Chem. Senses 32: 245–253, 2007

doi:10.1093/chemse/bjl052
Advance Access publication January 13, 2007

A Complex Relationship among Chemical Concentration, Detection Threshold, and Suprathreshold Intensity of Bitter Compounds

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Abstract

Detection thresholds and psychophysical curves were established for caffeine, quinine-HCl (QHCl), and propylthiouracil (PROP) in a sample of 33 subjects (28 female mean age 24 ± 4). The mean detection threshold (±standard error) for caffeine, QHCl, and PROP was 1.2 ± 0.12, 0.0083 ± 0.001, and 0.088 ± 0.07 mM, respectively. Pearson product–moment analysis revealed no significant correlations between detection thresholds of the compounds. Psychophysical curves were constructed for each bitter compound over 6 concentrations. There were significant correlations between incremental points of the individual psychophysical curves for QHCl and PROP. Regarding caffeine, there was a specific concentration (6 mM) below and above which the incremental steps in bitterness were correlated. Between compounds, analysis of psychophysical curves revealed no correlations with PROP, but there were significant correlations between the bitterness of caffeine and QHCl at higher concentrations on the psychophysical curve ($P < 0.05$). Correlation analysis of detection threshold and suprathreshold intensity within a compound revealed a significant correlation between PROP threshold and suprathreshold intensity ($r = 0.46$–0.4, $P < 0.05$), a significant negative correlation for QHCl ($r = -0.33$ to -0.4, $P < 0.05$), and no correlation for caffeine. The results suggest a complex relationship between chemical concentration, detection threshold, and suprathreshold intensity.

Key words: bitter taste, caffeine, individual differences, propylthiouracil, threshold

Introduction

Taste receptors located on taste cells in the surface regions of our oral cavity are activated when chemicals enter our mouths. An electrical impulse is initiated and transferred via afferent fibers to cortical levels of the brain where it is decoded and we experience a perception associated with the chemical. A taste quality is experienced when the chemical concentration in the oral cavity reaches a level that not only activates a receptor, but the signal sent from the receptor is strong enough to elicit a perception. For example, a chemical may be in solution at a concentration that the sample population could not detect. As the concentration of the chemical increases, a detection threshold will be reached, the level at which the chemical in solution may be discriminated from water. As the concentration of the chemical increases further, the recognition threshold is reached, the point at which the quality (e.g., bitter) can be identified. As the concentration of the chemical increases still further, the intensity of bitterness mutually increases to a theoretical asymptote where concentration increases no longer cause subsequent increases in intensity (Keast and Breslin 2003) (Figure 1).

Intuitively, you may expect an individual with low detection threshold (sensitive to the chemical) to experience higher intensities at higher concentrations of the chemical compared with a second individual with higher detection threshold (insensitive to the chemical). An example of this intuitive model is observed with phenylthiocarbamide (PTC), if you have a low detection threshold for PTC (sensitive) you will be sensitive throughout the entire psychophysical function for that compound (Bufe et al. 2005). However, such relationships are not the norm (Bartoshuk 2000; Mojet et al. 2003), presumably, due to both genetic and environmental factors influencing bitter taste and the complex nature of the organization of the oral peripheral and central cognitive system involved in bitter taste processing.

There is a large family of approximately 30 putative bitter taste receptors (TAS2R's) (Adler et al. 2000; Chandrashekar et al. 2000) located on bitter taste cells (Mueller et al. 2005). There are also many postreceptor transduction mechanisms including α-gustducin (McLaughlin et al. 1992), a phospholipase β subtype (Rossler et al. 1998), and transient receptor

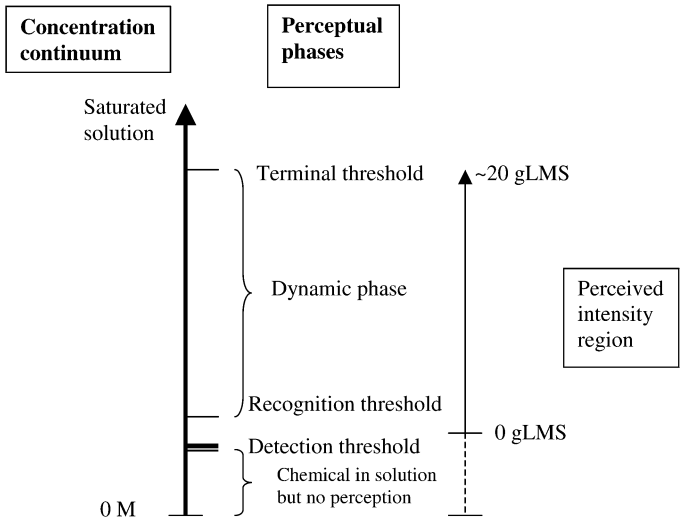

Figure 1 Schematic illustration of the relationship between chemical concentration, detection threshold, and suprathreshold intensity using gLMS. The left-hand side of the bold black *y* axis represents chemical concentration from 0 molar (0 M) solution to a saturated solution. The right-hand side of the bold black *y* axis represents the perceptual relationship to increasing concentration. The far right vertical axis represents the gLMS scale from no perception to a theoretical terminal threshold.

