Modifying the Bitterness of Selected Oral Pharmaceuticals with Cation and Anion series of Salts

Russell S. J. Keast[1,2] and Paul A. S. Breslin[1,2]

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Purpose. NaCl has proven to be an effective bitterness inhibitor, but the reason remains unclear. The purpose of this study was to examine the influence of a variety of cations and anions on the bitterness of selected oral pharmaceuticals and bitter taste stimuli: pseudoephedrine, ranitidine, acetaminophen, quinine, and urea.

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

Results. The cations (all associated with the acetate anion) inhibited bitterness when mixed with pharmaceutical solutions to varying degrees. The sodium cation significantly ($P < 0.003$) inhibited bitterness of the pharmaceuticals more than the other cations. The anions (all associated with the sodium cation) also inhibited bitterness to varying degrees. With the exception of salicylate, the glutamate and adenosine monophosphate anions significantly ($P < 0.001$) inhibited bitterness of the pharmaceuticals more than the other anions. Also, there were several specific inhibitory interactions between ammonium, sodium and salicylate and certain pharmaceuticals.

Conclusions We conclude that sodium was the most successful cation and glutamate and AMP were the most successful anions at inhibiting bitterness. Structure forming and breaking properties of ions, as predicted by the Hofmeister series, and other physical-chemical ion properties failed to significantly predict bitterness inhibition.

KEY WORDS: bitter taste; bitterness blocking; salts; taste psychophysics; pseudoephedrine; ranitidine; acetaminophen.

INTRODUCTION

Excessive bitterness of the active compounds in oral liquid formulations is a major taste problem facing the pharmaceutical industry. Bitterness of formulations can influence pharmaceutical selection by physicians and patients and affect compliance with prescribed regimens (1,2). Consequently, many methods to inhibit or block bitterness, both chemical and physical, have been employed. A few examples are: the addition of sweeteners, lipids and emulsifiers, carbohydrates, proteins and flavors, and the encapsulation of the active compound (3); none of these methods is fully successful. Moreover, the problem of excessive bitterness in oral liquid formulations is a particularly pressing issue given the recent Federal requirement for pediatric formulations of pharmaceuticals to be produced. Oral liquid dosage of pharmaceuticals, as opposed to pills or tablets, is the most tolerated form of delivery for infants, children and adult patients with difficulty swallowing solids.

It is our opinion that the bitter taste of pharmaceuticals is an ongoing formulation problem in part because the transduction mechanisms for bitter taste are complex and not fully elucidated. If we had a full understanding of bitter taste transduction, we should be able to decrease bitter taste perception by affecting selected transduction mechanisms. Recently, up to 80 putative G-protein-coupled bitter receptors (4–6) have been identified. In addition to multiple trans-membrane receptors, there are other means of activating bitter taste signal cascades, such as blocking or opening ion channels/pumps (e.g., quinine hydrochloride) (7) and direct activation of a G-proteins or enzymes [e.g., caffeine (8)] [for a review of bitter taste transduction pathways see Dulac (9), Brand (10), and Spielman (11,12)]. Given the potential diversity in bitter taste transduction sequences, it is unlikely that a single, universal, bitter blocker will be discovered. Thus, the problem of how to suppress bitterness by physiological-chemical interactions continues to be unresolved.

Certain ions, however, have been shown to suppress bitterness well. Sodium is known to selectively inhibit the bitterness of specific compounds (13–16). In this study, we systematically examined the influence of five cations on bitter pharmaceuticals to determine if the sodium cation is unique or if bitterness inhibition is a property shared with other cations. In addition to the cations, we investigated the influence of anions on bitterness. In this study we compare 12 anions, including known bitterness inhibitors, adenosine monophosphate (AMP) (17) and glutamate (18), to determine the degree to which anions possess bitterness inhibiting properties beyond those of the cations.

While there has been no systematic study investigating the influences of a variety of salts on bitter taste perception, there is a large body of published research on the physical-chemical properties of salts. Much of this research concerns salts in the Hofmeister series (19) (Fig. 1). The Hofmeister series of salts are sequenced, in part, according to their electronegativity at moderate concentrations (10 mM to 1M) and neutral pH, and their ability to alter the structure of pure water and/or proteins [for a review see Collins (20)]. We examined whether the chemical-bond making or bond breaking properties of cations and anions employed here, predicted their ability to inhibit bitter taste according to their ranking in the Hofmeister series.

Three of the most common over-the-counter pharmaceuticals were used as bitter stimuli in this research: pseudoephedrine (bronchodilator), ranitidine (antiulcerative) and acetaminophen (analgesic, antipyretic). In addition, two older pharmaceuticals commonly used in bitter taste research, quinine-HCl (antimalerial), and urea (osmotic, diuretic), were employed to make comparisons with the existing taste literature.

The overall aim of this research project was to discover which of these sets of cations and anions were most effective

[1] Monell Chemical Senses Center, 3500 Market St, Philadelphia, Pennsylvania 19104, USA.

