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Bitterness inhibition using zinc sulfate and Na-cyclamate: Oral peripheral and central cognitive strategies to modify bitterness

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**Running Head**: Bitterness inhibition using zinc

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Abstract

**Purpose:** Zinc sulfate inhibits the bitterness of quinine-HCl, and we investigated whether bitterness inhibition properties were generalized to other bitter compounds. The usefulness of zinc as a bitterness inhibitor in complex formulations is compromised because it inhibits the sweetness of most sweeteners {Keast, 2004 #1383}. However, we also investigated whether a combination of zinc and Na-cyclamate (sweetener zinc does not inhibit) was an effective mixture to inhibit bitterness.

**Method:** Human psychophysical taste evaluation using a whole mouth exposure procedure was used.

**Results:** Zinc sulfate significantly inhibited the bitterness of quinine-HCl, Tetralone, and denatonium benzoate (DB) (p<0.05), but had no effect on the bitterness of sucrose octaacetate, pseudoephedrine (PSE), and dextromethorphan. Differential suppression of bitterness indicates that the effect of the zinc ions is at the oral periphery rather than a cognitive effect of any taste of zinc per se. The second experiment looked at the influence of zinc sulfate and a sweetener on bitter solutions. The bitter compounds used were DB and PSE. The sweet compounds were sucrose (sweetness is inhibited by 25mM zinc sulfate) and Na-cyclamate (sweetness is not inhibited by zinc sulfate). As predicted, the combination of zinc sulfate and Na-cyclamate was the most effective at inhibiting the bitterness of the DB (86%) (p<0.0016), and while the combination inhibited the bitterness of PSE, the effect did not differ from the effect of Na-cyclamate alone.

**Conclusion:** Zinc sulfate differentially inhibits the bitterness of a number of compounds. The addition of a sweetener, Na-cyclamate, to zinc sulfate further enhances the bitterness inhibition properties.
Bitterness inhibition using zinc

Keywords

Zinc, Na-cyclamate, bitterness, sweet taste, human psychophysics
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) and physically removing these compounds from foods would negate the health benefits. Similarly, excessive bitterness of the active compounds in many oral liquid formulations is a major taste problem facing the pharmaceutical industry. In both situations, the addition of a bitterness suppressor would minimize the risk of the product having a bad taste.

The human bitter taste system is complex. It is subserved by approximately two dozen putative G-protein coupled receptors, the TAS2R’s \{Adler, 2000 #137;Chandrashekar, 2000 #83\}, and several post-receptor transduction mechanisms \{Wong, 1996 #19;Kinnamon, 1996 #18;Huang, 1999 #56;Rossler, 1998 #993\}. 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, like Na+, inhibit the bitterness of a relatively large number of bitter agents.

There are two general ways which bitterness can be suppressed, in the peripheral oral receptor systems or more centrally where cognitive/perceptual interactions may occur. At a cellular level in the oral periphery there is the potential for an inhibitor to interfere with taste receptor cell function or transduction mechanisms; the signal sent to the processing regions of the brain is modified at the source of the signal. For example, the bitterness of certain compounds is suppressed by sodium salts whether or not these salts elicit taste \{Kroeze, 1985 #301;Bartoshuk, 1982 #576;Breslin, 1995 #103;Keast,
2002 #563;Keast, 2002 #626}. Alternatively, central cognitive effects can occur when different quality taste stimuli (above threshold) are mixed together and the intensity of the mixture is less than the sum of the individual component taste intensities. This is labeled mixture suppression {Pangborn, 1960 #833}. In mixture suppression, the taste signals are transmitted to the brain and it is the processing of the signal in the brain that result in a perception of intensity that is less than additive. An example of mixture suppression results when bitter and sweet tasting compounds are mixed together {Kroeze, 1985 #301}.

