. The rats were initially trained to drink from the cylinders with deionized water in both cylinders for 4 days to ensure stable ingestive behavior. Deionized water was then presented for 2 more days for data analysis. Sixteen rats were tested with IMP mixed in deionized water. Eight rats were tested with 30 μM amiloride added to all solutions (including deionized water) and 8 without amiloride. Sixteen rats were similarly tested with GMP. Each concentration (0.0, 0.0005, 0.001, 0.005, 0.01, 0.05, 0.1, 0.5, 1.0, 2.5, 5, 10, and 15 mM) of IMP or GMP was presented twice, counterbalanced for position, and in ascending order.

Results and discussion

The data for each subject were first converted to preference ratios for each concentration. This was done by dividing the volume of each stimulus ingested over the 2 days by the total volume ingested from both bottles and then multiplying by 100. Thus, a ratio of 50% would indicate no preference for the substance over water. These scores were then subjected to a 3-way ANOVA for mixed designs to compare the intake of IMP with the intake of GMP, with and without amiloride, over the 13 concentrations. These analyses indicated that only the concentration variable significantly altered preference ratios, $F(12,336) = 86.87$, $P < 0.001$ (Figure 2). *T*-tests comparing the preference ratios of each concentration indicated that there was a significant increase in preference scores of IMP and GMP over water when the concentration of each nucleotide was 0.5 mM, $t(15) = 2.923$, $P < 0.02$, or greater (all P values < 0.01). Neither the specific nucleotide nor the presence of amiloride affected preference scores.

All rats showed a clear preference for both nucleotides. However, in spite of the low detection threshold seen in the first experiment, these animals did not show any preference for either IMP or GMP until concentrations reached 0.5 mM. Like earlier reports (Delay et al. 2000), rats increasingly preferred IMP as concentrations were increased. A similar concentration–response function was observed for the GMP groups. The addition of amiloride did not appear to alter preferences for either nucleotide, suggesting that these preferences were most likely related to the nonsodium taste elicited by the nucleotides. In sum, both nucleotides appear to elicit quite similar taste preferences in all these rats.

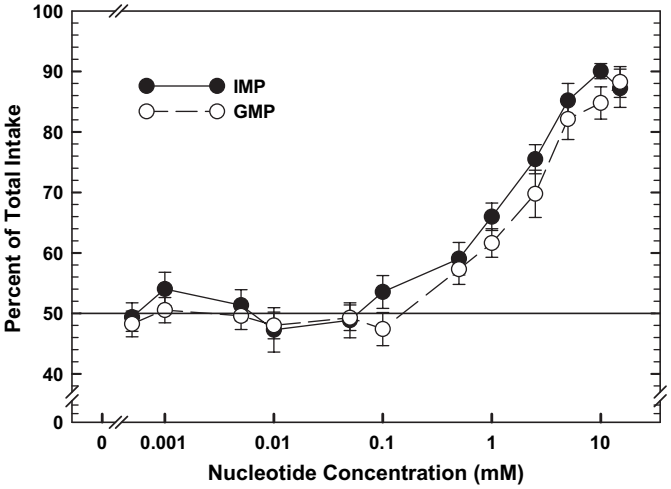

Figure 2 Mean preference ratio (±standard error of mean) of each concentration of IMP (solid line, filled circle) and GMP (dashed line, open circle) in 24-h 2-bottle preference tests are shown. The concentration–response functions for the 2 nucleotides are not significantly different from each other. Intake of both nucleotides was significantly greater than water at 0.5 mM and higher.

Experiment 3: CTA

The 2-bottle preference tests showed that both nucleotides possess quite similar positive hedonic properties to rats, but the differences between the concentrations at which well-trained rats can detect nucleotides (experiment 1) and the concentrations at which naive rats begin to show preference for these substances are quite striking. On the surface, this might suggest that the stimulus qualities that induce taste preference may not emerge unless the concentration of these substances is greater than 0.1 mM, but this conclusion may be incorrect for at least 2 reasons. First, daily intake of a substance during 24-h, 2-bottle preference testing can be biased by postingestive effects of a substance (Spector 2003). In this case, any dietary or other postingestive effects of IMP or GMP might influence intake measures even if the animal could not identify the hedonic qualities of the taste stimulus. Second, it is also possible that the rat can detect the taste qualities of a substance, but these qualities may not be sufficiently intense or sufficiently attractive to change intake behavior. CTA methods, often used to study synergistic interactions between umami stimuli, are particularly effective at enhancing the importance of weak but identifiable concentrations of a taste stimulus for a rat. To further compare the taste qualities of IMP and GMP, we used CTA methods combined with brief-access testing procedures to minimize postingestive effects on consumption.

