Suppression of Bitterness by Sodium: Variation Among Bitter Taste Stimuli

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Abstract

Taste interactions between salts (NaCl, LiCl, KCl, L-arginine:L-aspartic acid, Na-acetate and Na-gluconate) and bitter-tasting compounds (urea, quinine HCl, magnesium sulphate, KCl, amiloride HCl and caffeine) were investigated. In each study binary combinations of three or four concentrations of one bitter compound with four concentrations (0, 0.1, 0.3 and 0.5 M) of one salt were rated for bitterness and saltiness using the method of magnitude estimation. In most cases, perceived bitterness was suppressed by salts, although the degree of suppression varied. In general, bitterness suppression was not accompanied by an equivalent reciprocal suppression of saltiness. Only $MgSO_4$ and amiloride had suppressing effects on the saltiness of NaCl at the intermediate concentrations and no bitter compound affected the saltiness at the high concentrations of NaCl. Since salt suppressed the bitterness of urea effectively, a detailed analysis of suppression of the bitterness of urea by different salts was conducted. Those studies indicated that the key component in this effect was the sodium or lithium ion for two reasons: first, all three sodium salts and the lithium salt had a suppressive effect on bitterness, whereas KCl did not; secondly, the effect of a salt on suppression of the bitterness of urea was independent of its perceived saltiness; that is, NaCl, Na-acetate (which is perceived as less salty than NaCl), and Na-gluconate (which is perceived as less salty than Na-acetate) reduced bitterness comparably. These results suggest that there is a major peripheral component to the suppression of the bitterness of urea, and perhaps other bitter tasting compounds, by sodium. Chem. Senses 20: 609–623, 1995.

Introduction

When two compounds that elicit different taste qualities are mixed in solution, the mixture will often yield a taste sensation that is less intense than the simple sum of the component tastes. In two-component mixtures, each taste quality is usually perceived as less intense than when it is tasted separately (Kamen *et al.*, 1961; Bartoshuk, 1975; McBride, 1989; Kemp and Beauchamp, 1994). However, binary combinations of certain taste stimuli may result in asymmetrical changes. For example, Schifferstein and Frijters (1992a) recently reported that when quinine hydrochloride (QHCl), which usually elicits a bitter taste, is mixed in solution with sodium chloride (NaCl), which usually elicits a salty taste, the saltiness of the NaCl is relatively unaffected, while the bitterness of QHCl is suppressed from 50 to 70% (see also Frijters and Schifferstein, 1994), depending upon the concentrations involved (cf. Kroeze, 1982; Kemp and Beauchamp, 1994).

It is not known how NaCl decreases the bitterness elicited by QHCl, although there is evidence that the decrease occurs peripherally (Bartoshuk, 1979, 1980; Bartoshuk and Seibyl, 1982; Kroeze and Bartoshuk, 1985). Also unknown is whether the taste profile of other bitter-salty mixtures would

exhibit the same characteristics. There is growing evidence that there are multiple transduction pathways associated with bitter taste and that a single compound, such as QHCl, does not stimulate all of them equally (see below for references). For example, a single transduction process is believed to account for the wide variation in sensitivity across individuals to the bitter compounds propylthiouracil (PROP) and phenylthiocarbamide (PTC) which varies independently of quinine's bitterness in the same subjects (Fischer and Griffin, 1963; Mela, 1989; Schifferstein and Frijters, 1991). McBurney *et al.* (1972; McBurney, 1969) have also reported asymmetrical cross-adaptation among bitter compounds, a result consistent with multiple bitter transduction sequences. Yokomukai *et al.* (1993) and Cowart *et al.* (1994) have obtained data consistent with those of McBurney *et al.* (1972; see also Lawless, 1979). Taken together, these studies suggest that there may be at least three classes of bitter transduction sequences in humans, one implicated in the transduction of PTC- and PROP-like compounds, one sensitive to quinine- and caffeine-like compounds, and one sensitive to urea- and magnesium sulphate-($MgSO_4$)-like compounds.

The goal of Experiment 1 of this paper was to compare the interactions of NaCl with several bitter-eliciting compounds that may have different transduction sequences. In addition, more detailed studies were conducted with urea, a bitter compound that was effectively suppressed by NaCl. Specifically, Experiments 2 and 3 explored the effects of anion and cation substitution on bitter suppression, respectively.

Materials and methods

Subjects

Subjects between the ages of 21 and 30 were paid to participate in 12 studies after giving their informed consent. All were employees of the Monell Center. The number of subjects (12–27) in each study is given in Table 1; some subjects participated in more than one study. Each subject was coded with a random number.

Stimuli

The bitter agents and salts that were used are listed in Table 1. All solutions were prepared with deionized water. Solutions were kept at 5°C in a dark cold-room and were replaced at least every 2 weeks. Prior to testing, the stimuli were brought to room temperature with the aid of a water bath.

Intensity matching

The range of concentrations for the bitter stimuli in each series was selected so that perceived bitter intensities were matched across series, as determined by pretesting, except for potassium chloride (KCl) which would be prohibitively salty when matched to quinine for bitterness.

Table 1 The experimental design of all 12 studies in Experiments 1, 2 and 3

Experiment	No. subjects	Bitter compound (concentrations)	Salt (concentrations)
1A	21	QHCl (0, 0.1, 1 mM) S *	NaCl (0, 0.1, 0 3, 0.5 M) F
1B	18	Caffeine (0, 1.25, 10 mM) S	NaCl (0, 0.1, 0.3, 0 5 M) F
1C	12	MgSO4 (0, 0 3, 0.5 M) S	NaCl (0, 0.1, 0.3, 0.5 M) F
1D	15	Amiloride (0, 70, 100 μM) F	NaCl (0, 0.1, 0.3, 0.5 M) F
1E	27	KCl (0, 0.05, 0.10, 0.20 M) F	NaCl (0, 0.05, 0.10, 0.20 M) F
1F	20	UREA (0, 0.5, 1.0 M) F	NaCl (0, 0.1, 0.3, 0.5 M) F
2A	14	UREA (0, 0.5, 1.0 M) F	SA (0, 0.1, 0.3, 0.5 M) S
2B	15	UREA (0, 0.5, 1.0 M) F	SG (0, 0.1, 0.3, 0.5 M) A
2C	14	QHCl (0, 0.1, 1 mM) S	SA (0, 0.1, 0.3, 0.5 M) S
3A	14	UREA (0, 0.5, 1.0 M) F	KCl (0, 0.1, 0.3, 0.5 M) F
3B	12	UREA (0, 0.5, 1.0 M) F	LiCl (0, 0.1, 0.3, 0.5 M) F
3C	12	UREA (0, 0 5, 1.0 M) F	LALA (0, 0.1, 0.3, 0 5 M) S

*Abbreviations: QHCl = quinine hydrochloride; LALA = L-arginine-L-aspartate; SA = sodium acetate, SG = sodium gluconate; F = Fisher Co.; S = Sigma Co.; A = Aldrich Co.

