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Individual Differences in Perception of Bitterness from Capsaicin, Piperine and Zingerone

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Abstract

It was recently shown that in some subjects capsaicin can evoke bitterness as well as burning and stinging, particularly in the circumvallate (CV) region of the tongue. Because perception of bitterness from capsaicin is characterized by large individual differences, the main goal of the present study was to learn whether people who taste capsaicin as bitter also report bitterness from structurally similar sensory irritants that are known to stimulate capsaicin-sensitive neurons. The irritancy and taste of capsaicin and two of its most commonly studied congeners, piperine and zingerone, were measured in individuals who had been screened for visibility of, and reliable access to, the CV papillae. Approximately half of these individuals reported tasting bitterness from all three irritants when the stimuli were swabbed directly onto the CV papillae. Concentrations that produced similar levels of burning sensation across subjects also produced similar (though lower) levels of bitter taste. These results are consistent with the hypothesis that capsaicin and its congeners stimulate bitterness via a common sensory receptor that is distributed differentially among individuals. Additionally, bitter tasters rated gustatory qualities (but not burning and stinging) slightly but significantly higher than did bitter non-tasters, which suggests that perception of capsaicin bitterness is associated with a higher overall taste responsiveness (but not chemesthetic responsiveness) in the CV region.

Key words: capsaicin, chemesthesis, circumvallate papillae, human, psychophysics, taste

Introduction

Capsaicin has traditionally been studied as a purely trigeminal (Lawless and Gillette, 1985) or, more broadly, chemesthetic stimulus (Green *et al.*, 1990; Green, 1996). Recent evidence suggests, however, that capsaicin can also stimulate the gustatory system. Green and Schullery (2003) found that capsaicin elicits bitterness in addition to burning in ~50% of individuals, particularly when applied to the circumvallate (CV) papillae. Moreover, in the same study it was shown that sucrose can effectively suppress capsaicin-evoked bitterness, indicating that capsaicin acts like a typical bitter stimulus. Subsequently, Green and Hayes (2003) found that in the CV region capsaicin's bitterness did not self-desensitize even though its burning sensation did, suggesting that the bitterness and burn of capsaicin are mediated by different transduction mechanisms.

Although it is not possible to determine by psychophysical means what the transduction mechanism for capsaicin's bitterness is, it is possible to determine whether bitterness is limited to capsaicin or can be evoked by other structurally similar sensory irritants. If such irritants also taste bitter, it would imply that the transduction mechanism is not uniquely sensitive to capsaicin, and if the perception of bitterness were correlated across individuals, it would suggest that the irritants stimulate a common sensory receptor that is distributed differentially throughout the population. In addition, experiments with structurally similar irritants could provide a further test of the hypothesis that capsaicin bitterness and burn are mediated by different transduction mechanisms (Green and Hayes, 2003; Green and Schullery, 2003). This hypothesis would be supported if subjects who do not perceive bitterness report the same level of burning and stinging as those who do perceive bitterness. We therefore tested three irritants, capsaicin, piperine and zingerone, which are structurally similar and known to produce their chemesthetic effects via capsaicin-sensitive neurons (Liu and Simon, 1996; Liu *et al.*, 2000; Szolcsanyi and Bartho, 2001). Finally, because in the previous study the more pronounced bitterness reported for capsaicin in the CV region was not accompanied by a differential response to quinine on the front or back of the tongue (Green and Schullery, 2003), it remained possible that individuals who tasted capsaicin as bitter had a generally higher

sensitivity to bitterness. We therefore assessed overall taste responsiveness in the present experiment using four prototypical taste stimuli, including quinine.

Methods

Subjects

Subjects were recruited in and around the Yale University campus; all gave informed, written consent and received monetary compensation for participation. The initial eligibility criteria were that subjects must be non-smoking, fluent English speakers between 18 and 45 years of age with no known defect of smell or taste. Potential subjects were also excluded if they were pregnant, taking any prescription pain medication, had tongue, cheek or lip piercings, or had been diagnosed with a disorder involving either a loss of sensitivity or chronic pain. Because prior testing had shown that precise application to the CV papillae was necessary for accurate assessment of capsaicin bitterness, subjects who met the initial criteria were further screened for visibility and accessibility of the papillae. Of the 69 potential subjects screened (44 females and 25 males), 39 (30 females and nine males) were accepted into the study. The remaining 30 subjects were invited to take part in another study. Subjects were instructed not to eat or drink within at least 1 h of coming to the laboratory and to abstain from eating hot and spicy food for at least 24 h prior to each testing session.

