Physiological Evidence for Ionotropic and Metabotropic Glutamate Receptors in Rat Taste Cells

WEIHONG LIN AND SUE C. KINNAMON
Department of Anatomy and Neurobiology, Colorado State University, Fort Collins 80523; and Rocky Mountain Taste and Smell Center, University of Colorado Health Sciences Center, Denver, Colorado 80262

Lin, Weihong and Sue C. Kinnamon. Physiological evidence for ionotropic and metabotropic glutamate receptors in rat taste cells. J. Neurophysiol. 82: 2061–2069, 1999. Monosodium glutamate (MSG) elicits a unique taste in humans called umami. Recent molecular studies suggest that glutamate receptors similar to those in brain are present in taste cells, but their precise role in taste transduction remains to be elucidated. We used giga-seal whole cell recording to examine the effects of MSG and glutamate receptor agonists on membrane properties of taste cells from rat fungiform papillae. MSG (1 mM) induced three subsets of responses in cells voltage-clamped at −80 mV: a decrease in holding current (subset I), an increase in holding current (subset II), and a biphasic response consisting of an increase, followed by a decrease in holding current (subset III). Most subset II glutamate responses were mimicked by the ionotropic glutamate receptor (iGluR) agonist N-methyl-D-aspartate (NMDA). The current was potentiated by glycine and was suppressed by the NMDA receptor antagonist D-(−)-2-amino-5-phosphonopentanoic acid (AP5). The group III metabotropic glutamate receptor (mGluR) agonist L-2-amino-4-phosphonobutyric acid (L-AP4) usually mimicked the subset I glutamate response. This hyperpolarizing response was suppressed by the mGluR antagonist (RS)-α-cyclopropyl-4-phosphonophenylglycine (CPPG) and by 8-bromo-cAMP, suggesting a role for cAMP in the transduction pathway. In a small subset of taste cells, L-AP4 elicited an increase in holding current, resulting in taste cell depolarization under current clamp. Taken together, our results suggest that NMDA-like receptors and at least two types of group III mGluRs are present in taste receptor cells, and these may be coactivated by MSG. Further studies are required to determine which receptors are located on the apical membrane and how they contribute to the umami taste.

INTRODUCTION

Monosodium glutamate (MSG) is a natural component of many foods, including seafood, meats, milk and their by-products, mushrooms, and some vegetables. Both naturally occurring and purified MSG have been used to enhance the flavor of foods and increase food palatability. The taste induced by MSG is called “umami,” a Japanese term meaning delicious or savory (Ikeda 1909). It is believed that the appetitive taste of MSG and other amino acids reflects the requirement of protein in the diet of most animals.

Several studies have characterized the properties of umami taste (for review, Bellisle 1999). As a potent taste stimulus, MSG alters the activity of afferent nerve fibers and central gustatory neurons (Adachi and Aoyama 1991; Hellekant and Ninomiya 1991; Hellekant et al. 1997; Nakamura and Norgren 1993; Ninomiya et al. 1991; Plata-Salaman et al. 1992). In general, it is believed that glutamate is the primary stimulus for the umami taste (Schiffman and Gill 1987; Yamaguchi 1987, 1991; Yamaguchi and Kimizuka 1979).

Several studies have provided evidence that glutamate receptors similar to those in brain may be involved in the transduction of umami taste. Ligands of brain glutamate receptors, including aspartate and ibotenate, are potent taste stimuli; these compounds induce responses in the chorda tympani nerve (Faurion 1991) and elicit an umami taste in humans (for review, Maga 1983). Recent studies suggest that group III metabotropic glutamate receptors (mGluRs) may play a key role in this process. Molecular studies have shown that mGluR4 is specifically expressed in taste cells and not the surrounding epithelium (Chaudhari et al. 1996). In addition, behavioral studies show that the group III mGluR agonist L-2-amino-4-phosphonobutyric acid (L-AP4) generalizes to the taste of glutamate (Chaudhari et al. 1996). Further, physiological studies have shown that both MSG and L-AP4 elicit conductance changes (Bigiani et al. 1997) and alter intracellular Ca2+ levels (Hayashi et al. 1996) in taste cells, providing further evidence for a role of these receptors in the transduction process.