The matching procedure was as follows. Two concentrations of quinine hydrochloride (QHCl), 0.1 and 1.0 mM, were selected as the medium and high levels of bitter sensation, and all other bitter compound concentrations were selected to match these two in bitterness (except for KCl). Twenty individuals served as subjects and were run individually. To match the medium level of bitterness, each subject was presented with four pairs of solutions. One member of each pair was the medium level of QHCl and the other was a concentration of a different compound thought by the experimenter to appear close to the same level of bitterness. After sampling from both cups, a subject was asked to identify which one had the more bitter solution. This continued for all four pairs of cups. Subjects were instructed to rinse their mouth four times with room temperature de-ionized water before testing and twice between each sampling. If several (about five) subjects perceived either the QHCl or the other bitter compound as consistently more bitter on all four trials, then the concentration of the other bitter compound was adjusted appropriately and the testing restarted. When all five subjects did not select one of the compounds on four out of four trials, then all twenty subjects were tested with the four-trial procedure. A tally was kept of how many times each compound had been selected as more bitter. If either the QHCl or the other bitter compound was selected, on average, as more bitter 55% of the time or more by the 20 subjects, then the entire procedure was repeated with a new test concentration. The next test concentration was either half or double the former (as needed), and subsequent steps were halfway between those two steps again moving up or down as needed. When neither compound was perceived as more bitter on more than 55% of the trials, the two compounds were considered to be matched on average for bitter intensity, since binomial variance dictates that a two alternative procedure with 55% selection and 80 trials has a standard error of ±5.6 percentage points. The same procedure was used to match the higher QHCl concentration, 1.0 mM.

These intensity matches were obtained from twenty subjects and were an average response across the whole sample population. Therefore, each individual subject may have found that any pair of solutions for the two compounds were not matched in intensity, when, on the average, all 20 subjects found the two solutions comparable in bitterness intensity (see e.g. Yokomukai *et al.*, 1993; Cowart *et al.*, 1994). Matches were not calculated at the individual level as this would be prohibitively time consuming when trying to intensity match six different compounds for each subject.

Procedure

Each study consisted of judgments of the bitterness and saltiness of all possible combinations of three or four concentrations of a bitter compound with four concentrations of a salt, which resulted in a $3\times4 = 12$ solution matrix for all but KCl-NaCl mixtures, which were tested in a $4\times4 = 16$ solution matrix. In every case, one concentration of the bitter agent and the salt was 0.0 M.

The method of magnitude estimation was used to obtain ratings of the perceived intensities of saltiness and bitterness from every solution sampled. All subjects were familiar with the method and no modulus was given. Subjects were instructed to rate only the saltiness and the bitterness of each solution, and to ignore any other qualities.

Within any bitter-salt mixture series (containing only one salt and one bitter agent), each solution ($n = 12$ or $n = 16$) was sampled twice. Subjects were instructed to rinse and expectorate with deionized water four times over a period of roughly 2 min prior to testing. The solutions were presented in random order, without replacement. The two salty ratings and the two bitter ratings for each solution were arithmetically averaged to yield single ratings of saltiness and bitterness. Subjects were required to rinse twice thoroughly with deionized water during the approximate 60-s interstimulus interval. All samples were delivered in 10-ml volumes in polystyrene medicine cups.

Standardization of data

Experiment 1

To eliminate the variance produced by idiosyncratic number usage in the magnitude estimation task, the saltiness and bitterness ratings were standardized to the grand arithmetic mean of the saltiness ratings of NaCl in water for all subjects in all studies involving the use of NaCl. Each subject's individual mean saltiness rating was divided into the grand mean and the quotient was used as the multiplicative standardization factor for that individual's saltiness and bitterness ratings. This procedure equated mean ratings of salt in water across subjects while maintaining the individual relations between saltiness and bitterness for each subject.

Experiments 2 and 3

Because only one study in Experiments 2 and 3 included NaCl, whereas urea was used in all but one study (QHCl and Na-acetate, discussed below), the data from Experiments 2 and 3 were standardized to the urea data shown in Figure 3a (urea and NaCl). Each subject's individual mean urea

bitterness rating from each study was divided into the grand arithmetic mean for the bitterness of urea in water without salt. The resulting multiplicative standardization factor was used for both the saltiness ratings and the bitterness ratings. In the study depicted in Figure 3d (QHCl and Na-acetate) neither NaCl nor urea were employed. Since QHCl was employed in both Studies 3d and 1a (QHCl and NaCl), the bitterness ratings of QHCl in water from data shown in Figure 1a were used to standardize the data from Figure 3d using a method parallel to that described above.

Analysis

Since the distributions of standardized saltiness and bitterness ratings in each study were skewed in a manner that approximated a log-normal distribution, the data were transformed to logs before statistical analysis. Because there were frequent reports of either zero saltiness or bitterness, 1.0 was added to all ratings prior to transformation. This addition of 1.0 to all ratings has a larger impact on smaller numbers in log co-ordinates potentially resulting in less apparent suppression of bitterness at weaker bitter agent concentrations. However, in practice this was not the case, as weak concentrations were always affected more than the stronger bitter concentrations (see Results and Figures below).

Data from each study were analysed separately using a 2-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 analysed separately. When interaction effects were obtained, one-way ANOVAs were performed on the different levels of the mixture for each concentration step. Because we viewed water as belonging to the concentration continuum, some interaction effects may be due to the subjects' perception of bitterness of the salt in water or residual bitterness sensations reported when water was presented. All pairwise comparisons were performed with the Tukey HSD method. Different compounds were not directly statistically compared since tests of each compound were conducted neither on exactly the same subjects nor on a completely different set of subjects. To provide some basis for comparisons among compounds, however, the arithmetic mean percentage suppressions of the two bitter concentrations was presented in Figures 2, 4 and 6 with standard errors. Percentage 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 = {1 − [(bitter and salt)/ unmixed bitter]} for each subject at each concentration and then taking the arithmetic mean ± SEM (note that this calculation does not take into account baseline bitterness levels of water or unmixed salt).

There was never a significant suppression of saltiness for the highest concentration of NaCl. Therefore, percentage suppressions were not calculated for the saltiness of NaCl.

Results

All main effects of mixture and interaction effects for all experiments are presented in Table 2. Because in all cases saltiness and bitterness varied directly with concentration ($P < 0.01$), the main effect of concentration has been omitted from the table. Pairwise comparisons (Tukey HSD) with significant differences are indicated in the figures with asterisks (see Figures 1, 3 and 5).

Experiment 1: mixture of NaCl and various bitter compounds

Overview

Consistent with others (see Introduction), we found that most, but not all, of the bitter tasting compounds were suppressed by NaCl. The extent of that suppression differed among the bitter compounds, even though they had been, on average, matched for intensity. For example, NaCl suppressed the bitterness of urea 76 ± 6% (mean ± SE), but only suppressed the bitterness of $MgSO_4$ 4 ± 26%. These differences appear small in a logarithmic plot and so accompanying figures of the percent suppressions were included (Figures 2, 4 and 6). In most mixtures, saltiness was affected less than bitterness. The specific results for each bitter compound are presented below; statistical results are summarized in Table 2.

Quinine HCl (Figures 1a and 2a)

Bitterness: NaCl significantly suppressed the bitterness of quinine HCl, suppressing 41 ± 11% of the maximum bitterness sensation. The bitterness of 10^{-4} M QHCl was suppressed by the addition of all concentrations of NaCl, whereas the bitterness of 10^{-3} M QHCl was suppressed only by 0.3 and 0.5 M NaCl.

