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The Liaison of Sweet and Savory

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Abstract

The sense of taste provides humans with necessary information about the composition and quality of food. For humans, five basic tastes are readily distinguishable and include sweet, bitter, salty, sour, and savory (or umami). Although each of these qualities has individualized transduction pathways, sweet and umami tastes are believed to share a common receptor element, the T1R3 receptor subunit. The two G-protein–coupled heteromer receptors that comprise an umami stimulus receptor (T1R1–T1R3) and a sweetener receptor (T1R2–T1R3) constitute a potential link between these two qualities of perception. While the role of the individual monomers in each human heteromer has been examined *in vitro*, very little is known of the implication of this research for human perception, or specifically, how sweet and savory taste perceptions may be connected. Using a psychophysical approach, we demonstrate that lactisole, a potent sweetness inhibitor that binds *in vitro* to hT1R3, also inhibits a significant portion of the perception of umami taste from monosodium glutamate. Following the molecular logic put forward by Xu *et al.* (2004, Proc. Natl Acad. Sci. USA, 101, 14258–14263), our psychophysical data support the *in vitro* hypothesis that the shared T1R3 monomer moderates the activation of both T1R2 and T1R1 in humans and impairs suprathreshold perception, respectively, of sweetness and, to a lesser degree, umaminess in the presence of lactisole.

Key words: GMP, IMP, inhibition, lactisole, MSG, synergy, umami

Introduction

Sweet and savory (umami) tastes are stimulated via transduction pathways that include metabotropic T1R receptors. T1R3 is an important G-protein–coupled receptor included in both umami and sweet taste pathways; it has been demonstrated in rodents that both T1R1 (umami) and T1R2 (sweet) need to be expressed with T1R3 to have normal responses to stimuli representative of both taste qualities (Damak *et al.*, 2003; Zhang *et al.*, 2003).

Lactisole (Na, *p*-methoxy-phenoxy-propionate, Figure 1A) is a potent sweet taste inhibitor that has been recently shown *in vitro* to bind specifically to the human T1R3 transmembrane domains causing inhibition of the T1R2–T1R3 receptor's response to sweeteners (Xu *et al.*, 2004; Jiang *et al.*, 2005; Winnig *et al.*, 2005). Many saccharides, on the other hand, are believed to bind the T1R2 extracellular amino-terminal domain (Zhao *et al.*, 2003; Jiang *et al.*, 2005; Morini *et al.*, 2005); however, glucose and sucrose have been recently shown to bind to the isolated extracellular amino-terminal domains of both T1R2 and T1R3 (Nie *et al.*, 2005). Our understanding of sweet and umami transduction links these two tastes via the T1R3 subunit; thus, we hypothesize that compounds capable of activating or inhibiting this subunit (e.g., lactisole) will have a direct effect on suprathreshold perception of both taste qualities.

Xu *et al.* (2004) demonstrated that lactisole acts as a noncompetitive inhibitor of hT1R1–T1R3 *in vitro*. They reasoned that, to the degree that hT1R1–T1R3 is involved in monosodium glutamate (MSG) taste, lactisole should impair the detection of MSG in human subjects. In support of their *in vitro* findings, they found that human MSG detection thresholds were elevated in three subjects by approximately fourfold using an ascending series threshold procedure. Since the detection threshold measures are not necessarily related to suprathreshold perception, it is not clear whether lactisole would inhibit suprathreshold perception of umaminess (Bartoshuk, 2000; Mojet *et al.*, 2005). Therefore, we extended their logical approach to determine whether lactisole would inhibit the suprathreshold perception of umami taste from MSG and other stimuli. Here we show that lactisole is a weak inhibitor of suprathreshold umami perception from MSG (Figure 1A) but has no inhibitory effect on mixtures of MSG with 5′ ribonucleotides such as 5′-inosine monophosphate (IMP) and 5′-guanosine monophosphate (GMP).

Materials and methods

Twelve adult subjects (6 female, 6 male, mean age 26 ± 1) were paid to participate in 12 sessions after providing