Yet, other investigators have provided evidence for the presence of ionotropic glutamate receptors (iGluRs) in taste cells. These data have come from bilayer studies, in which taste epithelial membranes were incorporated (Brand et al. 1991; Teeter et al. 1992). MSG and N-methyl-D-aspartate (NMDA) both activated a cation conductance in the bilayers, suggesting that the receptor may be an NMDA receptor. In addition, Ca2+ imaging studies have shown increases in intracellular Ca2+ in response to NMDA in isolated taste cells (Hayashi et al. 1996).

Using the whole cell patch-clamp technique and pharmacological agents, we examined the following questions in the present study. 1) Are both NMDA and mGluRs present in taste cells of fungiform papillae? 2) What intracellular signaling pathways are involved? 3) Which ions are involved in responses to NMDA and L-AP4? Preliminary accounts of this work have been published in abstract form (Lin et al. 1996; Lin and Kinnamon 1996).

METHODS

Isolation of taste buds

Four to 12-wk-old male Sprague-Dawley rats were used. Taste buds were freshly isolated from fungiform papillae with a method adapted from Behé et al. (1990). Briefly, rats were killed with CO2, and the
tongue was dissected and placed into cold Tyrode’s solution. Approximately 0.3–0.8 ml of an enzyme mixture containing 3 mg dispase, 0.7 mg collagenase B (Boehringer Mannheim, Indianapolis, IN), and 1 mg trypsin inhibitor (type I-S; Sigma Chemical, St. Louis, MO) in 1.0 ml of Tyrode’s was injected beneath the lingual epithelium of the tongue. The tongue was then incubated in Ca²⁺- and Mg²⁺-free Tyrode’s for 30 min, or until the epithelium could be gently separated from the underlying muscle and connective tissue. The stripped lingual epithelium was pinned serosal side up in a silicone elastomer (Sylgard)-covered Petri dish and incubated in Ca²⁺- and Mg²⁺-free Tyrode’s for 20 min. Taste buds were removed by gentle suction with a glass pipette and plated onto Cell-Tak (Collaborative Research, Bedford, MA)–coated glass slide chambers. The chambers were formed by affixing a Sylgard ring (2-mm wall thickness with an opening diameter of 1.5 cm) to the Cell-Tak–coated slide.

Solutions and chemicals

Normal Tyrode’s was used as a standard bath solution, containing (in mM) 140 NaCl, 5 KCl, 1 MgCl₂, 1 CaCl₂, 10 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid buffer (HEPES), 10 glucose, and 10 sodium pyruvate (pH 7.4 with NaOH). The Ca²⁺- and Mg²⁺-free Tyrode’s for isolating taste buds contained 2 mM bis-(o-aminophenox)-N,N,N,N'-tetraacetic acid (BAPTA; Molecular Probes, Eugene, OR). The 70 mM Na⁺ or Na⁺-free Tyrode’s was obtained by replacing Na⁺ with equimolar N-methyl-D-glutamine (NMDG). The pH was adjusted to 7.4 with HCl. For Ba²⁺-Tyrode’s, NaCl was replaced by 100 mM BaCl₂ and 50 mM NMDG. Low Cl⁻ Tyrode’s contained 140 mM Na⁺ gluconate instead of NaCl. Bath solutions were gravity-fed into the 0.5-ml recording chamber. The standard intracellular pipette solution contained (in mM) 140 KCl, 1 CaCl₂, 2 MgCl₂, 10 HEPES, 11 EGTA, 1 ATP, and 0.4 GTP (pH 7.2 with KOH). Low Cl⁻ pipette solution contained 130 mM K gluconate and 10 mM KCl in place of 140 mM KCl.

MSG, guanosine 5’-o-(2)-thiodiphosphate (trilithium salt, GDP-β-S) and 8-bromo adenosine 3’5’-cyclic monophosphate (8-bromocAMP) were from Sigma Chemical. The agonists and antagonists of glutamate receptors were obtained from Tocris Cookson (Ballwin, MO); these included NMDA, MK-801, (R)-2-amino-5-phosphonopentanoic acid (AP5), 1-AP4, (R,S)-α-methyl-4-phosphonophenylglycine (MPPG), (RS)-α-cyclopentyl-1-4-phosphonophenylglycine (CPPG), and (S)-α-amino-3-hydroxy-5-methyl-4-isoxazolopropionic acid (AMPA). Solutions were gravity-fed into the recording chamber; solution exchange was complete in <10 s. Most of the chemicals used were bath applied except GDP-β-S, which was included in the pipette solution.

