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Variability in Human Bitter Taste Sensitivity to Chemically Diverse Compounds Can Be Accounted for by Differential TAS2R Activation

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Abstract

The human population displays high variation in taste perception. Differences in individual taste sensitivity may also impact on nutrient intake and overall appetite. A well-characterized example is the variable perception of bitter compounds such as 6-n-propylthiouracil (PROP) and phenylthiocarbamide (PTC), which can be accounted for at the molecular level by polymorphic variants in the specific type 2 taste receptor (TAS2R38). This phenotypic variation has been associated with influencing dietary preference and other behaviors, although the generalization of PROP/PTC taster status as a predictor of sensitivity to other tastes is controversial. Here, we proposed that the taste sensitivities of different bitter compounds would be correlated only when they activate the same bitter taste receptor. Thirty-four volunteers were exposed to 8 bitter compounds that were selected based on their potential to activate overlapping and distinct repertoires of TAS2Rs. Taste intensity ratings were evaluated using the general Labeled Magnitude Scale. Our data demonstrate a strong interaction between the intensity for bitter substances when they activate common TAS2Rs. Consequently, PROP/PTC sensitivity was not a reliable predictor of general bitter sensitivity. In addition, our findings provide a novel framework to predict taste sensitivity based on their specific T2R activation profile.

Key words: bitter taste, gLMS, hypertaster, hypotaster, TAS2R, PROP

Introduction

Dietary habits are often influenced by the hedonic value of foods. Human appetite for highly palatable energy-rich foods may lead to overconsumption, one of the principal causes of obesity (Yeomans et al. 2004). However, particularly since the discovery and characterization of the genes responsible for taste sensing (Hoon et al. 1999; Adler et al. 2000; Chandrashekar et al. 2000; Matsunami et al. 2000), it has also become apparent that the human population displays an enormous diversity in taste perception. In recent years, genetic polymorphisms identified in taste receptors have been associated with differences in

human sensitivity to sweet (Fushan et al. 2009), umami (Raliou et al. 2009), fatty acids (Keller et al. 2012) and bitter compounds (Soranzo et al. 2005; Pronin et al. 2007; Roudnitzky et al. 2011; Allen et al. 2013). In addition, it has been suggested that individuals with higher taste acuity might be able to convey stronger and faster signals to the hypothalamus which in turn would result in an early onset of satiety and better control of food intake (Stewart et al. 2011; Shafaie et al. 2013). Moreover, as taste receptors are now recognized as expressed in cells and tissues throughout the body, the functional implications of these receptor variants may also extend beyond the oral cavity (Behrens and Meyerhof 2011; Foster et al. 2014). Accordingly, it is important to gain an understanding of the molecular mechanisms of taste and taste sensitivity, as there is the potential to apply this knowledge to modulating hedonic value of foods in the context of trying to lower obesity incidence.

Bitter taste evolved as a sensing mechanism to detect and avoid consumption of a wide range of potential toxins present in food (Sternini 2007). Consequently, high sensitivity in bitter perception may have direct survival implications (Behrens and Meyerhof 2013). In humans, the detection of thousands of bitter compounds is mediated through a family of 25 bitter receptors (TAS2Rs) from the G protein-coupled receptor (GPCR) superfamily (Adler et al. 2000; Chandrashekar et al. 2000; Matsunami et al. 2000). TAS2Rs are genetically diverse and highly polymorphic genes (Drayna 2005; Kim et al. 2005), and these receptor variants often display altered functionality (Soranzo et al. 2005; Pronin et al. 2007; Roudnitzky et al. 2011).

The best characterized example is TAS2R38, which mediates the detection of thiouracil compounds such as 6-n-propylthiouracil (PROP) and phenylthiocarbamide (PTC) (Wooding 2006; reviewed in Hayes et al. 2013). In 2003, the molecular basis for the variability in PROP/PTC taste intensity was elucidated (Kim et al. 2003), more than 80 years after the initial suggestion that the trait could be genetically linked (Blakeslee 1932). Thus, most of the variability in PTC sensitivity can be accounted for by 3 single nucleotide polymorphisms that form 2 common haplotypes, giving rise to receptor variants that contain the amino acids proline, alanine, and valine in positions, 49, 262, and 296, respectively, or alanine, valine, and isoleucine, and which hence have been designated PAV and AVI. These haplotypes underpin the broad segregation of the population into PROP and PTC "tasters" and "nontasters" (Bartoshuk et al. 1994). Interestingly, a subset of tasters perceives PROP as intensely bitter, leading to the coining of the now ubiquitous term "supertaster" (Bartoshuk 1991; Bartoshuk et al. 1994). Although the notion of hypertasting originally referred to the heightened PROP sensitivity, it has since been generalized to include the influence of this phenotype on other taste and somatosensory stimuli (Bartoshuk et al. 1998; Prescott and Swain-Campbell 2000; Hayes and Duffy 2007; Hayes and Keast 2011).

