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Biochemical Studies of Glutamate Taste Receptors: The Synergistic Taste Effect of L-Glutamate and 5′-Ribonucleotides

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Unique features of the taste effects of monosodium glutamate (MSG) could make MSG taste an important system for a better understanding of the biochemical basis of taste sensation. Our approach to the glutamate taste at the biochemical level derives, on the one hand, from observations of its taste effects in food systems and, on the other, from previous studies in our laboratory on the biochemical basis of taste. The approach we are employing is essentially biochemical, utilizing measures of the initial interaction of the stimulus with its receptor sites.

Dried bonito (*katsuo-bushi*), black mushroom (*shiitake*), and the seaweed sea tangle (*Laminaria sp.*) have been used extensively as condiments in Japanese cuisine. These materials impart a characteristic taste that is called *umami* in Japanese (49,52), meaning "delicious" or "savory" taste (27). Chemical studies have shown that the taste-active substances from these foods belong to two separate classes of chemical compounds. In the early 1900s, the taste-active substance was isolated from sea tangle and identified by Ikeda (20) as a salt of L-glutamic acid. In contrast, the taste-active ingredient from dried bonito was found by Kodama (26) to be the histidine salt of IMP (29,30), and that from black mushroom was more recently shown to be GMP (37,45). The taste properties of MSG and ribonucleotides have been investigated by psychologists and food scientists (3,25,27,29–31,36,45,49,50,52), but the mechanism of action of these compounds in exciting the taste receptors has not been studied. Investigators consistently describe the "distinctive" or "unique" taste of MSG, and certain other amino acids have also been noted (23) to possess some of this characteristic taste.

The fact that these condiments are typically used in combination in Japanese cookery has a certain theoretical interest. Psychophysical taste evaluations have conclusively shown that a powerful synergism exists in mixtures of certain 5′-ribonucleotides and MSG; the taste intensity of such a mixture is greater than the sum of the tastes of the two components. Yamaguchi (49) initially used mixtures of IMP and MSG to study this synergism, and subsequently (50) extended the work to include several additional purines and their derivatives. Because of the potent

synergistic effect, Cagan (11) recently noted that: "to understand the mechanism of action of MSG we may actually need to understand the synergistic action of MSG and ribonucleotides rather than the effect of MSG alone."

The use of new approaches and methodologies has led to a number of advances in our basic understanding of the initial events underlying taste sensation. The stimulus-receptor interaction in taste has been investigated in our laboratory (7–10,12–15,28) and elsewhere (32,40). Our recent studies have included the development of improved methods enabling the direct measurement of the initial interaction, which presumably reflects the complex formed (15,28,46). In addition, several testable hypotheses were recently proposed (11) that focused attention on the possible sites of action and the underlying mechanisms responsible for the taste effects of MSG and of MSG-ribonucleotide mixtures. The loci noted were (a) receptor site interactions, (b) transduction processes within receptor cells, (c) peripheral synaptic transmission, and (d) neural transmission and central processing. When considering item (a), receptor site interactions, two fundamentally different types of effects at this level were described: first, effects on binding affinities and, second, effects on the accessibility of receptor sites.

The results of the studies noted above (7–10,12–15,28) have made it feasible to undertake a biochemical investigation of the glutamate taste. We have focused our attention on the binding interaction of L-glutamate to receptor sites in taste tissue and on the effects of GMP and other ribonucleotides on this interaction. Specifically, we wished to establish if the synergism could be accounted for by a peripheral mechanism, and if this could be measured biochemically using the binding of a radioactively labeled ligand. Our evidence demonstrates that L-glutamate binds preferentially to taste receptor tissue, and that GMP and certain other 5′-ribonucleotides cause a marked enhancement of L-glutamate binding (46). We propose that the basis of the synergistic effect is due to a change in the receptor properties, caused by the 5′-ribonucleotide, that affects the binding interaction of L-glutamate with the taste receptors. A corollary of this hypothesis is that the flavor enhancement of foods by MSG is due to a combination of its unique taste character coupled with the enhancement of its taste effect by low levels of 5′-ribonucleotides, such as those that may be present endogenously in the food.