Materials and methods

Subjects

The subjects for these experiments were 32 male albino Sprague–Dawley rats of the same description and housed in the same manner as in experiment 1.

Apparatus

A computer-controlled Davis MS80 Lickometer system (DiLog Instruments, Tallahassee, FL) was used for CTA testing. Rats were tested in an enclosed Plexiglass operant chamber with a metal grid floor. An oval-shaped opening covered by a metal shutter was located at one end of the chamber. Eight stimulus tubes with lick spouts were mounted on a moveable platform behind the opening. The rats had access to a taste solution when the shutter was opened. A lick was counted when a rat licked from the metal spout and completed a 64 nA contact current. To reduce olfactory cues, air flowed into the operant chamber from a tube mounted on the far wall of the chamber and exited the chamber through the oval-shaped opening. Masking noise (70 ± 5 dB, A scale) was also presented during these sessions.

Procedures

Behavioral training and testing were carried out in the lickometer for 7 consecutive days and followed the procedures previously described (Chaudhari et al. 1996; Stapleton et al. 1999). During the first 3 days, rats were trained to drink deionized water from the lickometer. Each session consisted of 32 trials and lasted 15–20 min. The rat initiated a trial by making contact with the delivery spout. Licks emitted during each 10-s trial were counted. Rats were given up to 60 s to begin a trial before the shutter closed and the next stimulus was presented. A 5-s intertrial interval followed each trial. Each rat was given access to a water bottle for 1 h beginning 45 min after the end of the session.

On the fourth day, rats were presented with 10 mM IMP (n = 16) or 10 mM GMP (n = 16) as the conditioned stimulus (CS). During the conditioning session, the nucleotide was randomly presented 16 times amidst water trials. CS and water solutions contained 30 μM of amiloride. Immediately after drinking the CS, the 8 rats randomly assigned to each experimental group received injections of 0.3 M LiCl (intraperitoneal [i.p.], 127 mg/kg, 1 ml/100 g body weight) as an unconditioned stimulus (US) to induce gastric distress and thus a conditioned aversion to the CS. The 8 rats assigned to each control group received injections of 0.9% NaCl (i.p., 1 ml/100 g body weight) as a US. The next 2 days were "recovery" days in which the rats were presented only deionized water. On the seventh day, rats were tested with 0.01, 0.1, 0.5, 1, and 10 mM IMP or GMP to assess the strength of the taste aversion. These rats were also tested with water and 2 control substances, 100 mM sucrose to determine if the CTA generalized to a prototypical sweet substance, and 25 mM N-methyl-D-aspartate (NMDA) to determine if rats were avoiding all detectable taste stimuli. Rats readily learn a CTA to NMDA, but this aversion does not generalize to MSG (Chaudhari et al. 1996; Stapleton et al. 1999; Nakashima et al. 2001). All stimuli were presented twice in random order with 1–3 water "rinse" trials between each taste stimulus. All solutions contained 30 μM of amiloride.

Results and discussion

The lick rate for each stimulus was first normalized to water trials by dividing the mean lick rate of the stimulus during the 10-s trial by the mean lick rate for the water rinse trials, then multiplying by 100. An ANOVA for mixed designs was used to compare the lick rates for nucleotides (2 levels), type of injection (2 levels), and test stimuli (8 levels) presented during testing. This analysis indicated that the type of injection, $F(1,60) = 184.32$, $P < 0.001$; the test stimulus, $F(7,165) = 29.36$, $P < 0.001$; and the interaction between these 2 variables, $F(7,165) = 24.16$, $P < 0.001$, had significant effects on lick rates. This analysis did not detect any significant effect related to the nucleotide variable. Two-way ANOVAs of the data for each nucleotide, followed by simple effects tests, compared the normalized lick rates of the LiCl-injected animals with those of NaCl-injected rats. These analyses indicated that 0.5 mM was the lowest concentration at which LiCl-injected rats showed significantly lower normalized lick rates than NaCl rats for both IMP, $F(1,14) = 7.13$, $P < 0.025$, and GMP, $F(1,14) = 13.93$, $P < 0.005$ (Figure 3). LiCl-injected rats conditioned to avoid either nucleotide significantly altered their behavior compared with controls when presented with concentrations of 0.5 mM or higher of either IMP or GMP, suggesting that rats identified the taste qualities of IMP and GMP at concentrations at least as low as 0.5 mM. It is possible that these qualities might be detectible at lower concentrations if the deprivation state of the rats in this experiment motivated the rats to ignore these qualities or if the strength of aversive conditioning was increased (e.g., more conditioning sessions). Regardless, when compared under identical CTA learning and testing conditions, the learned aversion to each nucleotide significantly altered drinking at the same concentrations and to the same degree as stimulus concentration increased.