Similarly, there is a substantial body of literature relating PROP sensitivity to other behaviors, including dietary preferences, risk of alcoholism, the control of food intake and risk of obesity and the prediction of taste thresholds for other compounds (Anliker et al. 1991; Looy and Weingarten 1992; Pelchat and Danowski 1992; Drewnowski and Rock 1995; Hong et al. 2005; Shafaie et al. 2013). Several early studies addressed the influence of PROP sensitivity on the taste sensitivity of other bitter agonists, albeit pre-dating the identification of the taste receptors as molecular mediators of taste (Hall et al. 1975; Bartoshuk 1979; Ly and Drewnowski 2001). Nonetheless, the evidence that PROP status is the sole predicting factor determining sensitivity to other bitter and non-bitter tastes has been controversial (Hayes et al. 2008).

Notwithstanding the advances in the molecular pharmacology of taste receptors, the role of specific TAS2Rs as determinants of human taste sensitivity to different bitter compounds has been largely overlooked. There are several notable cases where variations of *TAS2R* genes influence the taste sensitivity to different bitter compounds (Kim et al. 2003; Pronin et al. 2007; Hayes et al. 2011; Roudnitzky et al. 2011). For example, the taste sensitivity for the related compounds PROP and PTC is highly correlated (Bartoshuk et al. 1994; Hayes et al. 2008), which can be reconciled at the molecular level by their shared activation of TAS2R38 (Bufe et al. 2005). As the culmination of a series of studies conducted in the past decade, there is now a wealth of information about bitter agonists and their respective TAS2R activation profiles (reviewed in Behrens and Meyerhof 2013). It is clear that TAS2Rs vary in their ability to respond to agonists, resulting in both specific and promiscuous receptors, while the same agonists can also activate multiple receptors (Meyerhof et al. 2010). Incorporating and synthesizing these findings, in this study we sought to investigate the relationship between taste sensitivity to PROP and other bitter compounds. Accordingly, using suprathreshold bitterness perception testing for bitter compounds selected for their ability to activate different receptors, we examined the link between taste sensitivity and TAS2R activation profiles.

Materials and methods

Participants

Thirty-five volunteer staff and students of the University of Queensland (14 males and 21 females) aged between 18 and 51 years old (mean age 27.4 years ± 1.5 SEM) were recruited to perform taste testing. Participants were of diverse ethnic origin: 15 South-East Asians, 8 Caucasian-Australians, 7 Chinese, 3 Middle-Eastern, and 2 South-Asians (Indian subcontinent). All participants gave informed, written consent prior to the commencement of the study, and were selected after successfully completing the eligibility questionnaires and the initial training session. Volunteers with known illnesses, under medication, pregnant or lactating or reporting any kind of food allergies were not recruited for the current study. A single female volunteer was also excluded from the analyses due to not completing the second trial. All the procedures were approved by the University of Queensland Human Ethics Committee (Project number 2012001239) and complied with the Declaration of Helsinki for Medical Research involving Human Subjects.

Bitter taste compounds

Eight compounds or natural extracts known to taste bitter to humans were selected based on their chemical diversity and differential activation of TAS2Rs (Meyerhof et al. 2010) (Table 1). Where possible, the test concentrations for bitter taste compounds were selected based on previous literature from human psychophysical studies (references are provided for each compound below). Alternatively, preliminary trials with a small panel of volunteers were performed prior to establish suprathreshold test concentrations with similar intensity. The bitter taste compounds used were: PROP (0.32 mM, Fluka P3700000, Sigma–Aldrich) (Galindo-Cuspinera et al. 2009), sinigrin (1 mM, purchased as hydrated form, S1647 Sigma–Aldrich) (Krul et al. 2002), saccharin (0.8 mM, purchased as sodium salt hydrate, S1002 Sigma–Aldrich) (Pronin et al. 2007), caffeine (2 mM, purchased as anhydrate powder, F05075, Melbourne Food Depot) (Ly and Drewnowski 2001), quassia extract (6 ppm; active principle quassin, 65818 Sensient Technologies) (Scragg and Allan 1994), quinine (0.03 mM, as hydrochloride salt, 65707 Sensient Technologies), and gentian extract (30 ppm; active principle amarogentin, Integria health care). PTC paper strips were purchased from EISCO labs (Product FSC1031, Phenylthiourea Paper Strips). Sodium chloride

