Behavioral Taste Similarities and Differences among Monosodium L-Glutamate and Glutamate Receptor Agonists in C57BL Mice

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Summary Monosodium L-glutamate (MSG) and 5'-ribonucleotides elicit umami taste in humans and probably in some species of animals. Previous studies suggest that taste-mGluR4 and NMDA receptor may be involved in taste transduction for umami, but behavioral responses in rats do not support the involvement of NMDA receptor. In the present study, behavioral similarities and differences among MSG, mGluR4 agonist L-(+)-2-amino-4-phosphonobutyrate (L-AP4), and NMDA receptor agonist N-methyl-D-aspartate (NMDA) were compared in C57BL mice by using a conditioned taste aversion paradigm. Mice conditioned to avoid either MSG or 10 mM L-AP4 appeared to avoid MSG, disodium 5'-inosinate (IMP), a mixture of MSG and IMP, and L-AP4, but not NMDA. Aversive conditioning to either sucrose or NMDA was generalized only to a mixture of MSG+IMP or NaCl. However, aversive conditioning to L-AP4 at 1 mM was generalized to NMDA and the umami substances. Lick rates for L-AP4 increased by mixing with (RS)- α -cycloprophy-4-phosphonophenylglycine (mGluR4 antagonist) when animals were conditioned to avoid MSG or L-AP4. Lick rates for NMDA also either decreased or increased by mixing with glycine (NMDA receptor coagonist) or D(-)-2-amino-5-phosphonopentanoic acid (NMDA receptor antagonist) when animals were conditioned to avoid L-AP4 or NMDA. In sucrose-conditioned mice, gurmardin (a sweet inhibiting peptide) suppressed the avoidance of sucrose and a mixture of MSG and IMP, but not L-AP4 and NMDA. The results suggest the possibility that to C57BL mice MSG may taste similar to L-AP4 but different from NMDA, although both types of glutamate receptors as well as gurmardin-sensitive sweet receptor may be involved in perception of umami taste.

Key Words monosodium L-glutamate, L-(+)-2-amino-4-phosphonobutyrate, N-methyl-D-aspartate, conditioned taste aversion paradigm, mice

Monosodium L-glutamate (MSG) and 5'-ribonucleotides such as disodium 5'-inosinate (IMP) and disodium 5'-guanylate (GMP) are widely distributed in natural foods (1). In humans and probably in certain species of animals, MSG and 5'-ribonucleotides elicit umami, the fifth basic taste distinct from sweet, salty, sour, and bitter tastes. The most conspicuous feature of umami is a synergism, indicating the magnitude of umami for a mixture of MSG and 5'-ribonucleotide is far more than the sum of each component.

Previous electrophysiological studies have proposed that taste responses to MSG may be mediated at least partially through Na⁺ channels sensitive to amiloride and sweet receptors sensitive to both pronase E, a proteolytic enzyme, and gurmardin, a plant peptide isolated from *Gymnema sylvestre* (2). Pronase E, entire sweet response inhibitor in rats and mice (3, 4), partially suppresses the chorda tympani (CT) nerve response to MSG and a mixture of MSG and IMP in mice (2). Gurmardin

almost completely suppresses the CT nerve responses to sucrose and a mixture of MSG and IMP in rats (5, 6). In contrast, in C57BL mice, sweetener responses of the CT nerve are suppressed by gurmardin to ~50% of control response (7) and recovered by β -cyclodextrin (β -CD) (8), whereas suppressive effects of gurmardin is minimal in the glossopharyngeal (GL) nerve (9). In C57BL mice, gurmardin has also been shown to partially suppress the CT, but not the GL nerve responses to MSG and a mixture of MSG and IMP (2).

