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Taste Preference Synergy Between Glutamate Receptor Agonists and Inosine Monophosphate in Rats

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Abstract

Monosodium glutamate (MSG) elicits a taste called umami and interacts synergistically with nucleotide monophosphates such as 5′-inosine monophosphate (IMP) to potentiate this taste intensity. Indeed, the synergistic interaction of nucleotide monophosphates and MSG is a hallmark of umami. We examined interactions between MSG and other taste stimuli, including IMP, by measuring the lick rates of non-deprived rats during 30 s trials. To control for non-linear psychophysical functions, the concentration of one taste stimulus in a binary mixture was systematically increased while the concentration of the second taste stimulus was decreased (stimulus substitution method). Synergy between two stimuli was detected if the lick rate for a binary mixture exceeded that expected from the sum of the lick rates for each stimulus alone. In initial experiments, taste synergy was observed when rats were presented with mixtures of MSG and IMP but not with mixtures of MSG and sucrose. In subsequent experiments, glutamate receptor agonists other than MSG were presented with IMP to test for taste synergy. No evidence of synergy was seen when rats were presented with mixtures of IMP and kainic acid or IMP and *N*-methyl-D-aspartate. However, taste synergy between IMP and L-AP4, a potent agonist at mGluR4 receptors, was observed. These results suggest that a metabotropic glutamate receptor similar to mGluR4 may be involved in the taste synergy that characterizes umami.

Introduction

L-Monosodium glutamate (MSG) is a natural component of many protein-rich foods and exhibits two qualities that influence taste perception. The first is that MSG elicits a taste, called 'umami', which is believed to be a separate taste quality on a par with sweet, sour, salty and bitter. The second is the ability of MSG to interact synergistically with certain other stimuli to potentiate the perceived taste intensity. This second quality may be related to the ability of MSG to enhance flavors in foods. In recent years there has been considerable effort devoted to studying chemosensory transduction in taste receptor cells for MSG taste. These efforts have resulted in the identification of a candidate glutamate taste receptor for umami that is a modification of the metabotropic synaptic mGluR4 receptor (Chaudhari *et al.*, 1996, 2000). This receptor has been termed taste-mGluR4. Other glutamate receptors, notably *N*-methyl-D-aspartate (NMDA) receptors, have also been proposed to underlie MSG taste (Brand *et al.*, 1991; Hayashi *et al.*, 1996).

Less is known, however, about the synergistic properties of MSG. MSG taste synergy has been studied either with human subjects who were asked to judge the flavor of foods with and without MSG or, more objectively, with psychophysical, behavioral and electrophysiological studies of responses to mixtures of MSG and other taste stimuli in humans and non-human animals (Maga, 1983; Yamaguchi, 1987; Kumazawa and Kurihara, 1990; Yamamoto *et al.*, 1991; Schiffman *et al.*, 1994). Synergy between the taste of MSG and another stimulus is indicated when responses to the mixture are greater than the sum of the responses to individual components presented alone. The results of studies to date indicate that there is a pronounced taste synergy between MSG and certain other stimuli, most notably nucleotide monophosphates such as 5′-inosine monophosphate (IMP) and 5′-guanosine monophosphate (GMP).

The mechanism underlying MSG taste synergy is not known. Interactions between nucleotide monophosphates and MSG have been demonstrated in recordings from gustatory afferent nerve fibers (Ninomiya and Funakoshi, 1989; Kumazawa and Kurihara, 1990; Hellekant and Ninomiya, 1991; Sako and Yamamoto, 1999), in patch clamp record-

ings of isolated taste buds (Lin and Kinnamon, 1998) and in lingual membrane preparations believed to contain the taste receptor for umami (Brand *et al.*, 1991). Sako and Yamamoto (1999) further showed in whole nerve recordings from the rat chorda tympani nerve that IMP and L(+)-2-amino-4-phosphonobutyric acid (L-AP4), an agonist for mGluR4 receptors (Tanabe *et al.*, 1993), interacted synergistically. Binding of L-[^{3}H]glutamate to its receptor in taste buds has even been reported to be enhanced by nucleotide monophosphates (Torii and Cagan, 1980). Thus, the processes responsible for taste synergy between MSG and nucleotide monophosphates may begin at the level of the molecular receptor for umami. Collectively, these findings suggest that MSG taste, at least in part, may be transduced by mGluR4 receptors and that these receptors may be involved in the taste synergy. However, confirmation of this hypothesis at the behavioral level is lacking. One approach is to identify ionotropic and metabotropic glutamate receptor agonists that can mimic the taste synergism between MSG and IMP. If mGluR4 receptors underlie MSG taste, then the mGluR4 receptor agonist L-AP4 might be expected to have a synergistic interaction with IMP.

