Cross-adaptation and bitterness inhibition of L-tryptophan, L-phenylalanine and urea: Further support for shared peripheral physiology

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Running head; Shared bitterness of L-trp, L-phe, and urea

Abstract

A previous study investigating individuals' bitterness sensitivities found a close association among three compounds: tryptophan (L-trp), phenylalanine (L-phe) and urea (Delwiche *et al.*, 2001). In the present experiment, psychophysical cross-adaptation and bitterness inhibition experiments were performed on these three compounds to determine whether the bitterness could be differentially affected by either technique. If the two experimental approaches failed to differentiate L-trp, L-phe and urea's bitterness, then we may infer they share peripheral physiological mechanisms involved in bitter taste. All compounds were intensity matched in each of thirteen subjects, so the judgments of adaptation or bitterness inhibition would be based on equal initial magnitudes and, therefore, directly comparable. In the first experiment, cross-adaptation of bitterness between the amino acids was high (>80%) and reciprocal. Urea and quinine-HCl (control) did not cross-adapt with the amino acids symmetrically. In a second experiment, the sodium salts, NaCl and Na gluconate, did not differentially inhibit the bitterness of L-trp, L-phe and urea, but the control compound, $MgSO_4$, was differentially affected. The bitter inhibition experiment supports the hypothesis that L-trp, L-phe and urea share peripheral bitter taste mechanisms, while the adaptation experiment revealed subtle differences between urea and the amino acids indicating that urea and the amino acids activate only partially overlapping bitter taste mechanisms.

Introduction

A primary function of the peripheral gustatory system is to discriminate between nutritive and toxic chemicals among potential ingesta. Presumably, bitter taste perception evolved to detect potential toxins (Glendinning, 1994). The gustatory system distinguishes many classes of compounds as bitter: inorganic salts (e.g., potassium chloride), amines (e.g., denatonium), amino acids (e.g., tryptophan), peptides, alkaloids (e.g., quinine), acetylated sugars (e.g., sucrose octa-acetate), flavanols/phenols (e.g., epicatechin), carbamates (e.g., phenyltuiocarbamide) and isohumulones, to name some. To have the ability to taste such divergent structures, mammals have evolved multiple peripheral mechanisms, which have an affinity for the chemical structures. Psychophysical experiments (McBurney, 1969; McBurney *et al.*, 1972; Lawless, 1979; Yokomukai *et al.*, 1993; Cowart *et al.*, 1994; Delwiche *et al.*, 2001) have supported multiple physiological mechanisms involved in bitter taste, while electrophysiological and biochemical experiments have elucidated several bitter taste transduction systems (for review of bitter taste see Spielman *et al.* (Spielman *et al.* 1992) and Dulac (Dulac, 2000)).

It is logical to assume that bitter compounds will share taste receptor cells (TRCs) or transduction mechanisms, as it seems improbable that each of the thousands of bitter compounds would have its own unique transduction sequence. Molecular cloning and functional studies (Adler *et al.*, 2000; Chandrashekar *et al.*, 2000; Matsunami *et al.*, 2000) have revealed a family of 40-80 putative bitter receptors (Tas2Rs), many of which

are co-expressed on the same cells, which indicates bitter taste cells will respond to a number of bitter stimuli (c.f., Caicedo and Roper, 2001). Further to this, Chandrashekar *et al.*, demonstrated that the bitter compounds, PROP and denatonium benzoate, could activate the same receptor, thereby showing that the Tas2Rs also share ligands (Chandrashekar *et al.*, 2000). Given this evidence: that taste cells can express multiple Tas2Rs and that one Tas2R can be activated by a variety of ligands, it is probable that the bitter response activated by a group of structurally related bitter compounds may be similar.

In addition to receptor mediated bitter taste transduction, bitter compounds may directly activate transduction components downstream of the G-protein coupled receptors. Many bitter compounds are lipophilic or amphipathic and have the ability to rapidly permeate through cell membranes, such as the cyclic di-peptide Leu-Trp and quinine (Peri *et al.*, 2000). Compounds such as quinine and certain peptides can directly activate mixtures of G-proteins in vitro. Therefore, direct activation of G-proteins could result in bitter taste transduction (Naim *et al.*, 1994; Chahdi *et al.*, 1998). Certain compounds, such as caffeine, may also directly interact with bitter taste transduction enzymes (Rosenzweig *et al.*, 1999).

In research testing the hypothesis that bitter tasting compounds share transduction mechanisms, hence bitter compound sensitivities, Delwiche *et al.*, examined individual differences in sensitivity to 11 bitter compounds in 26 subjects and identified several tight compound groupings (Delwiche *et al.*, 2001). Among them, three bitter

compounds, L-tryptophan (L-trp), L-phenylalanine (L-phe) and urea, correlated the most tightly as a function of individual sensitivities. Those who were very sensitive to one compound were very sensitive to the other two, independent of their sensitivity to the other eight compounds. This correlation of compound specific differences in sensitivity may be caused by shared TRCs or receptor/transduction mechanisms.