[2] To whom correspondence should be addressed. (e-mail: keast@monell.org; breslin@monell.org)

ABBREVIATIONS: NaAc, sodium acetate ; COMAc, combined cations; (NaAc, NH_4Ac, MgAc, KAc, CaAc) NH_4Ac , ammonium acetate; MgAc, magnesium acetate; Kac, potassium acetate; CaAc, calcium acetate; MSG, monosodium glutamate; NaAMP, adenosine monophosphate; NaCl, sodium chloride; NaSal, sodium salyiclate; NaGlc, sodium gluconate; Na_2GP, di-sodium glycerophosphate; Na_2Phos, di-sodium phosphate; NaProp, sodium propionate; COM, combined anions; (MSG, Cl, Glc, Sal, I) Na_2SO_4, di-sodium sulfate; NaI, sodium iodide; KI, potassium iodide; PHCL, pharmaceutical.

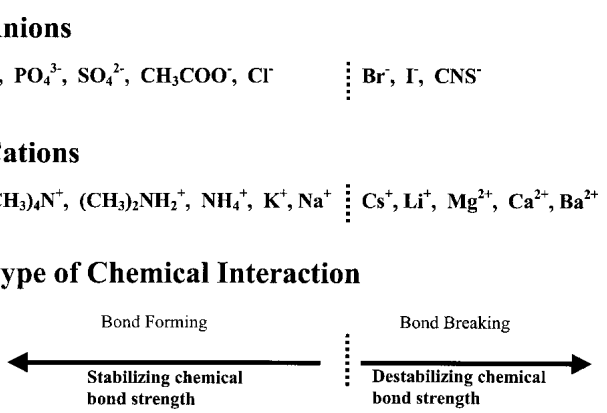

Fig. 1. Hofmeister series of cations and anions The Hofmeister series of salts are sequenced, in part, according to their electronegativity at moderate concentrations (10mM to 1M) and neutral pH, and their ability to alter the structure of pure water and/or proteins.

at inhibiting bitterness of the chosen pharmaceuticals, and, if possible, to infer why.

METHODS

Subjects

Subjects between the ages of 20 and 35 were paid to participate after providing informed consent on an Institutional Review Board approved form. All but one were employees of Monell Chemical Senses Center. Seventeen subjects (mean age 26 ± 5 years) participated in the intensity matching and cation experiment, and 14 subjects who participated in the cation experiment, (mean age 27 ± 4 years) were in the anion experiment. The subjects were asked to refrain from eating, drinking or chewing gum for at least one hour prior to testing.

Training

Subjects were initially trained in the use of the Labeled Magnitude Scale (LMS) following standard published procedures (21,22) except the top of the scale was described as the "strongest imaginable" sensation of any kind (23). The LMS 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 semi-logarithmically, based upon experimentally determined intervals (21,22) to yield ratio quality data. 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 by presenting them with exemplars. Salty taste was identified as the predominant taste quality from 150 mM NaCl, bitterness as the predominant quality from 0.05 mM quinine-HCl, sweetness as the predominant quality from 300 mM sucrose, sourness as the predominant quality from 3 mM citric acid, and savory the predominant quality from a mixture of 100mM glutamic acid monosodium salt and 50 mM inosine 5′-monophosphate. To help subjects understand a stimulus could elicit multiple taste qualities, 300 mM urea (bitter and slightly sour) and 50 mM NH_4Cl (salty, bitter, and slightly sour) were employed as training stimuli.

Materials

Ranitidine was purchased from ICN Pharmaceuticals (Aurora, Ohio), acetaminophen and pseudoephedrine were purchased from Aldrich (Milwaukee, Wisconsin). Quinine-HCl was purchased from Fluka Chemika (Buchs, Switzerland) and urea was purchased from Sigma Chemical (St. Louis, Missouri). All salts were of the highest purity available and were purchased from Sigma Chemicals; they were: monosodium glutamate (MSG), adenosine monophosphate sodium salt (NaAMP), sodium chloride (NaCl), sodium salicylate (NaSal), sodium gluconate (NaGlc), disodium glycerophosphate (Na_2GP), disodium phosphate (Na_2Phos), sodium propionate (NaProp), disodium sulfate (Na_2SO_4), sodium iodide (NaI), sodium acetate (NaAc), ammonium acetate (NH_4Ac), magnesium acetate (MgAc), potassium acetate (KAc), calcium acetate (CaAc) and potassium iodide (KI). The combined cation solution (COMAc) consisted of 20 mM of each of the following salts: NaAc, NH_4Ac, MgAc, KAc, and CaAc. The combined anion (COM) solution consisted of 20 mM of the following salts: MSG, NaCl, NaSal, NaGlc, and NaI.

All solutions were prepared with deionized (*di*) Millipore™ filtered water and stored in amber glass bottles at 4°C–8°C and brought up to room temperature prior to testing with the aid of a water bath. Solutions were made fresh every five days. Millipore™ filtered *di* water was used as the blank stimulus and the rinsing agent in all experiments.