Patch-clamp recordings

The whole cell patch-clamp technique was used (Hamill et al. 1981). The steady-state holding current was recorded at −80 mV, except as noted. In some experiments, changes in membrane potential were monitored under current-clamp conditions; responses were monitored at resting potential. The glass pipettes for recording were pulled from microhematocrit capillary tubes (Scientific Products, McGaw Park, IL) with a two-stage vertical puller (model PB-7; Narishige, Tokyo) or a Flaming/Brown micropipette puller (model P-97; Sutter Instrument, Novato, CA). Pipette resistance was 3–6 MΩ when filled with normal pipette solution and 4–8 MΩ when filled with the low Cl⁻ pipette solution. Membrane currents were low-pass filtered at 2 kHz and recorded with an Axopatch patch-clamp amplifier (model 200B, Axon Instruments, Foster City, CA). Voltage-activated Na⁺ and K⁺ currents were generated by applying depolarizing voltage steps from a holding potential of −80 mV; these were used to distinguish taste cells from nonsensory epithelial cells. Hyperpolarizing voltage pulses (20 mV) were used to monitor membrane conductance during whole cell recording. All voltage commands were generated by an Indec laboratory computer system (Sunnyvale, CA). Steady membrane currents were recorded on a strip chart recorder (Linear) as well as on videotape using a VCR (JVC) and analyzed subsequently.

RESULTS

Responses to glutamate

Taste cells from isolated taste buds were voltage clamped at −80 mV, and holding current and membrane conductance were monitored in response to bath application of MSG (1 mM). Responses were arbitrarily grouped into three subsets: a decrease in holding current and membrane conductance (subset I), an increase in holding current (subset II), and a biphasic response, i.e., an increase, followed by a decrease in holding current (subset III). A total of 108 cells of the 185 tested responded to MSG. Of these, 40 cells exhibited subset I responses, 38 cells subset II responses, and 30 cells subset III responses (Fig. 1). In general, it took longer for a subset I response to reach peak amplitude than a subset II response. The time taken to reach half of the response amplitude (T₁/₂ value) for subset I and II responses are 22.5 ± 1.1 and 2.7 ± 0.5 s, respectively (mean ± SE, n = 16, P < 0.001). There were no consistent differences in membrane resistance, capacitance, or expression of voltage-gated currents in these glutamate re-

FIG. 1. Whole cell responses to glutamate. A: bath application of 1 mM glutamate (MSG) induced 3 different types responses: a decrease in holding current and membrane conductance (top trace, subset I); an increase in holding current (middle trace, subset II), and a biphasic response with an increase followed by a decrease in holding current (bottom trace, subset III). B: number and percentage of taste cells exhibiting each subset of response.
GLUTAMATE RECEPTORS IN TASTE CELLS

and mGluRs are expressed in fungiform taste cells, we added a nonhydrolyzable GDP analogue, GDP-β-S (0.2 mM) to the recording pipette to inhibit G protein–mediated pathways. With GDP-β-S in the pipette solution, subset I responses were

FIG. 2. Involvement of G protein–coupled intracellular cAMP pathways. A: a biphasic response to glutamate (top trace) is converted to a subset II response (bottom trace) by intracellular perfusion with GDP-β-S. Note that the GDP-β-S suppressed the outward component of the biphasic response with time. B: the subset I response to glutamate (top trace) is suppressed by bath application of 1 mM 8-bromo-cAMP (middle trace). The effect is reversible (bottom trace).

sponding cells that could correlated with the type of glutamate response. We considered that responses to MSG were induced primarily by glutamate, because the addition of 1 mM MSG has a negligible effect on bath Na⁺ concentration. These results are consistent with a previous study of vallate taste cells that showed both increases and decreases of holding current in response to MSG (Bigiani et al. 1997).

