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Research Paper

Bitterness Suppression with Zinc Sulfate and Na-Cyclamate: A Model of Combined Peripheral and Central Neural Approaches to Flavor Modification

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Purpose. Zinc sulfate is known to inhibit the bitterness of the antimalarial agent quinine [R. S. J. Keast. The effect of zinc on human taste perception. *J. Food Sci.* **68**:1871–1877 (2003)]. In the present work, we investigated whether zinc sulfate would inhibit other bitter-tasting compounds and pharmaceuticals. The utility of zinc as a general bitterness inhibitor is compromised, however, by the fact that it is also a good sweetness inhibitor [R. S. J. Keast, T. Canty, and P. A. S. Breslin. Oral zinc sulfate solutions inhibit sweet taste perception. *Chem. Senses* **29**:513–521 (2004)] and would interfere with the taste of complex formulations. Yet, zinc sulfate does not inhibit the sweetener Na-cyclamate. Thus, we determined whether a mixture of zinc sulfate and Na-cyclamate would be a particularly effective combination for bitterness inhibition (Zn) and masking (cyclamate).

Method. We used human taste psychophysical procedures with chemical solutions to assess bitterness blocking.

Results. Zinc sulfate significantly inhibited the bitterness of quinine–HCl, Tetralone, and denatonium benzoate (DB) ($p < 0.05$), but had no significant effect on the bitterness of sucrose octa-acetate, pseudoephedrine (PSE), and dextromethorphan. A second experiment examined the influence of zinc sulfate on bittersweet mixtures. The bitter compounds were DB and PSE, and the sweeteners were sucrose (inhibited by 25 mM zinc sulfate) and Na-cyclamate (not inhibited by zinc sulfate). The combination of zinc sulfate and Na-cyclamate most effectively inhibited DB bitterness (86%) ($p < 0.0016$), whereas the mixture's inhibition of PSE bitterness was not different from that of Na-cyclamate alone.

Conclusion. A combination of Na-cyclamate and zinc sulfate was most effective at inhibiting bitterness. Thus, the combined use of peripheral oral and central cognitive bitterness reduction strategies should be particularly effective for improving the flavor profile of bitter-tasting foods and pharmaceutical formulations.

KEY WORDS: bitterness; human psychophysics; Na-cyclamate; sweetness; taste; zinc.

INTRODUCTION

Suppression of excessive bitterness is important for both the food and pharmaceutical industries. For example, in foods, there are many naturally occurring bioactive compounds that elicit bitterness yet have positive health effects (e.g., flavanoids and other phenols, amino acids, peptides, terpenes). Improving the taste profile of the food by removing these compounds eliminates their potential health benefit for the product. Similarly, the excessive bitterness of active compounds in many oral liquid, rapid dissolve, thin film, mist spray, and hard lozenge formulations is a major problem facing the pharmaceutical industry. For all these pharmaceutical applications, the addition of a bitterness suppressor would increase regimen compliance, particularly among children.

Solving this problem is complicated by the observation that the human bitter taste system is complex. It is subserved by approximately two dozen putative G-protein coupled receptors, the mammalian taste receptor protein families (TAS2Rs) (3,4), and several postreceptor transduction mechanisms (5–9). Given the potential diversity in bitter taste transduction sequences, it is unlikely that a single, universal, bitter blocker will be discovered. Nevertheless, some compounds or elements, such as Na, inhibit the bitterness of a relatively large number of bitter agents.

In general, there are two approaches to suppressing bitterness: peripheral physiological interactions with receptor cells (e.g., via receptor inhibitors) and central cognitive mixture suppression (e.g., via mixture with syrups and sweeteners). For example, sodium salts inhibit the bitterness

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ABBREVIATIONS: DB, denatonium benzoate; DEX, dextromethorphan; di, deionized; gLMS, general labeled magnitude scale; $Mg(OAc)_2$, magnesium acetate; $MgSO_4$, magnesium sulfate; NaOAc, sodium acetate; PSE, pseudoephedrine; QHCl, Quinine-HCl; SOA, sucrose octaacetate; TET, Tetralone; $ZnSO_4$, zinc sulfate.

of certain compounds whether or not the salts elicit a taste, indicating that the inhibition is peripheral rather than based on perceptual interactions (10–14). Alternatively, central cognitive effects can occur when different qualities of taste stimuli are mixed together and the perceived intensity of one or more of the components is diminished by the perception of the others. This is labeled mixture suppression (15) and is caused by cognitive interactions among taste qualities. As one example, mixture suppression occurs when bitter- and sweet-tasting compounds are mixed together (10), which is the predominant mode of bitterness suppression in liquid pharmaceutical formulations.