Intensity Matching Protocol

Bitter taste among individuals is highly variable (see the range of concentrations required to elicit moderate bitterness in Table I); what one individual perceives as weak bitterness, another may perceive as extremely bitter. In this research we fixed the perceived initial bitterness intensity (allowing individual concentrations of pharmaceuticals to vary) rather than fix the concentration of pharmaceuticals (allowing individual perceptual intensities to vary). This allowed us to compare whether a bitterness blocker was effective for all individuals, as well as to make fair comparisons of a blocker's efficacy from bitter pharmaceutical to bitter pharmaceutical.

The intensity matching procedure involved adjusting the concentrations until each subject rated the bitter intensity of every bitter stimulus as "moderate" on the LMS. The tasting protocol was whole mouth sip and spit. On each trial, subjects held 10 mls of solution in their mouth for five seconds and rated the taste qualities of the solution, prior to expectorating. Subjects wore nose-clips to eliminate olfactory input in this

Table I. Average Molarity and Range of Molarity of Target Compounds as Assessed in the Intensity Matching Experiment

	mM	Range
Pseudoephedrine	10.7 ± 5.3	1–25
Ranitidine	3.5 ± 1.7	1–7
Acetaminophen	43.8 ± 13.4	24.8–62.3
Quinine	0.24 ± 0.27	0.06–0.9
Urea	1500 ± 800	400–2800

Mean ± SD.

phase as well as the cation and anion salt series experiments. Subjects rated the bitter intensity of predetermined concentrations of pharmaceuticals (10 mM pseudoephedrine, 4 mM ranitidine, 50 mM acetaminophen, 0.1 mM quinine, 1.2M urea). Bitter taste intensity was recorded on a computerized LMS and transferred in real time via a printer to the technician making the solutions who altered the concentration of solutions up or down depending on the individual subject's response. The new solution was tasted and rated by the subject, and depending on the response, new concentrations were made until the bitter intensity was rated as "moderate". There was an interstimulus interval of approximately 60 s, during which time the subject was required to rinse their mouth with *di* water at least 4 times. As a test for successful matches, subjects were required to rate the intensity of the bitter compound as "moderate" on the LMS when randomly presented with a "matched" bitter stimulus during a separate test session. If the LMS rating did not match "moderate" (16 ± 4) allowing 25% variability during the test re-evaluations of the matched intensities, the subject was either retested again or excluded from the study after failing three retests for lack of reliability. Human sensory ratings are highly variable. If we limited the variability to 10% (16 ± 1.6) we would eliminate most subjects. Four of 21 subjects screened were excluded from the study by the 25% criterion. Mean and range of concentrations of pharmaceuticals determined in the intensity matching phase to elicit "moderate" bitterness are listed in Table I.

Cation Protocol

Solutions of pharmaceuticals were prepared for each subject at the concentration individually assessed in the intensity-matching phase. Because acetate reduces the perceived saltiness of cations relative to when they are associated with chloride (13), acetate was the fixed anion in the cation series. The acetate salts (100 mM): NaAc, NH_4Ac, MgAc, KAc, CaAc, and an equal molar cation combination of the five cations (COMAc, 20 mM of each cation giving a 100 mM cation cocktail) were added to the pharmaceutical solutions. As experimental controls, subjects were presented with: cations without pharmaceuticals, pharmaceuticals without cations, and *di* water as a blank. Each of six sessions was comprised of the pharmaceuticals in combination with 3 cations and the controls. All samples were presented in random order with an interstimulus interval of 2 min. Subjects rated five taste qualities (sweet, sour, bitter, salty, savory), each with its own screen on a computerized LMS. All cation-pharmaceutical combinations were presented in triplicate on separate test days as a test of reliability. All subjects participating in the experiment completed the cation phase prior to starting the anion phase.

Anion Protocol

The general protocol was the same as described in the cation experiment above. There were twelve anions used in this experiment: MSG, NaAMP, NaCl, NaSal, NaGlc, Na_2GP, Na_2Phos, NaProp, Na_2SO_4, NaI, and an equal molar anion combination of five anions (COM, 20 mM of each anion MSG, NaCl, NaGlc, NaSal, and NaI giving a 100 mM anion cocktail), were added to the pharmaceutical solutions. Potassium iodide (KI) was also included as a control for NaI to further assess the impact of the iodide anion on bitterness. Because sodium is a highly effective bitterness blocker it was the fixed cation in the anion salt series and all solutions were prepared to have 0.1N sodium. The objective was to determine whether any anions had bitterness blocking efficacy complimentary to sodium. Na_2Phos, Na_2GP, and Na_2SO_4 were all disodium salts therefore were prepared at 50 mM. As the pH of salt solutions were variable, a 10 mM potassium phosphate buffer (pH 6.6) was added to all solutions, which ensured solutions were within 0.6 pH units of neutrality with the exception of NaAMP which was pH 5. Buffer concentrations above 10 mM started to have a perceivable taste, whereas 10 mM potassium phosphate buffer had no effect on taste quality of solution (results not shown).