Metabotropic glutamate receptors in brain are coupled to G-protein–mediated intracellular pathways; activation of receptors in group III mGluRs inhibits the activity of adenylate cyclase and decreases the intracellular cAMP level (for review, Pin and Duvoisin 1995). To determine whether both iGluRs

FIG. 3. Whole cell responses to N-methyl-D-aspartate (NMDA). A: the presence of glycine (10 μM) in the bath potentiates the response to NMDA (1 mM). B: the response to NMDA is desensitized more slowly in Mg²⁺–free bath solution than in 10 mM Mg²⁺. C: the antagonist d(-)-2-amino-5-phosphonopentanoic acid (AP5; 50 μM) suppresses the responses to NMDA. Glycine was present in bath and NMDA solutions in B and C and in subsequent figures showing responses to NMDA.
abolished after 15–30 min of whole cell recording (n = 4). In addition, subset III responses were converted to subset II responses (n = 4; Fig. 2A) while subset II responses usually remained unchanged (n = 9). Because many group III mGluRs decrease cAMP in brain (Pin and Duvoisin 1995), we examined whether intracellular cAMP pathways are involved in taste cell responses to glutamate. Bath application of a membrane-permeable cAMP analogue, 8-bromo-cAMP (1 mM) suppressed subset I responses (7 of 10 cells; Fig. 2B) and the outward component of the subset III response (n = 3), while having no significant effect on subset II responses (n = 6). The results indicate that both iGluRs and mGluRs may be present in fungiform taste cells and that subset I responses may be coupled to G protein-mediated intracellular cAMP pathways.

On the basis of the results above, we utilized specific glutamate receptor agonists and antagonists in further experiments to identify possible subtypes of glutamate receptors in taste cells.

Responses to NMDA

Both glutamate and NMDA activate NMDA receptors in brain; glycine is usually required as a co-agonist for full activation of the receptor channel. Extracellular Mg$_{2+}$ blocks the channel at negative membrane potentials, resulting in voltage-dependent activation of the channel. The channel is permeable to Na$^+$, Ca$^{2+}$, and K$^+$ (for review, Collingridge and Watkins 1994). We examined whether NMDA receptors are present in fungiform taste cells and whether the receptors are similar to those in brain. Bath application of NMDA (1 mM) alone increased holding current in ~45% of cells tested when cells were voltage clamped at −80 mV (n = 111). The response generally mimicked subset II responses to glutamate. In the presence of 10 μM glycine, the amplitude of NMDA-sensitive currents increased from 5.2 ± 1.1 pA to 7.7 ± 1.0 pA (n = 10, Fig. 3A). The above experiments suggest that glycine is a co-factor for the taste cell NMDA receptor; thus it was added to all solutions in which NMDA was applied. Glycine (10 μM) applied alone usually did not change holding current.

Next, we tested blockage by extracellular Mg$^{2+}$. Because membrane depolarization removes the Mg$^{2+}$ block for brain NMDA receptors, we examined the NMDA-sensitive current at two holding potentials: −80 and −40 mV. Only 4 of 11 cells tested showed greater current amplitudes at −40 mV than at −80 mV, whereas in most cells, the peak currents induced at −80 mV were bigger than those at −40 mV (8.6 ± 0.8 pA and 5.9 ± 0.6 pA, respectively, n = 7, t < 0.05). Similar results were obtained from experiments conducted in extracellular Mg$^{2+}$-free solution, in which only three of nine cells showed an increase in the peak current. However, when cells were bathed in Mg$^{2+}$-free solution, the NMDA-induced current appeared to desensitize slower in six of nine cells tested. In contrast, when cells were bathed in 10 mM Mg$^{2+}$ solution, the
current desensitized much faster, although the peak current did not decrease \((n = 3)\). These data suggest that extracellular Mg\(^{2+}\) normally does not block the receptor channel, but once it opens, Mg\(^{2+}\) can partially occlude the channel (Fig. 3B). These results are consistent with the effect of MK-801, an open channel blocker. MK-801 blocked the current partially (data not shown), whereas 50 µM AP5, a specific antagonist of the channel, suppressed most of the current (Fig. 3C; \(n = 4\)). In addition, we tested the effect of cAMP on the NMDA-induced current. Similar to subset II responses to glutamate, 8-bromo-cAMP did not suppress the NMDA-sensitive current \((n = 3\), data not shown). To determine the reversal potential of the NMDA-sensitive current, we recorded the current at different holding potentials (Fig. 4, A and B). Unlike the NMDA-sensitive current in the brain, the current in taste cells did not reverse at 0 mV, but reversed at potentials considerably more positive than 0 mV \((n = 7)\). Due to the instability of recording at positive potentials, we could not obtain the actual reversal potential of the NMDA-sensitive current. Our results, however, suggest that the reversal potential is near the equilibrium potentials of Na\(^+\) and Ca\(^{2+}\). To test whether Na\(^+\) and Ca\(^{2+}\) both contribute to the receptor current, we replaced bath Na\(^+\) with the impermeant cation NMDG. The peak current induced by NMDA was reduced in 70 mM Na\(^+\) solution (from 6.42 ± 2.1 pA to 3.6 ± 1.1 pA; \(n = 3\)). However, the current was not totally eliminated, even in Na\(^+\)-free solution. When 100 mM NMDG was replaced with 100 mM Ba\(^{2+}\) in the bath solution, the current was partially recovered (Fig. 4C), suggesting that both Na\(^+\) and divalent cations carry the NMDA-sensitive current.