Saltiness: Only the addition of the highest QHCl concentration (10^{-3} M) suppressed the saltiness of the 0.1 M NaCl solution.

Table 2 *The summary of statistics for main effects and interactions: summary of statistical results**

	Test solutions	Bitterness main effect	Bitterness interaction	Saltiness main effect	Saltiness interaction
Experiment 1					
A	NaCl and quinine-HCl	$F(3,60)=3.91$, $P < 0.05$	$F(6,120)=9.47$, $P < 0.0001$	$F(2,40)=5.22$, $P < 0.01$	$F(6,120)=7.83$, $P < 0.0001$
B	NaCl and caffeine	$F(3,42)=3.79$, $P < 0.05$	$F(6,84)=8.00$, $P < 0.0001$	$F(2,28)=0.966$	$F(6,84)=6.29$, $P < 0.0001$
C	NaCl and $MgSO_4$	$F(3,33)=2.49$	$F(6,66)=4.09$, $P < 0.01$	$F(2,22)=5.71$, $P < 0.01$	$F(6,66)=14.22$, $P < 0.0001$
D	NaCl and amiloride	$F(3,42)=3\,00$, $P < 0.05$	$F(6,84)=7.90$, $P < 0.0001$	$F(2,28)=9.80$, $P < 0.001$	$F(6,84)=3.84$, $P < 0.01$
E	NaCl and KCl	$F(3,78)=51.24$, $P < 0.0001$	$F(9,234)=11.39$, $P < 0.0001$	$F(2,28)=9.80$, $P < 0\,001$	$F(9,234)=4.73$, $P < 0.0001$
F	Urea and NaCl	$F(3,57)=45.96$, $P < 0\,0001$	$F(6,114)=6.48$, $P < 0.0001$	$F(2,38)=5.60$, $P < 0.01$	$F(6,114)=4.95$, $P < 0.001$
Experiment 2					
A	Urea and Na-acetate	$F(3,39)=17.16$, $P < 0.0001$	$F(6,78)=3.79$, $P < 0.01$	$F(2,26)=17.66$, $P < 0.0001$	$F(6,78)=3.13$, $P < 0.01$
B	Urea and Na-gluconate	$F(3,42)=22.09$, $P < 0.0001$	$F(6,84)=4.73$, $P < 0.001$	$F(2,28)=11.33$, $P < 0.001$	$F(6,84)=5.68$, $P < 0.0001$
C	Quinine HCl and Na-acetate	$F(3,39)=0.60$	$F(6,78)=4.30$, $P < 0.001$	$F(2,26)=0.49$	$F(6,78)=1.29$
Experiment 3					
A	Urea and KCl	$F(3,39)=18.38$, $P < 0.0001$	$F(6,78)=10.85$, $P < 0.0001$	$F(2,26)=1.02$	$F(6,78)=2.13$
B	Urea and LiCl	$F(3,33)=11.58$, $P < 0.0001$	$F(6,66)=10.03$, $P < 0.0001$	$F(2,22)=1.15$	$F(6,66)=2.28$, $P < 0.05$
C	Urea and L-arginine-L-aspartate	$F(3,33)=5.13$, $P < 0.01$	$F(6,66)=6.33$, $P < 0.0001$	$F(2,22)=1.58$	$F(6,66)=2.28$, $P < 0.05$

*Pairwise Tukey HSD statistics (see Method) with significant results are shown in figures.

Caffeine (Figures 1b and 2b)

Bitterness: The 0.3 and 0.5 M NaCl concentrations significantly suppressed the bitterness of caffeine. NaCl was able to suppress 55 ± 6% of the maximum bitterness sensation.

Saltiness: Only the addition of the highest caffeine concentration (18 mM) suppressed the saltiness of the weakest NaCl concentration (0.1 M).

$MgSO_4$ (Figures 1c and 2c)

Bitterness: The addition of the strongest NaCl (0.5 M) decreased the bitterness of the lowest (0.3 M) concentration of $MgSO_4$. The NaCl was able to suppress only 4 ± 26% of the maximum bitterness sensation.

Saltiness: The addition of both 0.3 and 0.5 M $MgSO_4$ suppressed the saltiness of 0.1 M NaCl, but not of 0.3 or