Analysis

Data were expressed as arithmetic means ± SE Student *t* tests with Bonferroni corrected (*P* value divided by the number of *t* tests performed) criteria for significance were performed on percent bitterness inhibition data from subjects to examine any differences among cations ($P < 0.05/15 = P < 0.003$), among anions ($P < 0.05/45 = P < 0.001$) and the iodide salts ($P < 0.05/2 = P < 0.025$). Statistical significance of individual pharmaceutical cation/anion interaction was determined by 1 or 2 way analysis of variance (ANOVA) using the Statistica 4.5 package. Post-hoc pairwise comparisons were conducted with the Scheffé test. For the ANOVA's, *P* values <0.05 were considered statistically significant. All correlations were performed using Pearson's product moment matrix correlation.

RESULTS

Cation Influence on Bitterness

Results

The major taste associated with the various anions was: NaAc primarily salty, COMAc and KAc encompassed all tastes to varying extents, NH_4Ac was perceived to have all taste qualities except sweetness, MgAc was primarily sour with sweet and bitter qualities, and CaAc was primarily bitter with salty and sour qualities. Taste profiles of salt and pharmaceutical mixtures showed the major effect was a reduction of bitterness of the pharmaceuticals. Therefore, the following analysis concentrated on bitter taste.

There were significant differences among the cations overall bitterness inhibition properties on the pharmaceuticals (Fig. 2). NaAc (55%) was significantly more effective at inhibiting bitterness than NH_4Ac (36%, $P < 0.003$), MgAc (34%, $P < 0.003$) & KAc (33%, $P < 0.003$), and CaAc (18%, $P < 0.003$), but not COMAc (42%) which contains 20mM sodium. All cations significantly ($P < 0.003$) inhibited bitterness more than CaAc. There were no significant differences in bitterness inhibition among the cations COMAc, NH_4Ac, MgAc and KAc.

Figure 3 shows the mean bitter intensity ratings, standard error and significant differences for the five pharmaceuticals alone and in binary mixture with NaAc, COMAc, NH_4Ac, MgAc, KAc, and CaAc. The mean individual bitter intensities

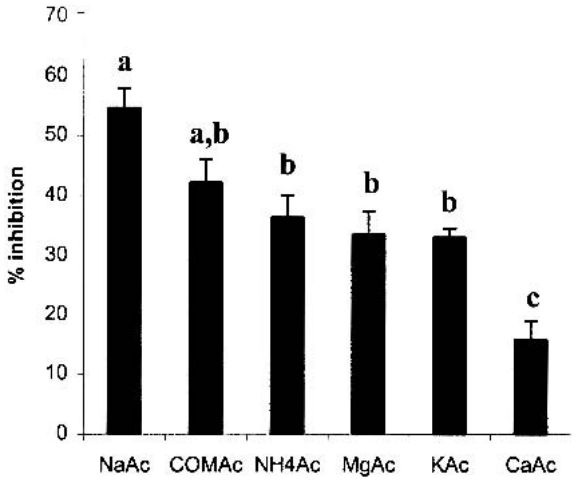

Fig. 2. Cations ability (%) to inhibit bitterness of pharmaceuticals The x-axis represents salt-pharmaceutical mixtures. Abbreviations of acetate salts (100mM) are: NaAc = sodium acetate, COMAc = combined cations (NaAc, NH_4Ac, MgAc, KAc, CaAc), NH_4Ac = ammonium acetate, MgAc = magnesium acetate, KAc = potassium acetate and CaAc = calcium acetate. The y-axis represents the % inhibition of bitterness for each salt averaged across all five pharmaceuticals. Different letters symbolize a statistically significant ($P < 0.003$) difference in bitterness inhibition efficacy between cations. Error bars represent standard errors.

were analyzed by a 5 × 7 (pharmaceutical × cation) repeated measures ANOVA.

There was a significant main effect of the bitterness of the different pharmaceuticals without salts [$F(4,64) = 4.04$, $P < 0.05$], indicating the initial bitterness of pharmaceuticals was different. Subjects rated pseudoephedrine as significantly more bitter than quinine ($P < 0.05$), but still within the established criteria for "moderate". Also, there was a significant main effect among the pharmaceuticals and their susceptibility to suppression by the cations [$F(4,64) = 3.08$, $P < 0.05$] (Fig. 3).

There was a significant interaction among the pharmaceuticals and cations [$F(24,384) = 3.03$, $P < 0.001$]. Thus, there was variation among individual cation's ability to inhibit the bitterness of various pharmaceuticals with the exception of KAc (compare columns in Fig. 3). NaAc inhibited the bitterness of ranitidine (73%) significantly more than the bitterness of quinine (36%, $P < 0.05$) or pseudoephedrine (51%, $P < 0.05$). And NH_4Ac inhibited the bitterness of ranitidine (61%) more than the bitterness of pseudoephedrine (26%, $P < 0.05$).

