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Oral Zinc Sulfate Solutions Inhibit Sweet Taste Perception

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Abstract

We investigated the ability of zinc sulfate (5, 25, 50 mM) to inhibit the sweetness of 12 chemically diverse sweeteners, which were all intensity matched to 300 mM sucrose [800 mM glucose, 475 mM fructose, 3.25 mM aspartame, 3.5 mM saccharin, 12 mM sodium cyclamate, 14 mM acesulfame-K, 1.04 M sorbitol, 0.629 mM sucralose, 0.375 mM neohesperidin dihydrochalcone (NHDC), 1.5 mM stevioside and 0.0163 mM thaumatin]. Zinc sulfate inhibited the sweetness of most compounds in a concentration dependent manner, peaking with 80% inhibition by 50 mM. Curiously, zinc sulfate never inhibited the sweetness of Na-cyclamate. This suggests that Na-cyclamate may access a sweet taste mechanism that is different from the other sweeteners, which were inhibited uniformly (except thaumatin) at every concentration of zinc sulfate. We hypothesize that this set of compounds either accesses a single receptor or multiple receptors that are inhibited equally by zinc sulfate at each concentration.

Key words: cyclamate, human psychophysics, sweet taste inhibition, zinc

Introduction

There is wide structural diversity in chemicals that elicit sweet taste, e.g. glucose, fructose (carbohydrates), sorbitol (sugar alcohol), saccharin, acesulfame-K (*N*-sulfonylamides), cyclamate (sulfamate), aspartame (dipeptide), D-phenylalanine (amino acid), thaumatin, monellin (proteins), stevioside (diterpenoid glycoside), and lead and beryllium salts (ions). A broad chemical diversity of agonists within a taste quality can implicate multiple receptor mechanisms, as it has with bitter taste (Adler *et al.*, 2000; Chandrashekar *et al.*, 2000). The results of selected psychophysical studies have been interpreted to be consistent with multiple sweet taste transduction mechanisms: individual differences in sensitivity to sweet compounds (Faurion *et al.*, 1980; Eylam and Kennedy, 1998), cross-adaptation experiments among sweet compounds (McBurney, 1972; Schiffman *et al.*, 1981; Lawless and Stevens, 1983; Froloff *et al.*, 1998) and sweetness synergy between pairs of sweeteners (Ayya and Lawless, 1992; Schiffman *et al.*, 1995, 2000; Schifferstein, 1996).

Recent advances in the molecular basis of sweet taste revealed that a human receptor-dimer hT1R2/T1R3 responded *in vitro* to many sweet tasting stimuli (Li *et al.*, 2002). Li *et al.* (2002) further show that *in vitro* threshold concentrations of a wide range of sweeteners are similar to the *in vivo* behavioral thresholds of rats (Nelson *et al.*, 2001). To date, this is the only known receptor complex demonstrated for sweet taste. Damak *et al.* (2003), however, reported that mice lacking T1R3 still responded to selected sugars, implicating a T1R3 independent sweet taste pathway. In contrast, Zhao *et al.* (2003) reported that the T1R2&T1R3 dimer and a T1R3 'stand alone' receptor are the only mechanisms responsible for sweet taste transduction in rodents and potentially in humans.

Sweet taste inhibitors have been used to draw inference into sweet taste transduction mechanisms. For example, Schiffman *et al.* (1999) found the sweet intensity of 12 of 15 sweeteners decreased using the sweet taste inhibitor ±2-(4-methoxyphenoxy)propanoic acid, sodium salt (lactisole). There was little reduction in sweet intensities of the remaining three compounds. They inferred that the three unaffected sweeteners access a sweet taste mechanism independent of the effects of lactisole. However, Li *et al.* (2002) found that hT1R2/T1R3 responded to compounds that lactisole did not block in Schiffman's experiment and Lindley (1991) found that lactisole was an inhibitor of all sweeteners tested. Dubois speculated that differences in temporal pattern of the sweeteners combined with differences in methodology may be the cause of variation in the literature (Dubois, 1997), but agreed with Schiffman that lactisole may be a selective sweetness inhibitor since it is not apparent that it is equally efficacious with all sweeteners.

Electrophysiological research on mice demonstrated that the application of zinc suppressed neural firing stimulated by sucrose, fructose, glucose, maltose and saccharin (Iwasaki and Sato, 1984, 1986). Consistent with this, Keast reported that zinc ions were potent inhibitors of sweetness elicited by glucose in humans without affecting salty, sour, or umami taste qualities (Keast, 2003). The mechanism responsible for the influence of zinc ions on sweet taste is unknown, but Keast (2003) hypothesized that it may form a complex with the extracellular portion of the sweet taste receptor hT1R2/T1R3, as zinc readily complexes with amino acids and proteins and has a high affinity for both thiol and hydroxy groups.

This aim of this study was to investigate the influence of a prototypical zinc salt, zinc sulfate, on the perceived sweetness of several sweeteners. The first experiment compared the effect of zinc sulfate and other salts on the sweetness of 12 chemically diverse sweeteners. The second experiment investigated the relationship between concentrations of zinc sulfate and sweetness inhibition of sweeteners. The third experiment investigated the effect of zinc sulfate on the sweetness resulting from the synergistic interaction between aspartame and acesulfame-K.