potential ion channels (Perez et al. 2002) to name a few. Any one bitter compound may access multiple transduction mechanisms. For example, caffeine is capable of translocating through cellular membranes and accessing second messenger systems associated with bitter taste (Peri et al. 2000), and quinine-HCl (QHCl) can also activate nonreceptor mechanisms associated with bitter taste cells (Kinnamon and Cummings 1992; Rosenzweig et al. 1999; Caicedo et al. 2003). Although there are multiple mechanisms on or within the bitter taste cell, the bitter quality we perceive is controlled by the taste cell, not the receptors; TAS2R's expressed on sweet taste cells confer appetitive quality to what should be an aversive chemicals (Mueller et al. 2005).

An electrical signal leaves the taste cell and is transferred via afferent fibers to the subcortical areas nucleus of the solitary tract, followed by the second-order synapse in the thalamus, before terminating in several regions of the insula (important in detection and suprathreshold intensity), frontal operculum cortex, and the orbital frontal cortex (important in hedonics). As the signal progresses upstream toward the cortical regions of the brain, greater selectivity of activation is observed and the neurons in the orbital frontal cortex may respond to only one taste quality. The cortical and subcortical regions of the brain integrate the signals and introduce plasticity into the gustatory system with feed-forward and feedback pathways in operation (Katz et al. 2002; Jones et al. 2006).

Presumably, differences in the quality and quantity of the multiple cellular mechanisms associated with bitter taste cells manifest in the large individual variation observed in bitter taste perception (Yokomukai et al. 1993; Bartoshuk et al. 1998; Delwiche et al. 2001; Keast and Breslin 2002b).

Even though there is large variation in bitter taste perception, there is some commonality to bitter taste elicited by multiple chemicals, and these associations have been supported in human psychophysical studies (McBurney 1969; Lawless 1979; Delwiche et al. 2001; Keast and Breslin 2002a). The most studied of all bitter chemicals that have commonality of bitterness are propylthiouracil (PROP) and PTC, primarily, because there is known heritable variability in bitter taste perception that is related to haplotypes of the *TAS2R38* gene (Duffy et al. 2004; Bufe et al. 2005). Other bitter compounds such as caffeine and QHCl have also been extensively studied, and commonality in suprathreshold bitterness has been established by phenotypic variation and genetic modeling (Hansen et al. 2006). However, there is no commonality between PROP bitterness and the bitterness elicited by QHCl and caffeine (Delwiche et al. 2001; Keast et al. 2003; Hansen et al. 2006).

In the present study, the objective was to assess the relationship between chemical concentration, detection threshold, and suprathreshold intensity within and between 3 bitter compounds. Caffeine and QHCl were selected as they share commonality in suprathreshold bitterness perception and therefore may have commonality at detection thresholds level. PROP was selected as it elicits bitterness independent of caffeine and quinine, and the bitterness of PROP has been linked to a single receptor, TAS2R38.

Materials and methods

Subjects

Subjects (n = 33, 23 ± 4 years old, 28 female) between the ages of 18 and 38 were University students in Melbourne, Australia. All subjects agreed to participate and provided informed consent on an approved Institutional Review Board form. The participants, all nonsmokers, were asked to refrain from eating, drinking, or chewing gum for 1 h prior to testing.

Subject training

Participants were initially trained in the use of the general Labeled Magnitude Scale (gLMS) following the published standard procedures (Green et al. 1993, 1996) except the top of the scale was described as the strongest imaginable sensation of any kind (Bartoshuk 2000). The gLMS is a psychophysical tool that requires participants to rate perceived intensity along a vertical axis lined with adjectives: barely detectable = 1.5, weak = 6, moderate = 17, strong = 35, very strong = 52, and strongest imaginable = 100; the adjectives are placed semilogarithmically, based upon experimentally determined intervals to yield data equivalent to magnitude estimation (Green et al. 1993, 1996). The scale only shows adjectives, not numbers, to the participants, but the experimenter calculates numerical data from the scale.