To compliment the close associations revealed by individual differences analyses, the present study was designed to determine whether this cluster of compounds, L-trp, L-phe and urea, could be differentiated perceptually by two additional psychophysical techniques: cross-adaptation and bitterness inhibition. Cross-adaptation studies can help determine whether compounds are likely to share TRCs/receptor/transduction mechanisms. In the gustatory system, when a compound cross-adapts a taste quality of another compound, this strongly suggests the compounds share a physiological process involved with that taste quality, most likely at the TRCs or the receptor/transduction level (McBurney, 1969; McBurney et al., 1972; Schiffman *et al.*, 1981; Lawless, 1982; Michel *et al.*, 1993; Smith and van der Klaauw, 1995; Froloff *et al.*, 1998), although more central adaptation affects cannot be ruled out. If the amino acids L-trp, L-phe and urea share bitter TRCs or receptor/transduction mechanisms, they should symmetrically cross-adapt each other's bitterness and affect the bitterness of unrelated compounds comparably.

As an additional test, it might be possible to differentially affect the stimuli with a bitter inhibitor and, thereby, infer that L-trp, L-phe and urea act on independent peripheral physiological mechanisms. Sodium inhibits the bitterness of different bitter

compounds to widely varying degrees (Frijters and Schifferstein, 1994; Breslin and Beauchamp, 1995; 1997). Furthermore, the bitterness inhibiting properties of sodium are peripheral, acting in the mouth, rather than a central cognitive effect of the perceived saltiness; mole for mole, sodium salts with little salt taste are comparably as effective at blocking bitterness as highly salty salts (Bartoshuk, 1979; Bartoshuk, 1980; Bartoshuk and Seibyl, 1982; Kroeze and Bartoshuk, 1985; Kemp and Beauchamp, 1994). Therefore, since sodium salts suppress the bitterness of urea and the effect is peripheral, other compounds that may share TRCs and/or receptor/transduction mechanisms with urea, such as L-trp and L-phe, should be suppressed to a similar extent.

Materials and Methods

1: CROSS ADAPTATION OF BITTERNESS

Subjects: Thirteen subjects (7 female, 6 male) between the ages 20 and 51 (mean 29.9 years) were paid to participate after providing their informed consent on an Institutional Review Board approved form. All but one were employees of Monell Chemical Senses Center. Each subject participated in 48 sessions over 3 months. They were asked not to eat, drink or chew gum 1 hour prior to each session.

Training: Subjects were initially trained in the use of the Labeled Magnitude Scale (LMS) following standard published procedures (Green *et al.*, 1993; Green *et al.*, 1996), except the top of the scale was described as the "strongest imaginable" sensation of any kind (Bartoshuk, 2000). The LMS is a psychophysical tool that requires subjects to rate the perceived intensity along a vertical axis lined with adjectives: barely detectable, weak, moderate, strong, very strong, strongest imaginable; the adjectives are spaced semi-logarithmically, based upon experimentally determined intervals (Green *et al.*, 1993; Green *et al.*, 1996) to yield ratio quality data. The scale 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 100 mM 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.

Stimuli: Tryptophan (L-trp), phenylalanine (L-phe) and urea were all purchased from Sigma (St Louis) and were Sigma-ultra grade. Quinine-HCl (QHCL) (>99%) was purchased from Fluka (Switzerland). Aqueous solutions were prepared every second day with deionized (*di*) Millipore™ filtered water and stored in amber glass at room temperature. All solutions were fully dissolved and there were no visible signs of undissolved solids or precipitation from solutions.

Tongue adaptation method; Intensity matching: An anterior tongue adaptation method was developed because whole mouth adaptation has been shown to be inconsistent and ineffective (Meiselman, 1968). This is likely due to the presence of posterior lingual and pharyngeal bitter receptors and inconsistency both within and between subjects in stimulating the same posterior receptors with repeated stimulation.

Most individuals in a sample population will perceive a single fixed concentration of a bitter compound differently (Yokomukai et al., 1993; Delwiche et al., 2001). Therefore, the concentration of bitter compounds were adjusted so that all subjects

judged the compounds to be of equal intensity on a large but well defined and controlled area of the tongue in order to compare them for psychophysical effects. Consequently, cross-adaptation was assessed with bitter compounds of equal intensities but different molarities.

Intensity matching: Subjects were required to rate bitter intensity of L-trp (0.06M), L-phe (0.17M), urea (2M) and QHCl (0.1mM) in separate sessions on the LMS. Both L-trp and L-phe were presented as saturated solutions and all subjects rated bitter intensities as "moderate" or weaker on the LMS. Whichever of the two amino acids, L-trp or L-phe, was rated least bitter, was then chosen as the compound to which the other compounds were matched for intensity, as the concentration (therefore the intensity) of the other amino acid could not be increased. Subjects (7 of 22 subjects screened) were not included in the study if they rated either L-trp or L-phe as less than "weak" on the LMS, since a study on bitterness adaptation must elicit bitterness to begin.

Subjects were instructed to extend their tongue out of their mouth so a significant portion of there anterior tongue (approximately 2.5cm) was exposed, then to form a good seal around their tongue with their lips, thereby isolating the anterior portion from the rest the oral cavity. Subjects completely immersed their exposed tongues into 30ml plastic medicine cup containing 25ml of stimuli so that their lips were in contact with the solution. After rating the intensity of the taste qualities (sweet, sour, bitter, savory and salty) on the LMS, subjects removed their tongue from the solution and rinsed with *di* water. There was a break of at least 60 min prior to the next test to eliminate any possible

adaptation or sensitization effects. The intensity matching procedure continued until individual concentrations of L-trp, L-phe, urea and QHCl were judged to be equal in bitterness magnitude for each subject. Subjects were not included in the study (2 of 15 subjects screened), if reproducibility for a particular compound was not within 25% of the determined LMS matched intensity over a series of at least three separate trials.