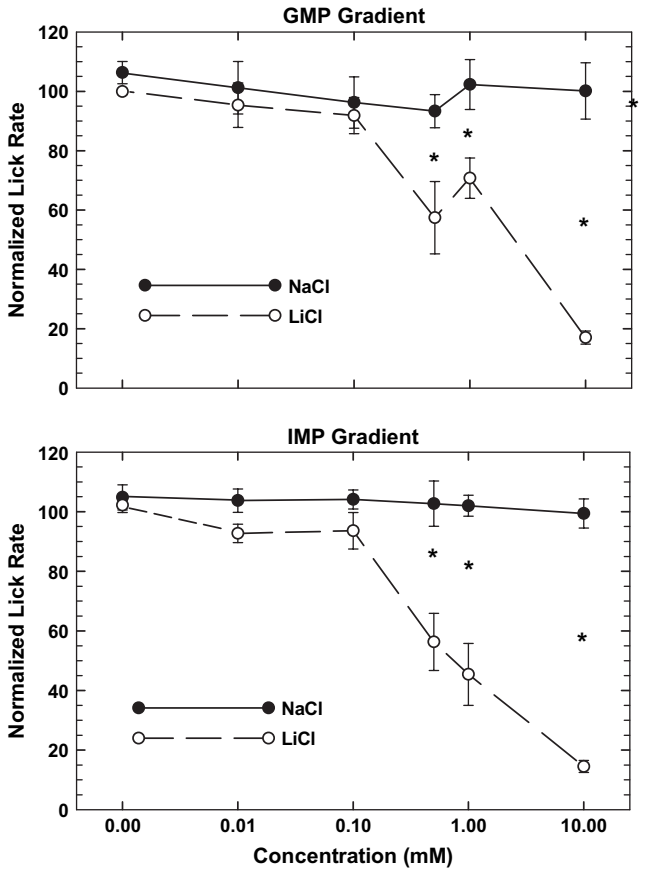

Figure 3 Mean (±standard error of mean) normalized lick rates for each concentration of GMP (upper panel) and IMP (lower panel) after the rats were conditioned with either NaCl (solid line, filled circle) or LiCl (dashed line, open circle) in the CTA experiment. Rats conditioned with LiCl injections showed significantly lower lick rates compared with rats conditioned with NaCl injections at 0.5 mM and higher for both nucleotides.

LiCl-injected rats conditioned to avoid either nucleotide responded to the control substances in much the same manner as previously reported for other umami stimuli. That is, these rats did not generalize their CTA to NMDA (all means >89%, Table 1). On the other hand, LiCl-injected rats showed strong generalization to sucrose when conditioned to avoid either nucleotide ($P < 0.001$, Table 1). Yamamoto et al. (1991) also reported that an aversion to IMP generalizes to sucrose in the presence of amiloride, and several studies have reported that a learned aversion to MSG or aspartic acid generalizes to sucrose in the presence of amiloride (Yamamoto et al. 1991; Chaudhari et al. 1996; Stapleton et al. 1999; Heyer et al. 2003). The findings of this experiment add further support to speculation that the downstream signal pathways for umami and sweet stimuli interact (Chaudhari and Kinnamon 2003). On the other hand, like sucrose or MSG (Chaudhari et al. 1996; Stapleton et al. 1999; Heyer et al. 2003), neither IMP nor GMP produced an aversion that generalized to NMDA in rats. These results suggest that IMP and GMP do not interact directly with the same receptor mechanisms as NMDA, although it is possible that they might if another substance such as glycine is also present (Nakashima et al. 2001).

Experiment 4: discrimination experiments

One of the strengths of CTA experiments is that an animal with an aversion to the taste of one substance will also avoid ingesting other substances with similar taste qualities, a

Table 1 Mean lick rates of control stimuli after conditioning to either IMP or GMP

CS	US	Sucrose	NMDA
IMP	NaCl	107.7(±6.4)	106.2(±4.5)
	LiCl	30.7(±10.7)*	89.3(±8.3)
GMP	NaCl	97.40(±7.5)	110.5(±7.7)
	LiCl	18.70(±3.2)*	94.7(±6.0)

*$P < 0.001$.

phenomenon known as stimulus generalization. Earlier CTA experiments (Ninomiya and Funakoshi 1989; Yamamoto et al. 1991) showed that the tastes of IMP and GMP are quite similar to MSG. However, 2 taste substances may possess similar qualities while also possessing dissimilar qualities that make one substance distinguishable from the other. Stimulus discrimination methods, which force the animal to focus on stimulus differences rather than similarities, are well suited to determine if differences in taste qualities exist between 2 substances. We used these methods to determine if, as often assumed, the tastes of IMP, GMP, and MSG are identical. Specifically, we conducted one set of discrimination experiments to determine if rats could differentiate between the tastes of IMP and GMP and a second set of experiments to determine if rats could discriminate between MSG and either IMP or GMP.