Participants were trained to identify each of the 5 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 0.50 mM QHCl, sweetness as the predominant quality from 300 mM sucrose, sourness as the predominant quality from 3 mM citric acid, and umami the predominant quality from a mixture of 100 mM Monosodium glutamate and 50 mM inosine 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. Sucrose and NaCl were presented at 3 concentrations (50, 200, and 400 mM) to ensure subjects could rank the solutions from least to most intense. All subjects were able to identify and rank taste solutions.

Stimuli and delivery

Caffeine and 6-PROP were purchased from Sigma Chemical (St Louis, MO) and were Sigma-ultra grade. QHCl was purchased from Fluka Chemika (Buchs, Switzerland).

All solutions were prepared with deionized (di) filtered water and were stored in glass bottles at 4–8 °C and were brought to room temperature (20 ± 3 °C) prior to testing. Filtered di water was used as the blank stimulus and the rinsing agent in all experiments.

All testing took place in specialized sensory testing facility comprising 7 individual computerized booths. Each subject was isolated from other subjects by vertical dividers, and there was no interaction between subjects.

Detection threshold determination for caffeine and QHCl, and *n*-PROP

A triangle forced-choice initially ascending procedure was used to determine detection threshold of caffeine, QHCl, and PROP for each subject. The range of concentration used is shown in Table 1: caffeine concentrations were modified from ''ISO 3972 method of investigating sensitivity of taste,'' QHCl concentrations were 0.2 log concentration steps, and PROP concentrations were 0.125 log concentration steps. Starting at the dilution step 3, solutions (10 ml) were presented in 30-ml plastic medicine cups in groups of 3. Subjects were instructed to hold the sample in their mouth for 3 s, then expectorate. Within each set of 3 solutions, 2 were water blanks and the 3rd was the bitter compound, and subjects had to identify which one was different (triangle test). The order of presentation was randomized and could have been any of 3 possible orders (A, blank, and B,stimulus): AAB, ABA, and BAA. If subjects failed to correctly identify the odd sample, the concentration was increased one step. If subjects correctly identified the sample on 2 occasions, the concentration was decreased one step. The level at which the sequence changed from ascending to descending or descending to ascending was termed a reversal. Four reversals were required, and the best estimate threshold for each subject was the geometric mean of the concentration where the last miss occurred and the next higher step. There was an interstimulus interval of approximately 60 s, during which time the subject was required to rinse with di water at least 4 times. Any one session included only one bitter compound and each session could take 30 mins to complete. The detection threshold method was repeated in a separate session to check reproducibility of detection thresholds, meaning a minimum of 6 sessions in total for each subject.

Construction of psychophysical curve for caffeine, QHCl, and *n*-PROP

The concentration ranges for constructing a psychophysical curve for the bitter stimuli are shown in Table 2. For caffeine and QHCl, subjects were presented with numbered trays that

Table 1 Concentrations and dilution steps used to determine subject detection threshold for caffeine, QHCl, and *n*-PROP in water

Caffeine [mM]	QHCl [mM]	PROP [mM]	Dilution step
0.28	0.00064	0.01	1
0.33	0.0009	0.014	2
0.42	0.0013	0.019	3
0.52	0.0017	0.025	4
0.66	0.0025	0.033	5
0.80	0.0035	0.045	6
1.03	0.005	0.059	7
1.3	0.007	0.079	8
1.57	0.01	0.1	9
1.84	0.014	0.14	10
2.11	0.02	0.19	11
2.38	0.028	0.25	12
2.65	0.04	0.33	13

The concentration series for caffeine was adapted from ISO3970, "method of investigating sensitivity of taste," the concentration series for QHCl was prepared with successive 0.15 log dilutions with filtered di water, and the concentration series for PROP was prepared with successive 0.125 log dilution steps.

Table 2 Concentrations of caffeine, QHCl, and *n*-PROP used to generate psychophysical curves

Caffeine [mM]	QHCl [mM]	PROP [mM]
0	0	0
3	0.05	0.05
6	0.1	0.25
12	0.15	0.75
24	0.2	1.25
48	0.25	2.5
	0.3	5.5

contained 7 randomized solutions (10 ml) of one bitter stimulus (6 concentrations from the psychophysical curve and one di water control). For PROP, the only difference was solutions were presented in ascending concentration order, rather than randomized order (Bartoshuk 2000). The 6 concentrations for each bitter stimulus ranged from below "weak" on the gLMS to maximum practical tasting limit. Each point on an individual psychophysical curve was tested at least 3 times.