Similarly, the bitterness of individual pharmaceuticals was differentially affected by the various cations (compare across rows in Fig. 3). MgAc was the most effective cation at inhibiting the bitterness of quinine, whereas NaAc was the most effective cation for all other pharmaceuticals. NaAc was the only cation to significantly inhibit urea's bitterness (55%), which supports previous research investigating sodium's influence on urea (13). CaAc was the only cation not to significantly inhibit the bitterness of any pharmaceutical.

The susceptibility of selected bitter compounds to inhibition by salts was highly correlated. For example, the bitterness inhibition of acetaminophen and urea by the salts COMAc, NH_4Ac, MgAc, KAc, CaAc ($r^2 = 0.93$, $P < 0.001$; $r^2 = 0.42$, $P < 0.05$; $r^2 = 0.8$, $P < 0.001$; $r^2 = 0.55$, $P < 0.05$; $r^2 = 0.93$, $P < 0.001$, respectively) were strongly correlated.

There were no significant correlations between bitterness inhibition efficacy of cations and physical parameters of the cations: molecular weight ($r^2 = 0.41$), logP value ($r^2 = 0.48$), enthalpy ($r^2 = 0.43$), entropy ($r^2 = 0.16$) and Gibbs energy of formation ($r^2 = 0.36$). Also, there was no relationship between bitterness inhibition efficacy of cations and Hofmeister series rank position.

Anion Influence on Bitterness

Results

The major taste associated with the various anions was: MSG, savory; NaAMP, savory, sour; NaCl, salty; NaSal, sweet; NaI and KI, bitter. All other anions had multiple taste qualities (24). Anions such as NaSal and NaProp have negligible saltiness, in fact only NaCl and NaGlc could be said to have salty as their dominant taste quality. As in the cation experiment, the taste profile of the anions was only slightly altered when mixed with the pharmaceuticals, the major change was an inhibition of bitterness of the pharmaceuticals, therefore the following analysis focused on bitter taste.

The iodide salts, NaI and KI enhanced the bitterness of the pharmaceuticals. Therefore, both were analyzed separately from the anions that inhibited bitterness to reduce variability within the analyzed data sets.

Overall there were significant differences in bitterness inhibiting properties among the anions (Fig. 4). Both MSG and NaAMP (both 67%) were significantly ($P < 0.001$) more effective at inhibiting bitterness across all pharmaceuticals than NaCl 56%, NaGlc 53%, Na_2GP 49%, Na_2Phos 48%, NaProp 47%, COM 46% and Na_2SO_4 33%. With the exceptions of NaProp and COM, all other anions significantly ($P < 0.001$) inhibited bitterness more than Na_2SO_4. There were no significant differences in bitterness inhibition among the anions NaCl, NaSal, NaGlc, Na_2GP, Na_2Phos, NaProp, and COM.

The mean bitterness intensity ratings, standard error and significant differences for the five pharmaceuticals in mixture with MSG, NaAMP, NaCl, NaSal, NaGlc, Na_2GP, Na_2Phos, NaProp, COM and Na_2SO_4 are shown in Fig. 5. The mean individual bitter intensities were analyzed by a 5 × 11 (pharmaceutical × anion) repeated measures ANOVA.

There was no significant difference in the initial bitterness of pharmaceuticals because the intensity matched concentration of pseudoephedrine was re-evaluated after the cation experiment, and decreased by 10% for every subject. Mean initial bitter intensities of pharmaceuticals from both the cation and anion experiment were analyzed by a 5 × 2 (pharmaceutical × experiment) repeated measures ANOVA: there was no significant difference between the bitterness levels in the cation and anion experiments [$F(1,16) = 3.04$, $p = 0.1$].

There was a significant pharmaceutical x anion interaction [$F(40,520) = 1.50$, $P < 0.05$], indicating that there were differences among individual anions and their effects on individual pharmaceuticals (Fig. 5).

As in the cation experiment, there was significant variation with an individual anion's bitterness inhibiting efficacy of various pharmaceuticals: NaSal inhibited the bitterness of