Materials and methods

Subjects

A total of 18 naive, male, Sprague–Dawley rats served as subjects. They were housed in the same manner as described for experiment 1.

Procedures

General. The animals were tested in the same apparatus and under the same general protocol as stated for the threshold experiments but with the following modifications. Six rats were randomly assigned to each of 3 experiments: IMP versus MSG, GMP versus MSG, and IMP versus GMP. Three randomly selected rats in each experiment were tested with one of the substances (e.g., IMP) as the S+ and the opposite substance (e.g., GMP) as the S−, and the other 3 were assigned to the opposite stimulus conditions. Initial training began with water as the S+ and the assigned S− substance. Within 2–4 days, all animals were performing at >90% accuracy. To ensure each animal was well versed with the S+/S− consequences, all animals were trained for a total of 12 days before discrimination training began. Discrimination training was initiated by changing the S+ condition from water to the opposite taste substance. During these sessions, 5 of 10 stimulus barrels contained different concentrations of the S− and 5 contained different concentrations of the S+. A different solution was randomly assigned to a storage barrel each day. The order of stimulus presentations within a session was randomized with a latin square procedure, and different orders were tested each day. Each rat received 20 days training with the assigned S+/S− combination before data collection began.

Concentrations tested. To compare the taste qualities of MSG with either IMP or GMP, we wanted to test concentrations of each nucleotide that spanned from a point below which the rats appear able to recognize the qualities of each nucleotide to a point at which rats strongly prefer each substance. Therefore, each nucleotide was tested in 2 ranges while the concentrations of MSG were held constant. For both nucleotides, the low range consisted of 0.01, 0.05, 0.1, 0.5, and 1.0 mM and the high range consisted of 1.0, 2.5, 5.0, 10, and 15 mM. The concentration range of MSG was 10, 25, 50, 100, and 150 mM. The low range of each nucleotide was tested first, followed by the high range of concentrations.

To compare the tastes of IMP and GMP, the same 2 ranges of concentrations were tested. The rats were first tested with the low concentration range and then with the higher range.

Amiloride conditions. Na^+ could affect each of these discriminations when MSG was one of the taste substances because the animals could use the intensity of Na^+ to identify the stimulus. Therefore, to control the cue function of Na^+ taste, animals were tested for 10 days in each of the following conditions: 1) without amiloride, 2) with amiloride (30 μM) in all solutions, and 3) when MSG was one of the tastants, with amiloride (30 μM) in all solutions and with NaCl added to the nucleotide solutions to neutralize the cue function of Na^+. That is, NaCl was added to each solution of nucleotide to match the Na^+ content of each concentration of MSG (Stapleton et al. 2002; Heyer et al. 2004). Thus, when we tested the high range of nucleotides, 8 mM of NaCl was added to 1 mM of IMP (a disodium salt) to match the sodium concentration of 10 mM MSG, 20 mM of NaCl was added to 2.5 mM of IMP to match 25 mM MSG, and so on. Rats in the IMP–GMP discrimination were tested first in the no amiloride, then the amiloride condition, but not the NaCl condition. After each amiloride condition, water-only sessions were conducted in which tubes were randomly assigned S+ and S− to test for nontaste cues. After the last water-control session, the rats in the IMP–GMP discrimination experiment were given one additional session with water as the S+ and their assigned S−.

Results and discussion

The percent correct detection of each stimulus during a test session was calculated and then averaged across sessions. Because a primary question of these experiments was to determine if these rats could identify any of the 3 substances more readily than the other substances, the discrimination scores for each compound within an experiment were subjected to several ANOVA procedures and then to *t*-test or simple effects tests as appropriate (Howell 1997).

During the water-control sessions in which water-filled tubes were randomly assigned as S+ or S−, rats correctly identified each tube between 38% and 55% of the trials. Analysis of these data did not reveal any evidence that the rats were using nontaste cues during these tests (all F values < 1.0).

GMP versus MSG

An initial analysis of the data obtained during the GMP/MSG discrimination experiment indicated that significantly

more stimuli were identified when the discrimination task involved the low range of concentrations of GMP than when the discrimination involved the high range of GMP, $F(1,5) = 22.81$, $P < 0.001$. Consequently, the data for each range of concentrations of GMP were analyzed separately with a 3-way ANOVA examining stimulus compound (2 levels), amiloride condition (3 levels), and concentration (5 levels) as within-subject variables.