Table 1. Chemically diverse bitter taste compounds display different bitter taste receptor (TAS2R) activation profiles

Receptor	Bitter taste compound							
	PROP	PTC	Sinigrin	Saccharin	Caffeine	Quassia	Quinine	Gentian
TAS2R1	—	—	—	—	—	—	—	30
TAS2R3								
TAS2R4	—	—	—	—	—	300	10	300
TAS2R5								
TAS2R7	—	—	—	—	300	—	10	—
TAS2R8	—	—	—	—	—	—	—	—
TAS2R9								
TAS2R10	—	—	—	—	300	300	10	—
TAS2R13								
TAS2R14	—	—	—	—	300	300	10	—
TAS2R16	—	—	100	—	—	—	—	—
TAS2R19								
TAS2R20								
TAS2R30	—	—	—	—	—	300	—	3
TAS2R31	—	—	—	80	—	—	10	—
TAS2R38	0.06	0.02	100	—	—	—	—	—
TAS2R39	—	—	—	—	—	—	10	300
TAS2R40	—	—	—	—	—	—	10	—
TAS2R41								
TAS2R42								
TAS2R43	—	—	—	170	300	—	10	30
TAS2R46	—	—	—	—	300	300	10	10
TAS2R50	—	—	—	—	—	—	—	100
TAS2R60								

Agonist concentrations (in μM) that activate the respective TAS2Rs in vitro are shown. Current TAS2R nomenclature is used.
Source: Meyerhof et al. (2010).

(NaCl, 100 mM) and green apples, used as a control taste modality and for palate cleansing, respectively (Johnson and Vickers 2004; Lucak and Delwiche 2009), were purchased from a local supermarket. Bitter taste compounds were diluted in spring water the day prior to a test session and stored at 4 °C. On the day of testing, solutions were served at room temperature (22 °C ± 2) as 10 mL portions in disposable cups, except for PTC, which was evaluated using a taste test strip. Testing was performed within individual booths in the Food Sensory Laboratory at The University of Queensland.

Experimental design and scaling methodology

A random within-subject design was adopted in the investigation. The study was performed over 1 training and 4 experimental sessions. Panelists were trained using 2 solutions, NaCl and PROP, which were prepared the day before the session and stored at 3–4 °C. During each experimental session, participants were provided with 4 randomly allocated bitter taste compounds, along with NaCl, apple and water to rinse between subsequent samples (Supplementary Table 1). All testing sessions lasted around 20 min and occurred from Tuesday to Friday between 9 AM until 12 PM, to control for the potential confounder of time on taste sensitivity. At the start of the first session each volunteer was weighed in the sensory lab. In addition, self-reported height was annotated to calculate BMI.

Participants used the general Labeled Magnitude Scale (gLMS) to report the intensity of the taste compounds, as has been described previously (Bartoshuk et al. 2003, 2004). The weighted scale includes the labels, with their corresponding numerical values shown in parentheses: "no sensation" (0), "barely detectable" (1.4), "weak" (6), "moderate" (17), "strong" (35), and "very strong" (53), and "the strongest imaginable sensation of any kind" (100) (Hayes et al. 2008).

Statistical analysis

Taste intensity data were recorded for all participants across the multiple sessions, along with relevant additional characteristics (including sex, age, and body mass index [BMI]). At the completion of the study, average intensity ratings were calculated for each taste compound and each participant. These ratings were used to categorize the individual taster status for each of the bitter compounds, according to the scaling previously used to classify PROP taster status (Tepper et al. 2001). The cut-off criteria for taster status classification (percent of the whole length scale) were: hypotaster ≤15.5, normal taster >15.5, and hypertaster ≥51.

The multivariate data containing the taste intensity scores for each of the 8 bitter compounds for each panelist was analyzed using principal component analysis (PCA). The data for each compound were standardized as although the bitter compounds were all scored using the same scale, there were marked differences in the average perceived intensity of the bitterness between different compounds. To investigate the similarity of the scores of the bitter compounds, agglomerative hierarchical clustering using single linkage and correlation coefficient distance was carried out. The clustering analyzed the 2 intensity scores for each panelist for each compound. The method commences with each of the bitter compounds separate as its own group and then progressively joins those which are most similar. As a result the calculation requires both a measure of similarity of the bitter compounds and a measure of defining the distance between clusters. The correlation matrix was used as the measure of similarity and the average linkage as the distance measure.