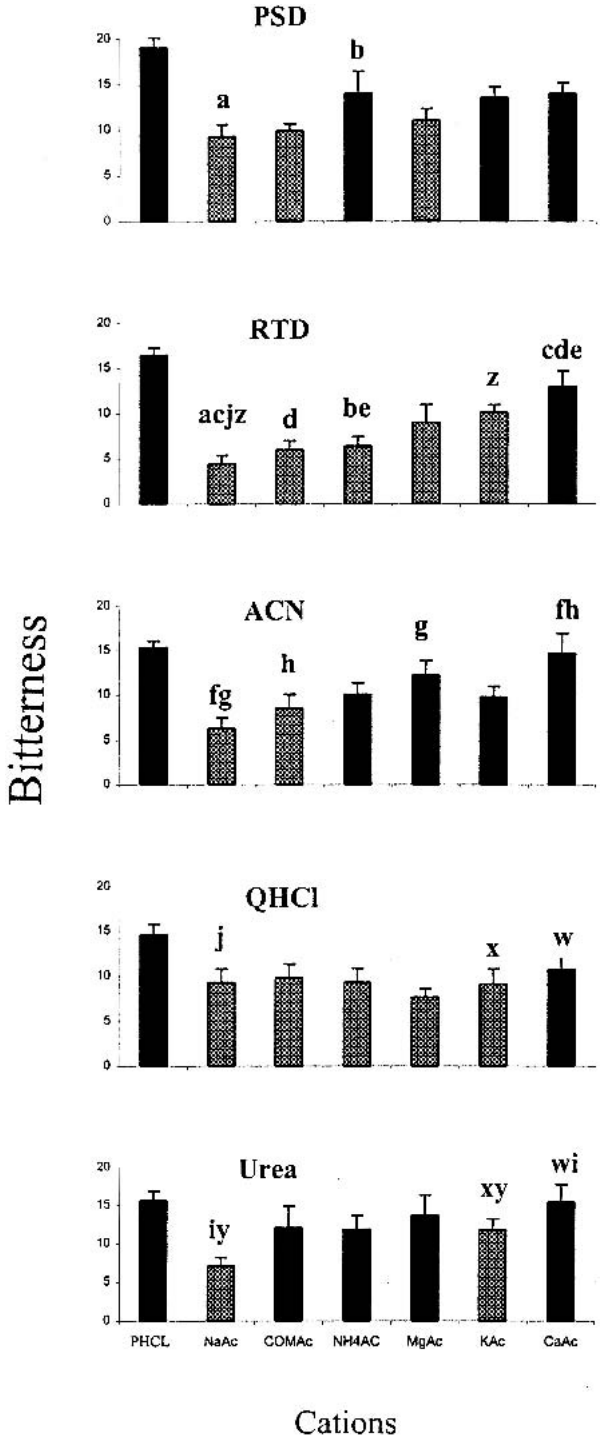

Fig. 3. Specific effects of cations on bitterness of pharmaceuticals Each panel represents one bitter compound. The Y-axis represents average bitterness rating on the Labeled Magnitude Scale (mean ± SE) and the X-axis lists the solutions tested. Abbreviations for pharmaceuticals are PSD = pseudoephedrine, RTD = ranitidine, ACN = acetaminophen, QHCl = quinine. Abbreviations of salts are the same as in Figure 2. Concentrations of salts are the same as Figure 2. The bar representing bitterness of the pure bitter compound is indicated by PHCL (pharmaceutical) on the X axis. Hatched bars indicate a significant ($P < 0.05$) reduction in bitterness when the cation was added to the pharmaceutical relative to PHCL. Corresponding letters above bars indicate differences in bitterness among cation-pharmaceutical mixtures. Within rows or columns, any bars that share a letter in common are different at the level indicated (a,b,c,d,e,f-,g,h,i,j $P < 0.05$; z,y,x,w $P < 0.1$).

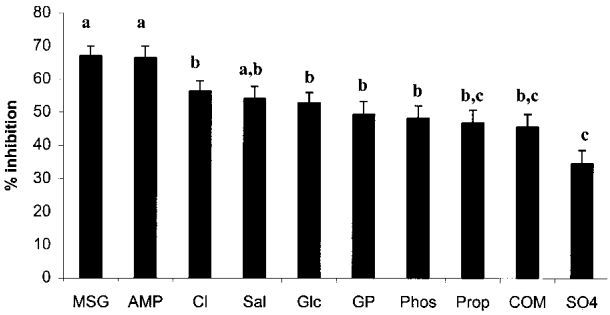

Fig. 4. Anions ability (%) to inhibit bitterness of pharmaceuticals The axes are the same as Figure 2 except different salts are represented. Abbreviations for sodium salts (0.1N Na) are: MSG = monosodium glutamate, AMP = adenosine monophosphate, Cl = sodium chloride, Sal = sodium salicylate, Glc = sodium gluconate, GP = di-sodium glycerophosphate, Phos = di-sodium phosphate, Prop = sodium propionate, COM = combined anions (MSG, Cl, Glc, Sal, I), SO_4 = di-sodium sulfate, I = sodium iodide, and KI = potassium iodide. Different letters symbolize a statistically significant ($P < 0.001$) difference in bitterness inhibition efficacy between anions. Error bars represent standard errors.

quinine (71%) significantly more than it did the bitterness of pseudoephedrine (41%) ($P < 0.05$). And MSG tended to suppress the bitterness of ranitidine (88%) more than it did the bitterness of pseudoephedrine (57%) ($p = 0.063$).

Both the iodide anions significantly ($P < 0.025$) increased bitterness across all of the pharmaceuticals, NaI 18% and KI 26%. Post-hoc analysis showed (Table II) interactions between the iodide salts and individual bitter compounds and the only significant increase in bitterness with a single compound was the addition of KI to urea (42%, $P < 0.05$).

There were no statistically significant correlations ($P < 0.05$) between bitterness inhibition efficacy of anions and physical parameters of the anions: molecular weight ($r^2 = 0.04$), log P value ($r^2 = 0.48$), enthalpy ($r^2 = 0.05$), entropy ($r^2 = 0.07$), and Gibbs energy of formation ($r^2 = 0.04$). There was no correlation between bitterness inhibition efficacy of anions and Hofmeister series rank position.