Cross adaptation of bitterness: Each subject was presented with eight 30ml medicine cups filled with 25ml of intensity-matched solutions in numbered trays. Solutions 1 and 8 were the "test" solutions while 2 through 7 were the "adapting" solutions (Figure 1). New adapting solutions were given to subjects every 30sec in case any saliva ran into the cup during adaptation and so the test cup (#8) would be experienced the same way the adapting cups (#2-7) were experienced. Sample 1 (pre-adaptation) was used as the reference against which sample 8 (post-adaptation) was compared. Subjects followed the tongue immersion methodology (described above) for sample 1; once rated, subjects rinsed their mouth with *di* water 4 times during a 60sec interstimulus interval. Subjects then followed the tongue immersion methodology for sample 2, but after rating the taste intensities, their tongue remained in the solution for 30sec. After 30sec subjects removed their tongue from solution 2 and repeated the immersion procedure with solution 3 through 7. The subjects tongue was not retracted into the oral cavity and no water rinsing occurred between samples 2-8. The procedure was the same for sample 8, except once sample 8 had been rated for taste intensity, subjects could retract their tongue into the oral cavity and rinse with *di* water. The procedure took 4 min per adaptation trial.

A factorial matrix design ensured that every compound was the adapting solution for every other compound, including itself. Water (*di*) was included in the matrix design as a control.

2: BITTERNESS INHIBITION BY SODIUM SALTS

Subjects: Thirteen subjects (5 male, 8 female) between the ages of 20 and 35 (mean 27.9 years) were paid to participate after providing their informed consent on an IRB approved form. All but two were employees of Monell Chemical Senses Center. Seven subjects who participated in the adaptation study also participated in the bitterness inhibition study. Each subject participated in 3 sessions over a period of 1 month. They were asked not to eat, drink or chew gum for 1 hour prior to each session.

Stimuli: L-Trp, L-phe, urea and magnesium sulfate ($MgSO_4$) were purchased from Sigma and were Sigma-ultra grade. QHCl (>99%) was purchased from Fluka (Switzerland). Sodium chloride (NaCl) and sodium gluconate (NaGlu) were purchased from Sigma and were Sigma-ultra grade. NaGlu was used in the experiment because of the reduced salty taste caused by the larger anion (Ossebaard and Smith, 1995); low saltiness allows us to distinguish between the peripheral inhibition of bitterness by sodium ions and the central cognitive inhibition of bitterness by perceived saltiness (Breslin and Beauchamp, 1995). Aqueous stock solutions were prepared every second day with *di* Millipore filtered water and stored in amber glass at room temperature.

Intensity matching: The bitterness inhibition experiment was a whole-mouth sip and spit procedure, thereby activating the fungiform papillae, as in the cross-adaptation experiment, as well as the foliate and circumvallate papillae. The foliate and circumvallate papillae have been shown to have a greater proportion of the putative bitter taste receptors (Adler *et al.*, 2000). Therefore, given the phenomena of spatial summation (Smith, 1971), we expected eqimolar solutions from the adaptation experiment to be more intense in the bitterness inhibition experiment. The intensity matching procedure involved adjusting the concentrations until the intensity of stimulus was rated as "moderate" on the LMS by each subject. The matching methodology follows: Subjects were instructed to wear nose clips to eliminate olfactory cues when sampling, and to rate the perceived total intensity of solution presented while the solution remained in the subjects mouth. Subjects rated the intensity of predetermined concentrations of bitter solutions (0.0198M L-trp, 0.04M L-phe, 0.6M urea, 0.2mM QHCl, 0.45M $MgSO_4$). Taste intensity was recorded on a computerized LMS and transferred in real time to the technician making 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 intensity was rated as "moderate". There was an interstimulus interval of approximately 60sec, during which time the subject was required to rinse with *di* water at least 4 times. When randomly presented with a "matched" bitter stimulus, subjects were required to rate the intensity of the bitter compound as "moderate" on the LMS. If the LMS rating (±25%) did not match "moderate" on subsequent evaluations of the matched intensities, the subject was retested or excluded

from the study. Five of 18 subjects screened were excluded from the study by this criterion because bitterness cannot be inhibited unless it is first elicited.

Methodology: Subjects, wearing nose-clips, were given trays of bitter compounds at concentrations individually assessed in the intensity matching phase. The solutions, which included bitter stimuli and water, were presented without salt or with 300mM of NaCl or NaGlu added. The testing protocol was as follows: Randomized solutions (10ml) were presented in 30ml plastic medicine cups and on numerically labeled trays. Subjects rinsed with *di* water at least four times over a 2 min period prior to testing. Each subject tasted, and then rated each solution for sweetness, sourness, saltiness, bitterness and savoriness, prior to expectorating. All subjects rinsed with *di* water 4 times during the interstimulus interval of 85 sec. The LMS was used as the rating method. Each sample was tasted only once per session and there were three sessions in total as a test of reliability.