More recently, molecular, electrophysiological, and behavioral studies have also proposed the involvement of at least two types of glutamate receptors: group III metabotropic receptor (mGluR4) and ionotropic (NMDA) receptor. Evidence for the possible involvement of mGluR4 initially derives from studies showing expression of mGluR4 specifically in rat taste buds and behavioral taste similarity among MSG and L-(+)-2-amino-4-phosphonobutyrate (L-AP4), an agonist for mGluR4 (10). Electrophysiological studies with isolated taste cells have also shown that L-AP4 mimics the hy-

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perpolarizing response elicited by MSG (11, 12), and that a mGluR4 antagonist (RS)- α -cyclopropyl-4-phosphonophenylglycine (CPPG) suppresses hyperpolarizing response elicited by L-AP4 (12). Recordings of the CT nerve responses also showed synergistic interaction to a mixture of L-AP4 and IMP or N-methyl-D-aspartate (NMDA, NMDA receptor agonist) and IMP (2). Furthermore, recent molecular studies have succeeded in cloning a variant form of brain-mGluR4 (taste-mGluR4) from rat taste buds and in functional characterization of the receptor expressed in CHO cells (13).

Possible involvement of NMDA receptor in taste transduction for umami has been suggested by the findings that MSG and NMDA activate a cation conductance in membrane vesicles from mouse tongue incorporated into bilayers (14, 15). Calcium²⁺ imaging studies also showed MSG- and NMDA-induced increase in intracellular Ca²⁺ concentration in isolated mouse taste cells (16). In addition, whole cell recordings of isolated rat fungiform taste cells have shown that NMDA elicits depolarizing response with either enhancement by glycine (NMDA receptor coagonist) or suppression by D(-)-2-amino-5-phosphonophenylglycine (NMDA receptor antagonist, D-AP5) (12). However, recent behavioral studies in rats suggest that the taste of MSG may be different from NMDA (17), and that a combination of NMDA and IMP, but not L-AP4 and IMP, does not result in a synergism (18).

The C57BL mouse is reported to show high behavioral discriminabilities between MSG and the four basic substances as compared with rats (6), hamsters (19) and other strains of mice (20–23). However, there is no available data on their behavioral responses to L-AP4 and NMDA, although their taste nerve responses have partially been analyzed (2). In the present study, therefore, we compared taste similarities and differences among MSG, L-AP4, and NMDA using a conditioned taste aversion paradigm in C57BL mice.

MATERIALS AND METHODS

Animals and stimuli. Animals were male or female C57BL/6 mice, weighing 18–25 g. Each animal was conditioned to avoid one of 5 compounds and distilled water, consisting of 5 experimental groups and one control group of 4–8 mice each. Three to four animals in each group were housed together and maintained on ad libitum food, but given access only to distilled water during the training and testing sessions.

The conditioning stimulus for each of the 5 experimental groups was one of the following: 30 mM MSG, 1 or 10 mM L-AP4 (Sigma Chemical Co., St Louis, MO, USA), 0.1 M sucrose or 50 mM NMDA (Nacalai Tesque, Inc., Kyoto, Japan) dissolved in distilled water. The conditioned stimulus for the control group was distilled water. Test stimuli used were as follows: 0.01–0.1 M sucrose (Suc), 0.1 M NaCl, 1 mM HCl, 0.1 mM quinine hydrochloride (Qui), 0.03–0.1 M MSG, 0.3 mM IMP, 0.001–0.1 M MSG+0.3 mM IMP, 0.01–10 mM L-AP4, 0.1 mM L-AP4+1 mM CPPG (Tocris Cookson, Bristol, UK), 0.01–50 mM NMDA, 10 μ M glycine (Gly), 0.01–

10 mM NMDA+10 μ M Gly, 1 mM NMDA+50 μ M D-AP5 (Tocris Cookson), and 10 mM NMDA+0.5 mM D-AP5. Solutions containing L-AP4, NMDA, CPPG or D-AP5 were titrated to pH 6.0–7.0 with 4N-NaOH to avoid potential complication elicited by acid taste.

