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Suppression of Bitterness Using Sodium Salts

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Abstract: Bitterness is an ongoing taste problem for both the pharmaceutical and food industries. This paper reports on how salts (NaCl, NaAcetate, NaGluconate, LiCl, KCl) and bitter compounds (urea, quinine-HCl, caffeine, amiloride-HCl, magnesium sulfate, KCl) interact to influence bitter perception. Sodium salts differentially suppress bitterness of these compounds; for example urea bitterness was suppressed by over 70% by sodium salts, while $MgSO_4$ bitterness was not reduced. This study indicated that lithium ions had the same bitter suppressing ability as sodium ions, however the potassium cation had no bitter suppression ability. Changing the anion attached to the sodium did not affect bitter suppression, however, as the anion increased in size, perceived saltiness decreased. This indicates that sodium's mode of action is at the peripheral taste level, rather than a cognitive affect.

A second experiment revealed that suppressing bitterness with a sodium salt in a bitter/sweet mixture causes an increase in sweetness. This suggests adding salt to a food matrix will not only increase salt perception, but also potentiate flavor by differential suppression of undesirable tastes such as bitter, while increasing more desirable tastes such as sweet.

Keywords: Bitter suppression · Human psychophysics · Sodium · Sweet enhancement · Taste

1. Introduction

Excessive bitterness is the major taste problem facing both the pharmaceutical and food industries. Oral pharmaceuticals are frequently unpalatable and regarded as an unpleasant oral experience to the majority of the population, especially children who consume liquid formulations. The active component of the pharmaceuticals is often extremely bitter; therefore masking bitterness by traditional means of adding sugar and some aroma active components is difficult and only partially effective. The food industry is dealing with an increasing demand for healthier foods. This means inherently bitter components such as natural antioxidants, flavonoids, bitter salts (calcium) are added to foods. Both industries would benefit from the discovery of a universal bitter blocker.

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One difficulty in discovering a compound that will universally block bitterness is that many different classes of compounds impart bitterness: inorganic salts, amino acids, peptides, alkaloids, acetylated sugars, isohumulones, phenols and carbamates. It is quite possible that a substance that inhibits bitterness of one compound will not influence the bitterness of a second. Knowing how many different classes of compound elicit a bitter response, it is not surprising that recent studies [1] suggest the perception of bitterness is mediated by many different molecular receptors on taste cells. This provides one rationale for differential bitterness blocking effectiveness among different compounds [2].

A common method of blocking bitterness is encapsulation; this effectively stops the bitter component interacting with the bitter receptor, however, encapsulation of a bitter component is impractical in many situations. Another approach widely used for reducing bitterness in pharmaceuticals and foods is the introduction of compound(s) that act to block or suppress bitterness. Many such compounds have been reported, particularly in the patent literature, but their efficacy is often questionable. Many patented bitter blockers have introduced the sodium cation associated with a variety of anions into the product. We suspect the sodium cation is primarily responsible for any bitter reduction observed.

Sodium salts have been shown to be potent inhibitors of some bitter compounds [3–6]. The mechanism or mode of action of the sodium cation on bitter perception is not know however, research shows that sodium acts at the peripheral taste level rather than a cognitive effect [7][8]. There have been no systematic studies investigating the effectiveness of a large variety of sodium salts and the range of bitter compounds for which they act as effective blockers.

We have been investigating bitterness blocking by sodium salts for two principle reasons. First, they serve as a model system to investigate (i) mechanisms of bitter blocking and (ii) variations in efficacy of simple blocker on several compounds. Second, as ubiquitous food and flavor ingredients, sodium salts presumably act in many instances as bitter blockers, even if they have not been added in a conscious attempt to reduce bitterness. In what follows, we provide a summary of

recent work on bitterness inhibition by sodium salts. Most of the work described has been previously published [6][9].

2. Results

2.1. *Sodium Salts as Bitter Blockers*

The objective of this experiment was to assess how sodium salts influenced bitterness of compounds that may have different bitter receptor/transduction sequences. We also investigated the influence of anions and cation on bitterness suppression.

2.1.1. *Mixture of NaCl and Bitter-compounds*

Consistent with previous findings (see Introduction), bitter tasting compounds were suppressed by NaCl. However, the extent of that suppression differed among the bitter compounds. For example, NaCl suppressed the bitterness of urea by 76%, while the bitterness of $MgSO_4$ was suppressed by only 4%. These results are summarized in Fig. 1; statistical analyses can be found in Breslin and Beauchamp [6]. In most mixtures, saltiness was affected far less than bitterness.