Finally, we examined the effect of NMDA on membrane potentials under current-clamp configuration. When cells were clamped with the standard pipette solution containing 140 mM KCl and bathed in the normal Tyrode’s solution, resting membrane potentials ranged from −20 to −70 mV. Only a few cells had resting potentials more negative than −50 mV, and some of these fired action potentials spontaneously. Spontaneously

![Figure 5](http://jn.physiology.org/) Responses to NMDA under current-clamp configuration. A: bath application of NMDA results in membrane depolarization and increased frequency of spontaneous action potentials; resting potential −70 mV. B: NMDA depolarizes a taste cell that does not fire action potentials spontaneously. In this particular cell, the response is much larger in Mg\(^{2+}\)-free solution (the resting potential: −65 mV).

![Figure 6](http://jn.physiology.org/) Most responses to L-2-amino-4-phosphonobutyric acid (L-AP4; 10 µM) mimic the subset I response to glutamate. A: the decrease in holding current is suppressed by 8-bromo-cAMP (1 mM). B: in current-clamp mode, L-AP4 hyperpolarizes the cells, and the metabotropic antagonist (RS)-α-cyclopropyl-4-phosphonophenylglycine (CPPG; 100 µM) suppresses the effect (the resting potential: −67 mV). C: responses to L-AP4 at different holding potentials and in low extracellular Cl\(^−\). The intracellular pipette solution contained 130 mM K gluconate and 10 mM KCl. Note that the L-AP4-induced current is Cl\(^−\) sensitive and is abolished near the Cl\(^−\) equilibrium potential.
active cells usually had relatively large voltage-gated Na$^+$
currents (peak current $\sim$2,000 pA) and small voltage-gated K$^+$
currents. NMDA depolarized these cells and increased the
frequency of action potentials (Fig. 5A, $n = 3$). In cells that
were not spontaneously active at rest, NMDA caused mem-
brane depolarization, but action potentials were not always
elicited (Fig. 5B; $n = 6$). In the particular cell shown in Fig.
5B, replacing normal bath solution to a Mg$^{2+}$-free Tyrode’s
dramatically increased responses to NMDA. Depolarizations
induced by NMDA were often followed by a small hyperpo-
larization during wash out of NMDA. Similar wash effects
were often observed with subset II and subset III glutamate
responses under voltage clamp.