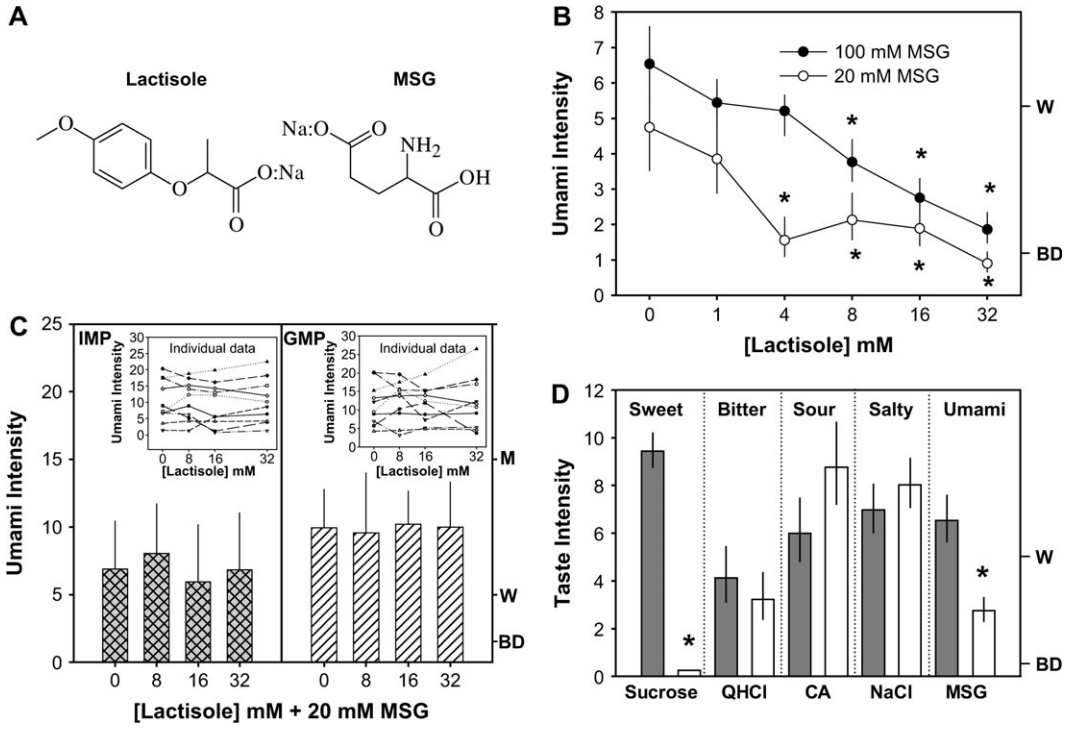

Figure 1 **(A)** Chemical structures of lactisole [Na, 2-(*p*-methoxy-phenoxy)-propionate] and MSG. **(B)** Effect of increasing levels of lactisole on the umami taste of MSG, GMs ± GeoSE (n = 12); asterisks denote a significant decrease on umami intensity as compared to baseline levels. **(C)** Effect of different concentrations of lactisole on synergism of 3 mM 5′ ribonucleotides with 20 mM MSG (n = 10), data from two subjects were dropped as their responses were inconsistent with replicate testing, top insets show individual data. **(D)** Effect of 16 mM lactisole (open bars) on standard exemplar solutions of five taste qualities (gray bars): 200 mM sucrose, 2.5×10^{-5} mM quinine–HCl, 2 mM citric acid, 100 mM NaCl, and 100 mM MSG. Data analysis: repeated measures ANOVA and Tukey's pairwise *post hoc* comparisons (n = 12). *Significant at $\alpha = 0.05$. The y-axes labels represent gLMS notation: BD, barely detectable; W, weak; and M, moderate.

informed consent on an Institutional Review Board approved form. The participants were asked to refrain from eating, drinking, or chewing gum for 1 h prior to testing. Subjects were trained in the use of a general Labeled Magnitude Scale (gLMS) following the published standard procedures (Green *et al.*, 1993, 1996; Bartoshuk, 2000). The top of the scale was described as the strongest imaginable sensation of any kind and ranges from 0 to 96. Participants were trained to identify each of the five taste qualities by presenting them with exemplars. Salty taste was identified as the predominant taste from 150 mM NaCl, bitterness as the predominant quality from 0.5 mM quinine–HCl, sweetness as the predominant quality from 300 mM sucrose, sourness as the predominant quality from 3 mM citric acid, and umami as the predominant quality from a mixture of 100 mM MSG and 50 mM IMP. To help subjects understand that a stimulus could elicit multiple taste qualities, 300 mM urea (bitter and slightly sour) was employed as training stimulus.

Lactisole solutions were neutralized to pH 7 by adding 0.1 N NaOH.

Intensity ratings of MSG and ribonucleotides

Five levels of lactisole (0, 1, 4, 8, 16, and 32 mM) were mixed with 20 mM MSG, 100 mM MSG, 3 mM IMP, 3 mM GMP, 20 mM MSG + 3 mM IMP, or 20 mM MSG + 3 mM GMP in all possible combinations. Solutions were tested in triplicate and in random orders using an interstimulus interval of 75 s. Subjects sampled each solution for 5 s, expectorated, and rated the five qualities of taste (bitter, sweet, salty, sour, and umami) on a gLMS, after which they rinsed four times before tasting another sample.