Stimulus delivery

An aliquot of 10 ml of each solution ($n = 7$) was presented in 30-ml polyethylene medicine cups (Dynarex, Orangeburg, NY) in randomized order (except PROP see above) on a numbered tray. Subjects rinsed with di water at least 4 times over a 2-min period prior to testing. Each subject tasted and then rated each solution for sweetness, sourness, saltiness, bitterness, and umami, prior to expectorating. All subjects rinsed with di water 4 times during the interstimulus interval of 90 s. The gLMS was used as the rating method. Each sample was tasted only once per session, and there were 3 sessions in total as a test of reliability of rating.

Psychophysical curves were constructed for the bitter compounds for each individual subject. These curves provided the opportunity to investigate perceived bitterness correlations as a function of individual sensitivities among bitter compounds at 6 different concentration levels and threshold concentrations. First, the intensity ratings were adjusted for bias in scale use.

Standarization of gLMS ratings with sweetness and weight ratings

The gLMS standardization was a modified version of Delwiche et al. (2001). Briefly, subjects rated the sweetness and total intensity of 10-ml samples of 5 concentrations of sucrose (50, 100, 150, 250, and 400 mM). Between each sample, subjects rinsed 4 times with di water. Subjects also rated the heaviness of 5 visually identical weights (opaque, sand-filled jars at levels 52, 294, 538, 789, and 1028 g). All ratings were made on the gLMS. Subjects were asked to rate the intensity of taste or heaviness, and all judgments were made within the context of the full range of sensations experienced in life. All stimuli were presented twice in blocks of ascending order. Subjects first rated the heaviness of weights and then the intensity of sucrose solutions.

There was a significant correlation between sucrose sweetness and heaviness ratings ($r^2 = 0.49$, $P < 0.05$). Because these sensory modalities were assumed to be unrelated, the significant correlation indicated that the gLMS ratings were prone to individual scale-use bias and required standardization across subjects.

To determine a standardization factor, each subject's average intensity for heaviness was divided by the grand mean for heaviness across weight levels and subjects. Each individual's bitter intensity ratings for caffeine, QHCl, and PROP were multiplied by his or her personal standardization factor for scale-use bias.

Statistical analysis

Data used for correlation analysis were the detection threshold concentrations and the individual bitterness intensity ratings (gLMS) at stated concentration levels. Correlation analysis (Pearson product–moment coefficients) was performed using SPSS version 12.0.1. Subjects who are termed insensitive to the bitter compounds tested have a higher detection threshold and lower intensity rating than sensitive subjects (lower detection threshold, higher intensity rating). When this data is analyzed, what is a positive correlation will have a negative sign. Therefore, in order to assess correlations between the detection threshold concentrations and suprathreshold intensities, positive r values were converted to negative and vice versa.

PASS statistical software (2005) was used to determine the power of this study. Assuming $r = 0.35$, $n = 33$, and $\alpha < 0.05$, the power of the study is 0.65. Ideally, a power of 0.8 should be achieved, and with $n = 33$ and $\alpha < 0.05$, the r value = 0.45. The study was large enough to assume a type II error is within acceptable range.

Results

Detection threshold

The mean detection threshold and standard error for caffeine, QHCl, and PROP was 1.2 ± 0.12, 0.0083 ± 0.001, and 0.088 ± 0.07 mM, respectively. The relationship between detection thresholds for caffeine and QHCl among subjects was investigated using Pearson product–moment correlation coefficient. There was no correlation between detection thresholds for caffeine, QHCl, and PROP ($n = 33$, $r = -0.006$ to -0.24, $P = 0.97 - 0.18$) (Figure 2).

Suprathreshold intensities

Psychophysical curves were constructed for caffeine, QHCl, and PROP, and there was much individual variation in bitterness perception (Figure 3A,B, and C). Even though bitterness intensity varied among subjects, as the concentration of QHCl and PROP increased, there was ordinal increases in bitterness intensity across subjects and, as expected, Pearson coefficient correlations revealed a significant relationship between all points on a bitter compound's psychophysical curve ([QHCl; $r = 0.61$–0.88, $P < 0.001$) (PROP; $r = 0.65$–0.924, $P < 0.001$]). Analysis of variance results showed significant differences between all incremental steps on the psychophysical curves ($P < 0.05$). This indicates that when a subject is given increasing concentrations of quinine or PROP (above detection threshold), there is an ordinal increase in bitterness intensity relative to intensity ratings across all subjects (a subject who was insensitive to the bitter