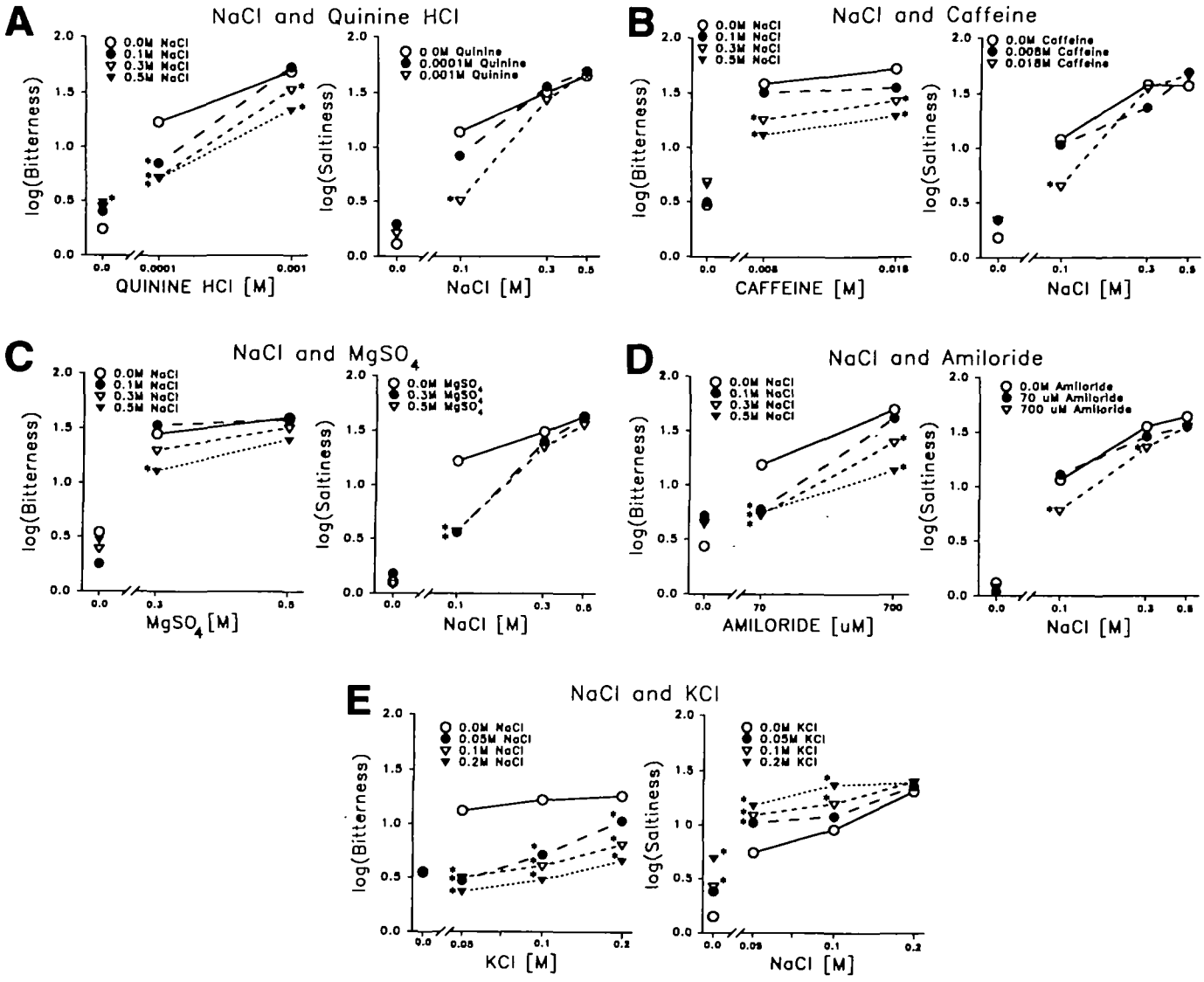

Figure 1 Graphs A–E depict the salt-bitter mixture interactions for NaCl and QHCl, NaCl and Caffeine, NaCl and $MgSO_4$, NaCl and Amiloride, and NaCl and KCl, respectively The left-hand column of panels shows the bitterness ratings for each study. The log standardized bitterness ratings were plotted as a function of bitter compound concentration. 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 log standardized saltiness ratings were plotted as a function of NaCl concentration 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.

0.5 M NaCl. $MgSO_4$ was the only bitter agent tested that at an intermediate bitterness level (not the highest concentration) suppressed the saltiness of NaCl (cf. amiloride Figure 1d).

Amiloride (Figures 1d and 2d)

Bitterness: The 0.3 and 0.5 M NaCl suppressed the bitterness of both amiloride concentrations, suppressing 69 ± 5% of the maximum bitterness sensation. All three levels of NaCl suppressed the bitterness of the lower amiloride (70 μM) concentration, whereas only the two high NaCl concentrations suppressed the bitterness of the 700 μM amiloride.

Saltiness: The highest amiloride concentration (700 μM) suppressed the saltiness of 0.1 M NaCl and 0.3 M NaCl, but not the saltiness of 0.5 M NaCl. Amiloride was the only bitter compound to suppress the saltiness of a higher NaCl concentration, 0.3 M NaCl.

KCl (Figures 1e and 2e)

Bitterness: NaCl at all concentrations suppressed the bitterness of all concentrations of KCl. The maximum bitterness suppression achieved by NaCl on the highest KCl concentration eliminated 78 ± 4% of the bitterness. However, this

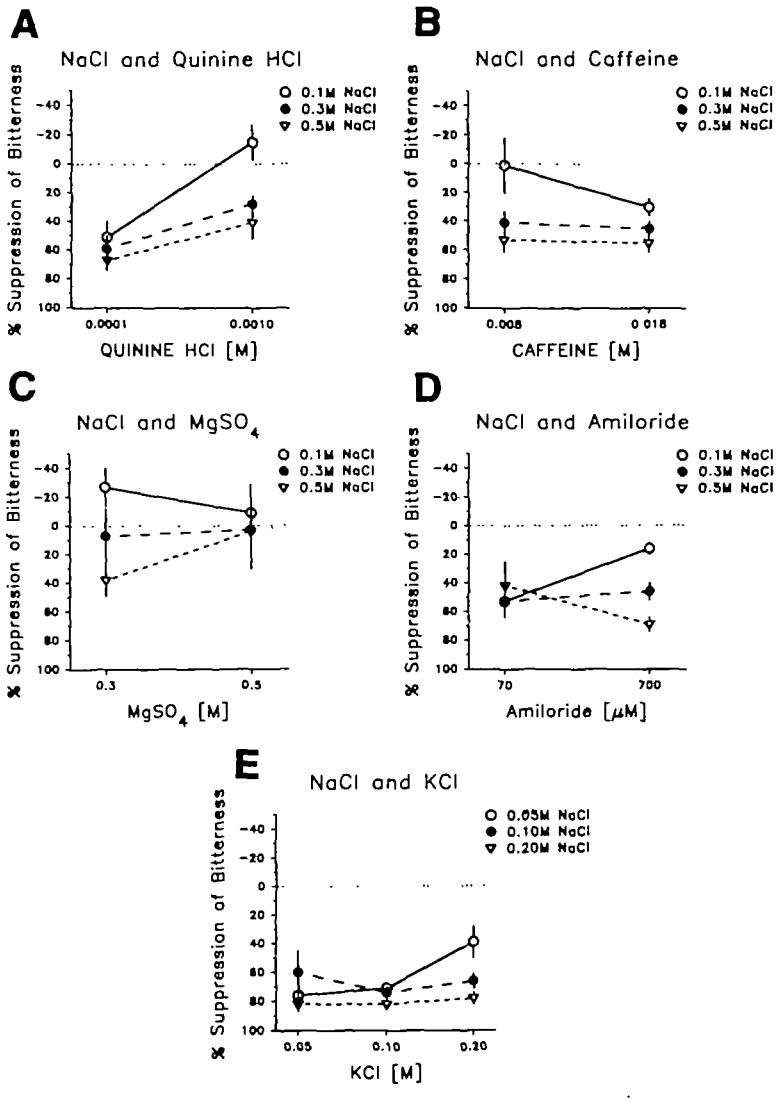

Figure 2 Graphs A–E are yoked to those of Figure 1 and follow the same layout except only suppression of bitterness is shown. The percentage suppression of bitterness is plotted as a function the concentration of the bitter compound. Only three functions mixed with 0.1, 0 3 or 0.5 M added salt are shown, since the percentage suppression is determined relative to no added salt.

maximum level of KCl bitterness was much lower than that obtained with the other bitter compounds employed, as noted previously.

Saltiness: Saltiness of ratings of KCl and NaCl mixture solutions were significantly greater than the saltiness ratings of the NaCl solutions alone. The 0.1 and 0.2 M KCl increased the saltiness of water, 0.05 and 0.1 M NaCl. Presumably because of its own inherent saltiness, KCl was the only bitter agent that when added to NaCl enhanced the saltiness of the NaCl mixture solution.

NaCl and urea (Figures 3a and 4a)

Bitterness: Both 0.3 and 0.5 M NaCl suppressed the bitterness of 0.5 and 0.1 M urea. The NaCl, when most effective, suppressed 76 ± 6% of the maximum bitterness sensation.

Saltiness: The addition of the highest concentration of urea (1.0 M) suppressed the saltiness of 0.1 NaCl, but no other concentration of NaCl.