Effect of lactisole on other taste qualities

Sucrose 200 mM (sweet), NaCl 100 mM (salty), citric acid 2 mM (sour), and quinine–HCl 2.5×10^{-5} M (bitter) were approximately intensity matched to 100 mM MSG using bench-top testing and were mixed, respectively, with 0 and 16 mM lactisole. Subjects tasted 10 ml of each solution and rated the five qualities of taste on a gLMS following the protocol described above. Each condition was tested twice.

Effect of lactisole on mixtures of sucrose with 5′ ribonucleotides

Sucrose 200 mM was mixed with 3 mM IMP or GMP and tested with or without 1 mM lactisole to assess the effect on sweetness inhibition. Subjects tasted each solution in duplicate and rated the five qualities of taste on a gLMS.

Statistical analysis

All data were log transformed and presented as geometric means (GMs) ± geometric standard errors (GeoSEs); zeroes were replaced with the lowest possible positive rating of 0.24. GeoSEs were calculated as follows: $10^{((\Sigma \log Xi)/n + SE \log Xi)}$ _ GM and GM – $10^{((\Sigma \log Xi)/n - SE \log Xi)}$ (Bishop *et al.*, 1975). Repeated measures analysis of variance (ANOVA) was performed on the transformed data followed by Tukey's pairwise *post hoc* comparisons $\alpha = 0.05$.

Results and discussion

Effect of lactisole on umami taste from MSG, IMP, and GMP

Lactisole inhibited the umami taste of MSG in a dose-dependent manner (Figure 1B; $F_{(5,55)} = 7.28$, $P < 0.0001$), albeit at concentrations approximately 16 times stronger than are required to suppress the sweetness of sucrose. MSG also elicits a salty taste (Zhao *et al.*, 2003), but there was no significant effect of lactisole on the saltiness of MSG. Relative to baseline intensities, 32 mM lactisole reduced the umami intensity of 100 mM MSG by 66% and 20 mM MSG by 69%. In contrast, there was no significant effect of lactisole on the savory taste of either IMP or GMP (Figure 2A, $P = 0.91$), which suggests that IMP and GMP may not bind to the same site on the receptor as MSG or even to the same receptor (Figure 3E) (Damak *et al.*, 2003; He *et al.*, 2004).

Effect of lactisole on other taste qualities

Since the concentrations of lactisole tested were quite high compared with the levels used with sweeteners (16–32 times stronger), they were assessed in mixtures with exemplars of the other taste modalities. As expected, high lactisole was a very effective inhibitor of sucrose's sweet taste and a moderate inhibitor of umami taste but had no significant effect on bitter, sour, or salty tastes (Figure 1D), although there were slight increases in sourness and saltiness. Lactisole is tasteless at neutral pH and at weak concentrations, but at high concentrations such as used here, lactisole tastes slightly salty due to its sodium ion, which could influence both saltiness and sourness ratings (Kamen *et al.*, 1961; Breslin, 1996).

Lactisole and umami taste interactions

A distinctive characteristic of human umami taste is its powerful positive interaction derived from mixing 5′ ribonucleotides with glutamate (Figure 3C,E,F) (Yamaguchi, 1967). To test the effect of lactisole on the umami interaction between MSG and 5′ ribonucleotides, increasing amounts of lactisole were added to constant mixtures of 20 mM MSG plus 3 mM of either IMP or GMP. We found no significant effect of lactisole on the umami taste of the mixtures (Figure 1C,