The main effects of BMI, session and sex on taste intensity, as well as the relationship between each binary combination of bitter taste compound, were tested using Pearson's correlations. Additional statistical analyses between groups were performed using 1-way

analysis of variance (ANOVA), as indicated. *P* values below 0.05 were considered significant.

Results

Variability in taste intensity for different bitter compounds

The taste intensity was evaluated for a selection of known bitter compounds that were previously associated with the activation of human bitter taste receptors (TAS2Rs). In addition to the archetypal bitter tastant PROP, we tested PTC, sinigrin, saccharin, caffeine, quassia extract, quinine and gentian extract. There was high level of concordance in the participants' intensity scores collected on repeated bitter taste compound trials (Supplementary Figure 1), which was also reflected in the overall retest correlation coefficient of 0.77 ($P < 0.05$). Consistent with the body of literature on variability in human taste perception, each participant displayed a unique tasting profile, where the taste intensity scores differed appreciably between the bitter substances tested (Supplementary Figure 2).

Taste intensity to other bitter compounds was independent of PROP taster status

Taste intensity values were used to classify the participants' taster status to PROP, according to established criteria whereby people are defined as hypertasters, normal tasters, and hypotasters (Tepper et al. 2001). In our cohort, there were 47% PROP hypertasters, 35% PROP normal tasters, and 18% PROP hypotasters (Figure 1A). Using the same criteria, we then classified the participants' taster status for the other bitter substances. Interestingly, PROP taster status was not significantly related to the taster status for the remaining structurally diverse bitter taste substances (Figure 1B, 1-way ANOVA, with Tukey's post-test). For each of the bitter tastants tested, there was considerable variation of participant-reported intensity values among the cohort, which is reflected in the number of panelists for each taster status (Table 2) and their individual intensity scores (Figure 2).

Relationships between the taste intensity of distinct bitter compounds

PCA was used to investigate the overall relationships between taste intensity scores and the different bitter compounds. The first 2 principal components accounted for more than 68% of the variability in the taste intensity scores (PC1 40.4%; PC2 28.3%). The loading plot of the first 2 components produced from the PCA (Figure 3A) showed a very close relationship between the 2 thiouracil compounds PROP and PTC, and another close relationship between caffeine, gentian, quassia, and QHCl. However, it is acknowledged that the sample size of the current trial may have limited the capacity to differentiate between these compounds.

The results of the cluster analysis confirmed the results obtained of both the Pearson's correlations and the PCA. The dendrogram produced (Figure 3B) shows a clear division into 2 trees with PTC, PROP (which have a very similar response) and sinigrin on 1 side and the remaining 5 compounds on the other. In addition, the 4 group solution separates sinigrin from PTC and PROP, and Saccharin from the QHCl, quassia, gentian, and caffeine.

Correlations between taste intensity for bitter compounds was related to TAS2R activation

We next performed correlations between the taste intensity values for all binary combinations of the 8 bitter substances (Table 3), and compared these to the respective profile of the bitter substance-mediated TAS2R activation (Meyerhof et al. 2010). In agreement with previous literature (Kim et al. 2003; Bufe et al. 2005) and our principal components analysis data, the PROP and PTC sensitivities were highly correlated ($P < 0.001$). In addition, sinigrin was also correlated with PROP ($P < 0.001$), consistent with the shared ability to activate the TAS2R38 receptor.

Indeed, for all of the 12 possible binary combinations where the bitter compounds reportedly activate the same TAS2R, the taste sensitivity scores were significantly correlated ($P < 0.05$). In cases where bitter substances do not activate the same receptor in vitro, 13 (out of 16) taste intensity scores were not correlated, with the exceptions being for saccharin and sinigrin, saccharin and quassia extract, and for sinigrin and caffeine (Table 3). Of these, the 2 sinigrin combinations showed the weakest correlations: 0.252 and 0.253 for saccharin and caffeine ($P < 0.05$), respectively. The correlation between saccharin and quassia extract was stronger, with a correlation score of 0.371 ($P < 0.01$).