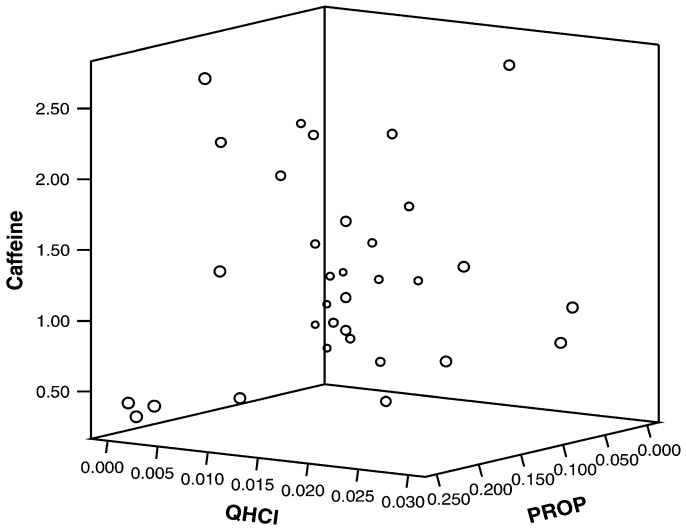

Figure 2 Detection threshold correlation. Detection threshold concentrations for caffeine, QHCl and *n*-PROP on a 3-dimensional plot. All concentrations are in millimolar, the *y* axis is caffeine, *x* axis is QHCl, and the *z* axis is PROP. Each point represents the threshold concentrations for 1 of the 33 subjects.

taste of the stimulus remains insensitive in relation to the other subjects for the concentrations tested). The strong correlation was also evident for caffeine but only at the higher concentrations 12–72mM (r = 0.61–0.96, $P < 0.001$). Whereas, at 6 mM caffeine, there was a strong correlation with 3 (r = 0.63, $P < 0.001$) and 12 mM caffeine (r = 0.61, $P < 0.001$) and weaker correlations with higher caffeine concentration (r = 0.43–0.46, $P < 0.05$). The bitterness intensity ratings of the subjects at lowest concentration of caffeine (3 mM) did not correlate with any of the concentrations above 6 mM (r = −0.06–0.2, P = 0.2–0.9). This indicates a low concentration and high concentration mechanism responsible for the perceived bitter taste of caffeine.

There were no significant correlations with subjects' intensity rating of caffeine and PROP (r = −0.06–0.1, P = 0.82–0.5) or QHCl and PROP (r = 0.07 – 0.3, P = 0.72–0.07), which is similar to other studies investigating correlations of bitter compounds with PROP bitterness (Delwiche et al. 2001; Keast et al. 2003; Hansen et al. 2006). Therefore, sensitivity to the bitterness of PROP does not predicate that the subject will be sensitive to the bitterness of caffeine or QHCl. At the 3 highest concentrations of caffeine and QHCl tested, there were significant correlations (r = 0.56–0.36, $P <$ 0.05). This supports previous research indicating perceptual and genetic similarities between the bitterness of caffeine and QHCl (Delwiche et al. 2001; Hansen et al. 2006).

Detection threshold and suprathreshold intensity among compounds

Table 3 shows Pearson product–moment correlation coefficient for detection threshold concentration and suprathreshold