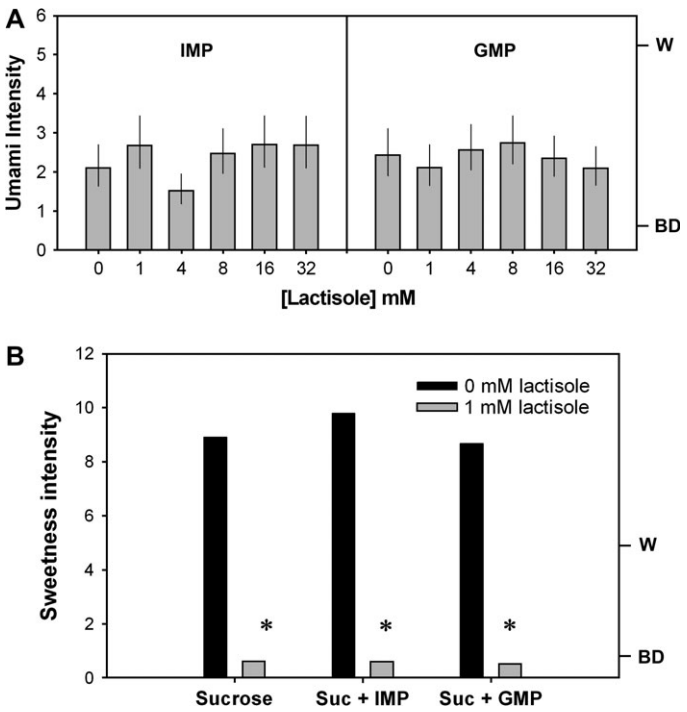

Figure 2 **(A)** Effect of increasing levels of lactisole on the umami taste of 3 mM 5′ ribonucleotides GM ± GeoSE. **(B)** Effect of 3 mM 5′ ribonucleotides on sweet inhibition of 200 mM sucrose by lactisole. Data analysis: repeated measures ANOVA and Tukey's pairwise *post hoc* comparisons ($n = 12$). *Significant at $\alpha = 0.05$. The *y*-axes labels represent gLMS notation: BD, barely detectable; W, weak; and M, moderate.

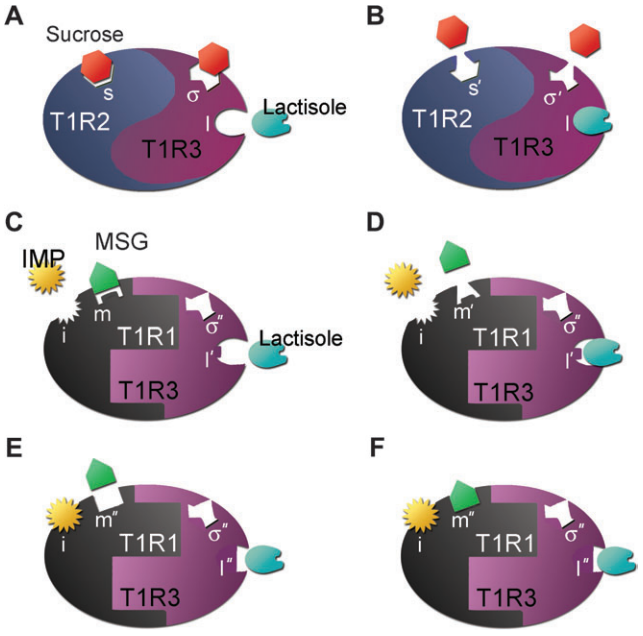

Figure 3 Human sweet [T1R2 (blue)–T1R3 (purple)] and umami [T1R1 (gray)–T1R3 (purple)] taste heteromer receptor schematics: inhibition of sucrose's (red) sweet taste by the compound lactisole (aqua) **(A, B)**; inhibition of MSG (green) umami taste by lactisole **(C, D)**; and modulatory effects of 5′ ribonucleotides, such as IMP (yellow), on MSG binding and IMP's blockade of lactisole's inhibition **(E, F)**.

$P = 0.86$). Note that 20 mM MSG without IMP is significantly inhibited as a linear function of added lactisole concentration (Figure 1B). As the GeoSE is relatively large, we have also displayed individual subjects' functions (Figure 1C, insets) to demonstrate the lack of inhibition by lactisole on umami interactions within individuals as well as in groups. The presence of the 5′ ribonucleotides prevented lactisole from inhibiting umami taste even at low perceived intensities (Figure 1C and 3C–E).

Although T1R3-independent mechanisms also appear to be involved in the perception of umami taste from MSG and 5′ ribonucleotides mixtures (Ninomiya and Funakoshi, 1989a,b; Chaudari *et al.*, 2000; Damak *et al.*, 2003; Sako *et al.*, 2003; He *et al.*, 2004), it has been repeatedly demonstrated that IMP enhances the cellular response to MSG in T1R1–T1R3 expression assays (Li *et al.*, 2002; Damak *et al.*, 2003; Xu *et al.*, 2004). Therefore, if lactisole inhibited the hT1R1–T1R3 receptor in the presence of 5′ ribonucleotides and this were the predominant mechanism of suprathreshold umami synergy in humans, we should have seen some slight reduction in perceived umaminess. To better explain these interrelationships in our data, we represented the present results in a schematic model of interactions (Figure 3) in which 5′ ribonucleotides modify the T1R1–T1R3 receptor such that lactisole is no longer able to inhibit activation by MSG. Note that this schematic is meant to represent the kernel of our present results and not literal changes in the receptor molecules.