Zinc salts are potent inhibitors of the bitterness of quinine and may have more general utility for inhibiting the bitterness of other compounds (1). One difficulty with using zinc ions more commonly as bitterness blockers is their capacity to inhibit many sweeteners and, thus, disrupt flavor balance in formulations. We reported that zinc ions inhibited the sweetness of 11 chemically diverse sweeteners, yet had no effect on the sweetness of Na-cyclamate (2) or other basic taste qualities elicited by other prototypical stimuli such as citric acid, NaCl, and monosodium glutamate (1). Although the reasons why zinc ions inhibit sweetness and bitterness are not known, zinc ions allosterically modify other transmembrane receptors, especially at histidine and cysteine residues, and may also alter taste receptor conformations rendering them unavailable for normal function (16–19).

To reduce excessive bitterness most effectively, both oral peripheral and central cognitive strategies should be employed in concert. Because zinc ions do not inhibit the sweetness of Na-cyclamate (2), a mixture of zinc and Na-cyclamate could be an effective tool for suppressing bitterness generally via a combination of oral peripheral (Zn^{2+}) and central cognitive (Na-cyclamate's sweetness) effects. The first aim of the present study was to determine whether zinc ions might inhibit the bitterness of compounds other than quinine–HCl in humans. The second aim was to assess the ability of combined oral peripheral and central cognitive inhibition (zinc sulfate and Na-cyclamate) to manage bitterness beyond the capacity of either compound alone.

MATERIALS AND METHODS

Subjects

Subjects (n = 20, 33 ± 5 years old, 10 females) between the ages of 21 and 50 were paid to participate after providing informed consent. All volunteers 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 provided informed consent on a form approved by the Office of Regulatory Affairs at the University of Pennsylvania and were then trained according to the procedure below.

Subject Training

Subjects were initially trained to use the general labeled magnitude scale (gLMS) following standard published procedures (20,21), except the top of the scale was labeled "strongest imaginable" sensation of any kind to help limit ceiling effects and intersubjective scaling differences (22). The gLMS is a psychophysical tool that requires 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 quasi-logarithmically, based on experimentally determined intervals to yield data equivalent to magnitude estimation. The scale only shows adjectives and not numbers 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, umami from 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 also employed as training stimuli.

Stimuli

The salts were zinc sulfate ($ZnSO_4$), sodium acetate (NaOAc), magnesium sulfate ($MgSO_4$), and magnesium acetate $Mg(OAc)_2$ purchased from Sigma Chemical (St. Louis MO, USA). The bitter compounds were quinine–HCl (QHCl) from Fluka Chemika (Buchs, Switzerland), Tetralone (TET) (family of iso-α-acids, the primary bittering compounds in beer) from Kalsec (Kalamazoo, MI, USA), sucrose octa-acetate (SOA), dextromethorphan (DEX), and denatonium benzoate (DB) from Sigma Chemical, and pseudoephedrine HCl (PSE) from Aldrich (Milwaukee, WI, USA). Aqueous solutions were freshly prepared every 2–3 days, using deionized (*di*) Millipore™-filtered water, prior to the initialization of the experiments. The solutions were stored in amber glass bottles and refrigerated.

Intensity Matching Bitterness of Compounds

The procedure involved presenting subjects with varying concentrations of bitter stimuli and assessing the average concentration required to elicit "moderate" bitterness on the gLMS. The protocol follows. Subjects were instructed to wear nose clips (GaleMed, Taipei, Taiwan) to eliminate olfactory input and to rate the perceived bitterness intensity of the solution while it remained in the mouth. Subjects rated the intensity of predetermined concentrations of bitter solutions (initial range of concentration is in parentheses): DB (5×10^{-9}–5×10^{-7} M), DEX (1×10^{-3}–1×10^{-2} M), PSE (5×10^{-3}–5×10^{-2} M), TET (1×10^{-6}–9×10^{-4} M), SOA (1×10^{-4}–1×10^{-3} M), and QHCl (5×10^{-5}–1×10^{-3} M). Taste intensity was recorded on a computerized gLMS. There was an interstimulus interval of

Table I. Molarity of Bitter Compounds and Salts Used in Experiment 1

Bitter compound (concentration M)	Salt (concentration M)
Denatonium benzoate (1.4×10^{-8} M)	$MgSO_4$ (25 mM)
Dextromethorphan (5.3×10^{-3} M)	$Mg(OAc)_2$ (25 mM)
Pseudoephedrine (2.7×10^{-2} M)	NaOAc (25 mM)
Tetralone (2.6×10^{-4} M)	NaOAc (300 mM)
Sucrose octa-acetate (4.1×10^{-4} M)	$ZnSO_4$ (25 mM)
Quinine–HCl (6×10^{-4} M)	

approximately 60 s, during which time the subject was required to rinse with *di* water at least four times. A group average concentration eliciting "moderate" bitterness was determined for each compound. Subjects were retested to verify that the concentrations of bitter compounds were perceived as moderately bitter on average across subjects. If the perceived bitterness rating did not match "moderate" (gLMS 16 ± 4) on subsequent evaluations, the concentration was adjusted up or down depending on whether more or less bitterness intensity was required. This procedure continued until a moderate bitter concentration was found. Final concentrations of the bitter compounds are shown in Table I.