2.1.2. *Effects of Anions and Cations*

To elucidate the respective bitter-suppressing roles of the anion and the cation in salts we held one ion constant and varied the other. Because urea was a compound whose bitterness was very effectively suppressed by NaCl, it was selected as the main bitter stimulus for this series of tests. Overall, we found that both sodium salts tested (NaAcetate and NaGluconate) were highly effective in suppressing the bitterness of urea (Fig. 2); NaAcetate also was very effective in suppressing the bitterness of quinine-HCl. LiCl suppressed bitterness of urea however, when the cation was changed to K^+, there was no evidence of bitter suppression (Figs 2 and 3).

2.2. *Sodium Salts Enhance Sweetness by Blocking Bitterness*

A widespread belief within the food industry that may potentially explain the popularity of sodium salts in foods is that they act as flavor potentiators (*i.e.* to increase the intensity of other desirable flavors) [10]. However, there is little evidence that this is so [4][11]. We hypothesized that rather than directly enhance a component(s) of food flavor, salts act to selectively suppress bitterness thereby enhancing favorable flavors such as sweetness. For example, in a mixture that is both bitter and sweet, bitterness and sweetness mutually suppress each other [8][12][13]. When a sodium-containing compound is added to the solution, we suggested that it may suppress the bitterness much more than the sweetness, thereby releasing the sweetness from suppression by bitterness. The resultant mixture would taste sweeter with salt.

We tested this hypothesis using a model aqueous system containing urea, sucrose, and NaAcetate [9]. Urea was selected as it is a bitter substance known to be strongly suppressed by sodium-containing compounds [6], while NaAcetate was selected because it does not have as strong a salt taste as does NaCl [6][14], thereby permitting a test of the flavor modifying effects of the sodium ion without a strong perceived saltiness. We hypothesized that if NaAcetate was added to a mixture containing urea and sucrose, the bitterness of urea would be suppressed more than the sweetness of sucrose. Therefore the resulting three-component mixture would have a heightened sweetness. Results consistent with this prediction would lend support to the argument that sodium-containing compounds, including NaCl, tend to potentiate food flavors by differentially suppressing flavor components.

There was a selective suppression of one of the two taste components by NaAcetate. The sucrose-urea mixtures, without NaAcetate, were relatively more bitter and less sweet than when NaAcetate was added. Therefore, the bitterness of urea was suppressed more than was the sweetness of sucrose (Fig. 4). Moreover, at the higher concentrations of sucrose (0.3, 0.5M) and urea (0.5, 1.0M), the absolute sweetness intensity was increased by adding either 0.1 or 0.3M of NaAcetate compared to when no NaAcetate was added. We postulate that this occurred as a result of releasing sweetness from suppression by decreasing the bitterness of urea. To further support the hypothesis, the addition of NaAcetate to sucrose in the absence of urea did not enhance sweetness. This is consistent with the literature that demonstrates that NaCl and sweeteners do not enhance one another, except at very weak NaCl concentrations [13][15].

3. Discussion

3.1. *Bitter Suppression*

We found that sodium salts are effective at suppressing bitterness, which is consistent with previous research [3][7][8][16]. However, the degree of average bitterness suppression conferred by the sodium salts varied widely across bitter substances. For example, the sodium salts substantially suppressed the bitterness of KCl, urea and amiloride, whereas sodium was less effective at suppressing the bitterness of quinine-HCl and caffeine. Sodium's differential suppression of quinine-HCl and urea bitterness was consistent with the previous research indicating that quinine-HCl and urea elicit bitter sensations through different taste receptor cells or through different transduction sequences on the same cells [17].

Results of this study (see also Breslin and Beauchamp [6] for more detailed discussion) suggest that the bitter-suppressing effect of the sodium ion is due to its chemical properties acting in the periphery, rather than its taste properties acting centrally. The active component in the bitterness suppression of urea is the sodium ion, independent of the anion and the perceived saltiness of the salt (Fig. 5). This conclusion is consistent with the results of several previous studies with quinine-HCl that also support the hypothesis that the suppression of bitterness by NaCl has a peripheral component [7][8].