Experiment 2: effects of anions

Experiment 1 demonstrated an asymmetrical pattern of taste suppression for most bitter compounds: bitterness was suppressed by NaCl, but there was less suppression of the saltiness by bitter compounds. We do not know why the suppression was asymmetrical nor do we know why NaCl was effective as a bitter suppressing agent. However, one

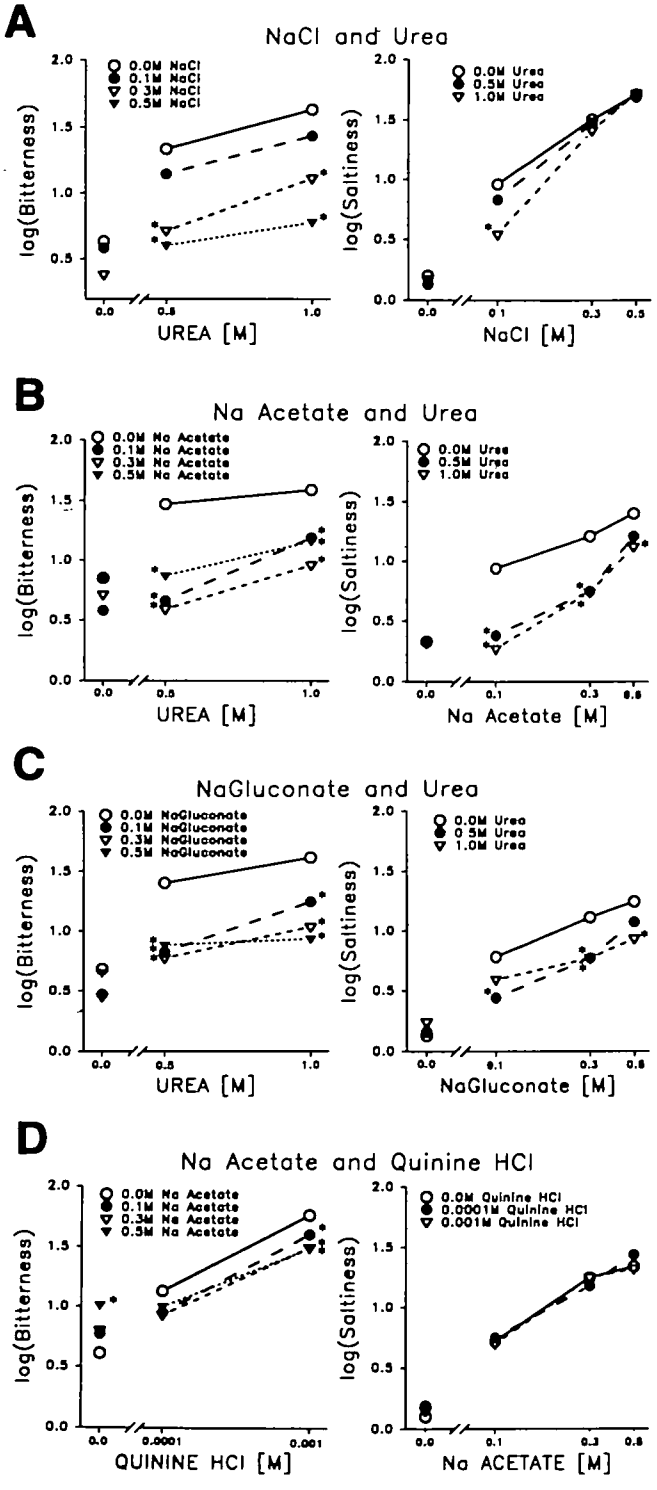

Figure 3 Graphs A–D depict the salt-bitter mixture interactions for NaCl and urea, Na-acetate and urea, Na-gluconate and urea, and Na-acetate and QHCl, respectively. See the caption to Figure 1 for more details.

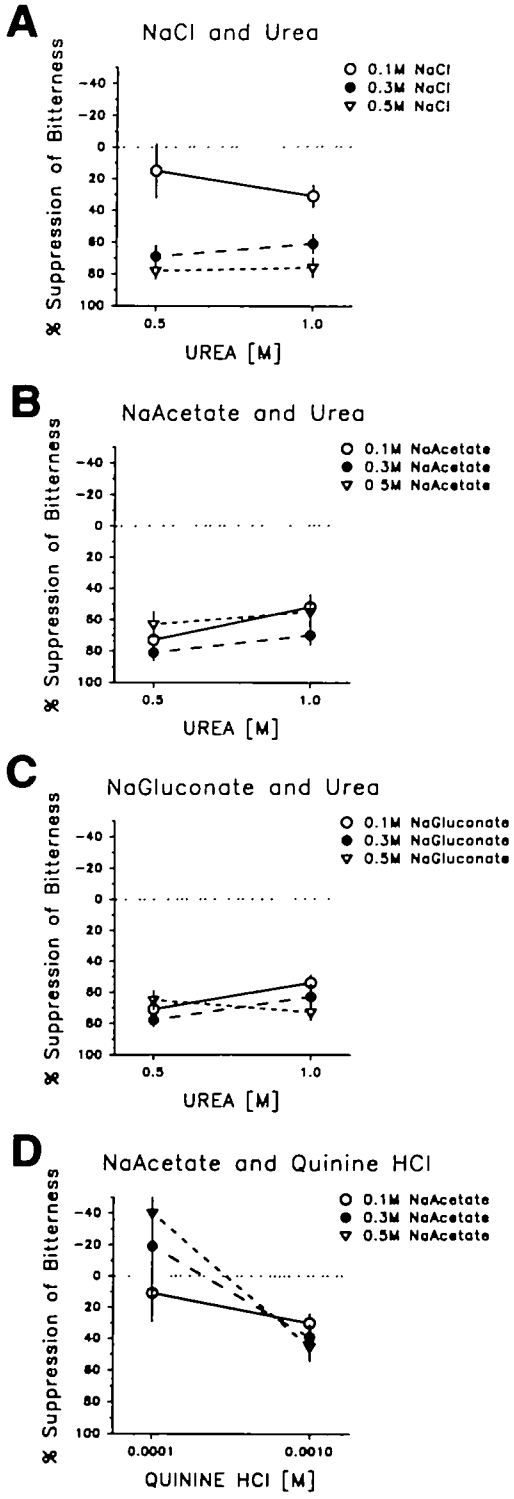

Figure 4 Graphs A–D are yoked to those of Figure 3 and follow the same layout except only suppression of bitterness is shown. The panels are similar to those in Figure 2.

strategy to elucidate the respective bitter-suppressing roles of the anion and the cation in salts is to hold one ion constant and vary the other. In Experiment 2 the anion was manipulated and in Experiment 3 the cation was manipulated. Because Experiment 1 showed that urea was a compound whose bitterness was most effectively suppressed by NaCl, it

was selected as the main bitter stimulus in both Experiments 2 and 3. QHCl was also employed in one study of Experiment 2 in order to compare the effects of a non-chloride sodium salt on another bitter compound.

Na-acetate and urea (Figures 3b and 4b)

Bitterness: Na-acetate at all concentrations suppressed the bitterness of urea at all concentrations up to maximum suppression of 55 ± 9% of the highest bitterness sensation. Although the bitterness levels were relatively small, Na-acetate itself elicited the highest levels of bitterness of all the sodium salts (compare y-axis values for points over the 0 M urea in Figure 3).

Saltiness: The 1.0 M urea suppressed the saltiness of Na-acetate at all concentrations. The addition of 0.5 M urea suppressed the saltiness of 0.1 M and 0.3 M Na-acetate, but not of 0.5 M Na-acetate.

Na-gluconate and urea (Figures 3c and 4c)

Bitterness: Na-gluconate at all concentrations suppressed the bitterness of all urea concentrations up to a maximum suppression of 73 ± 5% of the highest bitterness sensation.

Saltiness: The addition of 0.5 M urea suppressed the saltiness of 0.1 and 0.3 M Na-gluconate, but not of 0.5 M Na-gluconate. In addition, saltiness suppression was significant with 1.0 M urea only for the 0.3 and 0.5 M Na-gluconate solutions.