The analysis of the discrimination data of the experiment testing MSG against the low range (0.01–1 mM) of GMP (Figure 4, left panel) showed that accuracy was significantly better with the higher concentration levels of this experiment, $F(4,20) = 30.30$, $P < 0.001$. Amiloride conditions also had a significant effect on discrimination, $F(4,20) = 11.04$, $P < 0.005$. *T*-tests indicated that performance was significantly more accurate in the no-amiloride condition than in the amiloride condition ($P < 0.005$). Discrimination performance was also affected by an interaction between the taste compound and the amiloride condition, $F(2,10) = 5.77$, $P < 0.025$. As shown in Figure 5, MSG was correctly identified significantly less often in the amiloride and the amiloride + NaCl conditions than in the no-amiloride condition or GMP in any of the amiloride conditions (simple effects tests, P values < 0.02).

The analysis of the discrimination scores for the experiment with the high concentration range (1–15 mM) of GMP showed that these rats were affected significantly by the amiloride condition, $F(2,10) = 5.77$, $P < 0.025$, and by a significant interaction between amiloride and concentration conditions, $F(2,10) = 5.77$, $P < 0.025$ (Figure 4, right panel). To examine this interaction more closely, simple effects tests were used to compare discrimination scores in the 3 amiloride conditions obtained under each concentration level. These analyses showed that performance was consistently better in the no-amiloride condition than the amiloride condition at the 3 middle concentration levels (P values < 0.02) and that discrimination was significantly better in the amiloride + NaCl condition than in the amiloride-only condition ($P < 0.05$) at the middle concentration level.

IMP versus MSG

The analyses comparing the discrimination scores obtained under the 2 concentration ranges of IMP did not find any differences related to the concentration range of IMP or compounds tested. The data for the experiment testing the low concentration range (0.01–1 mM) of IMP and MSG were analyzed with a 3-way ANOVA for repeated measures to test whether the rats differentially detected IMP from MSG (taste compound variable) in the 3 amiloride conditions and the 5 concentrations of each stimulus. This analysis revealed a significant increase in correct-detection scores as the concentration of the substances increased, $F(4,20) = 5.46$, $P < 0.005$. It also revealed a significant concentration by compound interaction, $F(4,20) = 4.27$, $P < 0.025$, in which the accuracy of detecting IMP (poorest at the lowest concentration of IMP) increased to the same level as MSG as the concentration of each substance increased (Figure 6, left panel).

The results of the ANOVA of the discrimination scores for MSG and high concentration range (1–15 mM) of IMP found significant effects of the amiloride variable, $F(2,10) = 5.58$, $P < 0.01$, concentration, $F(4,20) = 12.83$, $P < 0.001$, and the interaction between amiloride and concentration, $F(8,40) = 4.98$, $P < 0.001$ (Figure 6, right panel). Simple effects tests comparing amiloride conditions

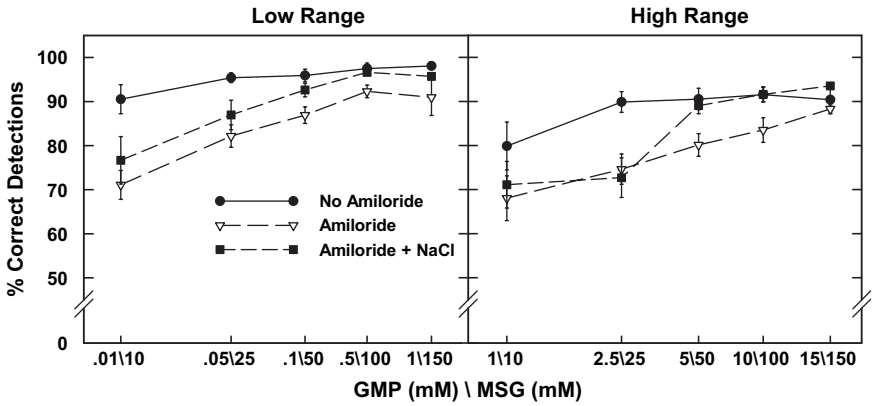

Figure 4 Mean (±standard error of mean) percent correct detections are shown for the 2 discrimination experiments involving GMP and MSG. The left panel shows the detection data for the experiment in which the concentration range of GMP was low (0.01, 0.05, 0.1, 0.5, and 1.0 mM), and the right panel shows the data for the experiment in which the concentration range of GMP was high (1, 2.5, 5, 10, and 15 mM). MSG was 10, 25, 50, 100, and 150 mM in both experiments. Both panels show the combined percent correct detection of MSG and GMP across concentrations in each amiloride condition. When GMP stimuli were of the lower concentration range, discrimination accuracy was better at the higher concentrations and in the no-amiloride condition (filled circles). When GMP stimuli were of the higher concentration range, rats were most accurate when amiloride was not present (filled circles). Rats had more difficulty discriminating between the tastes of these 2 substances when amiloride (30 μM) was added to all solutions (open triangles), especially at the lower concentrations tested in this experiment. Performance improved at the middle concentration when amiloride was added to all solutions and isomolar concentrations of NaCl were added to GMP (filled squares).

were performed on the data for each concentration. These tests showed that discrimination performance was better in the no-amiloride condition than the amiloride condition of all but the highest concentrations of IMP and MSG and better than discrimination in the amiloride + NaCl condition in the 2 lowest concentrations (all P values < 0.25 or less). Discrimination scores were also significantly better in the amiloride + NaCl condition than in the amiloride condition at 50 mM MSG and 10 mM IMP.