If, as we suggest, the suppression of several bitter compounds by salts is a peripheral phenomenon, how does sodium interact with the bitter transduction mechanism(s) to block bitter perception? Fig. 6 shows possible mechanisms or sites of action with which sodium may interact. First, sodium may have influence over certain G-protein coupled receptors; sodium may form an ionic shield around parts of the protein, which diminishes the receptor affinity for the bitter compound or slightly alters protein folding, thereby diminishing affinity for the bitter compound. Second, sodium may modulate various ion channels/pumps involved in the taste transduction sequence. Third, sodium may act to stabilize the cellular membrane, thereby limiting access of lipophilic bitter compounds to receptor sites imbedded in the membrane, or limiting direct access of those bitter compounds through the membrane to intracellular pathways. Last, sodium may interfere with specific second messenger systems (G-proteins or enzymes) responsible for bitter taste transduction from inside the cell.

What influence do other cations have on bitter perception and are there any anions that increase the bitter suppression efficacy of sodium? Currently we do not know the answer to this question, but re-

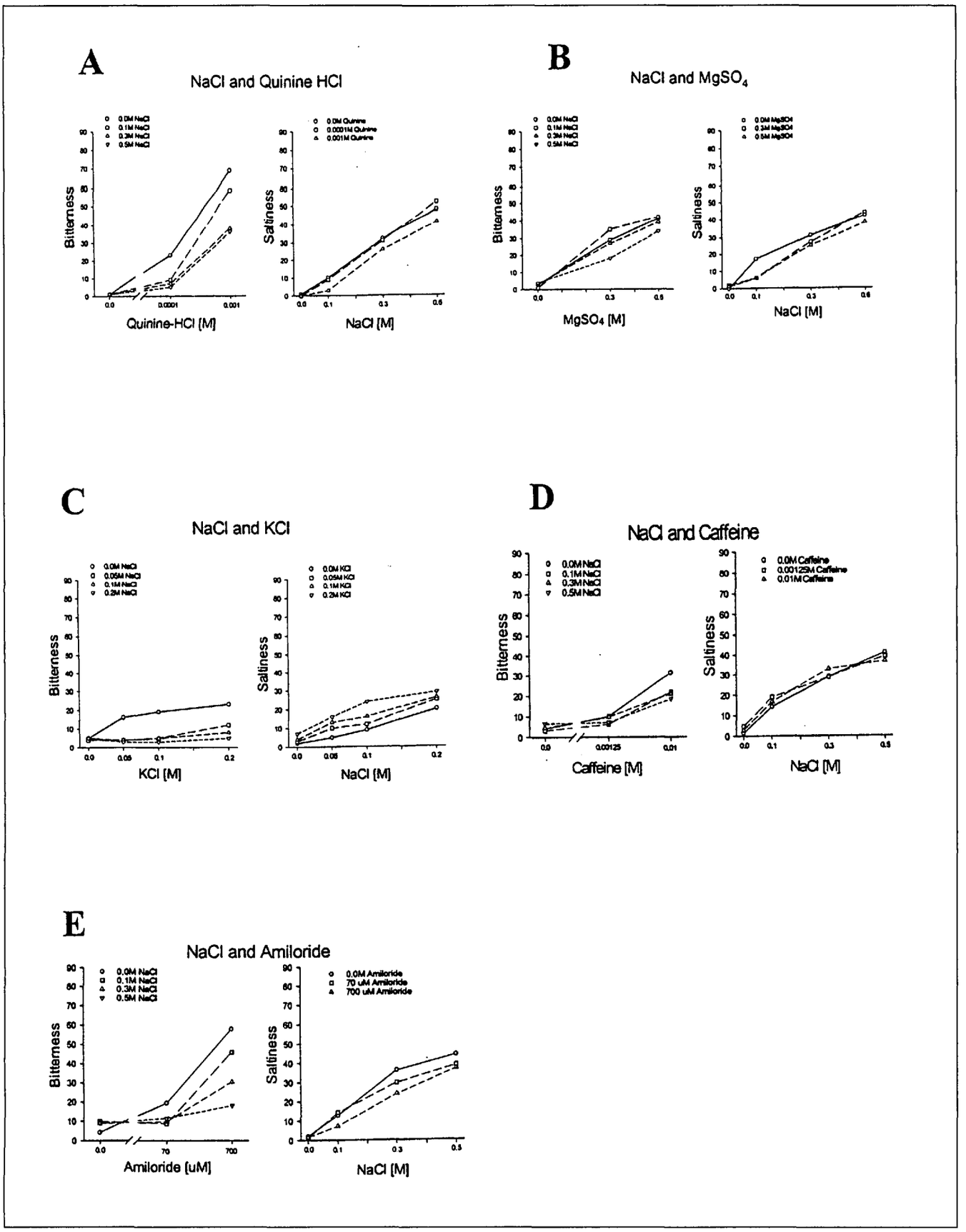


Fig. 1. Graphs A–E depict the salt–bitter mixture interactions for NaCl and quinine-HCl, NaCl and $MgSO_4$, NaCl and KCl, NaCl and caffeine, and NaCl and amiloride-HCl, respectively. The left hand column of panels shows the bitterness ratings for each study. The addition of varying amounts of NaCl to each level of the bitter compound is depicted by a separate curve for each sequential amount of NaCl that was added. The right hand column of panels shows the saltiness ratings for each study. The addition of varying amounts of bitter compound to each level of NaCl is depicted by a separate curve for each sequential amount of bitter compound that was added.