Na-acetate and quinine-HCl (Figures 3d and 4d)

Bitterness: The bitterness of 10^{-3} M QHCl was significantly suppressed by the addition of all concentrations of Na-acetate. Also, 0.5 M Na-acetate tasted bitter in water, again demonstrating that Na-acetate has some bitterness of its own. The Na-acetate, at its best, suppressed 41 ± 9% of the maximum bitterness sensation of QHCl.

Saltiness: QHCl did not significantly affect the saltiness of Na-acetate.

Experiment 3: effects of cations

Experiment 2 revealed that Na-acetate suppressed the bitterness of both urea and QHCl when in mixture and that Na-gluconate suppressed the bitterness of urea. The finding that the three different sodium salts (NaCl, Na-acetate, and Na-gluconate) were comparably effective at suppressing bitterness is consistent with the hypothesis that the anion

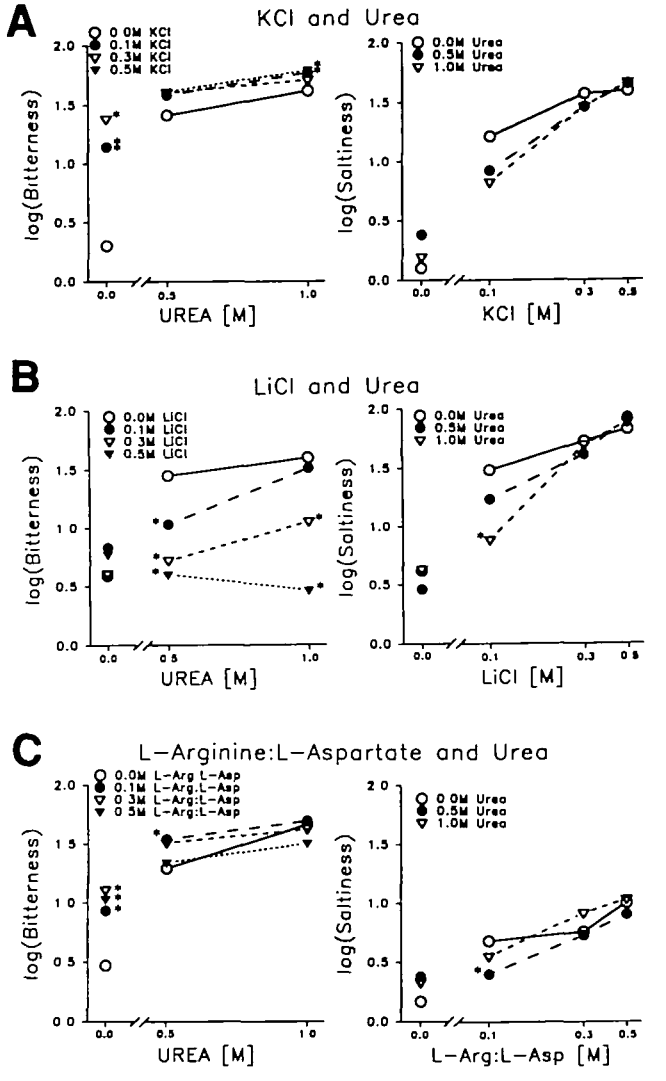

Figure 5 Graphs A–C depict the salt-bitter mixture interactions for KCl and urea, LiCl and urea, and L-arginine:L-aspartic acid and urea, respectively. See the caption to Figure 1 for more details.

was not as important as the sodium cation in the suppression of the bitterness of urea and QHCl by sodium salts. In Experiment 3 the cation was varied to evaluate its potential role in bitter suppression; both the cation and the anion were varied in L-ARG:L-ASP which is a non-sodium/non-chloride salt that elicits a salty taste (Lee, 1992).

KCl and urea (Figures 5a and 6a)

Bitterness: KCl when mixed with urea significantly increased the bitterness over the levels from urea alone, showing 56 ± 18% enhancement of the maximum bitterness sensation of urea alone. The KCl at all concentrations tasted bitter in water alone.

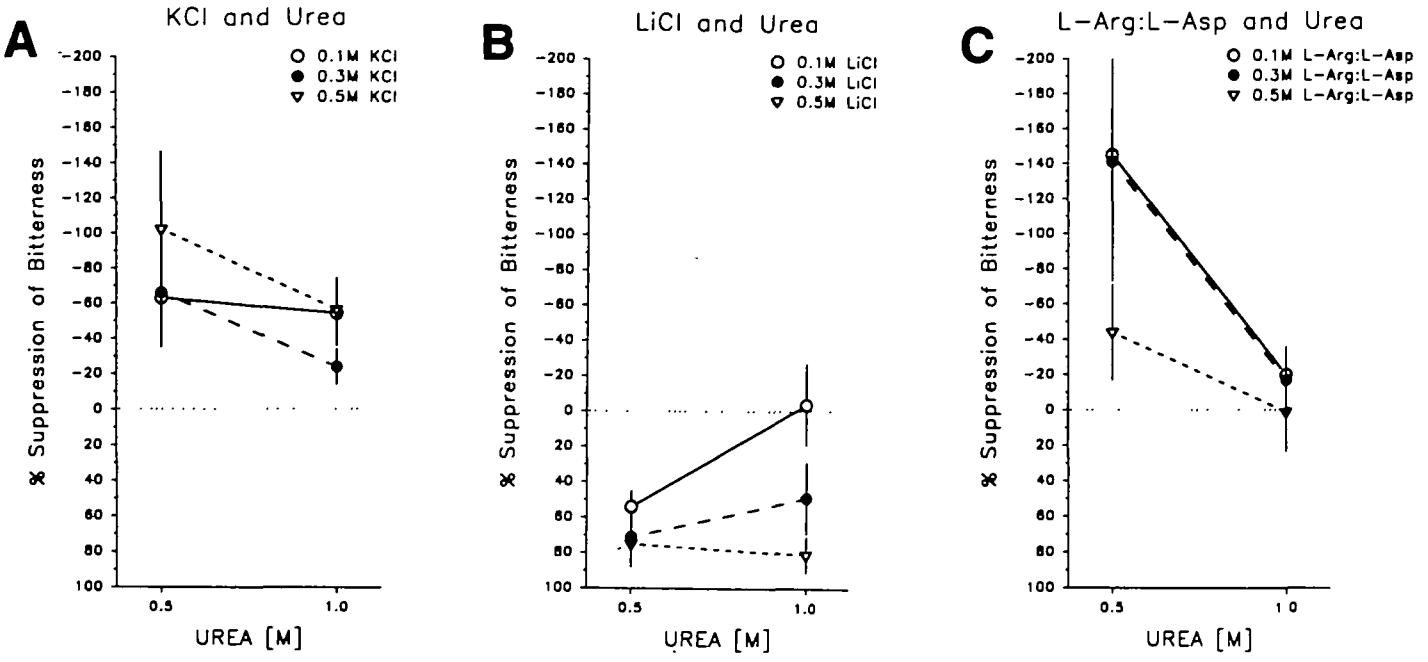

Figure 6 Graphs A–C are yoked to those of Figure 5 and follow the same layout except only suppression of bitterness is shown. The large negative suppression values mean that the addition of the salt increased bitterness relative to the no salt condition. The panels are similar to those in Figure 2

Saltiness: Urea did not significantly affect the saltiness of KCl.