Statistical analysis of experiments 1 and 2: Numerical results are expressed as means ± standard error. Statistical variation was determined by 1 or 2 way analysis of variance (ANOVA) using Statistica 4.5 software package. P values <0.05 were considered statistically significant. Bitter intensities pre- and post-adaptation were analyzed by 1-way ANOVA. Mean bitter intensity data from bitter inhibition experiment were analyzed by a 5 x 3 (bitter x salt) repeated measures ANOVA. All post-hoc pairwise comparisons were conducted with the Scheffé test.

Results

1: CROSS-ADAPTATION

Intensity matching: Table I shows the average molarity along with the range and average LMS score for each of the intensity matched stimuli used in this experiment. At their limits of solubility, the bitter intensity of L-trp and L-phe was rated between "weak" and "moderate" on the LMS for all subjects tested. As a result, an individual's bitterness rating of saturated L-trp or L-phe dictated the bitterness intensity to which the other compounds were matched. The results revealed that 8 of 13 (62%) subjects perceived the amino acids to be isointense at their maximum solubility. Given the variable nature of human taste sensitivities, the concentrations of L-trp and L-phe required to elicit iso-intense bitterness were remarkably similar over the majority of subjects, which of its own accord supports the findings of Delwiche *et al*. (Delwiche *et al*., 2001). That is, sensitivities to these two compounds correlate.

Adaptation: Overall, there was a significant effect of adaptation on bitterness of the compounds pre and post adaptation [$F(19,228)=41.4$, $p<0.001$)]. Figure 2 and Table II summarize the results of self- and cross-adaptation. Self-adaptation for the compounds tested were almost complete (96% L-trp, urea, and QHCl, 94% L-phe). In all cases, self-adaptation was greater than cross-adaptation of other compounds, which may indicate that each compound has at least partially independent peripheral bitter taste mechanisms. Water (*di*) was also used as an adapting stimulus and results show a significant increase in bitterness post-water adaptation for L-trp ($p<.05$), L-phe ($p<.001$) and urea ($p<.05$).

Cross-adaptation was not reciprocal in all cases. QHCl was chosen as a stimulus to control for spuriously finding symmetrical cross-adaptation as McBurney *et al*. (McBurney *et al*., 1972) has shown that urea can cross-adapt QHCl bitterness, but adaptation to QHCl only partially cross-adapts urea bitterness. Results from this experiment support McBurney's finding, since urea effectively cross-adapted 67% of QHCl's original bitterness ($p<0.001$), yet adaptation to QHCl only inhibited 26% of urea's bitterness ($p=0.87$).

Cross-adaptation between the two amino acids was homogeneous and symmetrical: adaptation to L-trp decreased L-phe bitterness by 80%, while adaptation to L-phe decreased L-trp bitterness 85%. Adaptation to urea decreased L-trp bitterness 82% and L-phe bitterness 77%. Overall urea was very effective at cross-adapting the bitterness of the three other compounds, while the other three compounds were more variable and less effective at cross-adapting urea's bitterness.

2: BITTERNESS INHIBITION

Intensity matching: The mean level of bitterness intensity for $MgSO_4$ was below the targeted "moderate" rating on the LMS. There was a significant difference in bitterness of $MgSO_4$ and L-trp and L-phe ($p<0.05$) (Table III). Attempts to increase the concentration of $MgSO_4$ during the matching phase produced significant irritation among the majority of subjects, therefore, we decided that the irritation produced by higher molarities of $MgSO_4$ would be too distracting to subjects. Even though $MgSO_4$ was significantly less bitter than L-trp or L-phe, it was imperative to have a control compound in the experimental design whose bitterness should not be inhibited by the addition of sodium salts (Breslin and Beauchamp, 1995). Prior research has shown that less bitter concentrations are more easily suppressed, so the bitterness of $MgSO_4$ in this study should have been easier to inhibit based on its intensity (Breslin and Beauchamp, 1995).

Inhibition: There was a significant overall inhibition of bitterness by salt [$F(2,24)=67.3$, $p<0.001$] and bitter x salt interaction [$F(8,96)=7.8$, $p<0.001$], which suggests some compounds were inhibited more than others. On average, NaCl and NaGlu significantly decreased bitterness (51% and 41% respectively, $p<0.001$). There was no statistical difference between the overall bitter inhibition ability of the two sodium salts ($p=0.13$).

Post-hoc tests revealed that NaGlu significantly reduced bitterness of L-trp ($p<0.001$), L-phe ($p<0.001$) and QHCl ($p<0.001$) and the suppression of urea was

marginal ($p=0.09$). NaCl significantly decreased the bitterness of L-trp ($p<0.001$), L-phe ($p<0.001$), urea ($p<0.05$) and QHCl ($p<0.001$).

Breslin and Beauchamp had previously reported 300mM NaCl inhibited the bitterness of urea (60%) and QHCl (40-60%), while $MgSO_4$ bitterness was not affected (Breslin and Beauchamp, 1995). The present experiment showed (Figure 3) that both NaGlu and NaCl were more effective at reducing the bitterness intensity of QHCl (45% and 56% respectively) than urea (37% and 42% respectively), while not affecting the bitterness of $MgSO_4$. NaGlu and NaCl also suppressed the bitterness of L-trp (52% and 64% respectively) and L-phe (54% and 66% respectively). Note that both bitterness inhibitors blocked the two amino acids symmetrically; NaCl inhibited bitterness L-trp 64%, L-phe 66% while NaGlu inhibited bitterness of L-trp 52% and L-phe 54%.