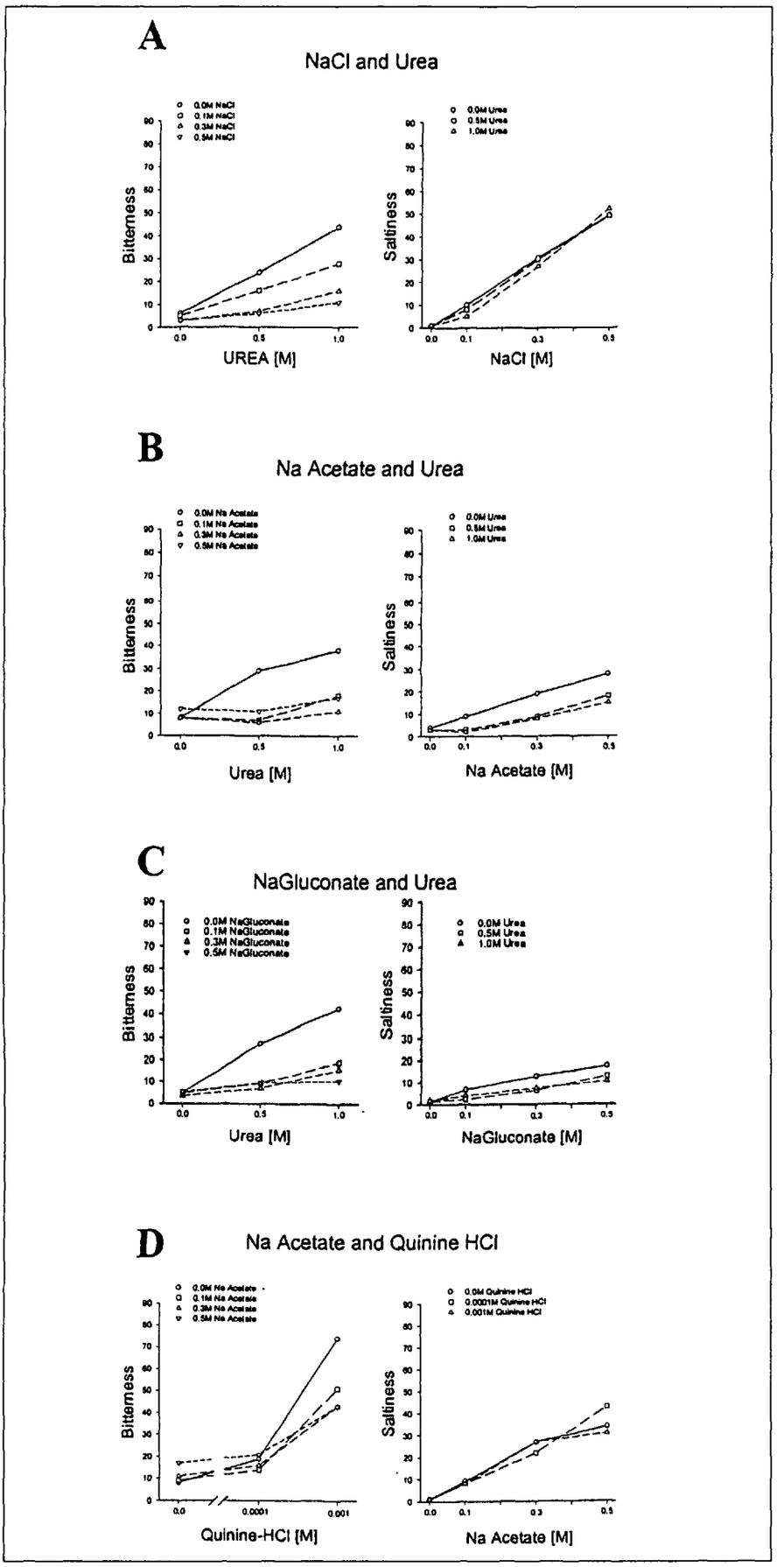


Fig. 2. Graphs A–D depict the salt-bitter mixture interactions for NaCl and urea, NaAcetate and urea, NaGluconate and urea, and