Materials and methods

Subjects

Subjects (n = 29, 32 ± 5 years old, nine female) between the ages of 20 and 51 were paid to participate after providing informed consent on an Institutional Review Board approved form. Thirteen were employees of the Monell Chemical Senses Center. The participants were asked to refrain from eating, drinking or chewing gum for 1 h prior to testing. Subjects did not participate in all experiments, but did complete the full experiment matrix for each experiment in which they were involved. All subjects were trained according to the procedure below.

Subject training

Subjects were initially trained using the general Labeled Magnitude Scale (gLMS) following standard published procedures (Green *et al.*, 1993, 1996) except the top of the scale was labeled as 'strongest imaginable' sensation of any kind (Bartoshuk, 2000). The gLMS is a psychophysical tool that allows subjects to rate the perceived intensity along a vertical axis lined with adjectives: barely detectable = 1, weak = 5, moderate = 16, strong = 33, very strong = 51, strongest imaginable = 96; the adjectives are spaced semi-logarithmically, based upon experimentally determined intervals, to yield data that parallel magnitude estimations. The scale shows only adjectives to the subjects, but the experimenter receives numerical data from the computer program. Subjects were trained to identify each of the five taste qualities and the oral sensation of astringency by presenting them with exemplars. Salty taste was identified as the predominant taste quality from 150 mM NaCl, bitterness as the predominant quality from 0.05 mM quinine HCl, sweetness as the predominant quality from 300 mM sucrose, sourness as the predominant quality from 3 mM citric acid, savory as the predominant quality from a mixture of 100 mM glutamic acid monosodium salt and 50 mM inosine 5′-monophosphate, and astringency as the predominant sensation of 0.5 mM tannic acid. To help subjects understand that a stimulus could elicit multiple taste qualities, 300 mM urea (bitter and slightly sour) and 50 mM NH_4Cl (salty, bitter, and slightly sour) were employed as training stimuli.

Stimuli

The salts were: zinc sulfate ($ZnSO_4$), sodium acetate (NaOAc), sodium salicylate ($NaC_7H_5O_3$), magnesium sulfate ($MgSO_4$), and magnesium acetate ($Mg(OAc)_2$); all were purchased from Sigma (St Louis, MO). Sweeteners purchased from Sigma (St Louis, MO) were: glucose, sucrose, aspartame, Na saccharin, fructose, Na-cyclamate, neohesperidin dihydrochalcone (NHDC), acesulfame-K and sorbitol. Sucralose was obtained from McNeil Nutritional (McIntosh, AL). Stevioside was obtained from Morita Kagaku Kogyo Co. Ltd (Jotoku, Osaka, Japan). Acesulfame-K was purchased from Fluka Chemika (Buchs, Switzerland). Thaumatin was obtained from Braes Group (London, UK). Aqueous solutions were freshly prepared every 2–3 days, using deionized (*di*) Millipore™ filtered water, several hours in advance of testing. The solutions were stored in amber glass bottles and refrigerated. The pH of the sweeteners and salts was measured with a pH meter (Jenko Electronics, Taiwan). The majority of sweetener–salt mixtures ranged from pH 5.1 to pH 6.7 (Table 1).

Intensity matching sweeteners

Sweetness was intensity matched by adjusting the concentrations of sweeteners until the average sweetness for the group was rated iso-intense to 300 mM sucrose on the gLMS. The matching methodology follows: Subjects were instructed to wear nose clips to eliminate olfactory cues when sampling and to rate the perceived sweetness of each solution while it remained in the mouth for 5 s. Subjects rated the intensity of predetermined concentrations of sweet solutions (700 mM glucose, 300 mM sucrose, 4 mM aspartame, 4 mM Na-saccharin, 500 mM fructose, 15 mM Na-cyclamate, 800 mM sorbitol, 0.5 mM NHDC, 10 mM acesulfame-K, 3 mM stevioside, 1 mM sucralose, 0.02 mM thaumatin). Taste intensity was recorded on a computerized gLMS and transferred in real time to the technician who would change the concentration of solutions depending on the individual subject's response. The new solution was tasted and rated by the subject, and depending on the response, new concentrations were made by rapid dilution from stock until the intensity was rated on average as isointense with 300 mM sucrose ±10%. There was an interstimulus interval of ~60 s, during which time the subject was required to rinse with *di* water at

Table 1 Specific pH of sweeteners and 25 mM salts and sweetener-salt mixtures used in the study