In this report, we describe a quantitative method to measure interactions between two taste stimuli in a behavioral paradigm with rats. The behavioral assay utilizes brief access taste tests to maximize gustatory and minimize post-ingestive influences on responding. The analytical nature of this method is based on a technique introduced by Rifkin and Bartoshuk (Rifkin and Bartoshuk, 1980) to investigate interactions between MSG and GMP in a human psychophysical study. Rifkin and Bartoshuk (Rifkin and Bartoshuk, 1980) noted that tests of interactions between taste stimuli in binary mixtures can be flawed if a range of concentrations for each component of the mixture is not tested. This can be particularly problematical if the dose–response relation (psychophysical function) for one or both of the mixture components is non-linear. They point out, for example, that mixing low concentrations of two stimuli (e.g. two sugars) with similar non-linear psychophysical functions may result in an artifactual appearance of synergy. Responses to the mixture may simply be equivalent to increasing the concentration of either stimulus and thus eliciting responses in a non-linear region of the psychophysical function, i.e. the response to the mixture may appear greater than the sum of the responses to each of the two components by virtue of the properties of the underlying psychophysical functions, rather than any inherent taste synergy. Rifkin and Bartoshuk (Rifkin and Bartoshuk, 1980) argue that a stimulus substitution method overcomes this problem. In the stimulus substitution method, a range of concentrations of each stimulus is tested in binary mixtures of A and B. In these mixtures the concentration of component A is increased as the paired concentration of component B is decreased. To our knowledge, no study employing the stimulus substitution method described by Rifkin and Bartoshuk (Rifkin and Bartoshuk, 1980) has been used to investigate taste interactions in non-human species. We modified the stimulus substitution method to test for taste synergy between two taste stimuli with non-deprived rats.

Using this method with brief access taste presentations, we tested for taste interactions between mixtures of IMP and MSG, sucrose and MSG, and IMP and certain glutamate receptor agonists, including two ionotropic glutamate receptor agonists, NMDA and kainic acid (KA) and the metabotropic receptor agonist L-AP4. Our results show a pronounced taste synergy between MSG and IMP but not between MSG and sucrose. We also found evidence of synergy between IMP and L-AP4, but not between IMP and either KA or NMDA. These results suggest that the mGluR4 receptor may be responsible for the taste synergy observed with MSG.

Materials and methods

Experiment 1: mixtures of MSG and IMP or MSG and sucrose

Subjects

Twenty-three male Sprague–Dawley rats (Harland Sprague-Dawley, Indianapolis, IN) served as naive subjects in the first experiment. All were 80–90 days of age on the first day of training and were housed in individual cages with food and water available *ad libitum* throughout the experiment. The colony was maintained on a 12/12 h light/dark cycle with the onset of lights at 7 a.m. All rats were handled for ≥10 min for at least 3 days prior to the beginning of the experiment. Each rat was tested at the same time each day during the light portion of the cycle.

Apparatus

All experimental procedures were conducted in Davis MS80 (Dilog Instruments, Tallahassee, FL) lick detection systems. This system comprised an eight-bottle unit for stimulus presentation, an operant chamber, a control unit and an IBM-compatible computer. The Plexiglas operant chamber was 15 cm wide, 30 cm long and 20 cm high and had a stainless steel front wall and floor. The 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, located on the outside of the front wall, opened. Taste stimuli were contained in 15 ml centrifuge tubes with drinking spouts. During a trial the tip of the drinking spout was positioned 3 mm behind the center of the opening in the front wall. The microcomputer controlled the shutter and the positioning of each centrifuge tube with its drinking spout. When the rat made contact with the spout, a circuit with a 64 nA current was completed and a lick was counted. To minimize olfactory cues, a small fan moved air into the back of the chamber and out through the opening around the drinking spout. A white house light provided ~10 lux illumination

throughout the test area. Masking noise (Radio Shack Sleep machine) was 70 ± 5 dB (20 μN/m^2) inside the operant chamber.

Procedure

Prior to the experiment, each subject was habituated to the testing environment and trained to drink from the Davis equipment for 3 days. To induce drinking, rats were presented with 0, 50 and 100 mM sucrose during the training sessions. Except for the order of presentation of taste stimuli, all procedures were identical during training and experimental sessions. After the first bottle was positioned, the shutter was opened. The rat had up to 100 s to initiate a trial before the shutter was closed and the next bottle was positioned for a new trial. The rat initiated a trial by making contact with the drinking spout. The number of licks during the subsequent 30 s was counted. The time between the presentation of each spout was 5 s. After the subjects completed training, 11 rats were randomly assigned to be tested with MSG and IMP and 12 rats were assigned to be tested with MSG and sucrose.

A critical feature of the stimulus substitution method described by Rifkin and Bartoshuk (Rifkin and Bartoshuk, 1980) is that a range of concentrations for each taste stimulus is tested and that as the concentration of one stimulus is increased across a series of test mixtures, the concentration of the second stimulus is decreased. Systematic deviation from the psychophysical function predicted when summing the responses to the individual taste stimuli represents synergy. Thus the procedure requires that one measure the lick rate (LR) of each rat for concentrations of each substance presented alone as well as the LR for each of the mixtures. To accomplish this, each animal was tested in three phases: pre-test, test and post-test. The pre-test and post-test phases were used to ensure that the rat preferred the taste stimuli and to establish the baseline LR for each stimulus. Taste mixtures were presented during the intervening 3-day test phase.