Discussion

Adaptation: Self-adaptation of a taste quality is a phenomenon that involves a reduction of the initial taste intensity with constant or repeated application of a taste stimulus. Cross-adaptation occurs between different stimuli. After adaptation to one stimulus, the taste intensity of a different second stimulus is reduced. Cross-adaptation is generally regarded as evidence that the two stimuli share taste physiology within the transduction sequence (McBurney et al., 1972; Lawless, 1982; Smith and van der Klaauw, 1995). L-trp and L-phe cross-adapted each other's bitterness in excess of 80%, compared to 96% and 94% self-adaptation respectively. Symmetrical cross-adaptation of bitterness between the two amino acids supports the theory that L-trp and L-phe share bitter TRCs or receptor/transduction mechanisms. It is worth noting that cross-adaptation was, in all cases, less than self-adaptation, and while the difference was not statistically significant, the trend suggests that the amino acids may have partially independent bitter taste mechanisms, albeit a small proportion (10-15%) of the total. Urea cross-adapted the amino acid bitterness by approximately 80%, but cross-adaptation was not reciprocal; adaptation to L-trp decreased urea's bitterness 58%, while adaptation to L-phe decreased urea's bitterness 69%. One-way ANOVA of cross-adaptation between the compounds revealed the difference between urea and L-trp was significant ($p<0.05$), but the asymmetry between urea and L-phe was not ($p=0.16$). This experiment supports the theory that urea has TRC's or receptor/transduction mechanisms in common with the amino acids, but in addition, urea appears to activate bitter taste mechanisms that are independent of the amino acids.

An important feature of the adaptation results was the general symmetry between the amino acids and their interactions with the other compounds tested, whether the amino acids were adapting- or test-stimuli (Figure 2 and Table 2). Further analysis of the results revealed two sub-populations of subjects that were demarcated by whether QHCl cross-adapted the amino acids more than the amino acids cross adapted QHCl or the opposite (Figure 4). Even within the two sub-populations, there was symmetry between the amino acids. For example, adaptation to QHCl suppressed bitterness of L-trp and L-phe 25% and 20% respectively for group A, or 87% and 84% respectively for group B. The consistency or symmetry observed between L-trp and L-phe in these two different groups is further evidence of shared bitter taste transduction mechanisms for the two amino acids.

For 9 of the 13 subjects (Group A), QHCl had limited efficacy when cross adapting the amino acids bitterness, but these compounds were able to cross-adapt QHCl bitterness. The remaining four subjects (Group B) conversely reported that QHCl was able to cross-adapt the amino acids bitterness, while the amino acids were less effective in cross-adapting QHCl bitterness. There is no simple explanation for the observed variation, and it may indicate variation in bitter taste transduction mechanisms among the subjects or relative proportion of amino acid:quinine receptors on the TRC's.

In addition, if urea replaces quinine at the center of Figure 4, then the differences between groups A and B disappear and the interactions appear as in Figure 2.

Results from this study replicate McBurney *et al*. who showed that urea was able to cross-adapt the bitterness of QHCl, while QHCl was not as effective at cross-adapting urea's bitterness (McBurney *et al*., 1972). Others have also inferred that urea and QHCl activate separate bitter taste transduction sequences (Lawless, 1979; Yokomukai *et al*., 1993), which was supported in the present study. The present observation that urea was able to cross-adapt QHCl's bitterness suggests that urea at least activates overlapping bitter TRCs, or receptors/transduction mechanisms involved in transducing QHCl's bitter taste.

One hypothesis regarding urea's ability to cross-adapt many bitter compounds relates to its ability to disrupt non-covalent interactions in proteins and enzymes, and permeate through cellular membranes (Lyall *et al.*, 1999). Urea could potentially modulate a wide variety of processes interfering with receptor protein conformation or altering enzyme activity involved in bitter taste transduction. Closer examination of results from McBurney *et al*. appear to support this hypothesis, and although the adapting concentration of urea (1M) used by McBurney *et al*. was not as concentrated as used in the present experiment, it appears that urea cross-adapted at least 50% of the bitterness of the majority of compounds (QHCl, quinine-SO_4, caffeine, KNO_3, $MgSO_4$, SOA), with the exception of PTC (McBurney *et al*., 1972).

Bitterness inhibition: Sodium salts' influence on bitterness is believed to occur in the peripheral taste system as a result of sodium's action on the gustatory physiology, rather than more central action caused by cognitive effects of perceived saltiness

(Bartoshuk, 1979; Bartoshuk, 1980; Bartoshuk and Seibyl, 1982; Kroeze and Bartoshuk, 1985; Kemp and Beauchamp, 1994; Breslin and Beauchamp, 1995). Keast *et al.* proposed 4 potential modes of action for sodium salts in the peripheral taste system: 1/ shielding of the receptor protein, 2/ moderating or modulating ion channels or pumps, 3/ stabilizing the cell membrane, 4/ interfering with second messenger systems after entering cells (Keast *et al.,* 2001). If L-trp, L-phe and urea were activating the same taste TRCs or receptors/transduction pathways, sodium salts should not differentially inhibit their bitterness. The findings show that sodium salts could not differentially inhibit the bitterness of L-trp, L-phe, and urea, but did differentially affect $MgSO_4$, although the impact of the sodium salts on urea's bitterness was less than previously observed (Breslin and Beauchamp, 1995). This may be due to the current use of very high concentrations (2.33M) of urea compared to the previously used concentration (1M).