	Water	$MgSO_4$	$Mg(OAc)_2$	NaOAc	NaSal	$ZnSO_4$
Water	6.7	6.5	6.8	7	5.5	4
300 mM sucrose	6.6	6.3	6.4	6.4	5.9	5.1
800 mM glucose	6.7	6.4	6.3	6.5	5.8	5.2
3.25 mM aspartame	6.2	6.3	6.1	6.4	5.9	5.1
3.5 mM saccharin	6.4	6.4	6.3	6.4	5.9	5.3
475 mM fructose	6.6	6.4	6.3	6.3	5.7	5.2
12 mM Na cyclamate	6.4	6.3	6.2	6.2	5.6	5.3
0.375 mM NHDC	6	6.4	6.4	6.5	5.7	5.2
1.5 mM stevioside	6	6.3	6.2	6.4	5.8	5.4
0.629 mM sucralose	6.6	6.4	6.3	6.5	5.8	5.4
1.04 M sorbitol	6.4	6.2	6.2	6.4	5.7	5.1
0.0163 mM thaumatin	4.5	5.9	6.2	6.4	5.3	4.6
14 mM acesulfame-K	6.2	6.4	6.4	6.4	5.8	5.4

Abbreviations for the 25 mM salts are: magnesium sulfate ($MgSO_4$), magnesium acetate [$Mg(OAc)_2$], sodium acetate (NaOAc), sodium salicylate (NaSal) and zinc sulfate ($ZnSO_4$). Abbreviation for the sweetener neohesperidin dihydrochalcone is NHDC.

least four times. Subjects who did not rate the intensity of 300 mM sucrose within ±30% of their previous ratings were dismissed as unreliable (2 out of 29 subjects).

The following concentrations were determined to be isointense with 300 mM sucrose on average, for the sample population: 800 mM glucose, 475 mM fructose, 3.25 mM aspartame, 3.5 mM saccharin, 12 mM Na-cyclamate, 14 mM acesulfame-K, 1.04 M sorbitol, 0.629 mM sucralose, 0.375 mM NHDC, 1.5 mM stevioside and 0.0163 mM thaumatin. Obvious deviations from published isointensity ratios between sweeteners (Schiffman and Gatlin, 1993; Dubois, 2000) may be due to (i) differences in the methods and scales used to intensity match compounds, (ii) individual differences in sweetener sensitivities among subjects in the studies and (iii) differences in the concentration of sucrose used to obtain the matches.

Experiment 1: The effect of zinc sulfate on sweetness

Subjects (n = 16, 31 ± 6 years old, 10 female) with nose clips on were given trays containing seven solutions: *di* water, one sweetener and five samples of the sweetener with 25 mM of each of the salts (e.g. 300 mM sucrose with 25 mM $Mg(OAc)_2$, 25 mM $MgSO_4$, 25 mM NaOAc, 25 mM $NaC_7H_5O_3$ and 25 mM $ZnSO_4$). There were 12 different trays (one for each sweetener) and each tray was tasted on three separate occasions, resulting in a total of 36 sessions. The testing protocol was as follows: solutions (10 ml) were presented in 30 ml plastic medicine cups (Dynarex, NY) on numerically labeled trays. The sweetener with $ZnSO_4$ was always presented last to avoid any potential carry over effects of its astringency or lingering sweetness blocking effects on other taste trials (Keast, 2003). The remaining six solutions were presented in random order. Subjects rinsed with *di* water at least four times over a 2 min period prior to testing. The subjects were instructed to draw the whole sample into their mouth, hold it in their mouth for 5 s and rate the solution for sourness, sweetness, bitterness, saltiness, savoriness, and astringency prior to expectorating (~6 s later). All subjects rinsed with *di* water four times during the interstimulus interval of 120 s. The gLMS was used as the rating scale.

Experiment 2: concentration effect of zinc sulfate on sweetness

Subjects (n = 15, 29 ± 5 years old, seven female), with nose-clips on, assessed the influence 5, 25 and 50 mM $ZnSO_4$ had on the sweetness of the following compounds: 800 mM glucose, 475 mM fructose, 3.25 mM aspartame, 3.5 mM saccharin, 12 mM sodium cyclamate, 14 mM acesulfame-K, 1.04 M sorbitol, 0.629 mM sucralose, 0.375 mM NHDC, 1.5 mM stevioside and 0.0163 mM thaumatin. A computerized data-collection program was used in all sessions with five gLMSs corresponding to the basic tastes (sweet, salty, sour, savory, bitter) on one screen, followed by astringency on a second screen. In any one session the subjects were presented with two solutions, the sweetener alone and with 5, 25, or 50 mM $ZnSO_4$ added. For example, subjects would rate the tastes and astringency of 800 mM glucose followed by rating the tastes and astringency for 800 mM glucose mixed with 5 mM $ZnSO_4$. The prototypical stimulus was always rated first because the sweetness blocking effects of zinc ions do not rinse away easily. Between the samples there was an interstimulus interval of 30 s during which subjects rinsed with *di* water at least four times. Ratings were

performed in triplicate for each concentration as a measure of reliability of rating. There were 108 sessions.