It was critical that ceiling effects on LRs were avoided during the test phase. Based on our own prior and previously published data (Smith *et al.*, 1992), it was estimated that steady responding for the entire 30 s trial would result in 200–220 licks/trial for most rats. Preliminary experiments were conducted to establish a concentration–response preference gradient for each taste substance. Only concentrations that did not elicit more than an average of 80 licks/trial in these experiments were used later to test for taste synergy. Based on this criterion, the rats tested with MSG and IMP were presented with 0, 10, 20, 30, 40, 50 and 100 mM MSG and 0, 1, 2, 3, 4, 5 and 10 mM IMP during the pre-test and post-test phases. During both phases, half of the rats were presented with all of the concentrations of one taste stimulus during each of 3 consecutive days, then with the concentrations of the second taste stimulus for the next 3 days. The other half of the rats were likewise presented with MSG and IMP, but in the opposite order. During the test phase, these rats were presented with the following mixtures (expressed as mM MSG/mM IMP): 50/0, 40/1, 30/2, 20/3, 10/4 and 0/5. In all three phases of this experiment taste stimuli were presented in random order each day and each rat was given a different order. Between one and three water 'rinse' trials separated the presentation of each stimulus during the session. A total of 16 trials, including the rinse trials, were presented each day.

A similar design was used with the rats tested for synergy between MSG and sucrose. However, the highest concentration of sucrose (and thus MSG) tested in this experiment was 40 mM, since in the preliminary experiments rats exceeded the 80 licks/trial criterion when presented with ≥50 mM sucrose. Therefore, rats were presented with 0, 10, 20, 30 and 40 mM sucrose and 0, 10, 20, 30 and 40 mM MSG during the pre-test and post-test phases. During the test phase, five mixtures of MSG and sucrose (expressed as mM MSG/mM sucrose) were randomly presented between rinse trials in each session: 40/0, 30/10, 20/20, 10/30 and 0/40. As before, the order of the taste stimuli for each rat was randomly assigned and between one and three water rinse trials separated the presentation of each stimulus during the 16 trial sessions.

Data analyses

Synergy between two taste stimuli is defined as when response rates of a rat deviate from (e.g. exceed) the predicted sum of LRs for the individual components of a stimulus mixture. When predicting LRs from drinking measured during short duration reactivity trials, it is important to identify any rat whose LRs are so high that ceiling effects might obscure evidence of synergy. To do this screening, we calculated the mean LR predicted for the taste mixtures of each rat. For these calculations we first determined the mean LR for each stimulus concentration during the pre-test and post-test sessions. Second, the predicted LR for each mixture was obtained by summing the mean LR for each component of the mixture. Then the predicted LRs for all stimulus mixtures were averaged. Since the maximum response rate during a 30 s trial is slightly over 200 licks, a rat was removed from the study if its average *predicted* LRs for all of the mixtures exceeded 150 licks/30 s.

The data for the remaining rats were then analyzed to answer two questions. First, did the rats show preferences for the individual taste stimuli when presented separately? To answer this question, the LRs for each stimulus concentration were analyzed by two-way analysis of variance procedures treating stimulus type and concentration as within-subject variables. Second, did the observed LRs to the mixtures exceed the predicted LRs based on simple summation? To answer this question, the data for each rat were converted into two preference ratios to normalize their LRs for each taste stimulus relative to their LRs for water during rinse trials, the predicted ratio and the behavioral

(observed) ratio. The predicted ratio was an adaptation of the Rifkin and Bartoshuk (Rifkin and Bartoshuk, 1980) model in which the response to a stimulus mixture is calculated as the simple sum of its elements, i.e. it represents *no synergy* between the two elements. More specifically, the ratio represents the sum of LRs for the combination of the two elements of a stimulus mixture during the pre-test and post-test phases relative to the LR for water (a neutral stimulus) during these sessions. The formula for computing this ratio is

$$\text{predicted ratio} = (\text{mean LR stimulus A} + \text{mean LR stimulus B})/(\text{mean LR water})$$

A ratio of 2.0 indicates no preference for the mixture over water.

The observed preference ratio was obtained from the LRs for each mixture and the LR for water during the test phase. This ratio represented the behavioral preference of a rat for a particular mixture. The formula for this ratio is

$$\text{behavioral ratio} = (\text{mean LR stimulus mixture})/(\text{mean LR water})$$

The ratio scores for each group of rats were then analyzed by two-way analysis of variance for within-subject designs to determine if the behavioral ratios deviated from the predicted ratios for any of the stimulus mixtures presented during the test phase. Simple effects tests were used as necessary to examine significant interactions (Howell, 1997). In addition, raw score LRs for the mixtures were analyzed by analysis of variance procedures for a repeated measures design.