Zinc salts are potent inhibitors of the bitterness of quinine and may inhibit the bitterness of other compounds {Keast, 2003 #1013}. One problem with using zinc ions as bitterness blockers in complex formulations such as foods and pharmaceuticals is its ability to inhibit the sweetness of many sweeteners. We reported that zinc ions inhibited the sweetness of 11 chemically diverse sweeteners, but had no effect on the sweetness of Na-cyclamate {Keast, 2004 #1383} or other basic taste qualities elicited by other prototypical stimuli such as citric acid, NaCl, and MSG {Keast, 2003 #1013}. Reasons for these effects are unclear. However, zinc ions readily complex with amino acids and proteins and could form complexes with taste receptors rendering them unavailable for normal function {Frederickson, 2001 #1463;Christianson, 1991 #1464}.

To most effectively inhibit excessive bitterness, both oral peripheral and central cognitive strategies should be employed. Since zinc ions do not inhibit the sweetness of Na-cyclamate {Keast, 2004 #1383}, a mixture of zinc and Na-cyclamate could be a very effective tool to inhibit bitterness generally via a combination of oral peripheral (Zn) and central cognitive (Na-cyclamate) effects. The first aim of the present study was to
determine whether zinc ions might inhibit the bitterness of compounds other than quinine-HCl. The second aim was to assess the ability of combined oral peripheral and central cognitive inhibition (zinc sulfate and Na-cyclamate) to modify bitterness beyond the capacity of either compound alone.
Materials and Methods

Subjects

Subjects (n=20, 33±5 years old, 10 female) between the ages of 21 and 50 were paid to participate after providing informed consent. All were employees of the Monell Chemical Senses Center. The participants were asked to refrain from eating, drinking or chewing gum for one hour 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 to use the general Labeled Magnitude Scale (gLMS) following standard published procedures {Green, 1996 #117; Green, 1993 #303} except the top of the scale was labeled as “strongest imaginable’ sensation of any kind {Bartoshuk, 2000 #289}. 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 upon experimentally determined intervals to yield data equivalent to magnitude estimation. The scale only shows adjectives 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 150mM NaCl, bitterness as the predominant quality from 0.05mM quinine HCl, sweetness as the
predominant quality from 300mM sucrose, sourness as the predominant quality from 3mM citric acid, umami from the predominant quality from a mixture of 100mM glutamic acid monosodium salt and 50mM inosine 5’-monophosphate, and astringency as the predominant sensation of 0.5mM tannic acid. To help subjects understand a stimulus could elicit multiple taste qualities, 300mM urea (bitter and slightly sour) and 50mM NH₄Cl (salty, bitter, and slightly sour) were also employed as training stimuli.

Stimuli

The salts were: zinc sulfate (ZnSO₄), sodium acetate (NaOAc), magnesium sulfate (MgSO₄), and magnesium acetate Mg(OAc)₂ purchased from Sigma (St. Louis). 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), Sucrose octaacetate (SOA), Dextromethorphan (DEX), and Denatonium Benzoate (DB) from Sigma Chemical (St. Louis, MO), and Pseudoephedrine (PSE) from Aldrich. 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, 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 (5x10^{-9}M-5x10^{-7}M), DEX (1x10^{-3}M-1x10^{-2}M), PSE (5x10^{-3}M-5x10^{-2}M), TET (1x10^{-6}M-9x10^{-4}M), SOA (1x10^{-4}M-1x10^{-3}M), and QHCl (5x10^{-5}M-1x10^{-3}M). Taste intensity was recorded on a computerized gLMS. There was an interstimulus interval of approximately 60sec, during which time the subject was required to rinse with DI water at least 4 times. A group average concentration eliciting ‘moderate’ bitterness was determined for each compound. Subjects were retested to verify 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±25%) 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 1.