In addition to investigating the relationship between the perceived bitter intensity and TAS2R activation profile, we also examined the

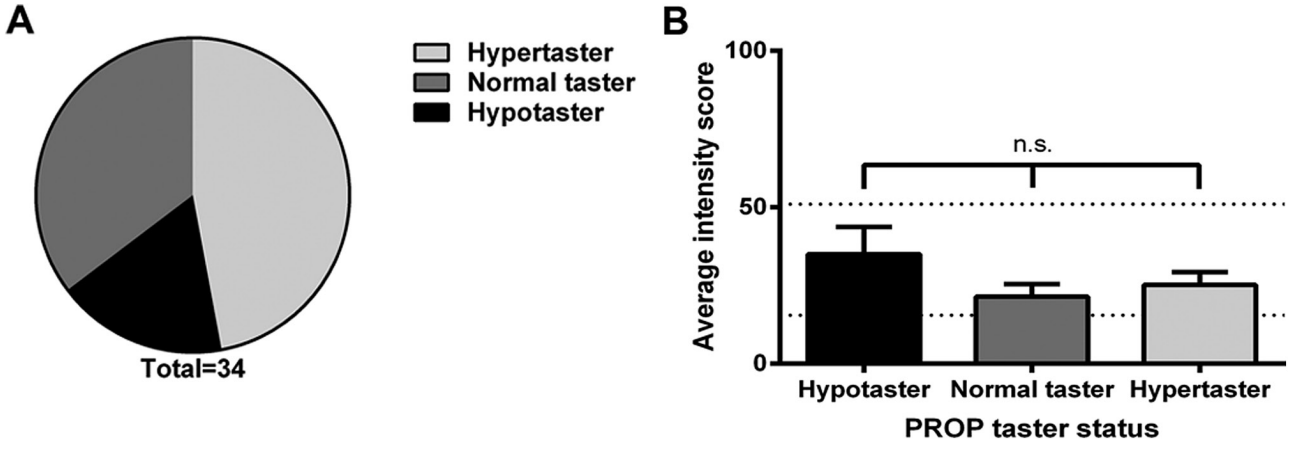

Figure 1. Taste sensitivity to the archetypal bitter compound PROP does not generalize to other bitter substances. (**A**) Participants were classified as a PROP hypotaster (≤15.5% of the whole length scale), normal taster (>15.5), or hypertaster (≥51) based on their taste intensity values. (**B**) The PROP classification was not a valid predictor of taster status for the remaining (non-PROP or PTC) substances. Data from $n = 34$ participants, 1-way ANOVA, *n.s.* non-significant.

Table 2. Differential taste sensitivity and taster status for bitter taste compounds

Taster status	Bitter taste compound							
	PROP	PTC	Sinigrin	Saccharin	Caffeine	Quassia	Quinine	Gentian
Hypertaster	16	15	3	7	1	9	11	6
Normal taster	12	10	13	13	14	16	9	14
Hypotaster	6	9	18	14	19	9	14	14
Total	34	34	34	34	34	34	34	34

Numbers of panelists are depicted, organized according to taster status.

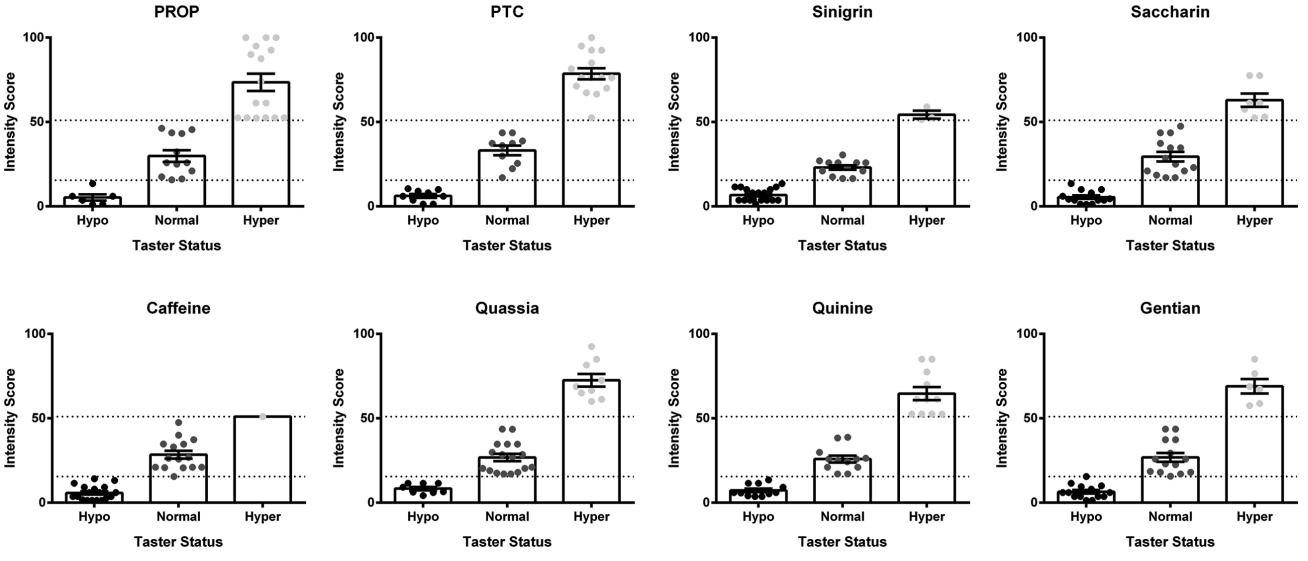

Figure 2. Taste intensity values were variable for all bitter substances tested. Participant-reported intensity values were used to classify participants as hypo-, normal-, or hyper-tasters, using the same criteria as for the PROP classification. Individual participants are depicted.