NaAcetate and quinine-HCl, respectively. See the caption to Fig. 1 for more details.

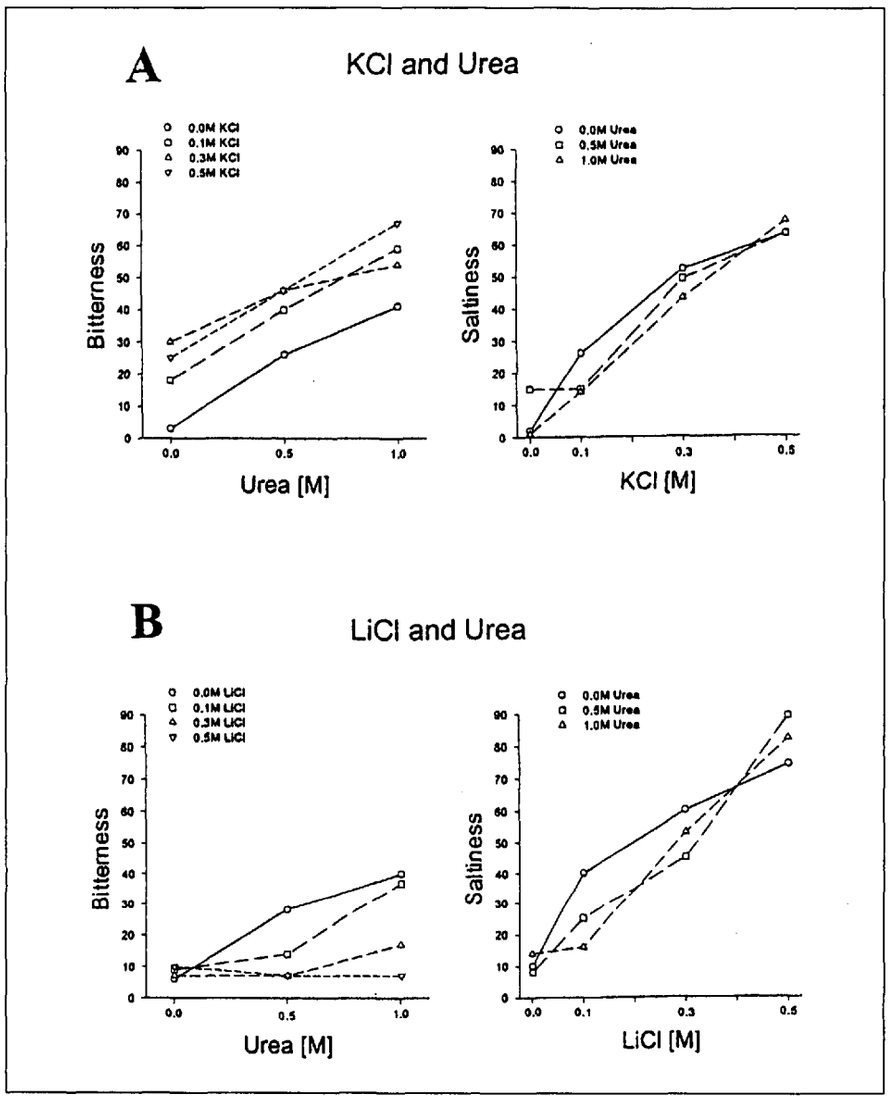
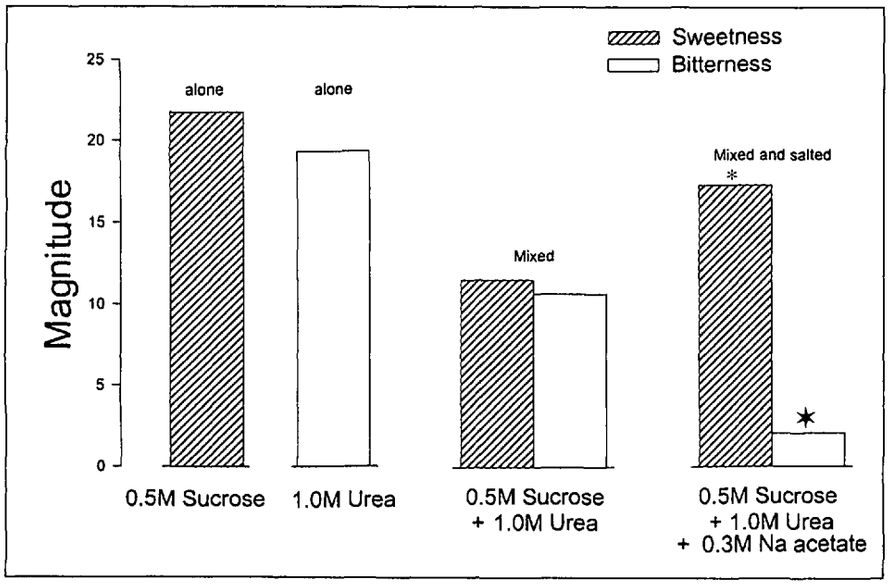