Responses to l-AP4

l-AP4 is a specific agonist for the group III mGluRs (for
review, Thomsen 1997). Previous studies showed that mGluR4 is
expressed in vallate taste cells and l-AP4 mimics responses to glutamate both physiologically (Bigiani et al. 1997) and behavior-
lorally (Chaudhari et al. 1996). Thus mGluR4 also may be ex-
pressed in taste cells of fungiform papillae. Bath application of
l-AP4 (10–20 $\mu$M) decreased the holding current in 30 of 93 cells
tested, mimicking the subset I response to glutamate. The pres-
ence of 8-bromo-cAMP (1 mM) suppressed the response (Fig.
6A). In addition, concomitant with this subset of response, l-AP4
induced either a hyperpolarization or had no effect on membrane
potentials for most cells tested in current-clamp mode. In a few
cells, l-AP4 decreased the firing rate of spontaneous action po-
tentials. The mGluR4 antagonists MPFG 500 $\mu$M and CPGG 100
$\mu$M suppressed responses to l-AP4 ($n = 4$ and 3, respectively; Fig.
6B). To determine the reversal potential of this l-AP4–
sensitive current, we recorded the responses at different holding
potentials. When the pipette solution contained 140 mM KCl, the
current reversed about 0 mV ($n = 2$, data not shown). However,
when the pipette solution contained 130 mM K gluconate and 10
mM KCl the current reversed about $-40$ mV, a potential near the
equilibrium potential of Cl$^-$ (Fig. 6C), suggesting that l-AP4 may
suppress a Cl$^-$ conductance. To further examine the role of Cl$^-$,
we conducted experiments in low Cl$^-$ (9 mM) Tyrode’s. Under
these conditions, the l-AP4 response was virtually eliminated.
This result was unexpected, because the driving force for Cl$^-$
should be larger under these conditions. It is possible that extra-
cellular Cl$^-$ is involved in the channel gating, as has been shown
in other systems (Pusch et al. 1995). Further experiments are
required to address this issue thoroughly.

In general, the results above are consistent with previous
studies on effects of l-AP4 in vallate taste cells (Bigiani et al.
1997). However, we recorded an increase in holding current in
response to l-AP4 in 16 of 93 cells (Fig. 7A). Unlike the effect
of l-AP4 described above, the amplitude of this response
increased with increasing concentrations of l-AP4 ranging
from 10 $\mu$M to 1 mM in the bath. These data are consistent
with the dual effects of l-AP4 reported previously in vallate
taste cells. Although most l-AP4–sensitive taste cells showed
a decrease in intracellular Ca$^{2+}$, a few cells showed increases
in intracellular Ca$^{2+}$ in response to l-AP4 (Hayashi et al.
1996). The inward currents elicited by l-AP4 were not signif-
ically suppressed by the metabotropic antagonist CPGG (100
$\mu$M; $n = 2$). In addition, this subset of l-AP4 response usually
was accompanied by membrane depolarization ($n = 3$, Fig.
7A), and replacement of extracellular Na$^+$ with NMDG elimi-
nated the response (Fig. 7A). To examine this response further,
we examined the effect of 8-bromo-cAMP on the l-AP4 re-
sponse. Curiously, bath perfusion of 8-bromo-cAMP (1 mM)
alone caused a decrease in holding current and membrane con-
ductance ($n = 7$; Fig. 7B). In the presence of 8-bromo-
cAMP, the l-AP4–induced inward current was transiently po-
tentiated in four of the seven cells (Fig. 7B). In these cells,
l-AP4 appeared to transiently remove the block of the conduc-
tance. This conductance appears to have similar properties to
the direct cyclic nucleotide conductance recently described in
frog taste cells (Kolesnikov and Margolskee 1995). Further
experiments will be required to examine this hypothesis.

To determine whether both mGluR4 and the NMDA recep-

![Figure 7](http://jn.physiology.org/doi/fig/10.1152/jn.1997.278.1478)

**FIG. 7.** In some cells, l-AP4 induces an increase in holding current and membrane depolarization. A, top trace: voltage-clamp recording in response to 0.1 mM l-AP4. Middle trace: current-clamp recording in response to 1 mM l-AP4. Bottom trace: response is eliminated by lowering the extracellular Na$^+$ concentration. All 3 traces are from the same cell. B: 8-bromo-cAMP (1 mM) decreases holding current and membrane conductance and potentiates the effect of l-AP4 (10 $\mu$M). Two 40-s intervals were removed from the bottom trace due to the extended length of the recording.
tor channels are located in the same taste cells, we applied glutamate, l-AP4, and NMDA sequentially to several taste cells. Data were pooled from all subsets of glutamate responses and are shown in Fig. 8. In a total of 32 cells that responded to glutamate, 11 cells (34%) responded to both l-AP4 and NMDA, 8 cells (25%) responded to NMDA only, 9 cells (28%) responded to l-AP4 only, and 4 cells (13%) responded to neither NMDA nor l-AP4. The lack of response to both NMDA and l-AP4 suggests that other subsets of glutamate receptors may be expressed in taste cells. Therefore we also tested some taste cells for responses to AMPA, a specific agonist of the AMPA receptor. Bath application of AMPA (100 μM) induced responses similar to the subset II response of glutamate, i.e., an increase in holding current in a small subset of cells. Further study is needed to determine whether AMPA receptors play any role in taste transduction.