When results for inhibition of urea's bitterness were analyzed according to the concentration of urea required to elicit iso-intense bitterness, subjects who required between 3.6M and 2.5M (n=5) reported the inhibition of urea's bitterness was only 12% with the addition of NaCl. Subjects who required a urea concentration of 2.4M or below (n=8) reported NaCl reduced bitterness by 58%, which was similar to bitterness reduction of the amino acids by NaCl (Figure 5). This analysis supports earlier research (Breslin and Beauchamp, 1995) that demonstrated a 60% reduction in urea's (1M) bitterness when NaCl was added. Perhaps concentrated urea solutions (>2.4M) influence TRCs sensory activity; these concentrated solutions, as well as urea's ability to permeate cellular

membranes and disrupt protein configuration, may decrease the effect sodium has on bitter taste transduction.

Conclusions

Self- and cross-adaptation results showed that the two amino acids were very similar but that the amino acids and urea were not identical adapting stimuli; cross-adaptation between the L-trp and L-phe was symmetrical and nearly complete, whereas cross-adaptation of urea and the amino acids was not symmetrical, indicating that urea has a portion of independent taste mechanisms involved in its bitter taste. The bitterness inhibition experiments were unable to differentiate between urea and the amino acids but did show they differed from $MgSO_4$. Since the adaptation experiment activated only fungiform papillae taste cells and the bitterness inhibition experiment activated whole mouth taste cells, the two experiments cannot be directly compared. Rather, each experiment should be viewed as an independent test of whether the amino acids and urea can be psychophysically distinguished.

Three independent psychophysical techniques, namely correlation of individual sensitivities (Delwiche *et al*., 2001), cross-adaptation and bitterness inhibition, illustrate a close perceptual association between L-trp, L-phe and to a lesser degree urea, which suggests shared peripheral physiological mechanisms involved in bitter taste transduction.

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Tables

Table I Mean molarity, range and average bitter intensity for stimuli in anterior tongue adaptation experiment

	Mean molarity	Range	Average LMS value
L-trp	0.053	0.03-0.06	13.9 ±0.4
L-phe	0.12	0.075-0.15	13±0.3
Urea	1.77	1.0-3.0	12.2±0.4
QHCl	0.00022	0.0001-0.0003	14.2±0.3

± standard error

Table II Summary of self and cross adaptation results (also see Fig. 2). Results are LMS intensity ratings of bitterness ± standard error. Pre indicates value prior to adaptation (solution 1 from Fig. 1) and post indicates value post adaptation (solution 8 from Fig. 1).

Adapting Solutions										
Test solutions	Water		Tryptophan		Phenylalanine		Urea		Quinine	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Tryptophan	13.5±1	15.9±1.4	14±1	0.5±0.1	14.2±1	2.8±1	13.2±0.6	2.4±0.5	14.5±1	8±1.3
Phenylalanine	12.8±1	15.6±1	12.7±0.7	1.8±0.4	12.7±1	0.7±0.2	12.9±1	2.6±0.5	13.9±1	8.4±1.5
Urea	12.2±0.5	14±0.7	11.9±0.6	5±1	12.3±0.7	3.7±0.6	12.8±1	0.4±0.2	11.7±0.6	8.6±0.5
Quinine	15±1	16±1	14±0.8	7.6±1	13.4±1	7.4±1	13.8±0.8	4.4±0.7	14.6±0.9	0.5±0.2

Table III Mean molarity, range and average intensity of bitter compounds in whole mouth bitter inhibition experiment

	Mean molarity	Range	Average LMS
L-trp	0.058	0.04-0.06	17.8±1.92
L-phe	0.14	0.111-0.17	18.3±1.55*
Urea	2.33	1.0-3.6	15.6±1.39
$MgSO_4$	0.78	0.3-2.5	13.1±1.31*
QHCl	0.00019	0.00006-0.0003	17.5±1.86

± standard error

* significant difference $p<0.05$

Figures

Figure 1 Schematic diagram representing test and adapting stimuli order for the anterior tongue adaptation experiment.

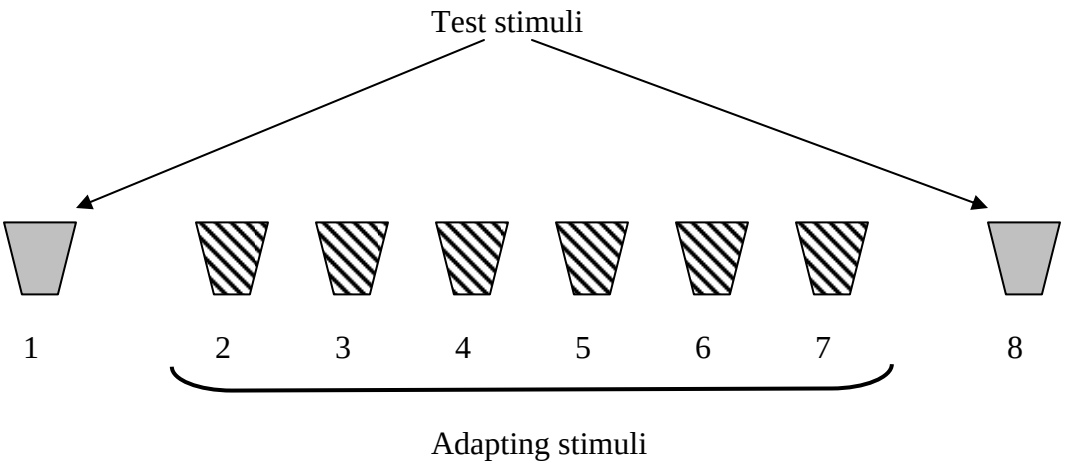
Figure 2 Summary of self and cross adaptation results. The arrowhead is pointing to the test compound while the origin is from the adapting stimulus. Double arrowheads indicate experimental controls of self-adaptation. Next to each arrow is indicated the percentage decrease of each test compound bitterness intensity after adaptation. Significant differences are indicated by *$P<0.01$, ** $P<0.001$.