IMP versus GMP

The detection rates of these animals were at near-chance levels during this discrimination experiment, whether or not amiloride was added to the solutions (Figure 7). Z-scores comparing each animal's performance with chance (50% accuracy) in each concentration range and amiloride condition were computed. None of these scores exceeded 1.04 in any of these conditions. To further examine whether rats could distinguish between the tastes of IMP and GMP, the discrimination data for each range of concentrations were analyzed with 3-way ANOVAs for repeated measures designs to assess the effects of nucleotide (2 levels), amiloride conditions (2 levels), and concentrations (5 levels). The only significant effect found in either of these analyses was a compound by concentration interaction in the data for the high concentration range, $F(4,20) = 4.40$, $P < 0.025$. In general, as the concentrations within this range increased, identification of GMP improved, whereas the identification of IMP decreased. Importantly, all these animals were performing at over 90% accuracy when the S+ was water prior to the beginning of the discrimination experiment. When these animals were retrained with water as the S+ and the assigned nucleotide as the S− for one session after the experiment, 5 of the 6 rats achieved over 80% accuracy by the second half of this session (the data for the sixth animal were lost before they could be analyzed), indicating these animals were still able to perform the discrimination task in spite of their inability to differentiate between the tastes of IMP and GMP. Taken together, these data indicate that the rats in this experiment found the tastes of IMP and GMP at least quite similar or nearly identical.

To further compare the taste properties of these nucleotides, 2 additional ANOVAs compared the data of the IMP/MSG discrimination experiment with the discrimination data of the GMP/MSG experiment. One ANOVA compared the detection scores when the test concentrations of the

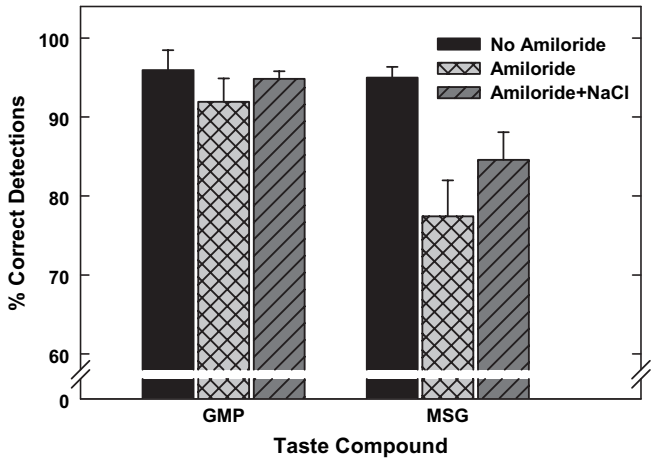

Figure 5 Mean (±standard error of mean) percent correct detection of GMP and MSG in the 3 amiloride conditions: no-amiloride condition, amiloride (30 μM) condition, and the amiloride + NaCl condition.