**DISCUSSION**

In this study we present electrophysiological evidence that NMDA receptors and at least two types of group III metabotropic glutamate receptors are expressed in rat fungiform taste cells. These findings, coupled with molecular, biochemical, and behavioral studies (Chaudhari et al. 1996) suggest that these receptors may play an important role in the transduction of umami taste.

**Responses to glutamate**

In our study, glutamate induced three different subsets of responses. Because these responses were pooled from all taste cells tested, we could not rule out the possibility that the multiple responses occur selectively in different cell types. However, we did obtain results from single taste cells that responded to both NMDA and l-AP4, suggesting that both receptors are often present in the same cells. We suggest that subset III responses represent compound responses that involve co-activation of NMDA and mGluR4 receptors. Several observations are consistent with this interpretation. First, cells exhibiting subset III responses usually responded to both NMDA and l-AP4, suggesting that both types of receptors are present in these cells. Second, the time to half-maximal activation was considerably longer for responses elicited by l-AP4 than for those elicited by NMDA; thus the inward current should precede the outward current. Finally, GDP-β-S and 8-bromo-cAMP suppressed only the glutamate-activated outward current in cells expressing subset III responses. These data are consistent with other studies showing both increases and decreases in intracellular Ca^{2+} or holding currents in response to glutamate (Bigiani et al. 1997; Hayashi et al. 1996). The data presented in this study showed that the percentage of cells responding biphasically (28%, Fig. 1B) is lower than the percentage of cells that respond to both l-AP4 and NMDA (34%, Fig. 8). This difference could be due to the fact that some responses to l-AP4 involved increases in holding current. Taste cells could express receptors for this novel response of l-AP4 in addition to NMDA receptors. Such a combination would not likely result in a biphasic response to glutamate.

**Responses to NMDA**

In vallate taste cells from mice, NMDA increased the membrane conductance (Teeter et al. 1992) and increased intracellular Ca^{2+} levels (Hayashi et al. 1996). Our data are in agreement with these studies. Our data on fungiform taste receptor cells differ somewhat from those of Bigiani et al. (1997), who showed that only a small fraction of vallate taste cells in rats showed increases in holding current in response to glutamate. In our study, most glutamate-sensitive taste cells exhibited some inward current in response to glutamate, and many also responded to NMDA. It appears that there may be differences in glutamate transduction in different species and even among papillae of the same species.

Our study extended the findings of previous investigators to show that NMDA receptors in taste cells share many properties with brain NMDA receptors, such as co-activation with glycine and suppression by AP5 (for review, Collingridge and Watkins 1994). However, the blocking effect of extracellular Mg^{2+} on the NMDA-sensitive current is somewhat less effective in taste cells than in brain. We observed blockage by Mg^{2+} in some cells, whereas in most of the cells tested, the amplitude of the peak current was not reduced in the presence of Mg^{2+}, although the desensitization of the channel was much faster. The lack of Mg^{2+} block may allow these channels to participate in the transduction of glutamate in vivo.

**Responses to l-AP4**

Most of the responses to l-AP4 involved a decrease in holding current and membrane conductance, concomitant with membrane hyperpolarization in some cells. These results are consistent with previous findings in taste cells of vallate and foliate papillae (Bigiani et al. 1997; Hayashi et al. 1996). We showed further that the response is suppressed by cAMP and by the metabotropic antagonist CPPG, supporting the idea that mGluR4 is expressed in rat taste cells (Chaudhari et al. 1996). Suppression by cAMP is to be expected, because activation of mGluR4 decreases intracellular cAMP levels in brain (Pin and Duvoisin 1995). In addition, MSG decreases cAMP levels in tissue from rat vallate and foliate papillae (Zhou and Chaudhari 1995). One difference between our results on fungiform taste cells and those of Bigiani et al. (1997) on foliate and vallate taste cells is the conductance that is modulated by l-AP4. In
vallate and foliate taste cells, a cation conductance is closed by L-AP4 stimulation, whereas in fungiform taste cells, a Cl⁻ conductance is closed. Although 8-bromo-cAMP suppresses the effect of L-AP4 stimulation, we do not believe that cAMP modulates the Cl⁻ conductance directly because changes in membrane conductance were usually not observed in these cells in response to 8-bromo-cAMP application.