Procedures. Experimental protocols for a conditioned taste aversion paradigm were essentially same to that of Ninomiya and Funakoshi (22). Briefly, each animal was placed in a test box on day 1 of training and given free access to distilled water during a 1-h session. Lick rates were measured by a lickometer with a laser beam lick sensor (Yutaka Electronics Co., Gifu, Japan) and recorded on a pen recorder. From days 2–5, training session time was reduced from 1 h to 30 min. During this period, the animal was trained to drink distilled water on an interval schedule, consisting of 10-s periods of presentation of the distilled water alternated with 20-s inter-trial intervals, resulting in 30–50 trials during the 30-min session. On day 6, each animal was given access to one of the five conditioning stimuli during the interval schedule for more than 20 trials, and then given an intraperitoneal injection of LiCl (230 mg/kg) to induce gastrointestinal malaise. Control mice drank distilled water before the LiCl injection. The seventh day was a recovery period, but the training of drinking distilled water for 30 min was still carried out on this day. On days 8–10, lick rates for 10 s were measured for each test stimuli and distilled water. Lick rates were also measured before and after treatment of the tongue with 30 μ g/mL gurmarin for 10 min, and after treatment of the tongue with 15 mM β -CD (Tokyo Chemical Industry Co., Tokyo, Japan) for 10 min. Gurmarin and β -CD were dissolved in 5 mM phosphate buffer, pH 6.8. The mean lick rate across days 8–10 was obtained for each of the test solutions in each animal.

The behavioral data were quantified by expressing them as a percent suppression score based on the ratio of the lick rate to each taste stimulus given by an experimental group to that of the control group, as shown by the following formula: percent suppression = $[1 - (\text{lick rate of experimental group} / \text{lick rate of control group})] \times 100$. Statistical analysis was Student's *t*-test.

RESULTS

Conditioning to MSG, L-AP4 or NMDA

Figure 1 shows patterns of suppression of licking across 7 taste stimuli after the mice were conditioned to avoid 30 mM MSG, 10 mM L-AP4, 0.1 M sucrose or 50 mM NMDA. The dark columns indicate that lick rate after conditioning was statistically significantly smaller than that in the control group ($p < 0.05$ – 0.01 , *t*-test). The generalization pattern was similar between the 30 mM MSG and 10 mM L-AP4 groups. These animals avoided 30 mM MSG, 0.3 mM IMP, a mixture of 30 mM MSG and 0.3 mM IMP, and 10 mM L-AP4, but not 0.1 M NaCl, 1 mM HCl, 0.1 mM quinine hydrochloride and 10 mM NMDA. Aversive conditioning to either 0.1 M sucrose or 50 mM NMDA was generalized only to a mixture of 30 mM MSG and 0.3 mM IMP or to 0.1 M NaCl. However, aversive conditioning to L-AP4 at 1 mM was

Fig. 1. Patterns of suppression of licking across various taste stimuli after aversions were conditioned in C57BL mice to 30 mM MSG (A), 10 mM L-AP4 (B), 0.1 M sucrose (C) or 50 mM NMDA (D). The number of mice used in each group is shown in parentheses. The dark columns indicate that lick rate after conditioning with each taste stimulus was statistically significantly smaller than that in the controls conditioned with distilled water (*t*-test, **: $p < 0.01$, *: $p < 0.05$). Suc, sucrose; Na, NaCl; Qui, quinine hydrochloride; MSG, monosodium L-glutamate; IMP, disodium 5'-inosinate; L-AP4, L(+)-2-amino-4-phosphonobutyrates; NMDA, N-methyl-D-aspartate.

generalized to 0.01–10 mM NMDA as well as a mixture of 0.1 M MSG and 0.3 mM IMP (data not shown).

Effects of mGluR4 antagonist on behavioral responses

After acquisition of learned aversion either to 30 mM MSG (Fig. 2A) or 10 mM L-AP4 (Fig. 2B), lick rates for 0.1 mM L-AP4 increased to statistically significant levels by mixing it with 1 mM CPPG ($p < 0.001$, *t*-test). Similar antagonism by 1 mM CPPG was also observed when 1 mM L-AP4 was used as a conditioning stimulus ($p < 0.001$, *t*-test, data not shown).

Effects of NMDA receptor coagonist and antagonist on behavioral responses

Aversive conditioning of 10 mM L-AP4 did not generalize to 0.01–10 mM NMDA, but generalized to mixtures of NMDA with 10 μ M glycine ($p < 0.01$ –0.001, *t*-test, Fig. 3A). Glycine at 10 μ M also augmented an aversion to 0.01–10 mM NMDA in 50 mM NMDA-conditioned mice (Fig. 3C).