DISCUSSION

Sodium's Bitter Inhibiting Properties

Overall sodium was significantly the most effective cation at inhibiting bitterness of the pharmaceuticals. The effect of the sodium cation on taste has been reported in the psychophysical literature (13,14,16,25–30), including sodium's effect as a bitterness inhibitor for compounds such as quinine and urea (13–16,25,31). Sodium ions inhibit bitterness in the periphery, acting on oral physiology, rather than in the brain by a cognitive interaction of perceived saltiness on bitterness. Evidence for this comes from the observation that sodium salts with little salty taste are equally effective at blocking bitterness as highly salty sodium salts (13,15). The site of sodium's action in the periphery is unknown, but Keast (32) proposed 4 potential sites or modes of action for sodium inhibiting bitterness within the peripheral taste system: 1/ shielding of the receptor protein from bitter compounds, 2/ moderating or modulating ion channels or pumps, 3/ stabilizing the cell membrane, 4/ interfering with second messenger systems after entering receptor cells. Whatever sodium's

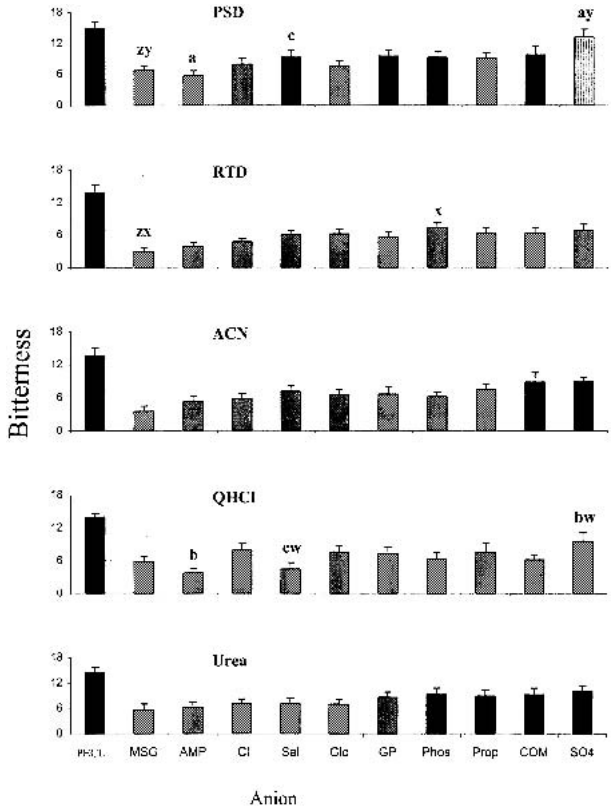

Fig. 5. Specific effects of anions on bitterness of pharmaceuticals Each panel represents one bitter compound. The Y-axis represents average bitterness rating on the Labeled Magnitude Scale (mean ± SE), and the X-axis lists the solutions tested. Abbreviations are the same as in Figures 3 & 4. The bar representing bitterness of the pure bitter compound is indicated by PHCL (pharmaceutical) on the X axis. Concentrations of salts were the same as Figure 4. Hatched bars indicate a significant ($P < 0.05$) reduction in bitterness when the anion was added to the pharmaceutical relative to PHCL. Corresponding letters above bars indicate differences in bitterness among anion-pharmaceutical mixtures. Within rows or columns, any bars that share a letter in common are different at the level indicated (a,b,c $P < 0.05$; z,y,x,w $P < 0.1$).

mode of action, its ability to suppress bitter taste is superior to that of other cations tested in this study.

MSG and AMP Bitter Inhibiting Properties

The anions were not neutral partners to sodium in blocking bitterness. MSG and NaAMP were significantly more effective at inhibiting bitterness of the pharmaceuticals than the other anions tested, which indicates an active role of the anion in bitterness inhibition. Psychophysical and electrophysiological research showed that the glutamate anion inhibits bitterness (18,33); however, the bitter blocking mode of action of glutamate is also unknown. The mechanisms of bitterness blocking by NaAMP are also unknown. In this study, we found commonality between MSG and NaAMP in regard to taste quality (savory) and bitter inhibiting properties, raising the possibility that these compounds share transduction components in the peripheral taste system.

Even though MSG (and NaAMP) exhibited superior bitterness inhibition in comparison to other anions tested, their associated savory taste may make them suitable in only a subset of formulations. Pharmaceutical flavorists may have to try low concentrations of MSG (or NaAMP) to find the minimum concentration that will inhibit bitterness.

Table II. Influence of Iodide Salts on Bitterness Intensity of Pharmaceuticals

	No salt	NaI	KI
Pseudoephedrine	15.7 ± 1.0	18.5 ± 0.4	17.9 ± 0.4
Ranitidine	14.9 ± 1.4	16.3 ± 0.5	18.5 ± 0.4
Acetaminophen	14.5 ± 1.4	17 ± 0.7	18.7 ± 0.4
Quinine	15.7 ± 0.9	18.5 ± 0.6	17.9 ± 0.5
Urea	14.4 ± 1.2	17.7 ± 0.5	20.5[a] ± 0.4

[a] Significant difference ($P < 0.05$) in bitterness between bitter compounds and bitter compounds with anion.
Mean ± SD (n = 14).