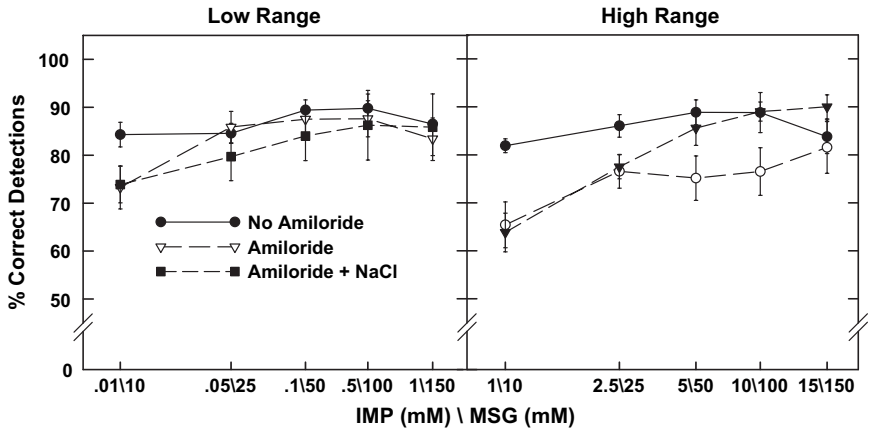

Figure 6 Mean (±standard error of mean) percent correct detections are plotted for the concentration-dependent correct detections of MSG and IMP in each amiloride condition when the concentration range of IMP was low (left panel) and high (right panel). The lower concentration range of IMP was 0.01, 0.05, 0.1, 0.5, and 1.0 mM (left panel) and the higher range was 1, 2.5, 5, 10, and 15 mM (right panel). MSG was 10, 25, 50, 100, and 150 mM in both experiments. When IMP stimuli were the lower concentration range, discrimination accuracy was high. When IMP stimuli were the higher concentration range, rats were most accurate when amiloride was not added to any solution (filled circles). Rats had more difficulty discriminating between the tastes of these 2 substances when amiloride (30 μM) was added to all solutions (open triangles). Performance improved at the middle concentrations when amiloride was added to all solutions and NaCl was added to IMP to match the concentrations of Na^+ in MSG (filled squares).

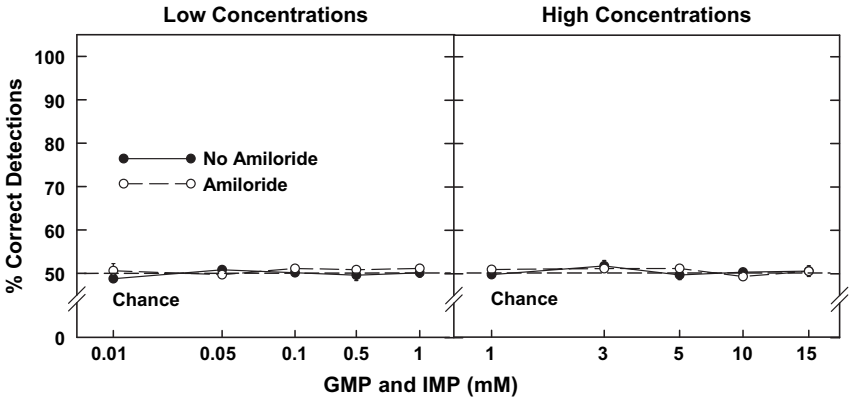

Figure 7 Mean (±standard error of mean) percent correct detections of isomolar concentrations of GMP and IMP in 2 sets of discrimination experiments are shown. The concentration range of both nucleotides were 0.01, 0.05, 0.1, 0.5, and 1.0 mM (left panel) in one set of experiments and 1, 2.5, 5, 10, and 15 mM (right panel) in the second set of experiments. Rats could not discriminate between the tastes of the 2 nucleotides above chance level regardless of the concentration range tested or whether amiloride was present (solid line) or not (dashed line).

nucleotides were 0.01–1 mM (low range), and the second compared the data obtained when the concentrations of the nucleotides were 1–15 mM (high range). Even though both ANOVAs detected the significant main effects and interactions identified by the individual analyses described above, no additional significant differences between these experiments were detected.

To summarize, rats can discriminate between the tastes of MSG and either IMP or GMP, especially when amiloride is absent, across all concentrations tested. Reducing the cue function of Na^+ by adding amiloride to all solutions increased the difficulty of each discrimination, but the rats were still able to distinguish between the nucleotides and MSG. Unexpectedly, adding NaCl to the nucleotide actually increased the ability of the rats to discriminate between MSG and both nucleotides rather than neutralizing the cue function of Na^+. This suggests that Na^+ might influence nucleotide transduction in some unknown manner, as is the case for some other substances (Mierson et al. 1988; Simon et al. 1989). Nevertheless, even though CTA studies indicate that these 3 substances possess similar taste qualities, the most important finding in these experiments is that rats are able to differentiate between MSG and either nucleotide, regardless of the amiloride condition.

The performance of the rats in the GMP/MSG discrimination experiment indicated that differences in perceived intensity were responsible for the better rate of detection when the low range of concentrations of GMP was tested. When the high concentration range was tested, a significant compound by amiloride interaction was revealed. Neither of these findings was detected in the data for the rats tested with IMP and MSG. These differences between the GMP/MSG and the IMP/MSG experiments may represent real differences in the tastes of the 2 nucleotides or possibly differences in the response strategies used by each group of rats. However, the near-chance detection rates of the rats in the IMP/GMP discrimination experiment strongly support the latter explanation that rats are unable to discriminate between the 2 nucleotides. Collectively, the findings of these discrimination experiments indicate that the tastes of IMP and GMP, if not identical, are very similar, but neither nucleotide elicits a taste identical to MSG.