Experiment 2: mixtures of either L-AP4, NMDA or KA and IMP

The second experiment was designed to ask whether one or more glutamate receptor agonists were capable of synergistic interactions with IMP. Previous experiments using taste aversion techniques (Chaudhari *et al.*, 1996) have shown that in rats an aversion to MSG generalized mildly to KA, an ionotropic glutamate receptor agonist, and strongly to L-AP4, a metabotropic glutamate receptor agonist (Tanabe *et al.*, 1993). These data suggest that KA, L-AP4 or both may mimic some of the taste properties of MSG. Others (Brand *et al.*, 1991; Faurion, 1991; Hayashi *et al.*, 1996) have suggested that other ionotropic glutamate receptors, specifically NMDA-like receptors, contribute to MSG taste transduction. All three agonists (KA, NMDA and L-AP4) were tested with IMP for taste interactions.

Subjects and apparatus

Thirty-one male Sprague–Dawley rats served as naive subjects in the second experiment. These rats were housed in the home colony and handled in the same manner as in experiment 1. The Davis MS80 apparatus was also used for the second experiment.

Procedures

Experimental procedures were comparable with those described for experiment 1. Preliminary experiments testing a wide range of concentrations were conducted to determine concentrations of KA, NMDA and L-AP4 for which rats show preferences and which could be used for tests of taste synergy. As above, only concentrations that did not elicit more than an average of 80 licks/trial in the preliminary experiments were selected for study in experiment 2. Consequently, one group of rats ($n = 10$) was tested with KA (0, 1, 2, 3, 4, 5 and 25 mM) and IMP (0, 1, 2, 3, 4, 5 and 10 mM) during the pre-test and post-test phases and with mixtures of KA and IMP during the test phase (mM KA/mM IMP: 5/0, 4/1, 3/2, 2/3, 1/4 and 0/5). A second group of rats ($n = 10$) was tested with identical concentrations of NMDA and IMP during the pre-test and post-test phases and mixtures of NMDA and IMP during the test phase. A third group of rats ($n = 11$) was tested with L-AP4 and IMP. Previous findings with taste aversion procedures (Chaudhari *et al.*, 1996) had shown that rats detect L-AP4 at much lower concentrations than either KA or NMDA and, in the preliminary experiments, rats showed preferences for L-AP4 at lower concentrations than other glutamate receptor agonists. Consequently, the third group of rats were tested with 0.0, 0.05, 0.1, 0.5, 1, 2 and 5 mM L-AP4 and with 0, 1, 2, 3, 4, 5 and 10 mM IMP during the pre-test and post-test phases. An examination of the LRs for L-AP4 after the pre-test phase indicated that the rats in this group did not show a preference for the lowest concentration of L-AP4 and thus may not have detected this stimulus. Therefore, the test phase was conducted with the higher concentrations of L-AP4 mixed with IMP (mM L-AP4/mM IMP: 5/0, 2/1, 1/2, 0.5/3, 0.1/4 and 0/5).

Data analysis

The LRs for rats were screened as in experiment 1 to exclude rats with an average LR 150 licks/30 s. Data for the remaining subjects were normalized and analyzed by the same procedures as described in experiment 1.

Results

Experiment 1

Eight of the 11 rats in the MSG/IMP group met the screening criterion. Analysis of the pre-test and post-test raw scores for these rats showed that LRs for both substances increased significantly as the concentration of MSG and IMP increased [$F(5,35) = 11.80$, $P < 0.001$; Figure 1]. Analysis of the raw data indicated that the LRs for the mixtures of MSG and IMP were significantly greater than the LRs for either substance alone [$F(5,35) = 18.82$, $P < 0.001$; Figure 2]. More importantly, analysis of the ratio data revealed a significant synergistic effect of the mixtures

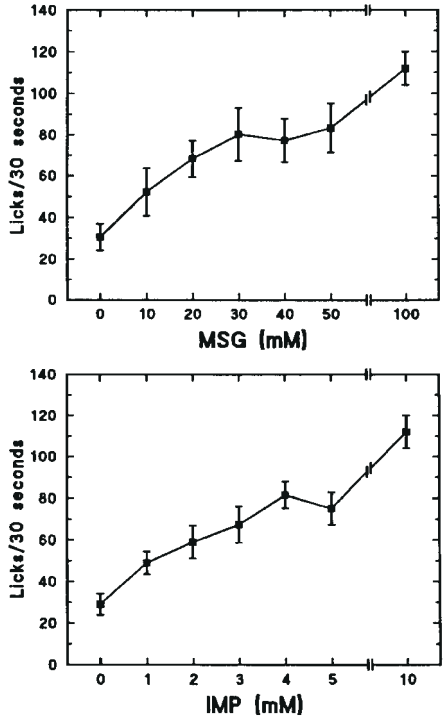

Figure 1 Mean (± SEM) number of licks during 30 s trials for each concentration of MSG and IMP presented during the pre-test and post-test phases of experiment 1.