When animals were conditioned to avoid 1 mM L-AP4, lick rates for 1 mM NMDA increased to a statistically significant level when it was mixed with 50 μ M D-AP5 ($p < 0.001$, *t*-test, Fig. 3B). Such an antagonism was also observed at 0.5 mM D-AP5 in lick rates for 10 mM NMDA when mice were conditioned to avoid 50 mM NMDA ($p < 0.001$, *t*-test, Fig. 3C).

Gurmarin inhibition of a synergism

Figure 4 shows the effects of gurmarin and β -CD on

Fig. 2. Effects of (RS)- α -cyclopropyl-4-phosphonophenylglycine (mGluR4 antagonist) on the lick rates for 0.1 mM L-AP4 when C57BL mice were conditioned to avoid 30 mM MSG (A) or 10 mM L-AP4 (B). CPPG, (RS)- α -cyclopropyl-4-phosphonophenylglycine. The mean lick rates for controls conditioned with distilled water are indicated by open triangles. The number of mice used in each group is shown in parentheses. Mean $\pm$ SE. ***: $p < 0.001$ (*t*-test).

the lick rates for various taste stimuli when mice were conditioned to avoid 0.1 M sucrose. Lick rates for 30–100 mM sucrose and mixtures of 10–300 mM MSG

Fig. 3. Effects of glycine (NMDA receptor coagonist) and D(-)-2-amino-5-phosphonopentanoic acid (NMDA receptor antagonist) on the lick rates for various taste stimuli when C57BL mice were conditioned to avoid 10 mM (A) or 1 mM (B) L-AP4 or 50 mM NMDA (C). The mean lick rates for controls conditioned with distilled water are indicated by open triangles. The number of mice used in each group is shown in parentheses. Gly, glycine; D-AP5, D(-)-2-amino-5-phosphonopentanoic acid. Mean $\pm$ SE. ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$ (*t*-test).

and 0.3 mM IMP statistically significantly increased by treatment of the tongue with 30 μ g/mL gurmarin ($p < 0.05$ –0.001, *t*-test). When the tongue was subsequently rinsed with 15 mM β -CD, lick rates for 30–100 mM sucrose and mixtures of 10–100 mM MSG and 0.3 mM IMP recovered to levels about 15–50% of those obtained after gurmarin ($p < 0.05$ –0.001, *t*-test). However, none of the lick rates for 0.1 M NaCl, 1 mM HCl, 0.1 mM quinine hydrochloride, 10 mM L-AP4, or 10 mM NMDA were affected by treatment of the tongue with gurmarin or β -CD (data not shown).

DISCUSSION

The present results in mice clearly show that an aversive conditioning to sucrose is generalized to a mixture of MSG and IMP. The results also show that generalization to a mixture of MSG and IMP is suppressed by gurmarin and recovered by β -CD. These findings confirm and extend previous behavioral and electrophysiological studies in rats and mice. In rats, sucrose behaviorally mimics the taste of MSG when amiloride is added to suppress Na^+ response (6, 10). Electrophysiological studies in rats (24) and mice (23) have shown that CT nerve fibers sensitive to MSG also respond either to NaCl or sucrose, and that some fibers showing a synergism are sensitive predominantly to sucrose. Pronase E has also been shown to suppress CT nerve responses to MSG and a mixture of MSG and GMP in C57BL mice to 72.6% and 43% of control, respectively (2). In rats, gur-

Fig. 4. Effects of treatment of the tongue with 30 μ g/mL gurmarin and 15 mM β -CD on the lick rates to various taste stimuli when C57BL mice were conditioned to avoid 0.1 M sucrose. The number of mice used is shown in parentheses. Lick rates are indicated by closed circles before gurmarin, open circles after gurmarin, and by open triangles after β -CD. Statistically significantly different between control and after gurmarin or between before and after β -CD. Mean $\pm$ SE. ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$ (*t*-test).