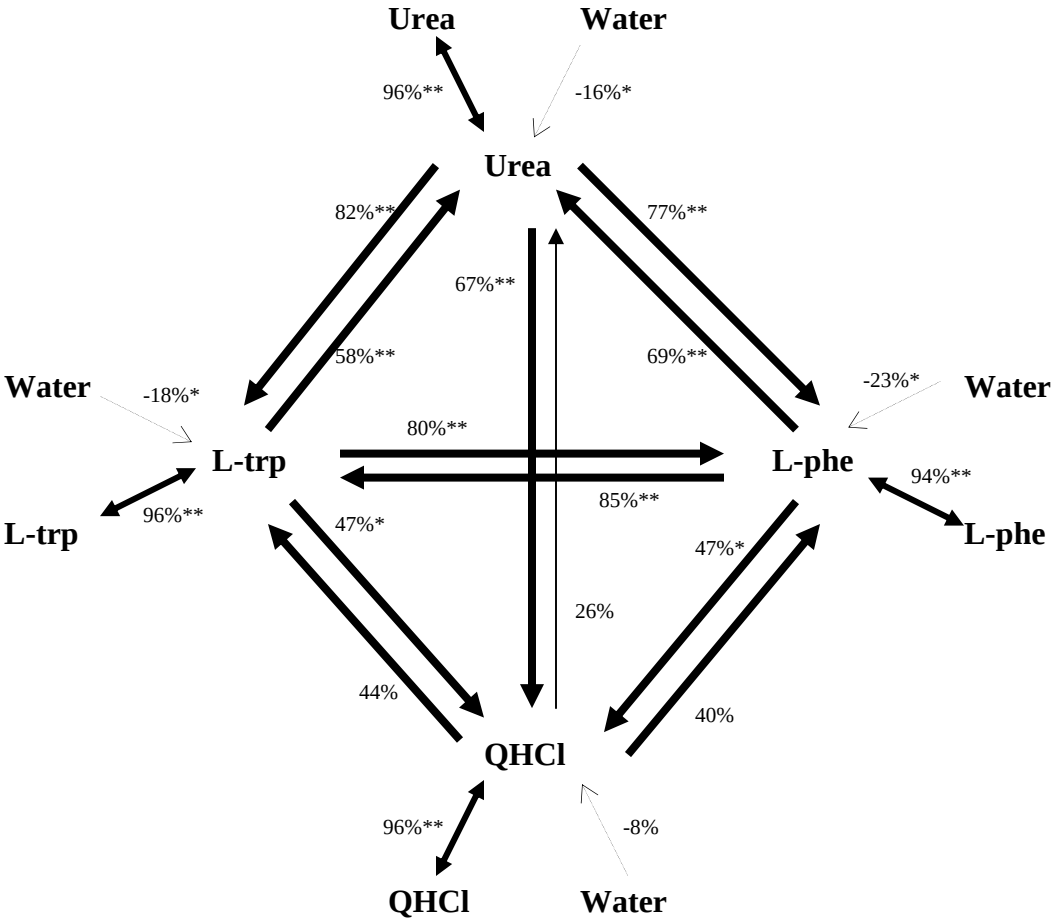
Figure 3 The influence of 300mM NaCl or NaGluconate (NaGlu) on bitterness of various compounds. The y-axis represents bitterness intensity. On the x-axis are shown results for five bitter compounds. Error bars represent standard error.

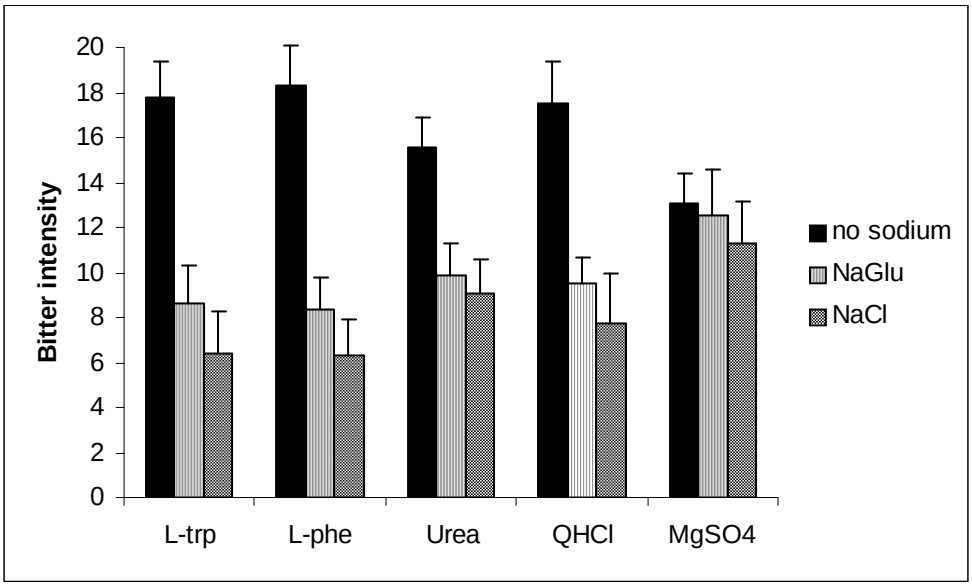
Figure 4 Schematic diagram of two distinct subject sub-populations for cross adaptation of QHCl, L-trp and L-phe. L-pheA and L-trpA 9 of 13 subjects (Group A), L-pheB and L-trpB 4 of 13 subjects (Group B). Arrows and percentages are as in Fig 2.