AMINO ACID BINDING TO TASTE RECEPTORS

In order to provide a background for our approach, previous studies from our laboratory on the taste system of the catfish are reviewed. The catfish *Ictalurus* has taste buds distributed over its body surface. The barbels ("whiskers") have the highest density of buds, and the rostral, dorsal, and dorsolateral surfaces also contain appreciable numbers (4,18). Behavioral studies (6,18) showed that the catfish uses its sense of taste to locate food, and electrophysiological measurements (5,16) from the barbel nerve established that the taste system is sensitive to a number of amino acids. In our laboratory (28) we measured the binding of amino acid taste stimuli to the catfish taste receptors *in vitro* by utilizing radioactively

labeled amino acids as ligands. In order to carry out these experiments, the taste epithelium is homogenized and fractionated by differential centrifugation to yield a sedimentable fraction (Fraction P2). This fraction, which contains plasma membranes (although not pure), is enriched in binding activity for taste stimulus amino acids (12,13,28). In recent studies (14) we further purified Fraction P2 to isolate the plasma membranes and demonstrated that the binding activity is in fact associated with this plasma membrane fraction. These results show that the initial discrimination of taste stimuli occurs at the outer surface of the receptor cells.

L-[^{3}H]Alanine has been used extensively as a ligand in our studies because the catfish is highly sensitive to this amino acid as a taste stimulus (16). This has allowed us to develop a convenient, reliable assay system by which to measure binding. We thereby established that binding is a measure of an early event in taste sensation, an event which we propose to be the initial discrimination step in the recognition of taste stimulus compounds. Several lines of evidence support the hypothesis: (a) binding is saturable and reversible, (b) the distribution of binding activity corresponds with the known distribution of taste buds, (c) amino acids that are electrophysiological taste stimuli also show binding activity (albeit in different orders of relative effectiveness), and (d) denervation, which is known to cause the degeneration of the taste buds (33,39,47), results in a decrease in binding activity of the denervated preparation.

Of the ligands we employ, the binding properties of L-alanine have been studied in greatest detail (28). The value of the K_D (dissociation constant) for L-alanine is 4.8×10^{-6} M, and the optimal pH for binding is 7.8. The reversibility of the binding was demonstrated both by measuring the dissociation rate of the ligand into ligand-free medium, and by displacement of the bound ^{3}H-ligand with a large excess of unlabeled ligand. The preparation from catfish taste tissue therefore contains receptor sites with which the taste stimulus L-alanine interacts in a reversible binding step.

ENHANCEMENT EFFECT AND "HIDDEN" RECEPTORS

During the course of studies to attempt to stabilize the binding activity of Fraction P2, a striking effect was observed (12,13) following the frozen storage of Fraction P2 in L-alanine. When Fraction P2 was maintained in a relatively high concentration (10 mM) of L-alanine (unlabeled) and then washed to remove the ligand prior to the assay for binding activity, the binding activity actually increased several fold. This enhancement effect shows an interesting degree of stereospecificity with respect to L- and D-alanine; it is similar to the stereospecificity of these isomers as taste stimuli measured electrophysiologically. D-Alanine averages 57% as effective a taste stimulus as L-alanine (16); D-alanine treatment of Fraction P2 enhanced the binding of L-[^{3}H]alanine to 50 to 60% of the level caused by L-alanine treatment (12,13). By analyzing the enhancement phenomenon using Scatchard plots, it was established (13) that the K_D for L-alanine is unchanged while the maximal binding of L-alanine increases by several fold. The effect, therefore, is not on the affinity of the taste

receptors for L-alanine, but rather on the number of sites available for binding this amino acid.

These results led to the hypothesis (12,13) that the enhancement due to L-alanine treatment is a result of exposing alanine receptor sites that were previously "hidden" or "buried," such as within the cell membrane. It was further postulated that the high ligand concentration causes a perturbation, presumably through a cooperative effect, of the receptor membrane complex such that additional binding sites become accessible to the ligand. Whether this involves a conformational change of the receptor molecules only, or a larger conformational change involving the membrane structure, will require further study.

MSG AND RIBONUCLEOTIDES

MSG is a taste stimulus in humans, being effective in the millimolar concentration range (25,31,36). MSG also shows the remarkable property of a synergistic taste effect in mixtures with certain 5′-ribonucleotides. Of the naturally occurring nucleotides studied, GMP is the most potent in evoking this effect (35,50).

Relatively few behavioral animal studies with MSG have been carried out, but those reported do agree in certain respects with human psychophysical studies. Although in an early study the food preference of rats was not affected by 1% MSG in the diet (44), rats do prefer solutions of MSG to water (19,38). Weanling calves ate more of a diet when it contained 0.2% MSG (48), and preliminary reports suggest that MSG affects the dietary intake of pigs (17,24). Pygmy goats appeared to have altered taste acuity to certain stimuli in the presence of MSG (34).