Experiment 1: The Effect of Zinc Ions on Bitterness

Subjects (n = 10, 32 ± 6 years old, 7 females) were given trays containing seven solutions: one *di* water, one bitter compound, and five samples of the bitter compound with each of the salts [e.g., 4.1×10^{-4} M SOA with 25 mM $MgSO_4$, 25 mM $Mg(OAc)_2$, 25 mM NaOAc, 300 mM NaOAc, and 25 mM $ZnSO_4$]. Magnesium salts were selected to act as divalent cation controls for $ZnSO_4$, and NaOAc was included at two concentrations (25 and 300 mM) because it is a known bitterness inhibitor at higher concentrations (11–13). There were six different trays (one for each salt), and each tray was tasted on at least three separate occasions, resulting in a total of 18 sessions on 18 separate days. The testing protocol was as follows. Solutions (10 ml) were presented in 30-ml plastic medicine cups (Dynarex, Orangeburg, NY, USA) on numbered trays. The bitter compound with added $ZnSO_4$ was always presented last to avoid any potential taste-altering carryover effects on taste (1). 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 pour the whole sample in their mouth while wearing nose clips, hold it in their mouth for 3–5 s, and rate the solution for sour, sweet, bitter, salty, umami, and astringent perceptions prior to expectorating. All subjects rinsed with *di* water four times during the interstimulus interval of 2 min. A gLMS was used as the rating tool.

Experiment 2: Oral Peripheral (Zinc Ions) and Central Cognitive (Sweet) Inhibition of Bitterness

Experiment 1 showed that $ZnSO_4$ inhibited the bitterness of DB, but had no effect on the bitterness of PSE. From a previous study, we know that the intensities of 300 mM sucrose and 12 mM Na-cyclamate were equi-intense and that zinc ions inhibit the sweetness of sucrose but do not inhibit the sweetness of Na-cyclamate (2). Therefore, by combining DB and PSE with sucrose and Na-cyclamate, we could assess the influence of zinc sulfate on bittersweet mixtures when the zinc could inhibit only the sweetness, only the bitterness, or both depending on which compounds were in solution. Figure 1 depicts the possible outcomes of the experimental design.

Subjects (n = 17, 30 ± 5 years old, 10 females) wore nose clips and rated the taste qualities of the following binary mixture solutions, as well as their individual components, both with and without added $ZnSO_4$ (25 mM): (1) DB and Na-cyclamate, (2) DB and sucrose, (3) PSE and Na-cyclamate, and (4) PSE and sucrose (Table II). A computerized data-collection program was used in all sessions with

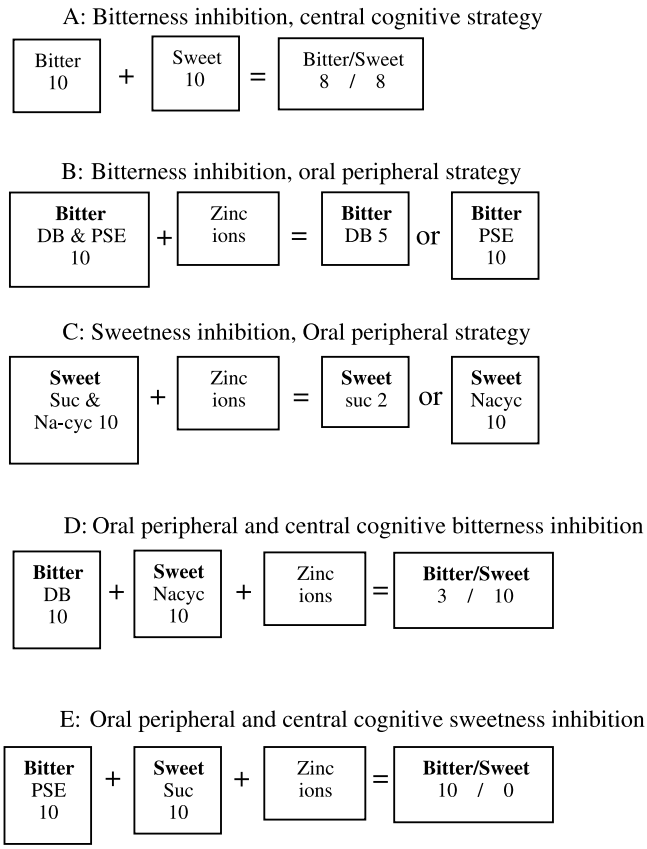

Fig. 1. Illustration of oral peripheral and central cognitive strategies to inhibit bitterness. Each equation is a hypothetical example of what happens to bitter or sweet intensity when they are mixed together and/or a zinc salt is added. Number(s) in each box represent hypothetical intensity levels. Equation A shows that the intensity of a bitter compound (10) and the intensity of a sweet compound (10) are mutually suppressed when they are mixed together (both 8) [mixture suppression (28)]. Equations B and C show the taste intensity of the bitter [denatonium benzoate (DB) and pseudoephedrine (PSE)] and sweet [sucrose and Na-cyclamate (Na-cyc)] compounds (all an intensity of 10) and the intensity of each component after a zinc salt has been added. Equation D illustrates the combined effect of oral peripheral (zinc ions) and central cognitive (sweetness) strategies to inhibit bitterness. Equation E illustrates that bitterness inhibition with zinc ions may not be effective, since zinc may inhibit sweetness and not inhibit bitterness.