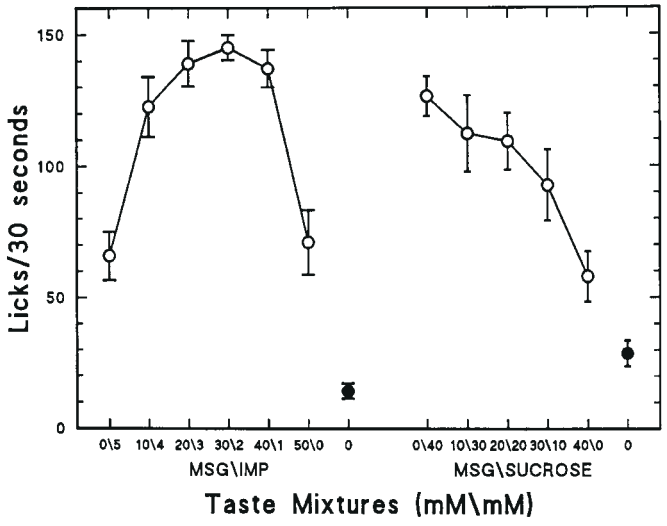

Figure 2 Mean (± SEM) number of licks (open circles) during 30 s trials for mixtures of MSG and IMP ($n = 8$) and for mixtures of MSG and sucrose ($n = 9$) during the test phase of experiment 1. Closed circles represent the mean (± SEM) lick rates on water rinse trials for each group during the test phase.

of MSG and IMP on LRs [$F(5,35) = 5.06$, $P < 0.01$] and a significant interaction between the ratio type variable and the mixture variable [$F(5,35) = 3.44$, $P < 0.025$]. Simple effects tests (alpha set at $P < 0.01$) showed that the behavioral ratios for the mixtures containing MSG and IMP were significantly higher than the predicted ratios (Figure 3). They were also significantly higher than the solutions in which a high concentration of either MSG or IMP was mixed with water only.

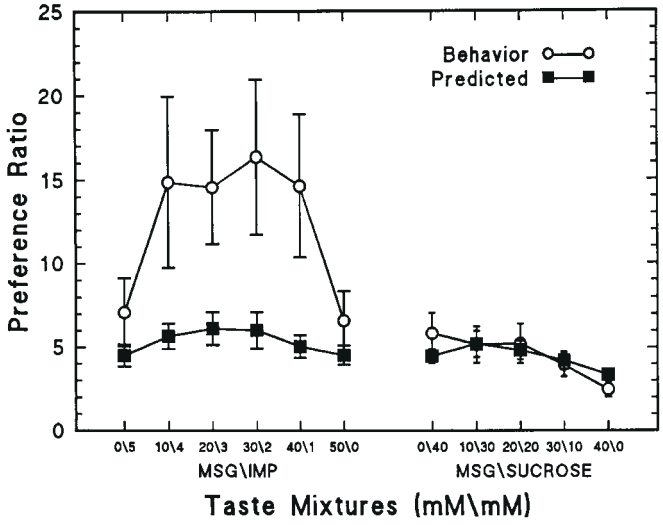

Figure 3 Mean (± SEM) preference ratios for mixtures of MSG and IMP ($n = 8$) and for mixtures of MSG and sucrose ($n = 9$). Predicted ratios (filled squares) represent predicted preference scores if there was no interaction (i.e. no synergy) between the two taste stimuli. A ratio of 2.0 indicates no preference for the stimulus mixture over water. Open circles represent the behavioral ratios measured during the test phase. Note that there are significant differences ($P < 0.001$) between predicted and behavioral ratios for mixtures of MSG and IMP (i.e. synergy), but not for mixtures of sucrose and MSG.

Nine of 12 rats tested with MSG and sucrose met the screening criterion. Again, all rats showed significant concentration-dependent increases in LRs for each stimulus [$F(4,32) = 28.18$, $P < 0.001$] during pre-test and post-test sessions. A significant stimulus × concentration interaction [$F(4,32) = 3.95$, $P < 0.025$] indicated that the rats preferred 30 and 40 mM sucrose to the comparable concentrations of MSG (Figure 4). However, in contrast to the findings with mixtures of MSG and IMP, no evidence of taste synergy could be detected ($F < 1.0$) for any of the mixtures of sucrose and MSG in the ratio scores (Figure 3). A significant main effect for mixture was seen [$F(4,32) = 8.97$, $P < 0.001$], indicating that the magnitude of both the behavioral and predicted ratios decreased as the concentrations of sucrose was decreased and MSG was increased. A similar effect was seen in the analysis of the raw score data [$F(4,35) = 7.36$, $P < 0.001$; Figure 2]. These results are consistent with the greater preference for sucrose than for MSG seen during the pre-test and post-test phases.