Differential Inhibition of Bitterness

Certain cations and anions exhibited specific inhibition of bitterness for selected pharmaceuticals. NaAc and NH_4Ac significantly inhibited the bitterness of ranitidine more than that of pseudoephedrine, while NaSal significantly inhibited quinine bitterness more than that of pseudoephedrine. Differential inhibition of bitterness may help elucidate receptors/mechanisms involved in bitter taste, as these interactions suggest that the cations/anions affect a part of the bitter taste signal cascade that is specific to one pharmaceutical and not another. Presumably, specificity of action of cations or anions is likely to occur at the receptor level, rather than downstream in the bitter signal cascade, since downstream events are likely to have more general effects. The differential inhibition of bitterness exhibited by some cations and anions should be further investigated. Pharmaceutical flavorists should explore a variety of salts to determine which salt has superior bitterness inhibiting properties for a particular bitter compound. Additionally, flavorists should utilize Na^+'s superior bitterness inhibiting properties and include it with the pharmaceutical formula when possible.

Potassium and Ammonium Ions

Not all cations differentially influenced bitterness; K^+ had a general or non-specific inhibitory effect on pharmaceutical bitterness. This general inhibitory effect suggests that K^+ affects a bitter taste mechanism(s) common to the pharmaceuticals employed in this study rather than having a specific mode of action at a receptor.

The K^+ and NH_4^+ cations have similar actions on in solution properties (34) and on cell membranes (35–37), while voltage clamp studies have shown differences between the two cations (38). In the present study, there were similarities between K^+ and NH_4^+ with regard to bitter inhibition for the pharmaceuticals. Neither salt significantly inhibited the bitterness of pseudoephedrine or urea, while both significantly inhibited the bitterness of ranitidine, acetaminophen and quinine. However, NH_4Ac and KAc's ability to inhibit ranitidine's bitterness were not identical. There was a significant difference between NH_4Ac and CaAc when inhibiting ranitidine bitterness, but KAc did not significantly differ from CaAc. And NH_4Ac inhibited ranitidine and pseudoephedrine

to different degrees while KAc inhibited their bitterness similarly.

Combined Cations

Two combinations of salts, one cation mixture (COMAc) and one anion mixture (COM), were assessed as bitterness inhibitors in this study. There was no significant difference between the bitterness blocking efficacy of NaAc and COMAc. The cation combination inhibited bitterness 20% more than predicted if bitter inhibition was a linear relationship with concentration of salts (e.g., 0.2 Na's inhibition + 0.2 NH_4's inhibition + etc.). This result suggests that there may be synergy among the cations leading to enhanced bitterness inhibition, or it may mean that sodium's concentration-bitterness inhibition function is hyperbolic and the 20 mM sodium in the combined cation mixture has comparable bitterness blocking efficacy to the 100 mM sodium. To assess whether there was synergy, sodium's concentration-bitterness inhibition functions would have to be generated for these pharmaceuticals to assess the bitterness blocking efficacy at low concentrations. The combination of anions was closer to the value a linear relationship between anion concentration and bitterness blocking efficacy would predict, although the actual value was 9% above the predicted value.

Hofmeister Series

In this study we also tested the hypothesis that the chemical-bond structure making or breaking ability of salts, indicated by their Hofmeister series position, would influence their bitterness inhibiting properties. The ranked Hofmeister series values for the cations ($NH_4^+ \rightarrow K^+ \rightarrow Na^+ \rightarrow Mg^{2+} \rightarrow Ca^{2+}$) and anions ($SO_4^{2-} \rightarrow PO_4^{2-} \rightarrow Cl^- \rightarrow I^-$) did not correlate with bitterness inhibiting efficacy when examining the ion's impact on each target compound. Nor was the Hofmeister series predictive when averaging across bitter targets. Therefore, we conclude that the chemical-bond structure making / breaking ability of cations and anions, does not predict bitterness inhibition in any obvious manner, based upon the stimulus set tested.

General Comments

In this research, each subject was given their own unique set of pharmaceutical concentrations to match the perceived bitterness across all subjects and all pharmaceuticals. This was done because bitter taste sensitivity is highly variable among individuals. In practice, however, the population receives a fixed concentration of a pharmaceutical in formulation. This means that some will perceive a formulation to be highly bitter and others will find it barely bitter. By fixing perceived bitterness across individuals in the present study, we have shown that we can inhibit bitterness with selected salt-pharmaceutical combinations for all tasters.

Overall the bitterness of these common over-the-counter pharmaceuticals could be inhibited by mixture with salts. Sodium and glutamate and AMP were the most effective ions at inhibiting bitterness. The salts are safe and effective at low concentrations, and should be applicable in pediatric and adult liquid formulations and should increase compliance with prescribed regimens with bitter pharmaceuticals.

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