Electrophysiological experiments with rats (2,21,41,43,51) and with cats (1,22) showed that MSG is a taste stimulus and, in addition, that mixtures of MSG and ribonucleotides are synergistic when recording from the chorda tympani nerve. As in humans, electrophysiological studies in rats (41) showed GMP to be the most effective of the naturally occurring nucleotides; IMP, UMP, and CMP were also effective.

Structure-activity relationships with nucleotides in human studies (35,50) have revealed several structural features that are necessary for their synergistic effect. For example, the importance of the oxygen function at position 6 of the purine ring has been established, as well as the importance of the phosphate ester being located at the 5′-position of the ribose ring. In addition, certain 2-substituted purine derivatives were shown to be even more potent than GMP. Electrophysiological studies in the rat employed chorda tympani recordings to establish that GMP and IMP were effective potentiators of the MSG response (2,21,41,43,51). Single-fiber recordings (43) showed potentiation by GMP, IMP, and, surprisingly, by AMP; UMP and CMP were relatively ineffective. One of the derivatives of IMP substituted at the 2-position (50) was also tested with rats and found to be highly effective (42).

The results from the animal studies, although not extensive, show that some species studied respond to MSG as a taste stimulus and to mixtures of MSG and ribonucleotides. Little is known, however, of such responses in the species of fish,

Ictalurus punctatus, which we have used extensively in our biochemical research on taste receptors. In addition, the catfish shows relatively small electrophysiological responses to L-glutamate (16).

Although the catfish has provided an experimental methodology and has yielded results leading to the hypothesis of "hidden" or "buried" receptors, it did not appear to be the most suitable experimental model for initiating direct studies of the binding of L-glutamate and of the synergistic effect with ribonucleotides. An additional animal model is available in our laboratory, dating from our earlier research on taste-binding interactions of sugars (9). The binding of ^{14}C-labeled sugars had been measured in bovine taste papillae preparations and in control tissue devoid of taste buds. More binding of the sugars occurred in the taste tissue than in the controls. The findings were recently confirmed and extended to show the involvement of the plasma membrane in the binding interactions with sugars (32,40).

In the intervening years, we have refined the approach, including methods of obtaining preparations with a higher enrichment of taste receptors (7,8), as well as adaptation of the binding methodology that we used for amino acid binding (28) to enable us to measure the binding of ^{3}H-labeled monellin to taste tissue preparations (15). Using the improved preparative procedures (7,8,46), coupled with the filtration binding assay, we have directly measured the initial interaction of L-[^{3}H]glutamate to bovine taste tissue preparations. We believe that our results may offer an explanation both of the site of action of MSG and of the mechanistic basis of the synergistic effect of MSG and ribonucleotides. The following is a preliminary report of the major findings, which will be published in detail elsewhere (46).

(a) The binding of L-[^{3}H]glutamate to the circumvallate (CV) preparation is several fold higher than to the control epithelium (EP) preparation devoid of taste receptors. This observation is consistent in the many experiments we have carried out, and representative data are shown in Table 1. Further, several features indicate

TABLE 1. *Binding of L-[^{3}H]glutamate to bovine circumvallate (taste) and epithelial (nontaste) tongue tissues*[a]

Preparation	L-Glutamate concentration	L-[^{3}H]Glutamate bound (nmoles/mg protein) 1.4 mM	6.9 mM	14 mM
Circumvallate	—	1.14	4.99	6.49
Epithelium	—	0.20	0.60	1.78

[a] The sidewall epithelium is peeled away from bovine circumvallate papillae and a homogenate is prepared (Circumvallate). The control preparation (Epithelium) consists of pieces of tongue epithelium taken from tongue regions devoid of taste buds and prepared in the same fashion. Differential centrifugation removes the low-speed pellet, and then the pellet that sediments at 7,000 × g (30 min) is used in each case. Binding is measured using L-[^{3}H]glutamate with a rapid filtration method. All samples are run in duplicate. The data for circumvallate are mean values from three experiments; those for the epithelium are from a single experiment.

that the binding to EP is qualitatively different than that to CV, and we suggest that the lower level of binding to EP is nonspecific. We conclude that the bovine circumvallate papilla has receptor sites for L-glutamate. Quantitatively, however, the amounts of ligand bound suggest that they reflect entrapment of fluid (containing radioactive ligand) in addition to direct complex formation with receptor sites.