Experiment 1: The effect of zinc ions on bitterness

Subjects (n=10, 32±6 years old, 7 female) were given trays containing seven solutions: one DI water, one bitter compound, five samples of the bitter compound with each of the salts (e.g., 4.1x10^{-4}M SOA with 25mM MgSO_4, 25mM Mg(OAc)_2, 25mM NaOAc, 300mM NaOAc, and 25mM ZnSO_4). Magnesium salts were selected to act as divalent cation controls for ZnSO_4, and NaOAc was included at two concentrations (25mM, 300mM) because it is a known bitterness inhibitor at higher concentrations {Breslin, 1995 #103;Keast, 2002 #563;Bartoshuk, 1982 #576}. 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 (10ml) were presented in 30ml plastic medicine cups (Dynarex, NY) on numbered trays. The bitter compound with added ZnSO₄ was always presented last to avoid any potential taste altering carry over effects on taste {Keast, 2003 #1013}. The remaining six solutions were presented in random order. Subjects rinsed with di water at least four times over a 2-minute 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 a few seconds, and rate the solution for sour, sweet, bitter, salty, umami, and astringent perceptions prior to expectorating. All subjects rinsed with di water 4 times during the interstimulus interval of 2min. The gLMS was used as the rating method.

**Experiment 2: Oral peripheral (zinc ions) and central cognitive (sweet) inhibition of bitterness**

Experiment one showed that ZnSO₄ inhibited the bitterness of DB, but had no effect on the bitterness of PSE. From a previous study we know the intensity of 300mM sucrose and 12mM Na-cyclamate were equi-intense and that zinc ions inhibit the sweetness of sucrose but do not inhibit the sweetness of Na-cyclamate {Keast, 2004 #1383}. Therefore, by combining these bitter (DB & PSE) and sweet compounds (sucrose & Na-cyclamate), both with and without ZnSO₄, we assessed the influence of zinc ions on bitter-sweet mixtures when it is a taste inhibitor of bitterness, or sweetness, or both bitterness and sweetness. Figure 1 schematically shows a theoretical outcome of the experiment design.
Bitterness inhibition using zinc

Subjects (n=17, 30±5 years old, 10 female), wearing nose-clips, assessed the influence of 25mM ZnSO₄ on the following bitter-sweet solutions: 1/ DB and Na-cyclamate, 2/ DB and sucrose, 3/ PSE and Na-cyclamate, 4/ PSE and sucrose. The individual components of the bitter-sweet mixtures (DB, PSE, sucrose, and Na-cyclamate) were also tested with 25mM ZnSO₄. A computerized data-collection program was used in all sessions with five gLMSs corresponding to the basic tastes (SWEET, SALTY, SOUR, UMAMI, BITTER) on one screen, followed by ASTRINGENCY on a second screen. In any one session the subjects were presented with two solutions, the bitter-sweet mixture or bitter or sweet component alone and the mixture or component with 25mM ZnSO₄. For example, subjects would rate the tastes and astringency of SOA/sucrose mixture followed by rating the tastes and astringency for SOA/sucrose mixture with 25mM ZnSO₄ added. The bitter-sweet mixture or bitter or sweet component was always rated first, followed by the bitter-sweet mixture with the ZnSO₄ to avoid any carryover effects zinc ions may have on taste. Between the samples there was an interstimulus interval of 2min during which subjects rinsed with di water at least four times. Ratings were performed in triplicate for each bitter-sweet mixture or component. There was a total of 24 sessions.

Statistical analysis

Numerical results are expressed as arithmetic means ± standard error. Statistical variation from experiment one was determined by two-way repeated measures analysis of variance (ANOVA) with post-hoc Tukey HSD. Statistical variation from experiment two was determined by repeated measures ANOVA and post-hoc analysis consisted of paired
t-tests with Bonferroni corrections. The software used to analyze data was SPSS 12.0.1 package. P values <0.05 were considered statistically significant. Statistical analyses of bitterness ratings are included in data presented from experiment one, and bitter and sweet intensity ratings are presented in experiment two. The other ratings were collected to minimize halo dumping effects {Clark, 1994 #991}, and the data were generally non-significant and not relevant to the findings in this paper.
Results