Experiment 3: the effect of zinc sulfate on synergy of sweetness

Aspartame and acesulfame-K exhibit synergy of sweet taste when mixed (McBride, 1988; Ayya and Lawless, 1992; Schiffman *et al.*, 1995; Schifferstein, 1996; Keast *et al.*, 2003), but the molecular mechanisms responsible for synergy are unknown. This experiment was included to determine whether the taste mechanisms responsible for sweet taste synergy would be affected by $ZnSO_4$. Sucrose was used as a control sweetener, since it does not synergize with either compound (Schifferstein, 1995; Keast *et al.*, 2003). Subjects (n = 14) intensity matched sweeteners (sucrose, aspartame, acesulfame-K) to gLMS 5 ('weak') prior to the experiment. The method for intensity matching was the same as that described above. The group mean concentration required for each of the sweeteners to elicit gLMS 5 intensity was determined: 140 mM sucrose, 4 mM acesulfame-K and 1 mM aspartame. Subjects tasted individual sweeteners and binary combinations of the three sweeteners with and without added $ZnSO_4$ (25 mM). There were only two samples per session (the sweetener followed by the sweetener with $ZnSO_4$ added) and all samples were tasted on at least three separate occasions giving a total of 36 sessions. The tasting procedure was the same as described above.

Standardization of gLMS ratings

The gLMS standardization methodology followed previously published methods from our laboratory (Delwiche *et al.*, 2001). A brief description follows. Subjects rated the loudness of six tones (generated by a Maico Hearing Instruments tone generator, presented via headphones at 4000 Hz for 2 s at levels of 0, 20, 35, 50, 65 and 80 dB) and the heaviness of six visually identical weights (opaque, sand-filled jars at levels of 225, 380, 558, 713, 870 and 999 g). All ratings were made on a computerized gLMS. Subjects were asked to rate the intensity of loudness or heaviness respectively and all judgments were made within the context of the full range of sensations experienced in life. Subjects first rated the intensity of the six weights, followed by the loudness of the six tones. Both weights and tones were presented twice in blocks of ascending order.

There was a significant correlation between loudness and heaviness (r^2 = 0.66, $P < 0.05$). Since these variables were expected to be unrelated, the correlation indicated that the gLMS ratings were subject to individual scale-use bias and required standardization across subjects.

To determine a standardization factor, each subject's average intensity for loudness was divided by the grand mean for loudness across decibel levels and subjects. This procedure for determining correction factors was repeated with heaviness ratings (averaging across weight levels). The two correction factors were averaged (weights and tones), and each individual's intensity taste ratings were multiplied by his or her personal standardization factor for scale-use bias.

Statistical analysis

Numerical results are expressed as arithmetic means ± SE. Statistical variation was determined by one or two-way analysis of variance (ANOVA) using Statistica 6.0 software package. *Post hoc* pair-wise comparisons were performed with Tukey's HSD. P-values < 0.05 were considered statistically significant. The calculation when stating percentage suppression was:

$$\%\ \text{suppression} = \frac{\text{intensity of sweetener \& salt}}{\text{intensity of the sweetener alone}} \times 100$$

Results

Experiment 1: the effect of zinc sulfate on sweetness

Results from a 12 × 6 (sweetener versus salt) two-way ANOVA revealed there was a significant main effect of sweeteners [$F(11,165) = 8.2$, $P < 0.0001$], indicating that the sweetness of compounds differed overall when pooled across salts. There was a significant main effect of salt [$F(5,75) = 74.6$, $P < 0.0001$], indicating that the salts differentially affected sweetness of the pooled compounds. There was a significant interaction among the salts and sweeteners [$F(55,825) = 4.9$, $P < 0.0001$] indicating differences in sweetness intensity of specific combinations of sweeteners and salts.

Post hoc pairwise comparisons showed the intensity matching procedure was effective, as there was no significant difference in sweetness intensity of the compounds without added salts. However, when salts were added the sweetness of thaumatin was significantly inhibited compared with the other sweeteners (Figure 1K). Pairwise comparisons showed that every salt significantly inhibited the sweetness of the protein thaumatin ($P < 0.001$), indicating chemical and not physiological interactions.

Zinc sulfate was the only salt to significantly affect overall sweetness ($P < 0.0001$; Figure 1A–L). Averaged across compounds in this study, $ZnSO_4$ inhibited sweetness by 75%, which was similar to the effect of $ZnSO_4$ on sweetness of glucose previously reported (Keast, 2003). However, the sweetness inhibiting effect of $ZnSO_4$ did not occur for every sweetener, as the sweetness of Na-cyclamate was not inhibited (Figure 1F). With the exceptions of thaumatin and $ZnSO_4$, there were no significant differences in sweetness among the 11 remaining sweeteners and any of their salt mixtures, i.e. none of the other salts significantly affected sweetness. The pH of solutions (Table 1) cannot account for this pattern of results seen in experiment 1.

