Basic Characteristics of Glutamates and Umami Sensing in the Oral Cavity and the Gut

Chemosensory Signal Transduction in *Paramecium*[1,2]

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ABSTRACT *Paramecia* are ciliated single-cell eukaryotic organisms that can respond to chemical cues in their environment. Glutamate is among those cues, which attract cells. We describe briefly here the following attributes of glutamate chemoresponse: *1*) Cells are attracted to L-glutamate relative to KCl at high concentrations of glutamate. *2*) There are at least two specific, relatively low affinity glutamate binding sites on the cell surface. Glutamate can be displaced from only one of the binding sites by inosine monophosphate (IMP), and quisqualate displaces glutamate from the second site, which is likely to be the glutamate receptor involved in attraction to glutamate. *3*) IMP is a repellent and does not act synergistically with glutamate, whereas guanosine monophosphate (GMP) does. *4*) Similarly, glutathione is an attractant, but glutamate and glutathione appear to use different transduction pathways. *5*) Glutamate hyperpolarizes the cell. The ionic mechanism is not yet verified, but is likely to involve a K conductance. *6*) Glutamate induces a rapid and robust increase in cAMP in the cell. Protein kinase A (PKA) is possibly involved in the transduction pathway because kinase inhibitors such as H7 and H8 inhibit glutamate response, but do not affect responses to other attractants, such as acetate and ammonium. Activation of PKA by the rapid rise in cAMP may sustain the hyperpolarization phosphorylation and activation of the plasma membrane calcium pump. *7*) Candidate glutamate binding proteins are being identified among the cell surface proteins with the use of affinity chromatography. J. Nutr. 130: 946S–949S, 2000.

KEY WORDS: • *glutamate receptors* • paramecium • *protein kinase A* • *glutamate*

Paramecia are ciliated single-cell eukaryotic organisms that can respond to chemicals in their environment. Glutamate is an important attractant chemical cue, probably signifying the presence of bacteria, their food.

We have investigated the signal transduction pathway for glutamate in *Paramecium tetraurelia* by characterizing the behavior of cells, specific binding sites for glutamate and second messengers. Glutamate chemoresponse in *P. tetraurelia* has some attributes similar to the umami taste of monosodium glutamate and also some significant differences as described below.

MATERIALS AND METHODS

Cell culturing. *Paramecium tetraurelia* strain 51-S (sensitive to killer) were grown as described in Sasner and Van Houten (1989).

Behavioral assays. T-maze assays of chemoresponse were conducted as described in Van Houten et al. (1982).

Cyclic nucleotide assays. Assays for cAMP and cGMP were done using kits from Amersham (Piscataway, NJ), as described in Yang et al. (1997).

Membrane potential measurements. Measurement of membrane potential was done as in Preston and Van Houten (1987).

Binding studies. Binding of ^{3}H-glutamate to whole cells and cilia was carried out as described in Yang (1995) and as for biotin and cAMP extracellular binding measurements (Bell et al. 1998, Smith et al. 1987).

Inhibitor studies in behavioral assays. Cells were treated with H7 or H8 before T-maze assays of attraction to glutamate and other stimuli as described in Yang et al. (1997).

Chemicals. All chemicals were from Sigma (St. Louis. MO) or Calbiochem (San Diego, CA) unless otherwise indicated.

Affinity chromatography. Glutamate agarose beads (Sigma) were used for affinity chromatography generally as described for cAMP affinity chromatography (Van Houten et al. 1991). Cell surface proteins were harvested in salt/ethanol washes of cells (Capdeville et al. 1993, Preer et al. 1981) or Triton X100 detergent extracts of cell body membranes [pellicles, harvested as described in Bilinski et al. (1981)] and solubilized as described in Van Houten et al. (1991).