**Experiment 1: The effect of zinc ions on bitterness**

Results from a 6 x 6 (bitter v salt) two-way ANOVA revealed 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 in analysis showed that the intensity matching protocol was effective as there was no significant difference in the bitter intensity of compounds prior to the addition of the salts, but pooled across salts the bitterness of TET (24%), DB (26%), PSE (16%), and QHCl (32%) were suppressed more than the bitterness of SOA (7%) (p<0.05)(Figure 2). There was variation among the salts ability to inhibit bitterness: ZnSO₄ (40%), 300mM NaOAc (38%), and Mg(OAc)₂ (20%) significantly suppressed bitterness (p<0.05) whereas 25mM NaOAc and MgSO₄ had no effect on bitterness (Figure 3). Pairwise tests revealed that ZnSO₄ inhibited the bitterness of TET (43%), DB (63%), and QHCl (70%)(p<0.001) {Keast, 2003 #1013}, while ZnSO₄ did not significantly affect the bitterness of SOA, PSE, and DEX (Figure 4). NaOAc (300mM) significantly inhibited the bitterness of DB (45%), PSE (56%), DEX (48%), and QHCl (60%) (p<0.001), but failed to significantly inhibit the bitterness SOA and TET (results not shown). Mg(OAc)₂ did not significantly inhibit the bitterness of any individual compound.
Experiment 2: Oral peripheral (zinc ions) and central cognitive (sweet) inhibition of bitterness

When different quality suprathreshold compounds 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 {Keast, 2003; Pangborn, 1960} (Figure 1, equation A). When a bitterness suppressor is added to a bitter-sweet mixture, and the bitterness is suppressed, sweetness should be released from mixture suppression which can positively affect the taste of a product (Figure 1, equation D). If the sweetness is suppressed, bitterness will be released from mixture suppression and the taste will be negatively affected (Figure 1, equation E).

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 applied, resulting in a level of significance of p<0.0016 (0.05/31=0.0016).

Pairwise t-tests revealed that ZnSO₄ (60%), sucrose (40%) and Na-cyclamate (35%) inhibited the bitterness of DB (p<0.0016) (Figure 5a&b). Mixtures of ZnSO₄ & sucrose (55%) and ZnSO₄ & Na-cyclamate (86%) also significantly inhibited the bitterness of DB. There was no statistical difference in bitterness when zinc & sucrose (zinc inhibits sweetness), ZnSO₄, or sucrose were added to DB. The mixture of ZnSO₄ & Na-cyclamate (both oral peripheral and central cognitive bitterness inhibition strategies) 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) (Figure 6a&b). A mixture of ZnSO₄ & Na-cyclamate (33%) also inhibited the bitterness of PSE, however the mixture of ZnSO₄ & sucrose did not (zinc inhibits sweetness of sucrose, but not the bitterness of PSE). There was no statistical difference in bitterness when ZnSO₄ & Na-cyclamate, or sucrose, or Na-cyclamate was added to PSE.

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 applied, resulting in a level of significance of p<0.0014 (0.05/36=0.0014).

Pairwise t-tests showed that ZnSO₄ (81%) and a combination of DB & ZnSO₄ (98%) inhibited the sweetness of sucrose (p<0.0014) (Figure 5a&b). In addition, a combination of PSE & ZnSO₄ (94%) inhibited the sweetness of sucrose (p<0.0014) (Figure 6a&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) while it had no effect on the bitterness of other compounds (SOA, PSE, DEX) at the concentrations used. Since compounds of roughly equal bitter intensity differed markedly in their response to ZnSO₄, it is likely that zinc’s bitterness suppression occurred in the oral cavity. If, however, zinc ions had suppressed bitterness similarly across all equi-bitter compounds, it might have been due to a central cognitive effect (mixture suppression) of zinc’s taste/somatosensations. We believe that this suppression is due to the zinc ion. Evidence for this comes from the observation that MgSO₄ (sulfate anion) failed to significantly inhibit bitterness, ruling out a direct effect of the anion. We have also observed that other zinc salts inhibit bitterness as well (data not shown). In addition to suppressing bitterness, zinc ions are a potent inhibitor of sweetness {Keast, 2004 #1383}. Therefore, its practical utility as a flavor modifier of complex mixtures via bitterness inhibition would be confounded, as any masking of sweetness may cause additional flavor problems in foods and pharmaceutical formulations {Keast, 2003 #1013}. However, when zinc ions were combined with Na-cyclamate (the sweetener zinc ions do not inhibit), the excessive bitterness of DB was dramatically reduced (86%).