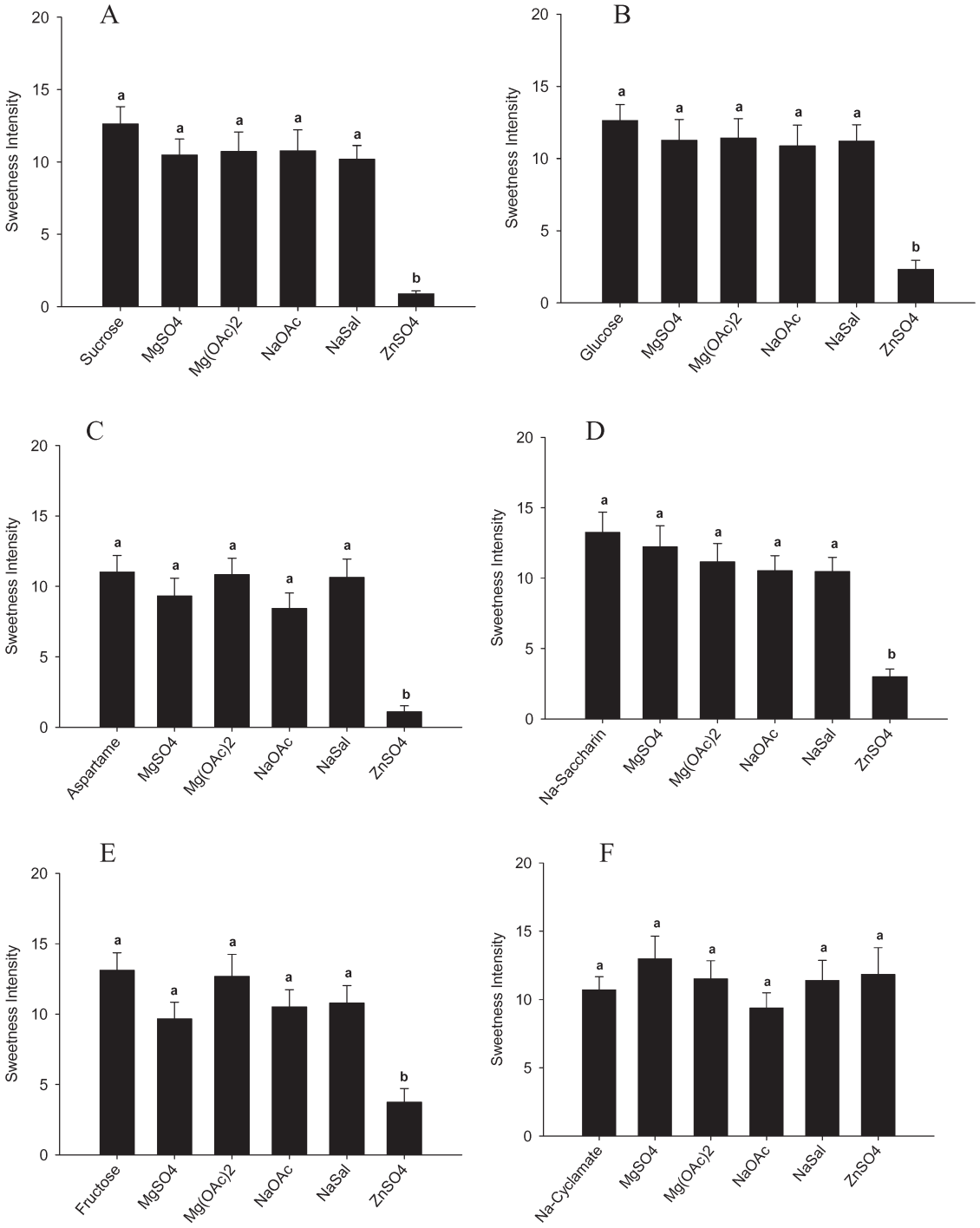

Figure 1 The effect of 25 mM salts on the sweetness intensity of chemically diverse sweet tasting compounds. Each bar represents the average rated sweetness intensities of the compounds and mixtures listed along the *x*-axis. The first bar of each panel represents the indicated sweetener without any salt. The remaining bars represent ratings of the same concentration of this sweetener with 25 mM of the indicated salt added. Concentrations and panel letters (in parentheses) for the sweet compounds are: 300 mM sucrose **(A)**, 800 mM glucose **(B)**, 3.25 mM aspartame **(C)**, 3.5 mM sodium saccharin **(D)**, 475 mM fructose **(E)**, 12 mM sodium cyclamate **(F)**, 0.375 mM neohesperidin dihydrochalcone (NHDC) **(G)**, 1.5 mM stevioside **(H)**, 0.629 mM sucralose **(I)**, 1.04M sorbitol **(J)**, 0.0163 mM thaumatin **(K)**, and 14 mM acesulfame–K **(L)**. Abbreviations for the 25 mM salts are: magnesium sulfate ($MgSO_4$), magnesium acetate [$Mg(OAc)_2$], sodium acetate (NaOAc), sodium salicylate (NaSal) and zinc sulfate ($ZnSO_4$). The *y*-axis represents average sweetness rating on the gLMS (arithmetic mean ± SE) for each sweet tasting compound. Different letters symbolize a statistically significant ($P < 0.0001$) difference in sweetness intensity. There was no difference in sweetness intensity of the compounds without salts.