LiCl and urea (Figures 5b and 6b)

Bitterness: Both 0.3 and 0.5 M LiCl suppressed the bitter taste of urea at 0.5 and 1.0 M levels, showing 81 ± 10% suppression of the maximum bitterness sensation of urea alone. The 0.1 M LiCl significantly suppressed the bitterness 0.5 M urea, but not the bitterness of 1.0 M urea.

Saltiness: The addition of the highest urea concentration (1.0 M) suppressed the saltiness of 0.1 M LiCl, but not that of other concentrations.

L-arg:L-asp and urea (Figures 5c and 6c)

Bitterness: The salt, L-arginine:L-aspartic acid (L-arg:L-asp), like KCl, also significantly increased the bitterness of urea. All concentrations of L-arg:L-asp tasted bitter, as well as salty, when alone in water. Also, the bitterness of the 0.5 M urea + 0.1 M L-arg:L-asp mixture was greater than that of the unmixed 0.5 M urea. The highest concentration of L-arg:L-asp barely altered the bitterness of 1.0 M urea by 1 ± 22%.

Saltiness: The addition of 0.5 M urea suppressed the saltiness of 0.1 M L-arg:L-asp, but not that of any other concentration.

Discussion

Bitter suppression

Sodium chloride suppressed the bitter sensation elicited by several compounds when mixed in solution with them. The amount of bitterness suppression tended to vary directly with the concentration of NaCl and inversely with the concentration of the bitter agent. These general observations are consistent with the findings of others (Bartoshuk, 1975, 1977, 1979, 1980; Kroeze, 1980; Bartoshuk and Seibyl, 1982; Bartoshuk and Gent, 1985; Kroeze and Bartoshuk, 1985; Schifferstein and Frijters, 1992a).

The degree of average bitterness suppression varied widely across bitter substances. For example, the bitterness of KCl, urea and amiloride were suppressed up to about 78% by high concentrations of NaCl. In contrast, the suppression of the bitterness of quinine HCl and caffeine appeared to be weaker, roughly 48% suppression, (compare Figure 2a and b with others) and previous reports had indicated that NaCl had no suppressing effect on the bitterness of caffeine (Kamen *et al.*, 1961). NaCl, on average, had no significant effect on the bitterness of the high concentration of $MgSO_4$, and although the bitterness of urea and QHCl used in these studies was judged approximately the same as that of $MgSO_4$, NaCl was more effective at suppressing the bitterness of

the high concentration of urea (~76 ± 6% suppression) than of QHCl (~41 ± 11% suppression), as can be seen from a comparison of Figures 2a and 4a. The baseline bitterness for water and/or the salt solutions when the bitter tasting compound was not present were usually above zero, either due to lingering bitterness from previous trials or due to bitterness elicited by the salt, particularly for certain bitter tasting salts such as for KCl, Na-acetate, and L-arg:L-asp.

McBurney *et al.* (1972) have suggested, based on the results of cross-adaptation studies, that QHCl and urea may elicit bitter sensations through different transduction sequences, a conclusion consistent with findings reported by Yokomukai *et al.* (1993) and Cowart *et al.* (1994). The differential suppression of bitter we have observed would seem to provide further support for this hypothesis, although unlike McBurney *et al.* (1972) and Yokomukai *et al.* (1993) we did not observe similar patterns of responses to urea and $MgSO_4$.

To investigate whether suppression of the bitter taste of urea was due to the Na^+ ion, the Cl^- ion or both, and whether the perceived saltiness of the compound was associated with its ability to suppress bitterness, the anion and/or cation of the salt stimulus was varied. If the presence of the chloride ion or the perceived saltiness were responsible for suppression of bitterness, then KCl should suppress the bitterness of urea, since it has a strong salty taste as well as a weak bitter taste (Murphy *et al.*, 1981). However, the KCl-urea mixtures were more bitter than was urea alone. To determine if there was a noticeable suppression of bitterness, despite the overall increase in bitterness from the added bitterness of KCl, we calculated the ratio of the actual bitterness rating to the urea + KCl mixture divided by the bitterness rating to the urea alone plus the bitterness rating of KCl alone, $[urea_{alone} + KCl_{alone}/(urea + KCl)_{mixed}]$. The total bitterness of any particular mixture was 92 ± 6% of the sum of the average bitterness of KCl alone plus the average bitterness of urea for the 1.0 M urea, and 85 ± 17% of their sum for the 0.5 M urea. Thus, relative to NaCl, KCl had a much weaker bitter suppressing effect.

L-arg:L-asp is an ionic salt of the base, L-arginine and the acid, L-aspartic acid. It, too, was chosen because it elicits a mild salty taste (as well as a slight bitter taste) and has been implicated in the salt taste system as a compound that enhances the saltiness of NaCl, while containing neither sodium nor chloride (Lee, 1992). However, its effects on bitterness resembled those of KCl; it increased the bitterness of the solution when mixed with urea. Most likely the enhanced bitterness comes from the base, L-arginine, which is reported to have a slight bitter taste (Stone, 1967; Schiffman *et al.*, 1975).

Thus, the two salty-tasting compounds (KCl and L-arg:L-asp) that contained neither sodium nor lithium had little efficacy suppressing the bitterness of urea. This suggests that the suppressing effects of NaCl (and LiCl) are due to their chemical properties acting in the periphery, rather than to their taste properties acting centrally. This conclusion is strongly supported by the results of Experiment 2 which indicated that the active component in the bitterness suppression of urea is the Na^+ ion, independent of the anion and the perceived saltiness of the salt. Specifically, NaCl, Na-acetate, and Na-gluconate were comparably effective in suppressing urea bitterness (Figures 3 and 4), even though these sodium salts were substantially different in saltiness. For example, 0.5 M NaCl was roughly twice as salty as 0.5 M Na-acetate, and three or four times as salty as 0.5 M Na-gluconate (Figure 3), as has previously been reported (Kahlenberg, 1901; DeSimone and Price, 1976; Bartoshuk, 1980; Weiffenbach and Ryba, 1993). Figure 7 makes this bitter suppression/saltiness comparison graphically. It depicts the mean saltiness ratings for all three concentrations of NaCl, Na-acetate and Na-gluconate in the top panel and their effectiveness as bitterness suppressors when mixed with 1.0 M urea in the bottom panel. At a given concentration saltiness was independent of bitter-suppressing efficacy. A very similar observation may be made between the bitter suppressing efficacy of NaCl and Na-acetate on quinine HCl.

The results of several previous studies with QHCl also support the hypothesis that the suppression of bitterness by NaCl has a peripheral component (Bartoshuk, 1979; 1980; Bartoshuk and Seibyl, 1982; Kroeze and Bartoshuk, 1985). In the first of these studies, examining mixture suppression, Bartoshuk (1979) employed the strategy of adapting the subjects to one of the components of the mixture and then having the subjects taste the mixture. If the suppression is a central phenomenon, then adapting to one of the mixture components will prevent the adapted stimulus from eliciting a perceived taste and hence from suppressing. In general, peripheral adaptation to one component (e.g. HCl) tended to release the suppression of the other component (e.g. sucrose); however, there was one notable exception. Adaptation to NaCl did not release the suppression of QHCl in the NaCl−QHCl mixture, suggesting a peripheral locus of interaction.