Table II. Molarity of Bitter Compounds and Sweeteners Used in Experiment 2

Bitter compound (concentration M)	Sweet compound (concentration M)
Pseudoephedrine (2.7×10^{-2} M)	Sucrose (3×10^{-1} M)
Denatonium benzoate (1.4×10^{-8} M)	Na-cyclamate (1.2×10^{-2} M)

five gLMSs corresponding to the taste qualities (sweet, salty, sour, umami, and bitter) on one screen, followed by a scale for the somatosensory quality astringency on a second screen. In any one session, the subjects were presented with two solutions to rate, one with 25 mM $ZnSO_4$ added and one without. For example, subjects would rate the taste qualities and astringency of the SOA/sucrose mixture and then rate the tastes and astringency of the SOA/sucrose mixture with 25 mM $ZnSO_4$ added. The solution without $ZnSO_4$ was always rated first to avoid any lingering taste effects zinc ions may have. There was an interstimulus interval of 2 min during which subjects rinsed with *di* water at least four times. Ratings were performed in triplicate for each bittersweet mixture or component. There were 24 test sessions.

Statistical Analysis

Numerical results are expressed as arithmetic means ± standard error. Statistical analysis of results from experiment 1 was determined with two-way repeated measures analysis of variance (ANOVA), and *post hoc* pairwise comparisons were made with the Tukey HSD test. Statistical analysis of results from experiment 2 was determined with a repeated measures ANOVA, and *post hoc* analyses consisted of paired *t* tests Bonferroni corrected for multiple tests. The analyses were conducted with the SPSS 12.0.1 package. The p values < 0.05 were considered statistically significant. Statistical analyses of bitterness intensity ratings are included in data presented from experiment 1, and bitterness and sweetness intensity ratings are presented for experiment 2. Ratings of the other qualities were collected to minimize halo dumping effects (23). These ratings were generally not statistically different across conditions, low in magnitude, and not relevant to the objectives of this research project.

RESULTS

Experiment 1: The Effect of Zinc and Other Ions on Bitterness

Results from a 6 × 6 (bitter *vs.* salt) two-way ANOVA revealed that there was a significant main effect of bitter compounds [$F(5,145) = 6.6$, $p < 0.0001$] and of salts [$F(5,145) = 26$, $p < 0.0001$]. There was a significant interaction among the bitter compounds and salts [$F(25,725) = 9.9$, $p < 0.0001$] indicating that some salts interact with bitter compounds differently than other salts. *Post hoc* pairwise tests showed that the intensity matching protocol was effective as there were no significant differences in the bitterness ratings of compounds prior to the addition of the salts, but when pooled across salt conditions, the bitterness of TET (24%), DB (26%), PSE (16%), and QHCl (32%) were suppressed more than the bitterness of SOA (7%) ($p < 0.05$) (Fig. 2). Percentages presented in parentheses following a compound represent either the mean bitterness inhibition of that compound when salts were added or the mean bitterness inhibition by the salt specified. There was a variation among the salts' abilities to inhibit bitterness in general: $ZnSO_4$ (40%), 300 mM NaOAc (38%), and $Mg(OAc)_2$ (20%) all significantly suppressed bitterness ($p < 0.05$), whereas 25 mM NaOAc and $MgSO_4$ had no significant effect on bitterness (Fig. 3). Pairwise tests revealed that $ZnSO_4$ inhibited the bitterness of TET (43%), DB (63%), and QHCl (70%) ($p < 0.001$) (1), whereas $ZnSO_4$ did not significantly affect the bitterness of SOA, PSE, and DEX (Fig. 4). NaOAc (300 mM) significantly inhibited the bitterness of DB (45%), PSE (56%), DEX (48%), and QHCl (60%) ($p < 0.001$), but failed to significantly inhibit the bitterness of SOA and TET (results not shown). $Mg(OAc)_2$ did not significantly inhibit the bitterness of any individual compound.