cohort to determine associations between sex, age, or BMI and bitter sensitivity. In these analyses, there were correlations between participant age and the taste intensity scores for PROP and PTC (Supplementary Table 2). There were also correlations between BMI and taste intensity scores for sinigrin and gentian extract. Finally, there were no significant differences for any of the taste intensity scores between male and female participants (n = 14 male and 20 female; Supplementary Figure 3).

Discussion

The present study has demonstrated a clear link between taste intensity and the activation of TAS2Rs and provides strong evidence that taste receptors are a principal determinant encoding human bitter taste sensitivity. Accordingly, the reported taste intensity scores were related when bitter compounds share 1 or more TAS2Rs, whereas there was no significant relationship if the compounds activate a different receptor repertoire. This idea is consistent with the body of literature concerning the genetics of human taste variation (Hayes et al. 2013) and the current understanding of taste receptor signaling (Foster et al. 2014).

More precisely, the well-known variable bitter sensitivity to PROP (and the similar compound PTC) has been linked to single nucleotide polymorphisms in *TAS2R38* (Kim et al. 2003; Bufe et al. 2005). In turn, this has led to the common classification of taster status and has introduced the concept of "supertasting" to the public lexicon (Bartoshuk 1991; Bartoshuk et al. 1994; Hayes et al. 2008; Garneau et al. 2014).

Interestingly, in several early reports, PROP taster sensitivity (or "taste blindness") was linked to the perception of the bitterness of caffeine and saccharin (Hall et al. 1975; Bartoshuk 1979). In particular, Bartoshuk (1979) found saccharin taste to be related to PROP only at low concentrations (when saccharin is predominantly sweet) but not at suprathreshold concentrations such as in our research. In general, our study consisted of applying the PROP taste classification criteria to the gLMS ratings for other bitter substances. As with the ratings for PROP, among our cohort we observed a spread of taste sensitivities for all bitter compounds. However, we did not see a generalized relationship between the taster status for PROP and the taster status for the other bitter compounds. This was reinforced by the binary correlations between gLMS ratings, which were instead consistent with a model whereby bitter intensity is associated with TAS2R activation profiles.

Beyond the delineation of PROP taste sensitivity, genetic association studies have linked PTC/PROP taster status to an enormous range of phenotypic traits (reviewed in Guo and Reed 2001). It should be acknowledged that many of these reported associations may be false positives due to population stratification or to chance (Guo and Reed 2001). More recently, the naturally occurring polymorphisms in *TAS2R38* have been associated with increased alcohol intake (Duffy et al. 2004), influencing food choice (Ullrich et al. 2004; Sandell and Breslin 2006), adiposity (Tepper et al. 2008) and even with a pathological role in respiratory tract infection (Lee et al.