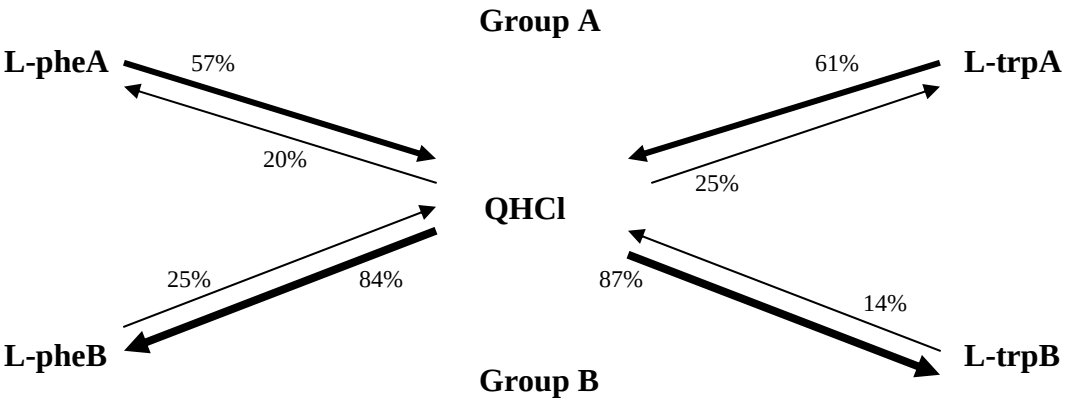
Figure 5 The effect of the concentration of urea on the bitterness inhibiting influence of sodium salts. The y-axis represents bitterness intensity. The x-axis represents the concentration of urea when intensity matched. Error bars represent standard error. The vertical hatched bars represent bitterness of urea when 300mM NaCl was added to the solution, and the stippled bars when 300mM NaGluconate was added.

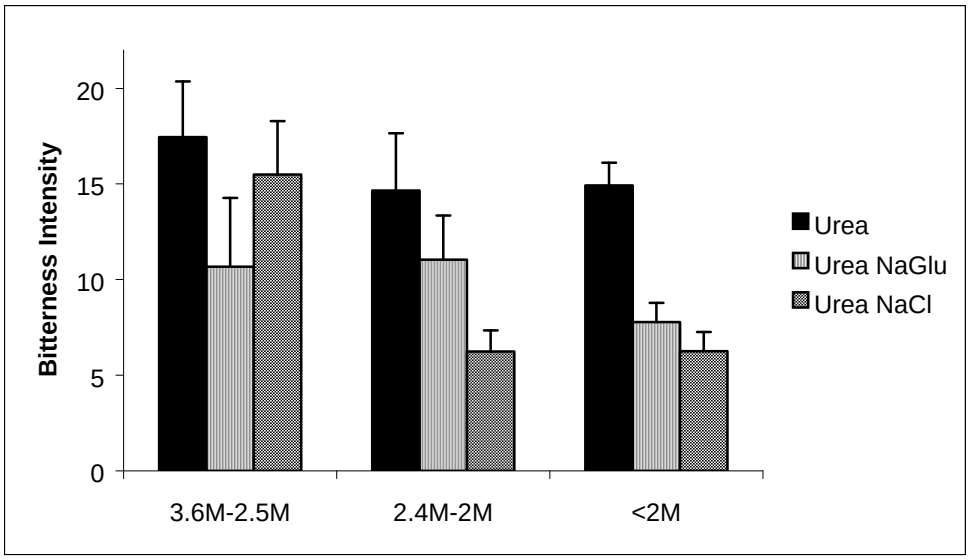
3


Test stimuli
1
2
3
4
5
6
7
8
Adapting stimuli


Urea
Water
96%**
-16%*
Urea
82%**
77%**
67%**
58%**
69%**
Water
-18%*
-23%*
Water
80%**
L-trp
L-phe
94%**
85%**
96%**
L-trp
L-phe
47%*
47%*
26%
44%
40%
QHCl
96%**
-8%
QHCl
Water


Bitter intensity
20
18
16
14
12
10
8
6
4
2
0
L-trp
L-phe
Urea
QHCl
MgSO4
no sodium
NaGlu
NaCl


Group A
L-pheA
57%
20%
61%
L-trpA
25%
QHCl
25%
84%
87%
14%
L-pheB
L-trpB
Group B


Bitterness Intensity
20
15
10
5
0
3.6M-2.5M
2.4M-2M
<2M
Urea
Urea NaGlu
Urea NaCl