marin completely suppressed CT nerve responses to a mixture of MSG and IMP dissolved in amiloride solution (6). In contrast in C57BL mice, gurmardin suppressed CT nerve responses to a mixture of MSG and IMP to ~65% of control, while it did not affect GL nerve responses to any of the taste stimuli, including umami substances (2). Taken together, these findings suggest that umami substances may stimulate gurmardin-sensitive sweet receptors of rat taste cells in the fungiform and a portion of the foliate papillae (innervated by the CT nerve). The findings also suggest that in C57BL mice responses to umami substances may be mediated through both gurmardin-sensitive sweet receptors and non-sweet receptor mechanisms of taste cells innervated by the CT nerve but through only the latter mechanisms of those in the vallate and foliate papillae (innervated by the GL nerve).

MSG- or L-AP4-conditioned C57BL mice exhibited aversion to solutions of MSG, IMP, a mixture of MSG and IMP, and L-AP4. Conditioning of MSG dissolved in amiloride solution in rats also resulted in aversion to a solution of L-AP4 (10). These data suggest that to mice and rats MSG may taste similar to L-AP4. Whole cell patch-clamp recordings of rat fungiform taste cells showed that hyperpolarizing responses elicited by L-AP4 were suppressed by the mGluR4 antagonist CPPG (12). Although not all experiments using isolated taste cells can exclude the possibility that bath-applied stimulant may act at either the apical or the basolateral membrane of taste cells, our behavioral data clearly show that the site of an antagonism between L-AP4 and CPPG is on the apical membrane of taste cells. Consistently, behavioral responses in rats (18) and the CT nerve responses in C57BL mice (2) showed a synergistic interaction between L-AP4 and IMP. In C57BL mice, the synergistic responses in the CT nerve were insensitive to both pronase and gurmardin (2). In addition, a threshold for L-AP4 was 30 μ M for taste-mGluR4 expressed in CHO cells (13), while a similar threshold (10 μ M) was obtained in behavioral response in C57BL mice. These findings suggest that taste transduction for umami may be mediated in part through L-AP4-sensitive taste-mGluR4.

Aversive conditioning to NMDA in C57BL mice is generalized to NaCl, but not to the other taste qualities, including umami. Similarly, MSG- or 10 mM L-AP4-conditioned mice display no aversion to solutions of NMDA. In rats, conditioning to MSG dissolved in amiloride solution did not result in aversion to NMDA (10, 17). These results suggest that both mice and rats may find the taste of NMDA quite different from that of MSG. However, conditioning to L-AP4 at 1 mM in C57BL mice is generalized to NMDA. In addition, avoidance of NMDA in 1 mM L-AP4- or NMDA-conditioned mice is either enhanced or suppressed by mixing it with glycine or D-AP5. Whole cell patch-clamp recordings of rat fungiform taste bud cells (12) have also shown that NMDA receptors share some properties with their brain form (25), such as co-activation by glycine and suppression by D-AP5. Although behavioral studies in rats showed no synergism between NMDA and IMP (18),

the CT nerve in C57BL mice showed synergistic responses to the mixture (2). In addition, several types of NMDA receptors were detected in rat lingual tissues (10). Collectively, these data suggest that NMDA receptors located on the apical membrane of taste cells may be involved in taste transduction for umami in both mice and rats.

Taste sensitivity to the umami substances in mice has been shown to be dependent on the strain, the taste nerves, and on age. Magnitudes of the synergism in the CT responses are greater in the order of C3H > C57BL > BALB mice (20). Taste information conveyed by the GL nerve fibers plays a relatively more important role in the qualitative discrimination between MSG and the four basic taste substances (21–23). Expression of mGluR4 in the vallate and foliate taste buds is higher in pre-weanling rats compared with adult rats (10), corresponding to greater responses of the GL nerve to MSG in juvenile mice (26). We are now on the way to examine if and to what extent such a difference in sensitivity to the umami substances would be reflected in behavioral responses.

In summary, the present results together with previous findings support the idea that both taste-mGluR4 and NMDA receptor as well as amiloride-sensitive Na⁺ channel and gurmardin-sensitive sweet receptor may be involved in taste transduction for umami substances in C57BL mice.

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