Experiment 2: Combined Oral Peripheral (Zinc Ions) and Central Cognitive (Sweetness) Suppression of Bitterness

When suprathreshold levels of compounds that elicit different qualities are mixed together, a general phenomenon called mixture suppression occurs, where the intensity of the mixture is less than the sum of the intensity of the components (15,24) (Fig. 1, equation A). When a bitterness suppressor is added to a bittersweet mixture, and

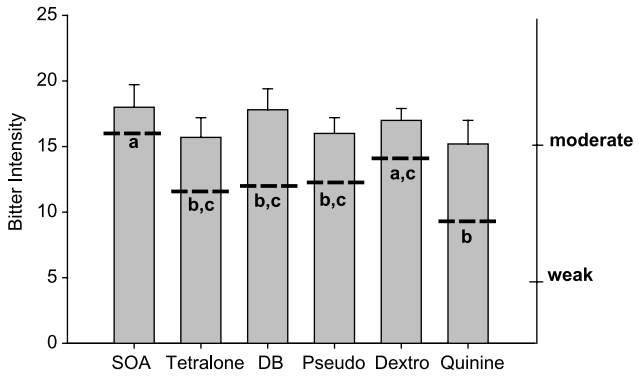

Fig. 2. Bitterness intensity of chemically diverse bitter-tasting compounds without and with addition of salts. Each bar represents the average bitterness intensity of the compounds listed along the *x*-axis. The gray bar is the bitterness of the compound without added salt, and the dashed black line indicates the bitterness of the compound when salts were added, averaged across salts. The *y*-axis represents the average bitterness rating (arithmetic mean ± standard error) on the general labeled magnitude scale (gLMS) for each bitter-tasting compound. The right-hand vertical axis lists the verbal descriptors from the gLMS. Concentrations and abbreviations for the bitter compounds were as follows: sucrose octa-acetate (SOA) (4.1×10^{-4} M), Tetralone (2.6×10^{-4} M), denatonium benzoate (DB) (1.4×10^{-8} M), pseudoephedrine (Pseudo) (2.7×10^{-2} M), dextromethorphan (Dextro) (5.3×10^{-3} M), and quinine–HCl (6×10^{-4} M). The salts were 25 mM magnesium sulfate, 25 mM magnesium acetate, 25 and 300 mM sodium acetate, and 25 mM zinc sulfate. Differences among letters over bars indicate that means are statistically different ($p < 0.001$) in intensity. The bitterness intensities of the compounds without added salts were statistically the same.

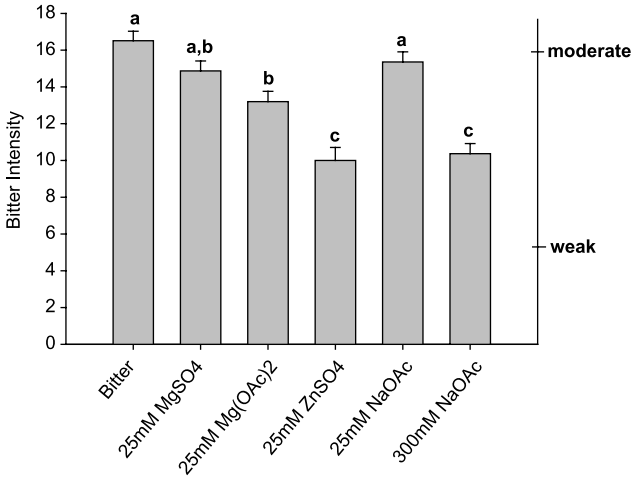

Fig. 3. Specific effects of salts on the bitterness of chemically diverse compounds (pooled across bitter compound). Each bar represents the average bitterness intensity ratings of the six diverse bitter compounds alone (first bar) and with the salts named on the *x*-axis added to them. The *y*-axis represents average bitterness rating (arithmetic mean ± standard error) on the gLMS for each condition. The pooled bitterness ratings were for the following six compounds: denatonium benzoate (1.4×10^{-8} M), dextromethorphan (5.3×10^{-3} M), pseudoephedrine (2.7×10^{-2} M), Tetralone (2.6×10^{-4} M), SOA (4.1×10^{-4} M), and quinine–HCl (6×10^{-4} M). The right-hand vertical axis lists the verbal descriptors from the gLMS. Abbreviations of sodium salts are as follows: magnesium sulfate ($MgSO_4$), magnesium acetate [$Mg(OAc)_2$], zinc sulfate ($ZnSO_4$), and sodium acetate (NaOAc). Differences among letters over bars indicate that means are statistically different ($p < 0.001$) in bitterness intensity.

the bitterness is suppressed, sweetness should be released from mixture suppression which can positively affect the taste of a product (25) (Fig. 1, equation D). If the sweetness is suppressed, bitterness will be released from mixture suppression and the taste will be negatively affected (Fig. 1, equation E).