Collectively, the results of experiment 1 indicate that the brief access taste test with stimulus substitution methodology measures a strong synergy between MSG and IMP, but no synergy between MSG and sucrose.

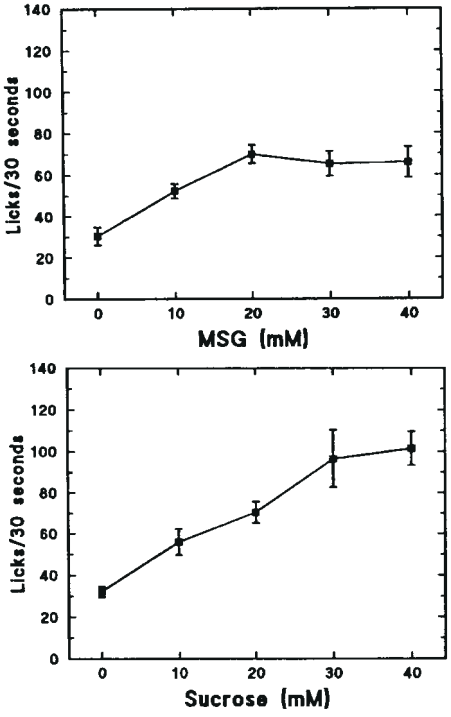

Figure 4 Mean (± SEM) number of licks during 30 s trials for each concentration of MSG and sucrose presented during the pre-test and post-test phases of experiment 1.

Experiment 2

KA

Data for seven of the 10 rats tested with KA and IMP were analyzed after meeting the screening criterion. These rats also showed a similar increase in preference for both of these substances as the concentrations were increased [$F(5,30) = 8.91$, $P < 0.001$; Figure 5A]. Analysis of the raw scores for the test phase did not detect any significant differences in LRs for the mixtures of KA and IMP (Figure 6). Interestingly, the analysis of variance procedures applied to the ratio data revealed that the behavioral ratios were significantly lower than anticipated from the predicted ratios [$F(1,6) = 19.42$, $P < 0.005$], suggesting a negative interaction between KA and IMP when mixed together (Figure 7). Neither the mixture variable nor the interaction mixture × type of ratio had a detectable effect on responding ($F < 1.0$).

NMDA

Of the 10 rats tested with NMDA and IMP, seven met the screening criterion. Pre-test and post-test LRs to these substances increased significantly over the ranges of concentrations tested [$F(5,30) = 9.05$, $P < 0.001$; Figure 5B]. Analysis of the raw scores for the test phase did not detect any significant differences ($F < 1.7$) in LRs for the mixtures of NMDA and IMP (Figure 6). When rats were presented with mixtures of NMDA plus IMP, there were no significant differences between the predicted ratios and the behavioral ratios (all $F < 1.0$), indicating an absence of taste synergy (Figure 7).

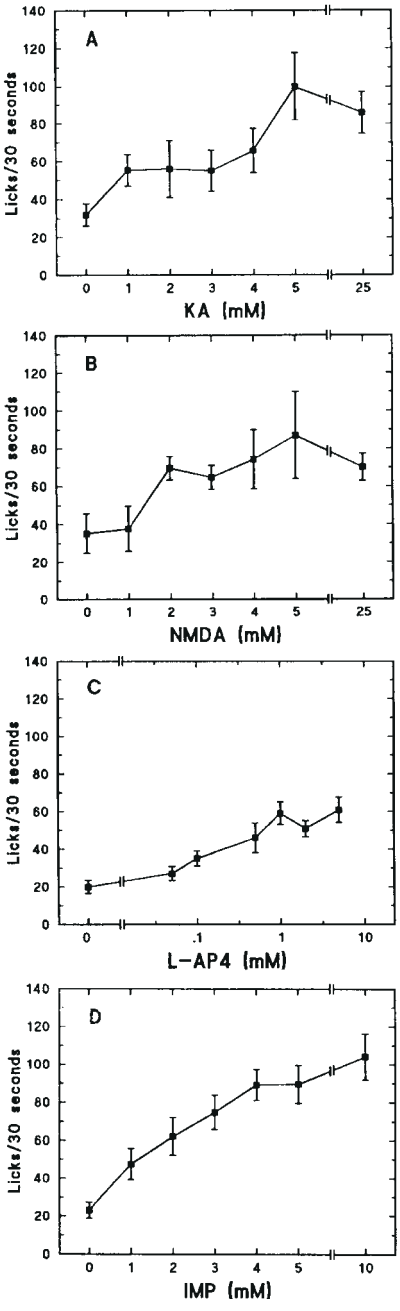

Figure 5 Mean (± SEM) number of licks during 30 s trials for each concentration of KA **(A)**, NMDA **(B)** and L-AP4 **(C)** during the pre-test and post-test phases of experiment 2. The means (± SEM) for IMP **(D)** represent data pooled from all three groups. Note that the abscissae are adjusted for the concentrations tested for each taste stimulus.