General discussion

Overall, the findings of these experiments indicate the taste properties of the 2 5′-ribonucleotides are very comparable to each other. Both nucleotides have quite similar 1) detection thresholds, 2) natural positive hedonic qualities (indicated by 2-bottle preference functions), 3) capacity to form learned negative associations (indicated by CTA functions), and 4) taste qualities that appear to make them nearly impossible for rats to differentiate from each other (indicated by discrimination experiments). On the other hand, even though these nucleotides have taste qualities that are similar to MSG (Ninomiya and Funakoshi 1989; Yamamoto et al. 1991), both nucleotides could be discriminated from MSG and thus do not elicit taste sensations identical to MSG.

Well-trained rats can detect IMP and GMP at concentrations much lower than other umami substances such as MSG or monosodium aspartate or other L-amino acids that can be potentiated by these nucleotides (Pritchard and Scott 1982; Nelson et al. 2002; Stapleton et al. 2002; Zhao et al. 2003; Delay et al. 2004; Taylor-Burds et al. 2004). However, the thresholds for these nucleotides are comparable with thresholds measured for L–2-amino-4-phosphonobutyric aicd (Delay et al. 2004), another umami substance that is a strong mGluR4 agonist (Chaudhari et al. 1996). In spite of the low detection threshold, neither nucleotide induced changes in preference ratios in 24-h preference testing or in avoidance scores in the CTA experiment unless IMP and GMP were presented at concentrations greater than 0.1 mM. Results of 2-bottle preference testing, often used to assess inherent hedonic properties of a substance, can be susceptible to

confounding by postingestive effects. However, the CTA experiment used a brief-access test format that eliminates most possibilities of postingestive effects and, through a learned association, changed the hedonic value of these nucleotides from positive to negative. It is possible that if the CS had been a lower concentration or more conditioning was conducted, these animals might have responded to a lower concentration of IMP or GMP. However, behavioral changes were detected at the same minimum effective concentration (0.5 mM) with both methods (2-bottle preference and CTA). These findings suggest that rats may not identify specific taste qualities that are the basis of the perceptual experiences elicited by these nucleotides unless concentrations are much higher than detection thresholds. That is, rats can detect either nucleotide at low concentrations but may not be able to recognize their umami qualities unless the concentration is >0.1 mM.

These experiments also expand our knowledge of the specific taste qualities elicited by IMP and GMP in rats. Previous CTA studies have shown that these nucleotides possess qualities similar to those elicited by MSG, especially when amiloride is added to solutions to reduce the saliency of the Na^+ ion (Ninomiya and Funakoshi 1989; Yamamoto et al. 1991). However, the discrimination experiments reported here show that neither IMP nor GMP has taste qualities that simply mimic MSG. These rats could distinguish between both nucleotides and MSG even when these discriminations were made more difficult by adding amiloride, indicating that either MSG or these nucleotides possess at least some unique taste qualities. This does not appear to be the case when comparing the qualities of IMP and GMP. Not only are thresholds, taste preference, and CTA results comparable but also rats in the discrimination experiments could not distinguish between the tastes qualities of the 2 nucleotides.

These behavioral results agree well with recent patch-clamp and Ca^{2+}-imaging data showing that GMP and MSG may not activate the same G-protein–coupled taste receptor cells of rats (Iseki et al. 2001; Lin et al. 2003). Lin et al. (2003) found that whereas many taste receptor cells responded to both MSG and GMP, there was also a substantial number of receptor cells that responded only to GMP or to MSG. Similar findings were reported for nerve single fiber and cells in the solitary nucleus (Hellekant and Ninomiya 1991; Scott et al. 1993; Sako et al. 2000). When the results of these studies are combined with the present study, it seems likely that although these nucleotides and MSG may activate a common set of taste receptors, it is also likely that they activate other, independent sets of receptors. On the other hand, if 2 substances activate the same population of taste receptors, one would predict that the tastes of the 2 substances would be virtually identical. The discrimination experiments comparing the tastes of IMP and GMP reported in this study clearly show that the 2 ribonucleotides elicit nearly identical perceptual qualities in rats and thus probably activate the same population of taste receptors.

The findings of these experiments have important practical implications. For example, even though both nucleotides have umami qualities, they are also capable of eliciting unique qualities not shared by the prototypical umami substance MSG. Whether this characteristic of IMP and GMP extends to other umami substances needs further examination. Maybe most important are the implications for studies of synergy. Researchers may want to consider whether they need to keep the concentration of IMP or GMP at levels below those capable of eliciting unique and recognizable taste qualities. Higher concentrations can contribute not only to the potentiation of the intensity of the umami experience through synergistic processes but also add new taste qualities to the gustatory experience. On the other hand, the findings of this study suggest that either nucleotide may be used interchangeably because to the rat, at least, they appear to elicit nearly identical perceptual experiences.

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