Fig. 3. Graphs A and B depict the salt-bitter mixture interactions for KCl and urea and LiCl and urea, respectively. See the caption to Fig. 1 for more details.



search is underway at our laboratory to determine if a variety of other cations and anions have bitter inhibition properties.

As we come to understand the bitter transduction mechanism(s) [18–20] we will begin to develop more specific hypotheses that can be tested.

3.2. *Sweet Enhancement*

Results of Experiment 2 demonstrated that by suppressing bitterness in a bitter-sweet mixture, salt enhances sweetness. These data support the hypothesis that a key role of salts in foods – in addition to adding desired saltiness – is to potentiate flavors [21] through differential suppression of bitter tastes and the release from suppression of palatable tastes, such as sweetness. People's desire for NaCl and other salts in foods as diverse as (often bitter) vegetables, oily foods and meats may be due in part to their ability to suppress off-flavors [18]. If so, this hypothesis would help explain one of the major reasons why it is so difficult to make low-sodium foods acceptable: not only are they lacking a desirable salty taste, but also off-flavors are more prominent than if sodium were present.

On a practical level, biophysical evidence [18–20] suggests that it may be extremely difficult or impossible to develop a salty-tasting substitute for salt that contains no sodium. Non-sodium substances would have to duplicate the differential flavor-suppressing effect of sodium salts, at the same time that saltiness was enhanced. In the search for salt substitutes it would be wise to take into account the multiple sensory functions of sodium in foods.

3.3. *Final General Comments on Bitter Blocking*

Excess bitterness can be a problem in pharmaceutical products that must be consumed orally and in some foods and beverages. The studies described here have clearly demonstrated that sodium salts are able to decrease the bitterness of

Fig. 4. The standardized reported magnitude of the taste of various solution mixtures is shown. The intensity of urea and sucrose at the highest concentrations were roughly the same (left). Statistical analysis revealed that in mixtures, the highest concentration of sucrose and urea (without NaAcetate) mutually and roughly equally suppressed their intensities (center). When NaAcetate was added, also at the highest concentration, intensity of bitterness greatly decreased, whereas sweetness intensity increase to levels that approximated the sweetness in pure deionised water. Asterisk denotes increase ($P<0.0001$) and star denotes decrease ($P<0.0001$) (reprinted with permission from *Nature*).