L-AP4

Data for 10 out of 11 of the rats tested with L-AP4 and IMP met the screening criterion and subsequently were analyzed for evidence of synergy. LRs during the pre-test and post-test phases increased systematically as the concentration of

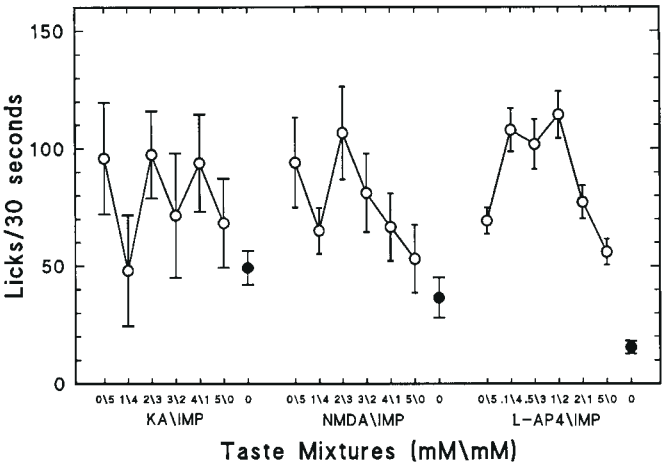

Figure 6 Mean (± SEM) number of licks (open circles) during 30 s trials for mixtures of KA and IMP (n = 7), NMDA and IMP (n = 7) and L-AP4 and IMP (n = 10) during the test phase of experiment 2. Closed circles represent the mean (± SEM) lick rates on water rinse trials for each group during the test phase.

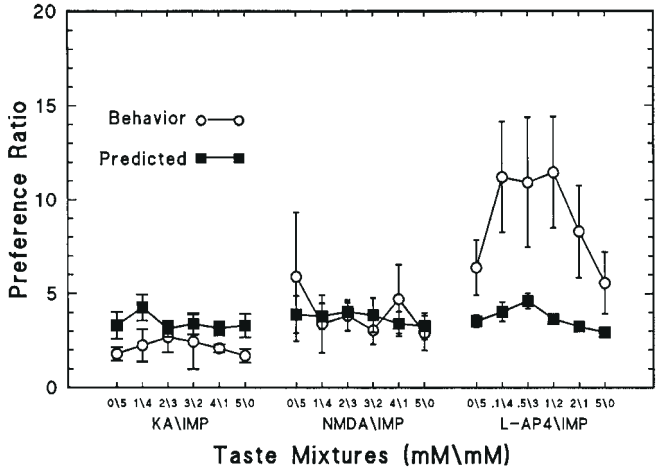

Figure 7 Mean (± SEM) preference ratios for mixtures of KA and IMP (n = 7), NMDA and IMP (n = 7) and L-AP4 and IMP (n = 10). Predicted ratios (filled squares) represent predicted preference scores if there was no interaction (i.e. no synergy) between the two taste stimuli. A ratio of 2.0 indicates no preference for the stimulus mixture over water. Open circles represent the behavioral ratios measured during the test phase. This figure shows a significant synergistic relationship between L-AP4 and IMP (P < 0.001), but not between KA and IMP nor between NMDA and IMP.

each stimulus increased [$F(6,54) = 29.31$, $P < 0.001$; Figure 5C], although the LRs of these rats indicated that they preferred IMP at the higher concentrations more than they preferred the higher concentrations of L-AP4 [$F(6,54) = 5.80$, $P < 0.001$]. The analysis of the raw scores for the test phase revealed significantly higher LRs for the 0.1/4, 0.5/3 and 1/2 mM L-AP4/mM IMP mixtures than LRs for the other mixtures [$F(5,45) = 9.55$, $P < 0.001$; Figure 6]. Analysis of the ratio data revealed significant main effects for ratio type [$F(1,9) = 13.60$, $P < 0.001$] and mixture concentration [$F(5,45) = 5.29$, $P < 0.001$] and a significant interaction between the two variables [$F(5,45) = 3.84$, $P < 0.01$]. Simple effects tests indicated that, as seen in Figure 7, the behavioral ratios were significantly higher for the mixtures of IMP and L-AP4 than the behavioral ratios for solutions of the individual stimuli mixed with water. They were also significantly higher than the predicted ratios for all mixtures. These results indicate taste synergy between IMP and L-AP4.

Finally, an analysis of variance was conducted to compare the LRs during water rinse trials during the test phase for each glutamate agonist (Figure 6). This analysis indicated that there were no significant group differences in these LRs [$F(2,22) = 3.01$, $P > 0.05$].

In summary, experiment 2 found no evidence of taste synergy between IMP and KA nor between NMDA and IMP. However, it revealed marked synergy between IMP and L-AP4.