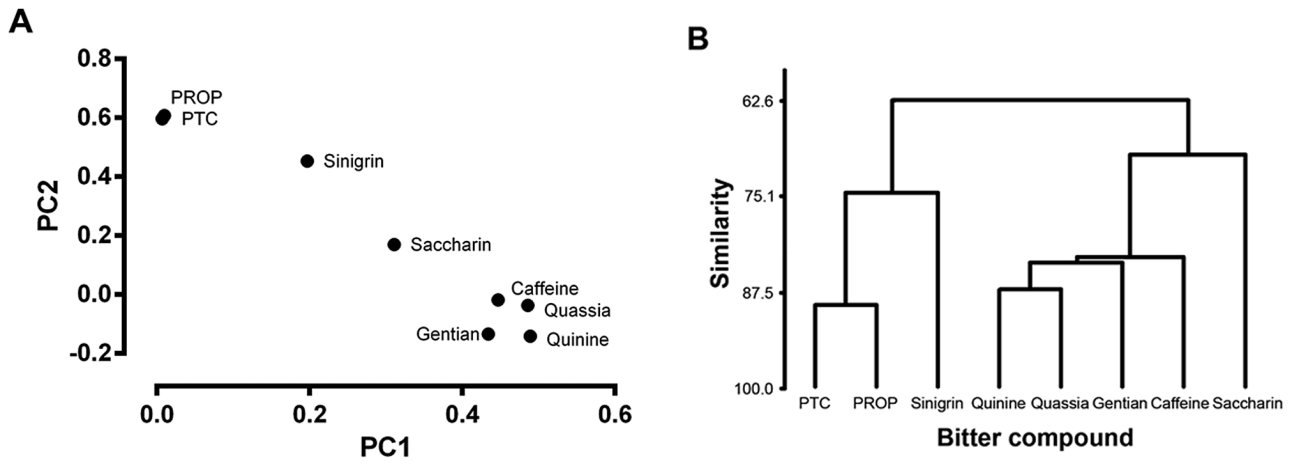

Figure 3. Relationships between taste intensity and distinct bitter compounds. (**A**) Principal components analysis shows strong relationships between taste sensitivity and different bitter substances, forming spatially distinct groupings. (**B**) Cluster analysis of the taste intensity scores also suggests a high degree of similarity between a subset of the bitter compounds.

2012). Moreover, variants in several other *TAS2R* genes have been associated with ingestive behaviors of bitter beverages (Hayes et al. 2011), artificial sweeteners (Roudnitzky et al. 2011), alcohol dependence (Hinrichs et al. 2006), as well as colorectal cancer (Campa et al. 2010) and cardiovascular disease (Shiffman et al. 2008; Akao et al. 2012). Nonetheless, there is not complete consensus in the literature on the influence of taste receptor genotype on phenotypic traits (Drewnowski et al. 2007). It has been suggested that neither the genetic variation in the taste receptors, nor the variable density of the fungiform papillae of the tongue are sufficient to explain the hypertaster phenomenon (Bufe et al. 2005; Hayes et al. 2008; Garneau et al. 2014). Additional mechanisms have been proposed that could account for the discrepancies, including differences in central nervous system processing (Green and George 2004), a genetic variant of the salivary trophic factor gustin, that could alter the function of the fungiform papilla (Calò et al. 2011; Melis et al. 2013) and the variable expression level of the bitter receptors themselves (Lipchock et al. 2013).

Our findings advocate for the key role of the taste receptor protein itself in the variations in taste sensitivity, as has been alluded to previously (Delwiche et al. 2001; Hansen et al. 2006). We observed that the known agonists for TAS2R38, PROP, and PTC, were highly correlated, in line with the previous literature (Pelchat and Danowski 1992; Bartoshuk et al. 1994; Bufe et al. 2005; Hayes et al. 2008). Sinigrin is a glucosinolate bitter compound found in brassica vegetables that activates TAS2R16, as well as TAS2R38 in vitro (Meyerhof et al. 2010). As such, sinigrin was also correlated with the other agonists for the same receptor ($P < 0.001$), supporting earlier psychophysical studies linking the avoidance of bitter vegetables genetic sensitivity to PROP (Drewnowski and Rock 1995; Drewnowski et al. 1997; Bell and Tepper 2006).

The gLMS ratings for quinine were significantly correlated to those of quassia extract, gentian extract and caffeine. These relationships can be neatly accounted for by the common TAS2R activation profiles for these bitter substances (quinine and quassia TAS2R4, -10,-14, and -46; quinine and gentian TAS2R4, -39, -43, and -46; quinine and caffeine TAS2R7, -10, -14, -43, and -46; TAS2R43 and -46 are activated by caffeine and gentian. This cluster of compounds showed no relationship to the PROP taste status, in the case of quinine confirming previous observations (Hall et al. 1975; Bartoshuk 1979; Keast and Roper 2007). Consistent with our hypothesis, we did not see any correlation between caffeine taste intensity scores and PTC or PROP, as these compounds activate different TAS2Rs. While this finding contradicts previous reports that have suggested a link between the 2 compounds (Hall et al. 1975; Ly and Drewnowski 2001), we note that the relatively low caffeine concentration used in our study could account for these differences.