RESULTS AND DISCUSSION

Glutamate is an attractant for *P. tetraurelia*, as demonstrated with T-maze assays of behavior (Yang et al. 1997). To characterize the chemoreceptors for glutamate attraction, we studied binding of ^{3}H-glutamate to whole cells by measuring displacement of ^{3}H-glutamate from whole cells by unlabeled glutamate or other unlabeled analogs. These studies revealed

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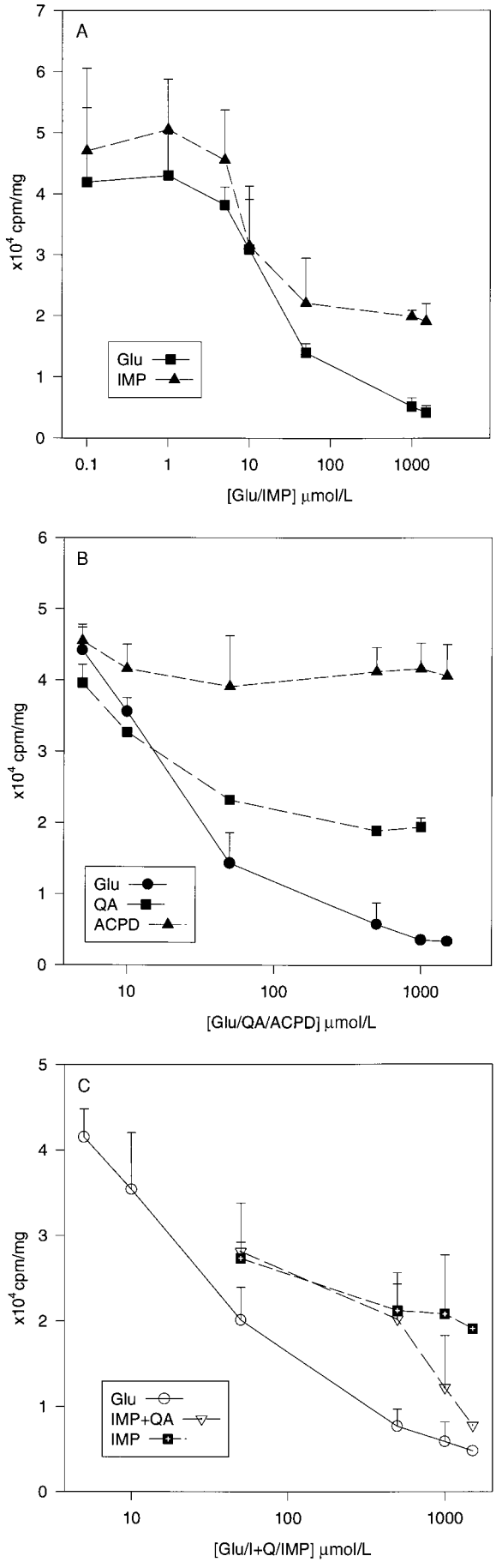

at least two low affinity binding sites with similar K_d of ~40 μmol/L (Yang 1995). In general, agreement was found between the half-maximal response for behavior and the K_d for binding of attractant stimuli (Smith et al. 1987, for example) with the half-maximal value for behavior somewhat higher, indicating that most binding sites must be occupied before a behavioral response is elicited. However, for glutamate, the half-maximal values for behavior and binding were different (1 mmol/L and 40 μmol/L, respectively). Although we cannot explain the differences at this time, the studies of interference in binding and behavior described below give confidence that the binding that we are measuring is the relevant binding for chemoresponse.

Because inosine monophosphate (IMP)[4] and other 5′-ribonucleotides act synergistically with glutamate in umami taste responses (Ugawa and Kurihara 1994), we tested for displacement of glutamate binding by these compounds. IMP displaced glutamate from about half of the *Paramecium* binding sites, but not from all of the specific sites (**Fig. 1***A*). Interestingly, quisqualate also displaced glutamate from some, but not all specific sites (Fig. 1*B*), and the combination of IMP and quisqualate was as effective as glutamate in displacing all of the specific ^{3}H-glutamate binding (Fig. 1C). Thus, IMP and quisqualate helped us to distinguish between two glutamate binding sites.

Glutamate chemoattraction is not affected positively or negatively by IMP, which might be expected if the glutamate binding site that is affected by IMP is not the chemoreceptor for glutamate attraction, but rather is the receptor for IMP chemoresponse (**Fig. 2***A*). However, glutamate completely interfered with chemoresponse to IMP, which surprisingly is a repellent (Fig. 2*B*). Quisqualate interfered with glutamate attraction, even at very low ratios of quisqualate to glutamate (data not shown; Yang 1995). Therefore, considering the results of the binding studies with these behavioral studies, we believe that quisqualate, unlike IMP, could be interfering with glutamate chemoresponse by interfering with binding of glutamate to the chemoreceptor sites that mediate attraction to glutamate.