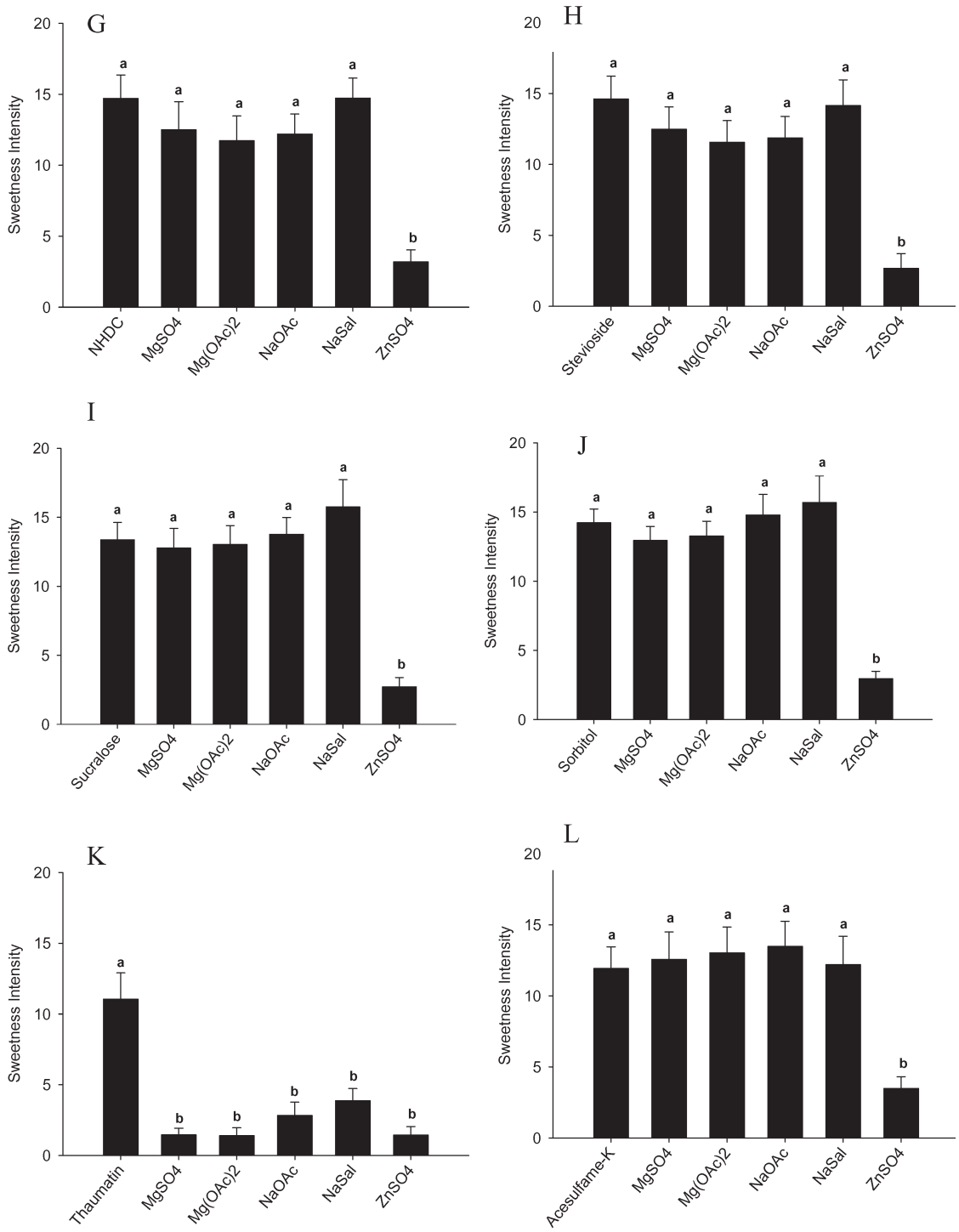

Figure 1 Continued.

Experiment 2: concentration effect of zinc sulfate on sweetness

Results from a 12 × 4 (sweetener versus zinc concentration) two-way ANOVA revealed there was a significant main effect of sweeteners [$F(11,154) = 9.1$, $P < 0.0001$], indicating that the sweetness of the compounds varied. There was a significant main effect of $ZnSO_4$ concentration [$F(3,42) = 204$, $P < 0.0001$], indicating that the concentration of zinc used in mixture affected sweetness. There was a significant interaction among the sweeteners and the concentration of $ZnSO_4$ [$F(55,825) = 4.9$, $P < 0.0001$] indicating differential effects on zinc concentration of specific sweeteners.

Post hoc pairwise comparisons of the sweeteners revealed that $ZnSO_4$ did not inhibit the sweetness of Na-cyclamate. However, $ZnSO_4$ inhibited the sweetness of the 11 other sweeteners equally (Figure 2). All concentrations of $ZnSO_4$ significantly inhibited sweetness ($P < 0.0001$) with 25 mM (74% sweetness inhibition averaged across sweeteners) and 50 mM $ZnSO_4$ (80% sweetness inhibition averaged across sweeteners) more effective at inhibiting sweetness than 5 mM $ZnSO_4$ (54% sweetness inhibition averaged across sweeteners; $P < 0.001$). There was no difference in sweetness inhibiting efficacy between 25 mM and 50 mM $ZnSO_4$.