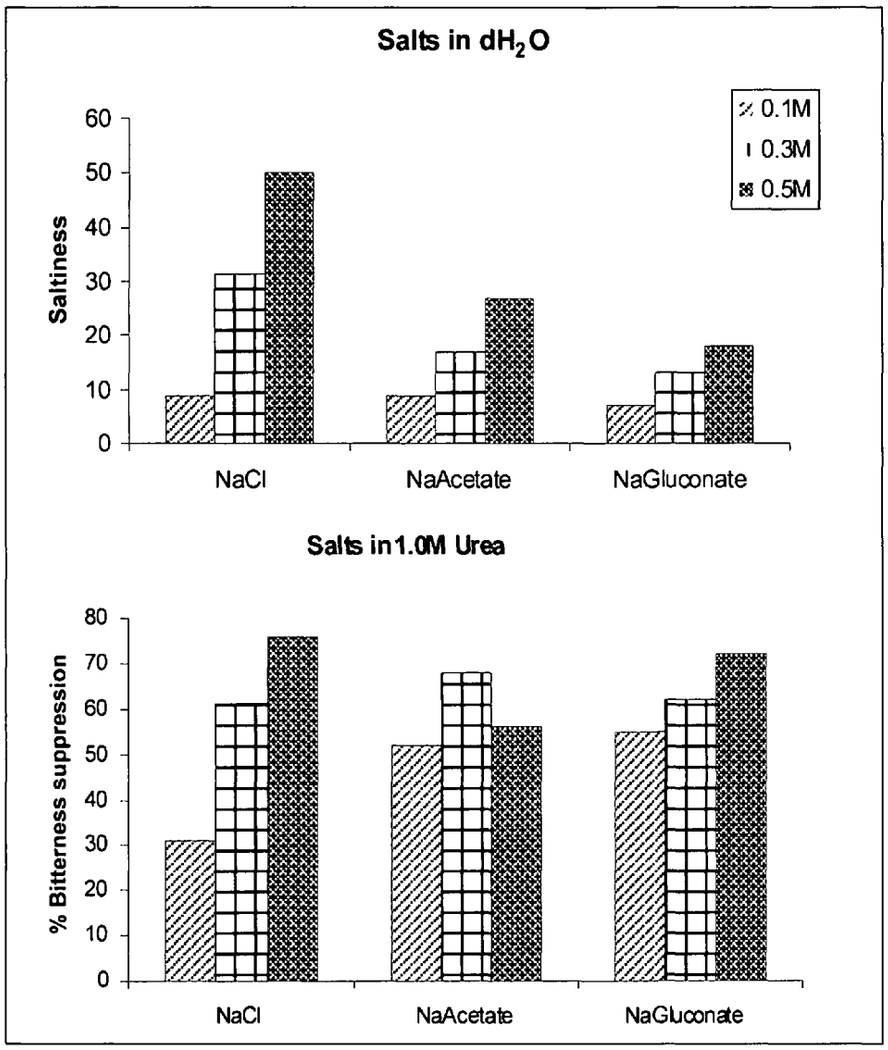


Fig. 5. The top panel shows the mean standardized saltiness ratings of 0.1, 0.3, and 0.5M NaCl, NaAcetate, and NaGluconate. The bottom panel depicts the percent suppression of the bitterness of 1.0M urea by these three salts, each at three concentrations.