Guanosine monophosphate (GMP) and glutamate act synergistically, i.e., cells are very attracted to concentrations of GMP and glutamate together that would not be attractive separately (**Fig. 3**). However, GMP does not displace glutamate from whole-cell binding sites and does not improve on the displacement of glutamate by IMP in binding studies (Yang 1995, data not shown). The mechanism of this synergy is not yet known.

Glutathione is an attractant for paramecia, and interferes

[4] Abbreviations used: GMP, guanosine monophosphate; IMP, iosine monophosphate; PKA, protein kinase A.

FIGURE 1 Binding studies of ^{3}H-glutamate to whole cells. (*A*) Washed cells were distributed equally to incubation solutions of unlabeled glutamate or K_2IMP (inosine monophosphate) at increasing concentrations before the addition of 0.018 nmol of stock L-^{3}H-glutamate. Cells were then incubated for 60 min (established as a time for equilibrium binding); the cells were collected by centrifugation for scintillation counting. Data points are the means ± 1 SD of three experiments each performed in duplicate (Yang 1995). (*B*) Cells were treated as above, with the exception that quisqualate (QA) or trans-1-amino-1,3-cyclopentane-dicarboxylic acid (ACPD) was substituted for glutamate. The concentrations were chosen to focus on the higher concentration ranges. (*C*) Cells were treated as in (*A*), with the exception that glutamate or K_2IMP or K_2IMP with quisqualate was used as the cold ligand.

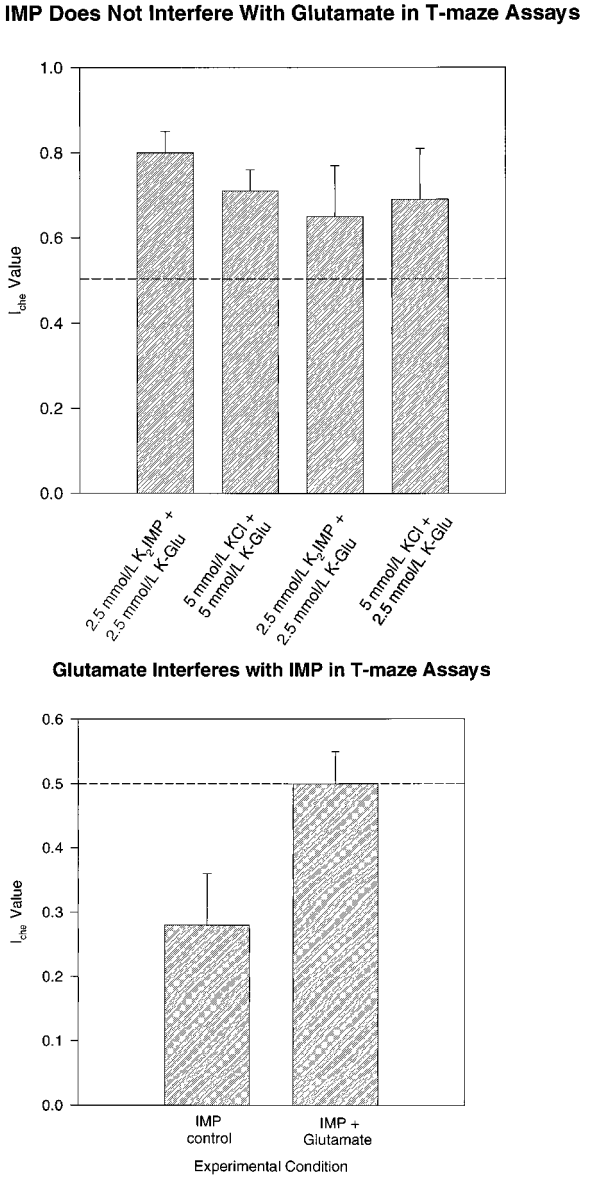

FIGURE 2 T-maze Assays of Interference with Attraction Behavior. T-maze assays were carried out to determine the effects of a ligand such as uniform distribution of IMP on response to a glutamate gradiant. T-mazes measure the time of the cells spent in arms of the T-maze that contain control or attractant in buffer. The measure of attraction or repulsion (I_{che}) >0.5 indicates attraction; <0.5 indicates repulsion. 0.5 should indicate a neutral response. 2A: K_2IMP when included in both the test and control arms of the T-maze does not interfere with attraction to glutamate. Note that the control T-mazes have additional KC1 in both arms of the T-maze to control for the added ionic strength of the interference test with additional IMP in both arms of the T-maze. 2B: Glutamate when included in both the test and control arms of the T-maze completely interferes with repulsion from IMP.