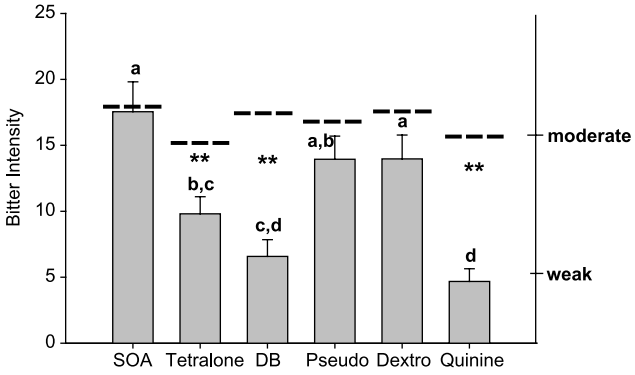

Fig. 4. The effect of 25 mM zinc sulfate on the bitterness of chemically diverse compounds. Each bar represents the average bitterness intensity of the compounds listed along the *x*-axis with the addition of 25 mM zinc sulfate, and the dashed black line indicates the initial bitterness of the compound without added zinc ions. The *y*-axis represents average bitterness rating (arithmetic mean) on the gLMS for each bitter-tasting compound and mixture. The right-hand vertical axis lists the verbal descriptors from the gLMS. The concentration and abbreviations for the bitter compounds are the same as in Fig. 1. ** indicates a significant difference ($p < 0.05$) in bitter taste intensity between the bitter compound with and without zinc ions. Differences among letters over bars indicate that means are statistically different ($p < 0.001$) in bitterness intensity between compounds when zinc sulfate had been added.

Bitterness

ANOVA results of bitterness intensity ratings of solutions containing DB and PSE showed a main effect of bitterness [$F(1,50) = 97$, $p < 0.0001$]. Thirty-one paired *t* tests were performed and Bonferroni correction was applied, resulting in a level of significance of $p < 0.0016$ (0.05/31 = 0.0016).

Pairwise *t* tests revealed that $ZnSO_4$ (60%), sucrose (40%), and Na-cyclamate (35%) inhibited the bitterness of

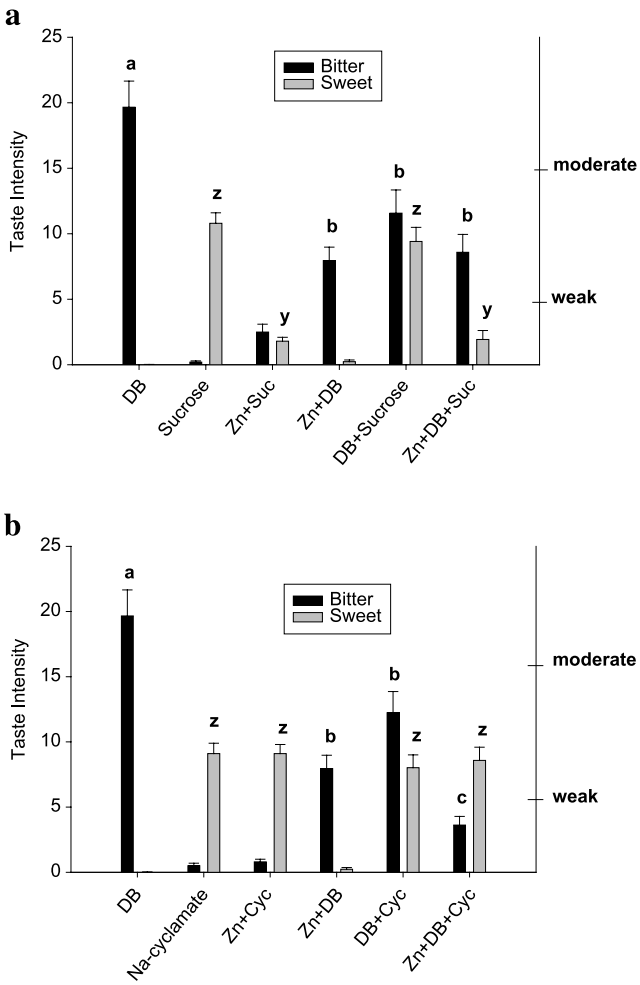

Fig. 5. The influence of zinc sulfate on bitterness and sweetness of mixtures of denatonium benzoate and sucrose, and denatonium benzoate and Na-cyclamate. Bold black bars represent bitter taste intensity; gray bars represent sweet taste intensity of the compounds and mixtures listed along the *x*-axis. The *y*-axis represents average taste intensity rating on the gLMS (arithmetic mean) for each compound or mixture. The right-hand vertical axis lists the verbal descriptors from the gLMS. The concentration and abbreviations for the bitter compounds are the same as in Fig. 1. The concentration and abbreviation for the sweeteners are as follows: Suc (sucrose) (300 mM) and Cyc (Na-cyclamate) (12 mM). The different letters a, b, and c symbolize statistically significant ($p < 0.0001$) differences in bitterness intensity among compounds or mixtures, whereas the letters y and z symbolize a statistically significant ($p<0.0001$) difference in sweetness between compounds or mixtures.