Second, adaptation to a NaCl−QHCl mixture had no effect upon the bitterness of QHCl administered alone (Bartoshuk and Seibyl, 1982). This would most likely

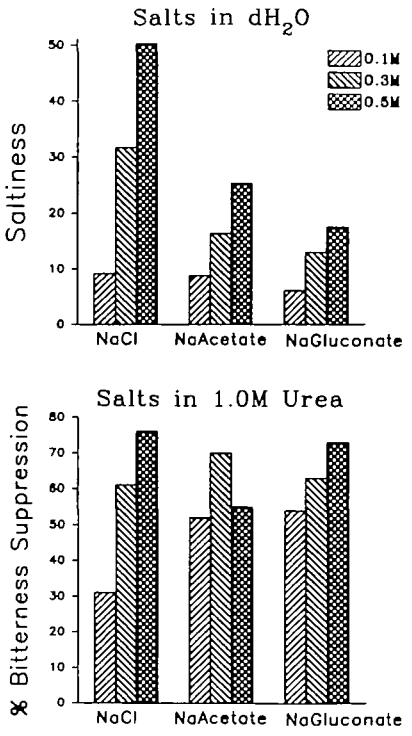

Figure 7 The top panel shows the mean standardized saltiness ratings of 0.1, 0.3 and 0.5 M NaCl, Na-acetate and Na-gluconate The bottom panel depicts the percent suppression of the bitterness of 1 0 M urea by these three salts at three concentrations.

occur if the QHCl was not able to interact normally with transduction elements while in mixture with NaCl. Third, Kroeze and Bartoshuk (1985) compared the mixture suppression of NaCl and QHCl when they were placed together on the same side of the tongue, and when they were placed simultaneously on opposite sides of the divided tongue. If the suppression were central, then equal suppression should occur in either case, but if the suppression were peripheral, then it should predominantly occur when the two were placed on the same side of the tongue. They found that much greater suppression occurred when NaCl and QHCl were placed together on the same side. In contrast, suppression in NaCl−sucrose occurred equally for the two situations.

If the suppression of several bitter compounds by salts is a peripheral phenomenon, how do sodium and lithium interact with the bitter transduction mechanism(s) to block bitter perception? An answer to this question will come when the bitter transduction mechanism(s) are better understood (see Kumazawa *et al.*, 1986; 1988; Brand and Shah, 1992; Spielman *et al.*, 1992). Since sodium's bitter-suppressing effects on these compounds were so varied, it is difficult to speculate what properties of the bitter compounds (polarity, charge, lipophilicity, etc.) or what aspect of the transduction sequence (ion channels, ion pumps, receptors, G proteins, enzymes, etc.) are involved in the differential suppression.

Saltiness suppression

As mentioned above, the mixture suppression was asymmetrical in that saltiness was suppressed little, relative to bitterness. On the average, $MgSO_4$ had a slight, but significant tendency to decrease the saltiness of NaCl, especially at the 0.1 M NaCl level. The suppression of saltiness was less with other bitter stimuli (with the exception of amiloride) although several bitter compounds, including QHCl, caffeine, $MgSO_4$ and urea, partially suppressed the saltiness of 0.1 M NaCl, the lowest concentration tested. This asymmetry is best seen with the urea and NaCl mixtures (Figures 1a and 2a). NaCl reduced the bitterness of urea 76%, while urea had little affect on saltiness, suppression being significant only for the 0.1 M concentration. KCl was unique among the stimuli tested in that it enhanced the perceived saltiness of the solution when mixed with NaCl. That is, in addition to NaCl suppressing the bitterness of KCl, there was also a large increase in the saltiness of NaCl solution when mixed with KCl. This effect is most likely a simple summation of the independent salty tastes of NaCl and KCl.

The main differences between the effects of urea on the saltiness of NaCl, Na-acetate, and Na-gluconate was that urea had a stronger effect upon the latter two compounds. This may be due to the fact that Na-acetate and Na-gluconate are perceived as less salty than is NaCl. It appears that weak salty sensations (as from Na-gluconate) may be suppressed by certain bitter compounds, but strong salt sensations (from NaCl at the same concentration as Na-gluconate) are more difficult to suppress (Ossebaard and Smith, 1995). Thus, the suppression of saltiness is not dependent on low concentrations of sodium ions; rather it is dependent on low intensities of perceived saltiness independent of number of sodium ions, suggesting a central locus for saltiness suppression. The suppression of strong sensations of saltiness was rarely seen. Indeed, only 700 μM amiloride was able to suppress the saltiness of the 0.3 M NaCl significantly, but not the saltiness of 0.5 M NaCl.

Since amiloride has received considerable attention as a salt taste suppressor (Schiffman *et al.*, 1983; Desor and Finn, 1989; McCutcheon, 1992; Tennissen, 1992; Ossbaard and Smith, 1995), it warrants further comment. Although amiloride suppressed higher NaCl concentrations (0.3 M) than any other bitter compound employed, it did not completely eliminate saltiness even at the lowest concentration

of NaCl. In view of the significant suppression shown by other bitter compounds here (e.g. $MgSO_4$), amiloride appeared to differ quantitatively rather than qualitatively from other bitter compounds in its salt suppressing capacity. Surprisingly, the saltiness suppressing capacity of amiloride was of much smaller magnitude than the ability of NaCl to suppress the bitterness of amiloride.

Methodological issues

Throughout this study, mixture effects were examined by asking subjects to rate saltiness and bitterness simultaneously. The manner in which gustatory ratings are obtained can impact upon the responses given by subjects (Frank *et al.*, 1993; Schifferstein and Frijters, 1992b; Stillman, 1993; Schifferstein, 1994a,b). For example, rather than asking subjects to rate saltiness and bitterness, as was done in the present study, they could have been asked simply to rate bitterness alone and then separately to rate saltiness on another day, or subjects could have been asked to rate saltiness and bitterness in addition to simultaneously rating sweetness and sourness and/or other sensations. The method by which the rating is obtained can result in certain response biases that can affect measurements of sensation intensity (Frank *et al.*, 1993). However, the point of the present paper is to make relative comparisons of bitterness suppression by various salts among compounds matched for bitterness intensity, rather than absolute statements of magnitude of effect. Since all compounds were evaluated using the same methodology, any major differences among compounds should not be attributed to response biases as a result of the method. However, there is a chance that side-tastes may tend to affect bitter intensity responses differentially as a function of how many qualities were rated. We have examined this idea and preliminary data (unpublished) suggest that three different instructions for rating sensations [e.g. rating one quality/session (saltiness or bitterness); rating two qualities/session (saltiness and bitterness); rating five qualities/session (saltiness, bitterness, sweetness, sourness, otherness)] all reveal the same general suppression effects of NaCl, Na-acetate and Na-gluconate on the bitterness of urea and quinineHCl, as those presented here.

Summary

Overall, the bitterness of a large array of compounds was suppressed by the addition of sodium salts. In contrast, the saltiness of the different salts was suppressed little by the addition of bitter compounds. Saltiness was suppressed only when the levels of perceived saltiness were low. The suppression of bitterness appeared to be dependent upon the presence of the sodium ion. Neither the anion of the salt nor the level of perceived saltiness were determinants of bitter suppression. The efficacy of the suppression of bitterness depended upon which compounds were eliciting the bitterness. The variability in the suppression of bitterness across bitter compounds provides additional evidence for multiple bitter transduction sequences.

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