with the glutamate chemoresponse, but not vice versa. The glutamate moiety of the glutathione molecule probably binds to the glutamate receptor and thereby interferes with the glutamate response. However, glutamate showed no significant interference with the glutathione chemoresponse, which likely means that glutamate is not the part of the glutathione structure involved in receptor binding. We suggest that there are two different receptors for glutamate and glutathione, and that during chemoresponse to glutamate, only the specific glutamate receptor is activated, whereas during chemoresponse to glutathione, both the glutamate and glutathione receptors may become bound and activated.

Cells in glutamate swim smoothly and quickly, which is indicative of a relatively hyperpolarized membrane potential; cells in IMP show opposite behaviors, implying that the cells are relatively depolarized. Hyperpolarization and depolarization and their concomitant effects on ciliary beat and swimming patterns are associated with attraction and repulsion, respectively (Van Houten 1994). Direct measurements of membrane potential of cells in glutamate relative to cells in control buffers confirm that cells hyperpolarize ~8–10 mV in 5 mmol/L K-L-glutamate relative to KCl (Yang 1995).

The hyperpolarization is probably not due to the uptake of glutamate; measurements are not made in Na^+ salts but rather in K^+ salts, which inhibit glutamate uptake from the medium (Yang 1995). The mechanism of the hyperpolarization is not yet known, but probably involves activation of a hyperpolarizing K^+ conductance initially to hyperpolarize the cell (Preston and Usherwood 1988). We do not know how the glutamate receptor couples to the activation of a K^+ channel. Interestingly, the hyperpolarization is sustained, and we speculate that the stimulus (glutamate), bound to receptors, activates adenylyl cyclase, which in turn activates protein kinase A (PKA) and the calcium pump of the plasma membrane. Calcium pump activation could then sustain the hyperpolarizing current (**Fig. 4**). [See Van Houten (1994 and 1998) for discussion.] The inhibitory effects of the kinase inhibitors H7 and H8 on the glutamate response support this possibility (Yang et al. 1997). Both of these inhibitors eliminate the attraction to glutamate but do not affect attraction to other stimuli such as acetate and ammonium.

There is evidence for ion channel–associated glutamate receptors (Brand et al. 1991, Hayashi et al. 1996) and also for metabotropic mGluR4 receptor in taste cells (Bigiani et al. 1997, Chaudhari et al. 1996). Similarly, there is evidence for both mGluR1 and 3 in catfish olfactory neurons (Medler et al.

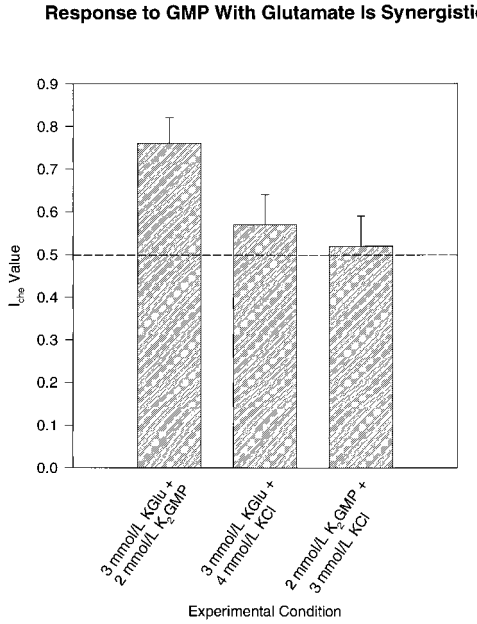

FIGURE 3 T-maze assay of guanosine monophosphate (GMP) and glutamate synergy. T-maze assays for K_2GMP vs. KCl, K-L-glutamate vs. KCl and a combination of K_2GMP + K-L-glutamate vs. KCl were conducted as described in Van Houten et al. (1982). A measure of attraction or repulsion (I_{che}) > 0.5 indicates attraction. Data are averages of nine T-mazes ± 1 SD.