There was a clear relationship between TAS2R activation and perceived bitter intensity, whereby all possible binary combinations of receptors that shared in vitro receptor activation were significantly correlated. These data were reinforced by the PCA and clustering analysis, which also suggest groupings of different taste compounds that align with the reported receptor activation profiles (Meyerhof et al. 2010). However, there were also 3 binary combinations of bitter compounds that were correlated despite these substances not activating the same TAS2Rs. In particular, the taste sensitivity scores for the saccharin-quassia combination were strongly correlated ($P < 0.01$). As we have reported previously, there is high linkage disequilibrium between TAS2R loci on chromosome 12, especially between the 5 member subfamily of receptors TAS2R30, 31, 43, 45, and 46 (Roudnitzky et al. 2011). Notably, both substances activate receptors within that cluster of TAS2R genes on chromosome 12: the TAS2R43 for saccharin and the TAS2R30 and 46 for quassia, which could account for the correlation. Alternatively, the positive correlations between substances might be related to non-TAS2R membrane targets, such as the sweet taste receptor (Max et al. 2001; Nelson et al. 2001), other enzymes and intracellular components (Peri et al. 2000). For most of the population saccharin is known to be primarily sweet with secondary bitterness at low concentrations (i.e., below 25 mM) (Galindo-Cuspinera et al. 2006). The overlap between sweetness and bitterness may have affected the bitter ratings. Equally, caffeine is known to target multiple other proteins, including the adenosine GPCRs, phosphodiesterases, ryanodine receptors, ionotropic glycine receptors, as well as salivary protein content (Duan et al. 2009; Yu et al. 2009; Dsamou et al. 2012). In this regard, the implications of potential multiple cellular targets of bitter substances and their influence on taste sensitivity have yet to be investigated.

Evidently, in taste intensity studies such as this, the choice of test concentrations is an important consideration. We either chose bitter

Table 3. Pearson correlations between binary combinations of bitter compound sensory scores

Bitter compound	PROP	PTC	Sinigrin	Saccharin	Caffeine	Quassia	Quinine
PROP							
PTC	0.784*** (1)						
Sinigrin	0.491*** (1)	0.473*** (1)					
Saccharin	0.222 (0)	0.134 (0)	0.252* (0)				
Caffeine	−0.038 (0)	0.024 (0)	0.253* (0)	0.315** (1)			
Quassia	−0.005 (0)	−0.012 (0)	0.220 (0)	0.371** (0)	0.660*** (2)		
Quinine	−0.132 (0)	−0.150 (0)	0.120 (0)	0.392** (2)	0.654*** (5)	0.744*** (4)	
Gentian	−0.150 (0)	−0.134 (0)	0.173 (0)	0.295* (1)	0.485*** (1)	0.616*** (3)	0.675*** (4)

Correlation coefficients are shown, with the number of common TAS2Rs activated in each binary combination of the 7 bitter compounds in parentheses.
$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

compound concentrations from the literature, or performed small pilot trials to establish our protocols. Given the range of intensity scores that we observed for each compound across our non-expert panelists, we feel that these test concentrations were appropriate. Nonetheless, the concentrations of caffeine and sinigrin were lower than have been tested in earlier studies (Fenwick et al. 1983; Ly and Drewnowski 2001). Similarly, while several panelists showed low ratings for all compounds, it was evident that the scales have been properly employed as the ratings were not restricted the upper or lower regions of the scale. In general, this type of study, testing for suprathreshold sensitivity of taste substances, is an accepted methodology for investigating taste phenotypes, which has been shown to be more reliable than using comparing differential detection thresholds for bitter compounds (Chang et al. 2006; Galindo-Cuspinera et al. 2009). One of the aspects which may have affected the ratings is the gustatory adaptation to bitterness (McBurney et al. 1972). However, the randomized block design of the experiment should have minimized it.

In addition to investigating the relationship of taste intensity to TAS2R activation, we also examined the influence of BMI and age on individual bitter compound gLMS ratings. Although previous studies have determined associations between genetic sensitivity to PROP and BMI (Tepper and Ullrich 2002; Bajec and Pickering 2010; Borazon et al. 2012), we did not see correlations in our cohort. We did note a modest link between age and PROP/PTC taste sensitivity, but as the participants were predominantly in the normal (healthy) BMI category, and fell within a narrow age range this finding would need to be recapitulated in a larger cohort. Moreover, the influence of aging on taste perception is most effectively studied by employing detection threshold methods of testing rather that supra threshold tests (Mojet et al. 2001). Interestingly, we did not see any significant difference between taste intensity ratings for the bitter compounds in male and female panelists, as has been previously reported (Bartoshuk et al. 1994).

In conclusion, this study synthesizes the long-standing observations on the genetic basis of the taste sensitivity to PROP with recent work defining the molecular receptive range of taste receptors. Here, we have shown a strong interaction between the intensity for distinct bitter substances when they are able to activate common TAS2Rs. These observations suggest that focusing solely on PROP and PTC sensitivity may be too reductive to understand the variability and complexities of taste sensitivity. Thus, our approach may provide a novel framework for the prediction of taste sensitivity to compounds that share TAS2R activation profiles.

Supplementary material

Supplementary material can be found at http://www.chemse.oxfordjournals.org/

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