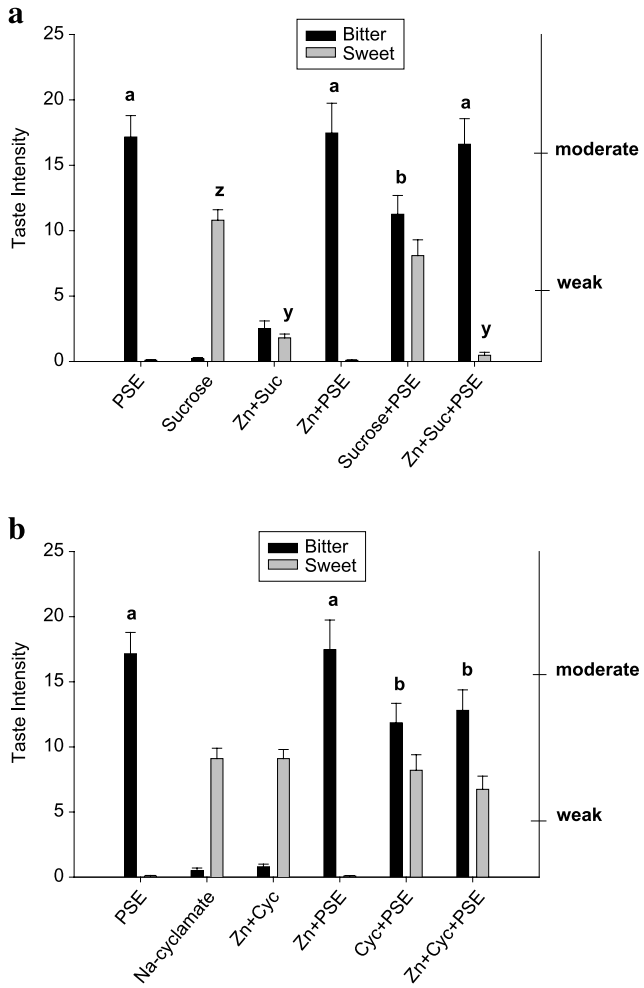

Fig. 6. The influence of zinc sulfate on bitterness and sweetness of mixtures of pseudoephedrine HCl and sucrose, and pseudoephedrine HCl and Na-cyclamate. This figure is organized to be directly parallel to Fig. 5a and b; only the chemicals have changed as indicated.

DB ($p < 0.0016$) (Fig. 5a and b). Mixtures of $ZnSO_4$ and sucrose (55%) and $ZnSO_4$ and Na-cyclamate (86%) also significantly inhibited the bitterness of DB. There were no statistical differences among the bitterness ratings of zinc and sucrose, $ZnSO_4$, or sucrose added to DB. The mixture of $ZnSO_4$ and Na-cyclamate was significantly more effective at inhibiting the bitterness of DB than any other mixture or component.

Pairwise *t* tests revealed that sucrose (36%) and Na-cyclamate (37%) inhibited the bitterness of PSE ($p < 0.0016$) (Fig. 6a and b). A mixture of $ZnSO_4$ and Na-cyclamate (33%) also inhibited the bitterness of PSE; however, the mixture of $ZnSO_4$ and sucrose did not. There were no statistical differences among bitterness ratings when $ZnSO_4$ and Na-cyclamate, sucrose, or Na-cyclamate were added to PSE.

Sweetness

ANOVA results of sweet intensity rating of solutions containing sucrose and cyclamate showed a main effect of sweetness [$F(1,50) = 124$, $p < 0.0001$]. Thirty-six paired *t* tests were performed and Bonferroni correction was applied, resulting in a level of significance of $p < 0.0014$ (0.05/36 = 0.0014).

Pairwise *t* tests showed that $ZnSO_4$ (81%) and a combination of DB and $ZnSO_4$ (98%) inhibited the sweetness of sucrose ($p < 0.0014$) (Fig. 5a and b). In addition, a combination of PSE and $ZnSO_4$ (94%) inhibited the sweetness of sucrose ($p < 0.0014$) (Fig. 6a and b). The sweetness of cyclamate was not inhibited by zinc ions.

DISCUSSION

Zinc sulfate was a potent inhibitor of the bitterness of specific compounds (DB, QHCl, TET), yet had little effect on the bitterness of other compounds (SOA, PSE, DEX) at the concentrations used, implying that zinc ions are selective bitterness inhibitors. In addition to inhibiting bitterness, zinc ions are also potent inhibitors of sweetness (2). Therefore, the practical utility of zinc salts as flavor modifiers via bitterness inhibition would be limited for complex flavors as in foods and pharmaceutical formulations (1). However, we demonstrate here that zinc may be used as a bitterness inhibitor in a complex bittersweet formulation without concomitant sweetness reduction when Na-cyclamate is incorporated as the sweetener because the sweetness of Na-cyclamate is not inhibited by zinc sulfate.