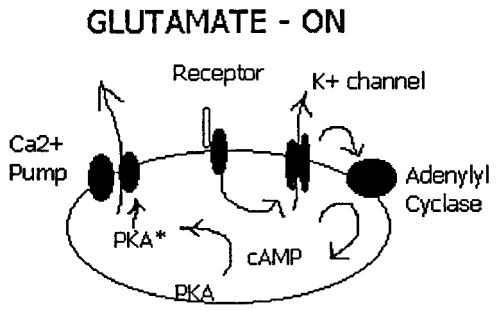

FIGURE 4 Cartoon of the proposed signal transduction pathway for glutamate chemoresponse. Starting the signal transduction pathway at the receptor, ligand binds to receptor and activates a K conductance. The K channel is drawn as a separate entity with unknown coupling to the receptor, but alternatively it may be part of the receptor. The ensuing hyperpolarization activates adenylyl cyclase and the subsequent rise in cAMP activates protein kinase A (PKA). PKA is known to activate the *Paramecium* and other plasma membrane calcium pumps, and the additional pump activity over and above the basal pump activity sustains an outward hyperpolarizing conductance. The K conductance has been explored to some degree by Preston and Usherwood (1988) and more recently by Preston and Van Houten (unpublished); the adenylyl cyclase may actually be the K channel (Schultz et al. 1992); the involvement of PKA and the pump has been supported by the work of Yang and others (Yang et al. 1997). However, the coupling mechanisms and involvement of G proteins have not yet been established.

1998). The pharmacology of the *Paramecium* glutamate receptor does not seem to fit perfectly with these models (Yang 1995). However, the *Paramecium* glutamate response does share some interesting characteristics with umami taste, specifically synergy with a 5′-ribonucleotide, GMP. It may also share a sustained hyperpolarization induced by glutamate in some rat taste cells (Bigiani et al. 1997). We have no evidence yet to determine whether the *Paramecium* glutamate receptor physically resembles the receptors implicated in taste (Bigiani et al. 1997, Hayashi et al. 1996) or in olfactory neurons (Medler et al. 1998). Considering that the bacterial glutamate receptor in the periplasmic space shares structural features with vertebrate metabotropic neurotransmitter receptors, it is quite possible that the glutamate chemoresponse system of *Paramecium* has retained these same features. The answer to this awaits the purification of the *P. tetraurelia* receptor. At present, we are using affinity chromatography to identify proteins from the cell surface that bind to glutamate (Bergeron, unpublished observations). There is a protein of 70 kDa that consistently elutes with glutamate from glutamate-agarose columns.

Several species of bacteria use extracellular cues to determine the density of their own populations and, thereby, judge the optimum time at which to express a set of genes or a behavior (Swift et al. 1996). A phenomenon called "quorum sensing" allows the bacteria to respond in almost a step function only when conditions are right, i.e., when some important cue is in adequate concentration. It is not unusual for paramecia to respond only to relatively high (10 μmol/L-1 mmol/L) concentrations of stimulus in their chemically noisy pond environments [see Van Houten (1994) for discussion]. We consider the *Paramecium* response at relatively high concentrations of stimuli such as glutamate to be a form of quorum sensing—not sensing of other paramecia but of their prey, bacteria, which are good sources of glutamate. It does not benefit a paramecium to dash off through the pond after a stray signaling molecule. It is more efficacious for the paramecia to wait until the bacterial population has risen, producing an elevation in glutamate concentrations to some threshold value before responding with an attraction response. [See Bell et al. (1998) and Van Houten (1994) for discussion.] It is tempting to speculate that the synergy between GMP and glutamate enhances locating and foraging on actively growing bacteria by enhancing sensitivity of chemoresponse, whereas the repellent signal from IMP warns against toxic conditions. Perhaps understanding the use of glutamate by *Paramecium* to accumulate near bacteria will provide some insight into the nuances among the glutamate receptors involved in sensory systems such as taste or olfaction in aquatic organisms and the receptors used in neurotransmission.

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