This combination of oral peripheral and central cognitive effects can be a potent inhibitor of bitterness.

Zinc ions as a tool to explore bitter taste transduction

The organization of the bitter taste system is complex with multiple putative receptor mechanisms. While 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 {Schiffman, 1999 #1379; Keast, 2003 #1013}. In this study we find that the bitterness of QHCl and DB is sensitive to zinc ions, while the bitterness of SOA, PSE and DEX was 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 (TAS2R’s), as zinc ions readily complex with amino acids and proteins and has a high affinity for both thiol and hydroxy groups {Christianson, 1991 #1464}. 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**

Zinc sulfate proved to be a potent bitterness inhibitor of specific compounds, yet the potential for zinc ions to perform a functional role as a bitterness inhibitor in foods or pharmaceuticals is minimized due to its effects on sweetness. Figure 6a shows that sucrose inhibits the bitterness of PSE, presumably through the cognitive phenomenon of mixture suppression {Kroeze, 1985 #301}. When ZnSO₄ is added to the PSE-sucrose mixture it inhibits the sweetness of sucrose but has no effect on the bitterness of PSE. The reduction in sweetness causes an enhancement of bitterness due to a release of bitterness from mixture suppression {Breslin, 1997 #101}. This is an example of how
Bitterness inhibition using zinc

addition of zinc ions to a food or pharmaceutical, whether for bitterness inhibiting properties or other functional or nutritional reasons, may result in a negative hedonic response by consumers.

Zinc ions and Na-cyclamate were the most effective combination at inhibiting the bitterness of DB (Figure 5b). The effect of zinc ions on the bitterness of DB is presumably at the cellular level in the oral periphery. Since zinc ions do not inhibit the sweetness of cyclamate {Keast, 2004 #1383}, the addition of Na-cyclamate’s sweet taste resulted in a central cognitive effect further reducing the bitterness of DB (mixture suppression). 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 Figure 5b 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.

Practical implications

Zinc is an essential nutrient (for review see {Brandao-Neto, 1995 #1005}) and zinc deficiency is one of the leading risk factors for morbidity and mortality (ranked 11th by the WHO) in developing regions of the world {Ezzati, 2002 #1006}. Zinc salts are added to a number of foods as nutritional supplements and to foods and oral care products for antimicrobial and anti-halotosis effects {Loesche, 2000 #980}, for functionality within a matrix {Ng, 1996 #982}, and for binding in dental-cements to support tooth structures {Pawlig, 2000 #983}. 
The addition of zinc ions to a food or oral care product will have negative effects on taste and flavor perception. Even though zinc ions may be used as a bitterness inhibitor for certain compounds, it also inhibits the sweetness of a wide variety of sweeteners. 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 appears more intense if the level of sweetness is increased, or a fruity aroma is reduced if the level of sweetness is reduced {Hornung, 1994 #1239}. The sweetness may be masking a bitterness that the zinc ions cannot inhibit and the loss of sweetness causes bitterness to be released from mixture suppression. 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 the potential taste and flavor problems can be managed if zinc ions are combined with Na-cyclamate.
Conclusion

Bitterness continues to be a problem for the pharmaceutical industry. We demonstrated that zinc sulfate differentially inhibits bitterness, and the effect is in the oral periphery rather than an effect of any zinc taste per se. Zinc salts are also potent inhibitors of sweetness of compounds, with the exception of Na-cyclamate. Therefore, zinc was mixed with Na-cyclamate; this approach combines two bitterness inhibition strategies; first the zinc ions inhibit bitterness in the oral periphery, and second the Na-cyclamate provides a sweet percept (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 bitterness of oral liquid formulations, providing zinc inhibits the bitterness of the active ingredient.