Experiment 3: the effect of zinc sulfate on synergy of sweetness

Results from a 3×2 (sweetener mixture versus zinc) two-way ANOVA revealed there was a significant main effect of sweetener [$F(2,26) = 16$, $P < 0.0001$], indicating that there was a difference in intensity between the sweetener mixtures. As expected, *post hoc* pairwise comparisons revealed that the mixture of acesulfame-K and aspartame was significantly sweeter than acesulfame-K/sucrose and aspartame/sucrose mixtures (Figure 3). There was a main effect of $ZnSO_4$ [$F(1,13) = 114$, $P < 0.0001$], indicating that $ZnSO_4$ had an effect on sweetness. Zinc sulfate inhibited the sweetness of all mixtures equally, with a mean suppression of 80%.

Discussion

Zinc sulfate is a potent inhibitor of the sweetness of most sweeteners, but does not affect salty, sour, or umami taste qualities (Keast, 2003) and differentially inhibits bitterness (Keast and Breslin, 2004). We believe that this suppression is due to the zinc ion. Evidence for this comes from the observation that $MgSO_4$ (sulfate anion) failed to significantly inhibit sweetness, ruling out a direct effect of the anion. We have also observed that other zinc salts inhibit sweetness as well (data not shown). However, $ZnSO_4$ did not inhibit the sweetness of Na-cyclamate. This result indicates that Na-cyclamate does not stimulate sweet taste via a zinc-sensitive mechanism, while at least 10 other sweeteners in this study and one of their synergistic interactions do. Other researchers have also suggested that cyclamate may access a receptor complex independent of the majority of other sweeteners (Chaudhari and Kinnamon, 2001).

At present we do not know whether the zinc insensitive mechanism for cyclamate is a separate binding site on a general sweet receptor, a distinct receptor type, or a downstream transduction event (direct cascade interaction with Zn). The fact that sweetness inhibition by $ZnSO_4$ was relatively homogenous across at least 10 sweeteners (and the one case of sweetness synergy between acesulfame-K and aspartame) suggests they could access the same zinc sensitive

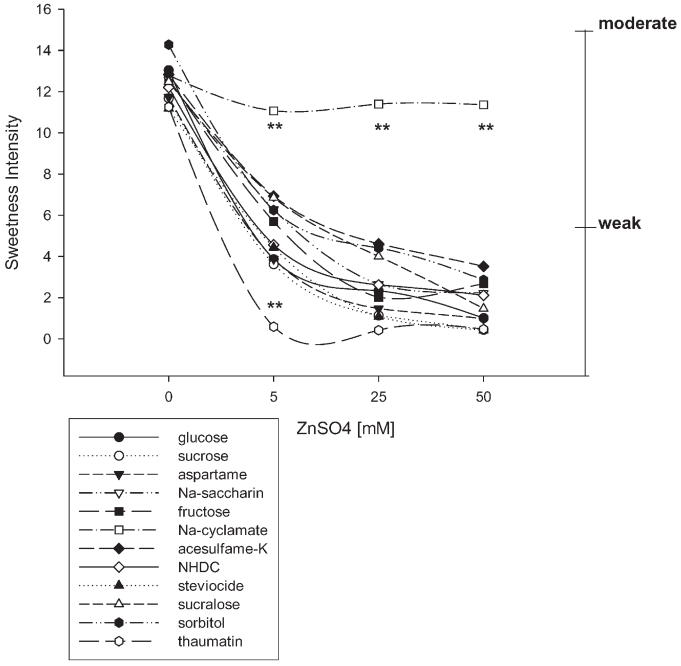

Figure 2 The effect of the concentration of zinc sulfate on sweetness of chemically diverse compounds. Each point on the graph indicates the sweetness ratings of a particular sweetener with 0 mM, 5 mM, 25 mM, or 50 mM $ZnSO_4$ added. The *y*-axis represents average sweetness rating on the gLMS (arithmetic mean) for each sweet tasting compound with $ZnSO_4$. The right-hand *y*-axis lists the verbal descriptors from the gLMS. **Significant difference ($P < 0.001$) in sweet taste intensity between one sweetener with $ZnSO_4$ and the other sweeteners at that concentration of $ZnSO_4$.