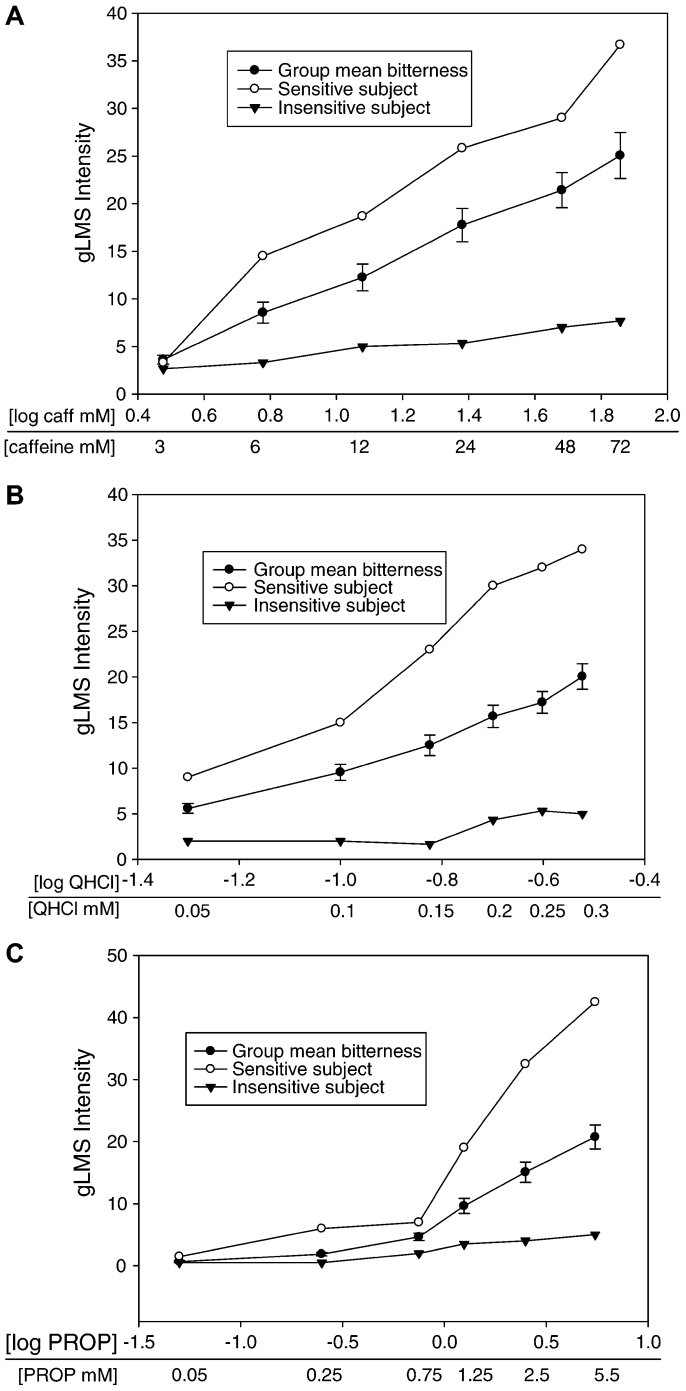

Figure 3 Psychophysical curves of the sample population mean and examples of an insensitive and sensitive subject for **(A)** caffeine, **(B)** QHCl, and **(C)** PROP. Included in each graph is a sensitive (highest curve) and insensitive subject (lowest curve) for that compound as well as the mean psychophysical curve. The *y* axis is a numerical measure of bitterness intensity from the gLMS. The *x* axis has 2 labels, the upper label in the log millimolar concentration for the particular compound and the lower label is the actual millimolar concentration. Error bars represent standard errors.

Table 3 Pearsons product–moment correlation between threshold and 6 suprathreshold intensity ratings for caffeine, QHCl, and 6-*n*-PROP

Stimulus No.	1	2	3	4	5	6
Caffeine	0.001, NS	0.15, NS	−0.2, NS	−0.09, NS	−0.08, NS	−0.05, NS
QHCl	−0.08, NS	−0.38*	−0.4*	−0.36*	−0.37*	−0.33*
PROP	0.26, NS	0.43**	0.43**	0.46**	0.4*	0.43**

NS, not significant. Concentrations of chemicals for stimulus number 1–6 are shown in Table 2.
*$P < 0.05$.
**$P \leq 0.01$.

intensities for the individual bitter compounds across subjects. There was no significant correlation between detection threshold and suprathreshold intensity ratings for caffeine. Surprisingly, there was a negative correlation between threshold of QHCl and suprathreshold intensity ratings of QHCl. This indicates that subjects who were sensitive to QHCl (low-threshold concentrations) generally found higher concentrations of QHCl less bitter, whereas subjects who were insensitive to QHCl (high-threshold concentrations) perceived higher concentrations of QHCl more bitter. There were positive correlations between PROP threshold and suprathreshold intensity rating (except at the lowest concentration on the psychophysical curve).

Discussion

The relationship between the concentration of a chemical and the perception of that chemical (intensity and liking) is complex (Amerine et al. 1965; Bartoshuk 2000; Mojet et al. 2005). The results from this study do not diminish that complexity; indeed, they add to complex relationship between chemical concentration, detection threshold, and suprathreshold intensity. As the concentration of a chemical increases from detection threshold to suprathreshold, there was a significant positive correlation for PROP, a significant negative correlation with QHCl, and no correlation for caffeine. The complexity may be due to multiple perceptual and peripheral mechanisms of bitter taste, and these multiple mechanisms may be activated at different concentrations. Figure 4 illustrates the positive and negative correlations among chemical concentration, detection threshold, and suprathreshold intensity observed in this study. As the statistics infer, Figure 4 is a generalization of results from this study and not all subjects will follow the model.