**Abbreviations**

gLMS = general Labeled Magnitude Scale  
di = deionized  
ZnSO$_4$ = zinc sulfate  
NaOAc = sodium acetate  
MgSO$_4$ = magnesium sulfate  
Mg(OAc)$_2$ = magnesium acetate  
QHCl = Quinine-HCl  
TET = Tetralone  
SOA = Sucrose octaacetate  
DEX = Dextromethorphan  
DB = Denatonium Benzoate  
PSE = Pseudoephedrine
Acknowledgments

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References
Tables

Table 1: Molarity of bitter compounds and salts used in experiment 1.

<table>
<thead>
<tr>
<th>Bitter Compound [concentration M]</th>
<th>Salt [concentration M]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Denatonium benzoate [1.4x10^{-8}M]</td>
<td>MgSO_4 [25mM]</td>
</tr>
<tr>
<td>Dextromethorphan [5.3x10^{-8}M]</td>
<td>Mg(OAc)_2 [25mM]</td>
</tr>
<tr>
<td>Pseudoephedrine [2.7x10^{-2}M]</td>
<td>NaOAc [25mM]</td>
</tr>
<tr>
<td>Tetralone [2.6x10^{-4}M]</td>
<td>NaOAc [300mM]</td>
</tr>
<tr>
<td>Sucrose octaacetate [4.1x10^{-4}M]</td>
<td>ZnSO_4 [25mM]</td>
</tr>
<tr>
<td>Quinine-HCl [6x10^{-4}M]</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Molarity of bitter compounds and sweeteners used in experiment 2.

<table>
<thead>
<tr>
<th>Bitter Compound [concentration M]</th>
<th>Sweet Compound [concentration M]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudoephedrine [2.7x10^{-2}M]</td>
<td>Sucrose [3x10^{-1}M]</td>
</tr>
<tr>
<td>Denatonium benzoate [1.4x10^{-8}M]</td>
<td>Na-cyclamate [1.2x10^{-2}M]</td>
</tr>
</tbody>
</table>
Bitterness inhibition using zinc

Figures

Figure 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. Numbers in each box is a represent a hypothetical intensity level. Equation A shows that the intensity of a bitter compound (10) and the intensity of sweet compound (10) are mutually suppressed when they are mixed together (both 8) (mixture suppression {Bartoshuk, 1975 #669}). Equations B & 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 as zinc may inhibit sweetness and not inhibit bitterness.

Figure 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 gLMS (general Labeled Magnitude Scale) 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: Sucrose octaacetate
(SOA) (4.1x10^{-4}M), Tetralone (2.6x10^{-4}M), Denatonium Benzoate (DB) (1.4x10^{-8}M),
Pseudoephedrine (Pseudo) (2.7x10^{-2}M), Dextromethorphan (Dextro) (5.3x10^{-3}M), and
Quinine-HCl (6x10^{-4}M). The salts were 25mM magnesium sulfate, 25mM magnesium
acetate, 25mM and 300mM sodium acetate, and 25mM zinc sulfate. Different letters
symbolize a statistically significant (p<0.001) difference in bitterness intensity. There
was no difference in bitterness intensity of the bitter compounds without added salts.

Figure 3 **Specific effects of salts on the bitterness of chemically diverse
compounds (pooled across bitter compound)**

Each bar represents the effect of the named salt (x-axis) on the pooled sweetness of six
chemically diverse bitter compounds. The x-axis lists the salts. The Y-axis represents
average bitterness rating (arithmetic mean ± standard error) on the gLMS for each salt
averaged across all six bitter compounds (Denatonium Benzoate (1.4x10^{-8}M),
Dextromethorphan (5.3x10^{-3}M), Pseudoephedrine (2.7x10^{-2}M), Tetralone (2.6x10^{-4}M),
SOA (4.1x10^{-4}M), and Quinine-HCl (6x10^{-4}M)). The right hand vertical axis lists the
verbal descriptors from the gLMS. The first bar is the average bitterness of all bitter
compounds without any added sodium salt. Abbreviations of sodium salts are:
magnesium sulfate (MgSO_{4}), magnesium acetate (Mg(OAc)_{2}), zinc sulfate (ZnSO_{4}),
sodium acetate (NaOAc). Different letters symbolize a statistically significant (p<0.0001) difference in bitterness intensity.