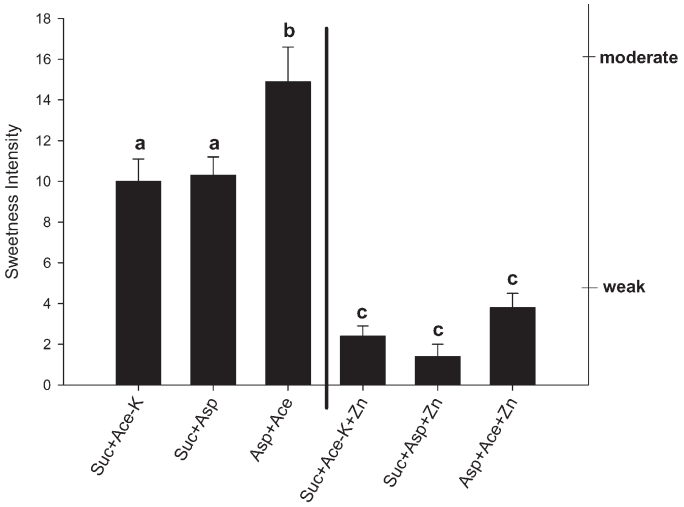

Figure 3 The influence of zinc sulfate on binary mixtures of sweeteners. Each bar represents sweetness of a binary mixture of sweeteners (left side) and the sweetness of the binary mixtures with 25 mM $ZnSO_4$ added (right side). The *x*-axis lists the binary mixtures. Abbreviations are: Suc (sucrose), Ace (acesulfame-K), Asp (aspartame) and Zn ($ZnSO_4$). The binary mixtures were sucrose and acesulfame-K, sucrose and aspartame, acesulfame-K and aspartame. The *y*-axis represents average sweetness rating on the gLMS (arithmetic mean ± standard error) for each sweet mixture. The right-hand *y*-axis lists the verbal descriptors from the gLMS. Different letters symbolize a statistically significant ($P < 0.0001$) difference in sweetness intensity.

mechanism. This would mean that there is one major receptor/complex that is responsible for the majority of sweet taste for these 10 or 11 compounds (Zhao *et al.*, 2003). An alternative hypothesis is that multiple sweet transduction mechanisms exist and are all comparably affected by $ZnSO_4$ at the three concentrations employed.

Concentration effect of zinc sulfate

The inhibition of sweetness by zinc ions reached maximum efficacy at 25 mM. At a concentration of 5 mM, $ZnSO_4$ was effective at inhibiting >50% of the perceived sweetness and this increased to 75% inhibition at 25 mM $ZnSO_4$. At 5 mM the sweetness of thaumatin was reduced significantly more than the other sweeteners, but all salts reduced the sweetness of thaumatin, which is a protein sweetener. Apart from thaumatin, the other salts had no influence on any of the sweeteners. The cause for the loss of sweetness of thaumatin is unknown, but is probably due to an ionic interaction between the protein and the salts rather than a physiological effect at a receptor. With the exception of Na-cyclamate (and thaumatin), 5 mM zinc did not differentially inhibit sweetness. This observation is consistent with the hypothesis that these nine sweeteners are transduced by a zinc-sensitive transduction mechanism that responds to them all. The minimum concentration required for a significant decrease in sweetness in humans is unknown, but it may be significantly lower than 5 mM, as Iwasaki and Sato (1986)report that 0.1 mM zinc inhibits nerve responses to sugars in mice.

Mode of action of zinc sulfate

Zinc ions have an affinity for thiol and hydroxy groups and will readily complex with proteins, peptides and amino acids. The oral influences of zinc ions are known to linger in the mouth with astringency remaining unaltered even after multiple water rinses (Keast, 2003). The effect of zinc ions on sweetness also persists and a pre-treatment of 25 mM $ZnSO_4$ continues to inhibit sweetness (except Na-cyclamate) more than a minute after rinsing with zinc (personal observation). Therefore, the ability of zinc ions to block sweetness occurs when sweeteners are mixed with zinc salts (as shown in this study) and also when zinc salt solutions are applied as a pre-rinse. The mode of action of zinc ions is likely related to its ability to bind to proteins, which could cause a change in the structural configuration of a receptor protein. The effect of zinc on sweetness would occur if it binds to a sweet taste receptor, changing the configuration of the receptor and altering the binding site making it unavailable for normal reception to most sweeteners.

Multiple mechanism of sweet taste?

We suggest there are at least two mechanisms responsible for sweet taste in humans, one primary sweet taste mechanism that is sensitive to zinc ions, and an independent zinc insensitive mechanism that is responsible for sweet taste of Na-cyclamate. Note that these two mechanisms could be different binding sites on a single receptor. If all sweeteners accessed the same receptor-binding site we would expect sweetness inhibition to be equal across all sweeteners. Note also that $ZnSO_4$ was responsible for an 80% reduction in sweet taste on average. Thus 20% sweetness remained that may be due to activation of a zinc insensitive mechanism. Alternatively, if the concentration of $ZnSO_4$ was increased beyond 50 mM, sweetness inhibition may have been complete.

Conclusions

Zinc sulfate, and possibly other zinc salts, is a potent inhibitor of the sweetness of most compounds used in this study. Zinc ions were unable to inhibit the sweetness of cyclamate suggesting that the sweetness of cyclamate is mediated through an alternative transduction mechanism to that used by the other sweeteners. The implications of this finding are that there is more than one transduction mechanism (receptor, binding site, etc) responsible for sweet taste transduction: one zinc sensitive mechanism that is responsible for the majority of sweet taste of the compounds tested, and at least one zinc insensitive mechanism that is activated by Na-cyclamate. As stated above, this does not, however, preclude the idea that all sweeteners activate the TAS1R2–TAS1R3 dimer.

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