(b) The binding of L-[^{3}H]glutamate to CV appears to be saturable. Under our conditions of measurement, binding begins to show saturation, but does not fully reach a plateau as the concentration of L-[^{3}H]glutamate increases. This is undoubtedly due to the interaction being weak; therefore, the concentrations of L-[^{3}H]glutamate required to saturate the binding sites are considerably higher than we have employed experimentally (up to 14 mM). The value of the K_D was estimated using both a double-reciprocal plot (Lineweaver-Burk) and a Scatchard plot, yielding a K_D for L-glutamate in the range of 17 to 20 mM.

(c) The addition of 5′-GMP dramatically increases the amount of L-[^{3}H] glutamate bound to CV and has no effect whatever on the already low level of L-[^{3}H]glutamate bound to EP. An example of the effect is illustrated in Table 2,

TABLE 2. *Enhancement by 5′-GMP of* L-[^{3}H]*glutamate binding to bovine circumvallate (taste) tissue*[a]

Preparation	L-[^{3}H]Glutamate bound (nmoles/mg protein)	
	No GMP	+ GMP
Circumvallate	3.44	18.2
Epithelium	0.60	0.80

[a] The tissues were prepared and assays carried out as described in Table 1. L-[^{3}H]Glutamate was present at 6.9 mM and 5′-GMP at 1.4 mM. The data shown are taken from a single experiment, in which samples were run in duplicate.

where a several-fold enhancement of the binding of L-[^{3}H]glutamate to CV is demonstrated. In further experiments, we have used Scatchard analyses to estimate the K_D for L-glutamate in the absence and presence of GMP. The K_D was unchanged by adding GMP, whereas the maximal amount of L-glutamate bound increased by sixfold. The latter observation can be explained by a hypothesis similar to that advanced to explain the ligand enhancement of binding with the L-alanine taste receptors from catfish (12,13). In the present case, GMP is postulated to cause the exposure of "hidden" or "buried" receptor sites for L-glutamate.

(d) The response to ribonucleotides shows a high degree of specificity. The 5′-ribonucleotides GMP, IMP, and UMP are each effective in enhancing the binding of L-[^{3}H]glutamate to the CV. AMP and CMP are ineffective. Also ineffective are guanine, GDP, GTP, adenine, ADP, and ATP. The specificity with respect to the nucleotide therefore shows a high degree of similarity, although not absolute, with human psychophysical responses.

SUMMARY

The ability of MSG to evoke a "distinctive" or "unique" taste sensation is well known. The taste is called *umami* in Japanese, which is translated as "delicious" or "savory." Furthermore, the remarkable synergistic effect of certain 5′-ribonucleotides in enhancing this taste is also clearly documented. Neither the site of action nor the biochemical mechanism is known. We have therefore investigated this question as an extension of our research into the initial steps in taste stimulus recognition; the binding interaction of stimulus molecules with sites in the receptor membranes of the taste cells appears to be critically involved in the initial discrimination of taste stimuli.

We have measured the binding of MSG by means of ^{3}H-labeled L-glutamate. Substantially greater binding of L-[^{3}H]glutamate occurs to the bovine circumvallate papillae preparations, which contain taste receptors, than to the epithelial preparations devoid of taste receptors. The low level of binding to the epithelium is qualitatively as well as quantitatively different than that to the circumvallate, and we conclude that the binding to the epithelial preparation is nonspecific. Our data demonstrate directly that 5′-GMP causes a marked increase in the binding of L-[^{3}H]glutamate to the circumvallate preparation. Furthermore, this effect occurs only with the taste tissue and not with the control, nontaste tongue epithelium. This response shows a high degree of specificity with respect to the nucleotide; GMP, IMP, and UMP are each effective, but AMP and CMP, as well as guanine, GDP, GTP, adenine, ADP, and ATP are ineffective. This system appears to be a useful experimental model for MSG taste and for the MSG-ribonucleotide synergistic taste phenomenon observed in humans and other species.

We propose that the site of action of the synergistic effect of ribonucleotides and MSG is at the peripheral level, acting on the taste receptor cell membrane. We further propose that the mechanism of action of the ribonucleotide is to expose additional receptor sites for L-glutamate. A major corollary of the hypothesis, therefore, is that MSG evokes its characteristic taste by binding to specific taste receptor sites, thereby itself acting as a taste stimulus. The effect of MSG becomes markedly enchanced by low levels of certain 5′-ribonucleotides, such as certain of those that occur endogenously in various foods.

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