Figure 4  **The effect of 25mM 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 25mM 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 is the same as in Figure 1. ** indicates a significant difference (p<0.05) in bitter taste intensity between the bitter compound with and without zinc ions. Different letters symbolize a statistically significant (p<0.0001) difference in bitterness intensity between compounds when zinc sulfate had been added.

Figure 5a&b  **The influence of zinc sulfate on bitter and sweet taste of mixtures of denatonium benzoate & sucrose and denatonium benzoate & 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 is the same as in Figure 1. The concentration and abbreviation for the sweeteners was: Suc (sucrose) (300mM), and Cyc (Na-cyclamate) (12mM). Different letters a,b,c symbolize a statistically significant (p<0.0001) difference in bitterness intensity between compounds or mixtures, while letters z,y symbolize a statistically significant (p<0.0001) difference in sweetness between compounds or mixtures.

Figure 6 a&b  **The influence of zinc sulfate on bitter and sweet taste of mixtures of Pseudoephedrine & sucrose and Pseudoephedrine & Na-cyclamate**

The graph description is the same as Figure 5
Bitterness inhibition using zinc

Figure 1

A: Bitterness inhibition, central cognitive strategy

$$\begin{align*}
\text{Bitter} & \quad 10 \\
+ & \\
\text{Sweet} & \quad 10 \\
= & \\
\text{Bitter/Sweet} & \quad 8 / 8
\end{align*}$$

B: Bitterness inhibition, oral peripheral strategy

$$\begin{align*}
\text{Bitter} & \quad \text{DB} \ & \ 	ext{PSE} \ & \quad 10 \\
+ & \\
\text{Zinc ions} & \\
= & \\
\text{Bitter} & \quad \text{DB} \ & \quad 5 \\
or & \\
\text{Bitter} & \quad \text{PSE} \ & \quad 10
\end{align*}$$

C: Sweetness inhibition, Oral peripheral strategy

$$\begin{align*}
\text{Sweet} & \quad \text{Suc} \ & \ 	ext{Na-cyc} \ & \quad 10 \\
+ & \\
\text{Zinc ions} & \\
= & \\
\text{Sweet} & \quad \text{Sucrose} \ & \quad 2 \\
or & \\
\text{Sweet} & \quad \text{Na-cyc} \ & \quad 10
\end{align*}$$

D: Oral peripheral and central cognitive bitterness inhibition

$$\begin{align*}
\text{Bitter} & \quad \text{DB} \ & \quad 10 \\
+ & \\
\text{Zinc ions} & \\
+ & \\
\text{Sweet} & \quad \text{Na-cyc} \ & \quad 10 \\
= & \\
\text{Bitter/Sweet} & \quad 3 / 10
\end{align*}$$

E: Oral peripheral and central cognitive sweetness inhibition

$$\begin{align*}
\text{Bitter} & \quad \text{PSE} \ & \quad 10 \\
+ & \\
\text{Sweet} & \quad \text{Sucrose} \ & \quad 10 \\
+ & \\
\text{Zinc ions} & \\
= & \\
\text{Bitter/Sweet} & \quad 10 / 0
\end{align*}$$
Figure 2
Figure 3
Bitterness inhibition using zinc

Figure 4

![Graph showing bitter intensity for different substances.]

- **SOA**
- Tetralone
- DB
- Pseudo
- Dextro
- Quinine

Bitter intensity scale:
- Weak
- Moderate
- Strong

Significant differences indicated by:
- a
- b,c
- c,d
- d
- ****
Figure 5a
Figure 5b
Figure 6a
Figure 6b