In contrast to the above-described effects of L-AP4, we observed that some cells responded to L-AP4 with an increase in holding current and membrane depolarization. This response was not reported in taste cells of rat vallate papillae (Bigiani et al. 1997), although in taste cells of mice vallate papillae, L-AP4 could either decrease or increase the intracellular Ca²⁺ level (Hayashi et al. 1996). Whether mGluR4 also mediates this response is not yet determined. Interestingly, taste cells express two different forms of mGluR4; a long form that is similar to mGluR4 in the brain, and a short form in which a significant portion of the extracellular N-terminus has been truncated. This short form may be specific to taste cells (Fedorov and Chaudhari 1998). Both forms of mGluR4 have been expressed in heterologous cells, and activation of both receptors causes decreases in intracellular Ca²⁺. Activation of taste-mGluR4 requires higher concentrations of glutamate for activation (Landin and Chaudhari 1999), similar to those required to elicit umami taste. Thus this receptor may be more important for glutamate taste transduction. The inward currents induced by L-AP4 in our study required higher concentrations of L-AP4 than the outward current. It is tempting to speculate that the inward current generated by L-AP4 results from activation of taste-mGluR4.

The present study suggests that both NMDA receptors and metabotropic glutamate receptors are present in taste cells and may be co-activated in response to glutamate taste stimulation. An important caveat in this interpretation is that glutamate was applied to the entire taste cell membrane, rather than selectively to the apical membrane as occurs in vivo. It is possible that some responses to glutamate observed in the present study were mediated by receptors located on the basolateral membrane rather than the apical membrane. The neurotransmitter in taste cells has not been identified, but in most other sensory receptors the transmitter is glutamate. Group III mGluRs are expressed on the presynaptic membranes of glutamatergic neurons in many regions of the brain, where they function to inhibit glutamate release (Pin and Duvoisin 1995). The inhibitory responses to L-AP4 could be mediated by such an inhibitory autoreceptor located on the basolateral membrane, possibly the brain form of mGluR4. Further studies will be required to determine the location of NMDA receptors and both forms of mGluR4 in taste cell membranes. However, a recently published paper showed that both L-AP4 and NMDA induced measurable responses in chorda tympani nerve recordings when these compounds were applied to the surface of a rat tongue (Sako and Yamamoto 1999). These data imply that receptors for both NMDA and L-AP4 are located at the apical membrane of taste cells, but further studies will be required to determine which type of L-AP4 response (hyperpolarizing or depolarizing) is mediated by receptors on the apical membrane.

One of the important features of umami taste is potentiation by 5′-ribonucleotides, such as guanosine 5′-monophosphate (5′-GMP) and inosine 5′-monophosphate (5′-IMP). This potentiation has been observed in afferent nerve recordings when MSG is applied together with 5′-GMP or 5′-IMP (Hellekant and Ninomiya 1991) and also when L-AP4 is applied together with the nucleotides (Sako and Yamamoto 1999). In preliminary studies, we have observed synergy between MSG and 5′-GMP in a subset of glutamate-responsive taste cells. Both subset I and II responses could be potentiated (Lin and Kinnamon 1996). Further studies will be needed to examine the mechanisms involved in the synergy.

We thank Drs. Nirupa Chaudhari and Stephen Roper for sharing unpublished data and for helpful discussions throughout the course of this study. This study was supported by National Institute on Deafness and Other Communication Disorders Grant DC-03013. Address for reprint requests: W. Lin, Dept. of Anatomy and Neurobiology, Colorado State University, Fort Collins, CO 80523.

Received 12 May 1999; accepted in final form 29 June 1999.

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