Discussion

This study employed an adaptation of a stimulus substitution method to test for interactions, especially synergy, between two taste stimuli when presented in mixtures to non-deprived rats in a brief access taste test. The findings of experiment 1 verify that this behavioral assay reliably measures taste synergy between IMP and MSG. The results of experiment 1 also show that there is no interaction between the tastes of MSG and sucrose in rats. Lastly, the data for experiment 2 reveal a significant taste synergy between IMP and L-AP4, but not between IMP and NMDA or IMP and KA.

Other studies have used one or two bottle preference tests where consumption of taste mixtures over 24 h periods is measured to test for synergy between taste stimuli. This introduces the possibility of post-ingestive effects confounding the results. However, Smith *et al.* (Smith *et al.*, 1992) found that preference concentration gradients can be reliably established in non-deprived rats by measuring LRs during relatively brief (30 s) exposures to each concentration of a taste stimulus. In the present study we combined a testing procedure similar to the one described by Smith *et al.* (Smith *et al.*, 1992) with a method for presenting taste mixtures introduced by Rifkin and Bartoshuk (Rifkin and Bartoshuk, 1980) to analyze taste synergy in humans. This method controls for non-linear concentration–response relations to taste stimuli and thus reduces the possibility of artifactual interactions between stimuli in binary mixtures. In experiment 1, lick responses of rats clearly showed a synergistic interaction between MSG and IMP over a range of concentrations for each substance. The lack of synergy between MSG and sucrose in experiment 1 was also important because it demonstrated that this type of response interaction is not an inherent property of the method under these conditions. Taken together, the findings of experiment 1 indicate that the modified stimulus substitution method in

a brief access taste test can accurately detect and reliably quantify synergistic relationships between taste stimuli in non-deprived rats.

The basis for synergy between MSG and IMP that characterizes the umami taste is unknown, but several lines of evidence suggest that a substantial component of taste synergy occurs in the peripheral sensory organs, perhaps at the receptor level. For example, electrophysiological recording studies have detected taste synergy between MSG and nucleotide monophosphates in gustatory afferent nerve fibers in several species [hamster (Faurion, 1991), rat (Yamamoto *et al.*, 1991; Sako and Yamamoto, 1999), mouse (Ninomiya and Funakoshi, 1989; Ninomiya *et al.*, 1992), chimpanzee (Hellekant and Ninomiya, 1991) and dog (Kumazawa and Kurihara, 1990)]. Patch clamp recordings show that MSG and IMP elicit membrane current responses in taste receptor cells and that mixtures of the two substances produce a synergistic effect in the membrane response (Lin and Kinnamon, 1998). Ion channel activity in membranes isolated from lingual taste papillae in rats also show synergistic activation by MSG and IMP (Brand *et al.*, 1991). Moreover, nucleotide monophosphates can enhance the binding of L-[^{3}H]glutamate to its receptor in taste buds (Torii and Cagan, 1980). Thus, even though perceptual processes for umami taste synergy may occur in the central nervous system, mechanisms located within taste receptor cells, perhaps even at the membrane receptor level, appear to underlie the initial taste synergism between MSG and IMP.

Regardless of the underlying mechanisms for umami taste synergy, agonists for the umami taste receptor(s), when mixed with IMP, might also be expected to induce synergistic drinking behavior similar to that for mixtures of MSG and IMP. It has been suggested that NMDA-like receptors may be responsible for transduction of MSG taste (Brand *et al.*, 1991; Faurion, 1991; Hayashi *et al.*, 1996). Recent behavioral and molecular studies, however, suggest that a novel variant of mGluR4, a metabotropic receptor, transduces MSG taste (Chaudhari *et al.*, 1996, 2000; Stapleton *et al.*, 1999). The behavioral results of experiment 2 in this study support this notion. These rats showed a preference for all three glutamate agonists during the pre- and post-test phases of the experiment. However, neither the ionotropic receptor agonist NMDA nor KA induced drinking behavior in rats that suggested any taste synergism when mixed with IMP. In contrast, mixtures of IMP and L-AP4, a mGluR4 receptor agonist, induced a significantly greater response than was predicted from summation of the LRs for the individual taste stimuli, indicating a synergistic interaction between L-AP4 and IMP. These behavioral results are in close agreement with recent whole nerve recordings of the chorda tympani nerve of rats, which also showed synergy between L-AP4 and IMP (Sako and Yamamoto, 1999). Moreover, human psychophysical studies showed taste synergy between L-AP4 and IMP (Kurihara and Kashiwayanagi, 1998). Collectively, those studies and the results of the present study support the hypothesis that the synergistic taste interactions of MSG and nucleotide monophosphates occur when mGluR4 receptors are activated.

In summary, the results of these experiments show that short duration taste preference tests that incorporate a stimulus substitution procedure can detect and quantify taste synergy between MSG and IMP in non-deprived rats. Tests of glutamate agonists mixed with IMP revealed synergistic patterns of drinking for binary mixtures of L-AP4 and IMP, but not for mixtures of NMDA and IMP nor KA and IMP. These findings support the hypothesis that the taste synergy that characterizes umami involves mGluR4 receptors.

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