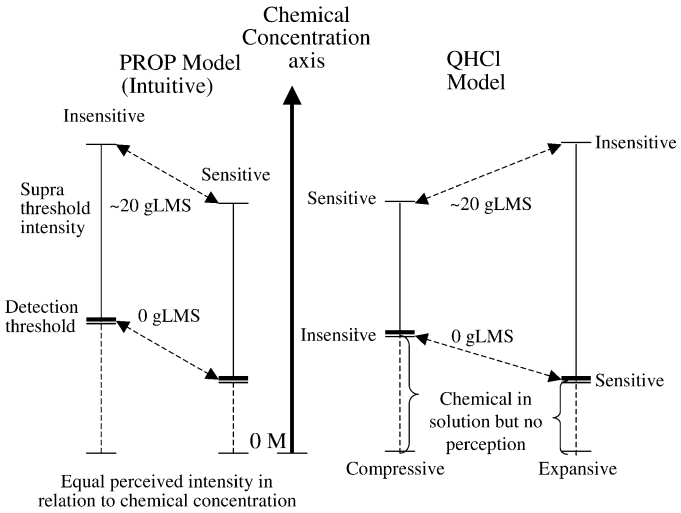

Figure 4 Schematic illustration of the association between chemical concentration, detection threshold, and suprathreshold intensity for PROP and QHCl. The bold black solid vertical line represents the chemical concentration. The thin solid vertical lines represent the gLMS intensity rating relative to the chemical concentration. The bottom of each thin solid line represents the detection threshold. The top of each solid line represents an intensity of ~20 on the gLMS scale. The vertical dashed line below the solid line represents the concentrations of chemical in solution without eliciting a noticeable difference from water. The left-hand side of the chemical concentration axis illustrates results observed for PROP, with sensitivity at detection threshold consistent over the concentration range tested. This is illustrated by an equal perceived intensity range relative to chemical concentration regardless of an individual's sensitivity to PROP. The right-hand side of the concentration axis illustrates the results observed for QHCl with subjects rating between 0 and 20 gLMS as either a compressed perceived intensity range relative to chemical concentration or an expansive perceived intensity range (far right) relative to the chemical concentration.

6-*n*-Propylthiouracil

In this study, PROP observed the intuitive model of sensitivity throughout a concentration range with sensitivity at low concentration predicting sensitivity at higher concentrations (Figure 4). However, in a comprehensive review of variation in taste perception, Bartoshuk (2000) has previously stated relying on detection thresholds for PROP may cause misclassification of subjects' "taster" status in the suprathreshold range. In support of Bartoshuk's observation, classifying PROP taster status on detection thresholds would have resulted misclassification of 4 of the 33 subjects at suprathreshold intensity, even though there was a significant correlation between detection threshold and suprathreshold sensitivity for PROP. The ability to taste PROP has been linked to the bitter receptor gene *hTAS2R38* (Duffy et al. 2004), and there is a very close association between absolute detection threshold and *hTAS2R38* haplotypes (Bufe et al. 2005). As there is one known receptor linked to perception

of PROP, it is not surprising to find a significant relationship between detection threshold and suprathreshold intensity. However, even for the PROP, there is speculation that additional genetic or environmental controls govern bitter taste perception as the PROP concentration increases (Bufe et al. 2005).

Quinine-HCl

In this study, there was a negative correlation between QHCl detection threshold concentration and suprathreshold intensity. Figure 4 illustrates that a subset of the sample population have a compressed perceived intensity range relative to chemical concentration, whereas a second subset of the population have an expansive perceived intensity range relative to the chemical concentration. There have been few reports of such negative correlations between threshold and suprathreshold sensitivity within taste, although Mojet et al. (2005) reported similar negative correlations for salt and umami qualities. The psychophysical data for QHCl suggests at least 2 perceptual mechanisms, an independent factor regulating threshold detection, which covaries with mechanisms associated with suprathreshold intensities. Multiple perceptual mechanisms of QHCl are supported by multiple peripheral mechanisms, including the ability of quinine to block K+ channels (Kinnamon and Cummings 1992), and in addition to sharing genetic factors associated with variation in perception with caffeine, QHCl has a putative specific genetic factor regulating only its bitterness perception (Bachmanov et al. 1996; Hansen et al. 2006).

Caffeine

Caffeine results were the most intriguing of the compounds tested. There was no correlation between detection sensitivity and sensitivity to caffeine at any point of the psychophysical function. Moreover, there was a specific concentration (6 mM) where perceived bitter taste could be differentiated—the lower concentrations elicited bitterness that was correlated among subjects, the same for the higher concentrations. However, the bitterness elicited by ≤6 and ≥6 mM concentrations did not correlate with each other. Overall, there were 3 perceptual shifts associated with caffeine concentration, which may indicate 3 different bitter taste mechanisms: one for detection threshold (very low concentrations, ≤1 mM); one for ~1 to <6 mM concentrations of caffeine; and one for >6 mM concentrations of caffeine. Multiple perceptual mechanisms for caffeine bitterness is supported by multiple independent putative mechanisms: caffeine can translocate through cellular membranes and has the ability to interfere with second messenger systems (Peri et al. 2000); the bitterness of caffeine has been associated with the bitterness of QHCl (this study and Delwiche et al. 2001); and there is a proposed small (2%) genetic link between PROP and caffeine (*hTAS2R38*) (Hansen et al. 2006).