Zinc Ions as a Tool to Explore Bitter Taste Transduction

As zinc ions selectively inhibited the bitterness of compounds that were of approximately equal bitter intensity, it is likely that zinc's bitterness suppression occurred via interactions with peripheral oral taste physiology. Had zinc's bitterness inhibition been equal for the matched bitter compounds, we might have inferred a central cognitive effect (mixture suppression). Rather, we believe that this bitterness suppression is a result of the specific actions of zinc cations on a peripheral component of bitter taste physiology, probably the bitter taste receptors. Evidence for this comes from the observation that $MgSO_4$ (sulfate anion) failed to significantly inhibit bitterness, ruling out a major effect of the anion and of charge, and we have also observed that other zinc salts inhibit bitterness as well (data not shown). Zinc ions are also known to modulate allosterically transmembrane receptors (GPCRs and ion channels) and can both activate or inhibit them depending on the receptor system (18,19).

The organization of the bitter taste system is complex with multiple putative receptor mechanisms. Although human psychophysical studies cannot directly test oral peripheral mechanisms of taste, such studies can provide information to help understand the taste system. For example, sweet taste inhibitors have been used in human psychophysical studies to help understand sweet taste transduction mechanisms (1,26). In this study, we find that the bitterness of QHCl, TET, and DB is sensitive to zinc ions, whereas the bitterness of SOA, PSE, and DEX is not. This suggests that these two groups of compounds access separate transduction mechanisms or perhaps different binding sites of the same mechanism. To inhibit bitterness, zinc ions may form a complex

with the extracellular portions of the bitter taste receptor/s (TAS2Rs), as zinc ions readily complex with amino acids and proteins and have a high affinity for both thiol and hydroxy groups (17). If zinc ions did bind to a TAS2R, the native configuration of the receptor could be changed and it would be unavailable for normal reception. Alternatively, zinc ions could form complexes with the bitter compounds that would render them insoluble and, thus, unable to access receptors; however, visual inspection of all solutions did not reveal any precipitation.

Zinc Ions and Na-Cyclamate Mixture as Bitterness Inhibitors

The combination of Na-cyclamate and zinc sulfate dramatically reduced the bitterness of DB by 86% (Fig. 5b). This combination of oral peripheral and central cognitive effects was more effective at suppressing bitterness than any other single or combined set of bitterness blockers and is noteworthy here because DB is an extremely potent bitter stimulus. The effect of zinc ions on the bitterness of DB is presumably at the cellular level in the oral periphery. Because zinc ions do not inhibit the sweetness of cyclamate (2), the addition of Na-cyclamate's sweet taste resulted in a central cognitive effect further reducing the bitterness of DB (mixture suppression).

Although zinc sulfate proved to be a potent bitterness inhibitor of specific compounds, the potential for zinc ions to perform a functional role as a bitterness inhibitor in pharmaceuticals or foods is minimized because of its effects on sweetness. Figure 6a shows that sucrose inhibits the bitterness of PSE, presumably through the cognitive phenomenon of mixture suppression (10). When $ZnSO_4$ is added to the PSE–sucrose mixture, it inhibits the sweetness of sucrose but does not inhibit the bitterness of PSE. The reduction in sweetness causes an enhancement of bitterness because of a release of bitterness from mixture suppression (25).

The loss of sweetness caused by the zinc ions has implications for the overall flavor of the product, as sweet taste can enhance congruent aromas. For example, a fruity aroma seems more intense if the level of sweetness is increased, or a fruity aroma can be reduced if the level of sweetness is reduced (27). The sweetness in a product may be masking a bitterness that the zinc ions cannot inhibit, and the loss of sweetness causes bitterness to be released from mixture suppression (as demonstrated in Fig. 6a). In such a situation, the consumer would perceive primary (loss of sweetness) and secondary (loss of aroma, unmasking of bitterness) effects of zinc on flavor, and the usually pleasant hedonic experience would be reduced. This study demonstrates that these potential taste and flavor problems may be managed if zinc ions are combined with Na-cyclamate.

The combinatorial effects of zinc ions and cyclamate make it an ideal mixture for bitterness inhibition, provided zinc inhibits the bitterness of the target compound. As Fig. 6b shows, when zinc ions are unable to inhibit the bitterness of a compound, the reduction in bitterness by the mixture is equivalent to the effect of Na-cyclamate alone. At this moment, Na-cyclamate is not approved for use as a sweetener in the USA, but is approved and used as a sweetener in many other parts of the world.

CONCLUSION

Bitterness continues to be a problem for the food and pharmaceutical industries. We demonstrate here that zinc sulfate differentially inhibits bitterness, and the effect is likely in the oral periphery rather than a cognitive effect of any zinc taste *per se*. Zinc salts are also potent inhibitors of sweeteners with the exception of Na-cyclamate. Therefore, the mixture of zinc and Na-cyclamate combines two bitterness inhibition strategies; first, the zinc ions inhibit bitterness in the oral peripheral taste system, and second, the Na-cyclamate provides a sweetness that masks bitterness (central cognitive). In combination, zinc and Na-cyclamate reduced excessive bitterness to a level that was barely perceived. This bitterness inhibition combination will dramatically reduce the bitterness of food products and oral liquid pharmaceutical formulations, provided that zinc inhibits the bitterness of the active ingredient.

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