Comparison with existing molecular models of umami taste

Our observation that low concentrations of lactisole completely inhibit suprathreshold sweetness of sugars is consistent with *in vitro* expression data of hT1R2–T1R3; however, the observation that lactisole does not readily inhibit suprathreshold umaminess of MSG is not consistent with *in vitro* expression data of hT1R1–T1R3 (Xu *et al.*, 2004). Therefore, it appears that this receptor either behaves differently *in vivo* or it is only a portion of the transduction repertoire for MSG (Ninomiya and Funakoshi, 1989a,b; Chaudari *et al.*, 2000; Damak *et al.*, 2003; Sako *et al.*, 2003; He *et al.*, 2004). Similarly, if T1R1–T1R3 is the principal mean by which humans experience the umami enhancement of MSG by mixture with 5′ ribonucleotide, then the present data also do not confirm the *in vitro* findings by Xu *et al.* (2004) who demonstrated that the synergy of 8 mM MSG with 0.2 mM IMP from expressed human T1R1–T1R3 receptors is completely inhibited by 10 mM lactisole. They determined that the hT1R1–T1R3 inhibitory concentration for 50% decrease for MSG + IMP was between 0.35 and 0.82 mM lactisole. Yet, we found no inhibition of the mixture with lactisole concentrations as high as 32 mM in human perceptual studies. Thus, either existing *in vitro* models of human umami taste synergy fail to model the suprathreshold behavior of the native *in vivo* human receptors or there are other transduction pathways involved with the MSG + 5′ IMP umami interactions (Damak *et al.*, 2003; He *et al.*, 2004). Although partial inhibitions may be difficult to detect perceptually, our methods clearly indicate partial inhibition of umami signal as illustrated by the inhibition of MSG alone. Yet, we find no evidence of any inhibition of MSG when mixed with IMP or GMP.

Conclusions

Our findings demonstrate that lactisole added at high concentrations inhibited the suprathreshold umami taste of MSG. To the degree that human umami and sweet taste perceptions are initiated by activation of T1R1–T1R3 and T1R2–T1R3 receptor heteromers, respectively, lactisole substantially inhibits activation of the T1R1–T1R3 umami taste receptor but with much lower affinity than it inhibits the T1R2–T1R3 sweet taste receptor. Although it is difficult to infer molecular mechanisms from psychophysical data, these findings are consistent with the *in vitro* observation that T1R3 is the key binding site for lactisole, and thus, lactisole inhibits both tastes qualities in humans. We further hypothesize that if lactisole is acting at a T1R3 site, then T1R3's inhibition by lactisole may be altered by the identity of the heteromer partner, as indicated by large inhibition differences between sucrose and MSG (Figure 3B,D) (Yamaguchi, 1967; Xu *et al.*, 2004).

The inhibitory effect of lactisole on umami taste was blocked when MSG was mixed with either GMP or IMP. Importantly, the addition of these 5′ ribonucleotides did not prevent lactisole from inhibiting the sweetness of sucrose (Figure 2B). Based on the *in vitro* observation of MSG and 5′ ribonucleotide synergy, if we assume that synergy occurs, at least in part, within the hT1R1–T1R3 receptor, then our data suggest that 5′ ribonucleotides bind to the T1R1 subunit but not the T1R2 subunit and alter the T1R1–T1R3 heteromer, preventing lactisole from inhibiting umami taste (Figure 3E,F) (Jensen and Spalding, 2004). Thus, we infer from lactisole's differential ability to inhibit both sweet (Figure 3A,B) and umami tastes (Figure 3C,D) and from 5′ ribonucleotide's ability to block lactisole's inhibition of umami but not sweet taste that the identity of a receptor subunit and/or its activation by ligands can alter the conformation of the partner subunit and hence its ability to be activated or inhibited (Figure 3).

We suggest based on these data and in conjunction with extant *in vitro* expression data that the T1R3 monomer, which is shared by a sweetener and an MSG receptor, may modulate the activation of both T1R1 and T1R2. We further believe that the popular molecular models of umami transduction, which are based on *in vitro* expression studies of hT1R1–T1R3, are insufficient to fully account for the psychophysical data presented here. Xu *et al.* (2004) found that human detection thresholds for MSG + IMP were elevated approximately sevenfold with the addition

of lactisole. Since we found no effect of lactisole on suprathreshold ratings of MSG + IMP, we infer that detection threshold ratings of MSG and IMP rely more on hT1R1–T1R3 while suprathreshold ratings rely on this heteromer, as well as additional receptors such as mGluR4, mGluR1, and other possible receptors (Chaudari *et al.*, 2000; Toyono *et al.*, 2003). In this study, we present a schematic model based on previous *in vitro* models that has been modified to accommodate our psychophysical data. Clearly, further research is necessary to describe fully the transduction repertoires that underlie umami perception in humans.

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