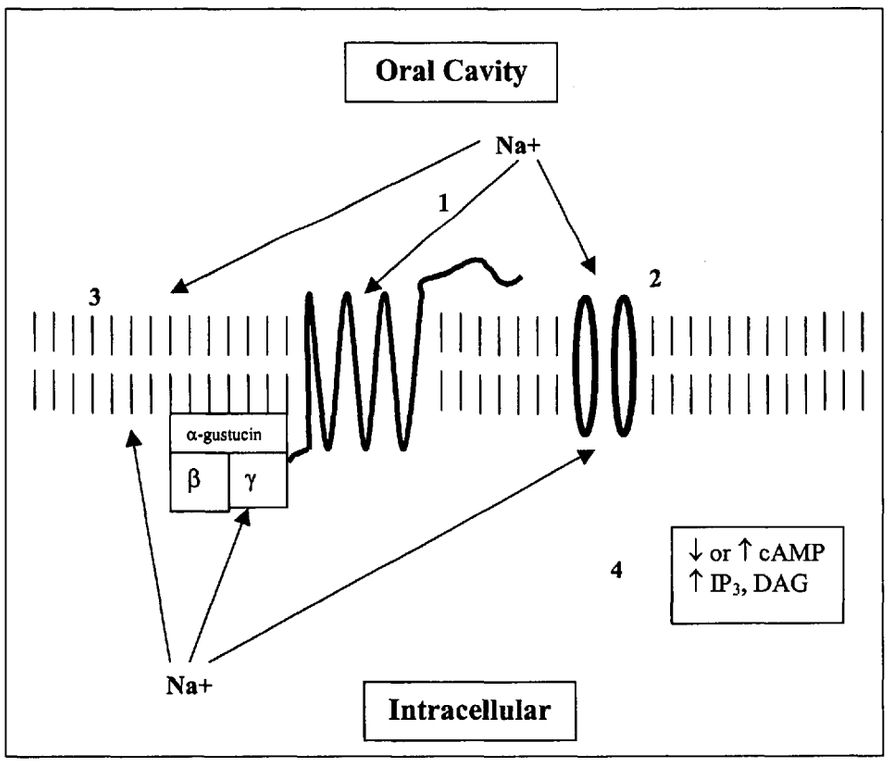


Fig. 6. Schematic diagram of potential sites of action of the sodium cation in bitter taste transduction. **1** G-protein coupled receptor. **2** Ion channels / pumps. **3** Membrane stabilization. **4** Na passing into the cell and affecting 2nd messenger systems (cAMP – cyclic adenosine monophosphate; IP_3 – inositol triphosphate; DAG – diacylglycerol)

many, but not all bitter compounds. It is highly likely that other bitter blocking compounds exist; indeed, other blocking compounds have previously been identified [22].

An important lesson from this work on sodium is that there will probably be no silver bullet – a compound that blocks bitterness perception of all bitter compounds. Thus in the future investigations will need to tailor specific bitter inhibitors to the bitter compound(s) of interest. As we learn more about the mechanisms of bitter perception, it should be possible to rationally identify or design specific blockers for specific uses. Also, use of receptor-based high throughput screening systems may make possible rapid discovery of, as yet unknown, candidate bitter-blocking compounds. Formulating additives to reduce off-flavors and bitter flavors in foods, pharmaceuticals and beverages should move from the flavorists' art to controlled parametric experimental protocols.

4. Experimental

4.1. *Bitter Suppression*

4.1.1. *Subjects*

Subjects were paid to participate in studies after giving their informed consent. Some subjects participated in more than one study.

4.1.2. *Stimuli*

A variety of bitter agents and salts were used. All solutions were prepared with deionized water. Solutions were stored at 5 °C in a dark cold-room and were replaced at least every two weeks. Prior to testing, the stimuli were brought to room temperature with the aid of a water bath.

4.1.3. *Intensity Matching*

In order to accurately assess the influence of sodium salts on bitter intensity, we wanted to ensure that the bitter intensity of the various stimuli were similar. A pretest was performed with 20 subjects and concentrations for the bitter stimuli in each series were selected so that perceived bitter intensities matched that of 0.1 and 1mM quinine-HCl. The exception was KCl, which was prohibitively salty when matched to quinine-HCl for bitterness. The matching procedure has been described previously [6].

4.1.4. *Procedure*

In each study (except for the KCl–NaCl mixtures) judgments of the bitterness and saltiness of all possible combinations of three or four concentrations of a bitter compound and four concentrations of a salt were evaluated. The matrix design included bitter compounds and salts without addition and deionized water as a control. Magnitude estimation was used to obtain ratings of the perceived intensities of saltiness and bitterness. Subjects were instructed to rate only the saltiness and the bitterness of each solution and to ignore any other qualities.

For each bitter-salt mixture series each solution was sampled twice. Subjects rinsed and expectorated with deionized water four times over a period of roughly 2 min prior to testing. The solutions were presented in random order, without replacement. Duplicate ratings for bitter and salty were averaged to yield single ratings of saltiness and bitterness. Subjects were required to rinse twice thoroughly with deionized water during the 60 sec interstimulus interval. All samples were delivered in 10 ml volumes in polystyrene medicine cups.

4.2. *Sweet Enhancement*

The methods were similar to those described for Experiment 1 with the exception that a NaAcetate was added to a bitter solution, a sweet solution, and a mixed bitter and sweet solution. Subjects were asked to taste every possible trinary mixture solution twice and to rate each for sweetness, bitterness, and otherness, where otherness constitutes the intensity of all gustatory sensations other than sweet and bitter, using the method of magnitude estimation.

4.3. *Standardization of Data and Analyses*

Data for each study were analyzed separately using a two-way within-subjects analysis of variance (ANOVA) [*Concentration* (3 or 4 steps) X *Added Compound* (3 or 4 levels)]. The two measurements of quality (saltiness and bitterness) were also analyzed separately. When interaction effects were obtained, one-way ANOVAs were performed on the different levels of the mixture for each concentration step. Percent suppression of bitterness was calculated by dividing the bitterness of the bitter-salt mixture by the bitterness of unmixed bitter compound concentration and then subtracting this value from 1, and multiplying by 100.

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