Detection threshold and suprathreshold intensity among compounds

In this study, there was no correlation between the detection thresholds of all 3 compounds; therefore, sensitivity to bitter compounds at threshold level was not common across subjects. This suggests that caffeine, QHCl, and PROP have independent mechanisms responsible for their detection at low concentration. This was not surprising for PROP, as previous research has established no common bitterness with caffeine and QHCl at suprathreshold level, a result that was replicated in the present study. Previous research has shown an association between caffeine and QHCl at suprathreshold intensities (Delwiche et al. 2001; Hansen et al. 2006), a finding that was also replicated in the present study. However, at lower concentrations, there was no correlation indicating that the commonality in bitterness between caffeine and QHCl may be due to a bitter taste mechanism activated at higher concentrations of the 2 compounds.

Organization of the bitter taste system

If a single receptor was responsible for detection and suprathreshold intensity, you would expect a strong correlation between chemical concentration, detection threshold, and suprathreshold intensity, and this was observed with PROP (Figure 4). However, if there are multiple taste transduction mechanisms that are activated at varying concentrations of the chemical, there may be no association between detection threshold and suprathreshold intensity, and this was observed with caffeine. A negative association may occur if a high-affinity receptor process was activated at very low concentrations of the chemical, but high enough to reach a detection threshold; then, as the concentration was increased, a lower affinity receptor mechanism was activated and was responsible for a perceived quality. If a subject had a larger quantity of 1 of the 2 receptor types, we may expect a negative association between detection threshold and suprathreshold intensity, and this was observed with QHCl (Figure 4).

The variation and lack of correlation in bitter taste perception may be due to multiple factors. Recent advances in our knowledge of the peripheral organization of the taste system strongly indicate that taste receptor cells are quality specific (Mueller et al. 2005; Huang et al. 2006). In addition to this, not all bitter taste cells contain all bitter taste receptors, but subsets of receptors are located on bitter taste cells (Chandrashekar et al. 2000). Variation in receptor subsets of receptors on bitter taste cells may influence bitter taste perception. For example, sweet and umami taste are activated by heterodimers of the TAS1R family, and it is not inconceivable the same dimer system could occur with the TAS2Rs on bitter taste cells. If a bitter taste cell lacks one part of a dimer, activation of that cell would not occur. There may also be single-nucleotide polymorphisms in TAS2Rs that result in differences in bitter taste perception (Bufe et al. 2005). Moreover, each TAS2R may have multiple

binding sites that are low or high affinity, and as the concentration of a compound increases the lower affinity receptor or active site of the receptor is activated (Galindo-Cuspinera et al. 2006).

Within an individual, the strength of an afferent signal may be magnified relative to other individuals. There may also be interindividual variations in the signal processing in the human brain, although our understanding of gustatory processing in the brain is still in its infancy (Small 2006).

Conclusions

There is a complex relationship between chemical concentration, detection threshold, and suprathreshold intensity of bitter compounds. The sensitivity of a person to detect very low concentrations of a compound is not necessarily associated with their sensitivity to the same compound when it is perceivably bitter. Moreover, in some situations, threshold sensitivity to a compound may be inversely related to the intensity of perceived bitterness of that compound. Such complexity has practical implications as threshold determination methods are increasingly (and incorrectly) used to infer suprathreshold intensity of specific compounds, for example, taste dilution analysis, (Frank et al. 2001; Ottinger et al. 2003). More broadly, this paper also continues to support that attempts to link threshold measures to food sensations and intake are at best misguided.

The bitter taste system may have distinct perceptual stages, one for threshold and at least one for suprathreshold intensities, and these perceptual stages may relate to distinct oral peripheral mechanisms. As the concentration of a compound increases, receptors that have a lower affinity for the compound may become involved in the process of taste transduction, resulting in perceptual phases that can be differentiated using psychophysical methods of evaluation.

Acknowledgements

We thank Professor Sing Kai Lo for his advice on the statistics undertaken in this study. Financial support was received from the School of Exercise and Nutrition Science, Deakin University. We thank all the subjects who took part in this study.

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Accepted December 8, 2006