Stimuli

The test stimuli were 97% synthetic capsaicin (Pfaltz & Bauer, Waterbury CT), 97% piperine (Aldrich, Milwaukee, WI) and 93% zingerone (Pfaltz & Bauer) in nominal concentrations of 100 μM, 70 mM and 0.3 M, respectively. Because the chemicals are insoluble in water, they were prepared in 95% ethanol solutions. Four classical gustatory stimuli (1.0 M sucrose, 0.5 M sodium chloride, 0.05 M citric acid and 1.0 mM quinine sulfate, all in aqueous solutions) were used to measure taste responsiveness and to give subjects experience rating taste intensity in a practice session (see below). Both the irritants and the taste stimuli were delivered to the tongue via pairs of saturated cotton-tipped swabs that were taped together to double the size of the stimulating surface. To present the tastants, the swabs were dipped into the appropriate solution immediately prior to presentation on each trial. The irritant swabs were prepared in advance by dipping the swabs into the ethanol solutions and allowing the ethanol to evaporate. The dry swabs were then wetted with deionized water immediately prior to presentation. The irritant concentrations are nominal because, as with stimulus application via filter paper disks, it is impossible to know the actual amount of stimulus delivered to the tongue from the swabs.

Practice session

Prior to the first data collection session all subjects participated in a short practice session which served (i) to screen subjects for the ability to reliably access and stimulate the CV papillae, (ii) to familiarize subjects with the Labeled Magnitude Scale (LMS; Green *et al.*, 1993, 1996) and (iii) to give practice rating taste stimuli on the front and back of the tongue. Access to the CV papillae was determined by the ability of the experimenter to see the papillae clearly and to contact them with blank, wetted swabs without inducing the gag reflex. Prior to rating actual stimuli, subjects were instructed on how to use the LMS and were given practice rating a standard set of common imagined oral sensations (e.g. the coolness of an ice-cold beverage; the bitter taste of black coffee), which served to encourage subjects to rate sensations in the context of everyday life rather than in the narrow context of laboratory stimuli. The scale was anchored at the top by 'strongest imaginable sensation of any kind' (referred to as the general LMS; Bartoshuk *et al.*, 2003).

After rating the 15 imagined sensations, subjects were instructed to rate the intensity of sweetness, sourness, saltiness, bitterness and burning or stinging using the LMS for the four prototypical taste stimuli. The instructions emphasized that the stimuli might include mixtures as well as individual taste stimuli and thus that careful attention should be paid to all possible qualities on every trial. Subjects then received the four stimuli twice each, once on the front and once on the back of the tongue, for a total of eight trials. The stimuli were applied alternately to the front and back of the tongue for 3 s on successive trials and the side of application was also alternated such that the four quadrants of the tongue were stimulated once before the first quadrant was stimulated a second time. Stimuli were applied for 3 s. On the front of the tongue the paired, saturated swabs were repeatedly drawn downward over a small area centered ~1 cm to the right or left of the tip of the tongue and on the back of the tongue they were applied in a circular motion to the CV papillae on one side. The taste stimuli were presented in one of three pseudorandom orders while the order in which each region was stimulated remained constant. An inter-stimulus interval of ~30 s allowed subjects to rinse at least twice between stimuli with 37°C deionized water.

Following application of the gustatory stimuli a single capsaicin stimulus was then applied for 10 s to the CV papillae on one side of the tongue. Immediately after application the subject began rating the resulting sensation every 30 s for a period of 3 min. Throughout this period the subject sat quietly with the mouth closed except to expectorate as needed.

Test session

As in the practice session, the four gustatory stimuli were applied unilaterally to the front and back of the tongue in one of three pseudorandom orders for a total of eight trials

before a single irritant stimulus was applied to one side of the back of the tongue. After rating the resulting sensation every 30 s for 3 min, the subject rinsed as needed to cleanse the palate and took a 7 min break to allow all burning and stinging sensations to disappear completely. Testing then resumed by repeating the procedure, beginning again with the prototypical tastants, this time on the contralateral side of the tongue. Thus within a single session two replicates were obtained on opposite sides of the tongue for each stimulus. The two remaining irritant stimuli were tested in separate sessions, with the order of presentation counterbalanced across subjects. All sessions were scheduled at least 24 h apart.

Data analysis

The arithmetic mean was calculated across each replicate within subjects. Because responses on the LMS tend to be log-normally distributed across subjects (Green *et al.*, 1993, 1996), the means were log transformed prior to statistical analysis. Repeated-measures analyses of variance (ANOVA), Tukey HSD post-hoc tests of significant interactions and cluster analyses were all performed using Statistica 6.1 (StatSoft Inc. Tulsa, OK).

Results

As in earlier studies (Green and Hayes, 2003; Green and Schullery, 2003), subjects were grouped for an initial analysis according to whether or not they rated the bitterness of capsaicin above 'weak' on the LMS. Grouping subjects in this way indicated there was a high correlation among ratings of bitterness for the three irritants. However, because use of the arbitrary criterion of 'weak' could have eliminated some individuals who reliably perceived low-level bitterness, a cluster analysis was also performed to evaluate subject groupings. The tree diagram (city block) in Figure 1 shows two distinct clusters of subjects, indicating both a uniform response across the three stimuli as well as a clear inter-group difference. A second analysis using an alternative linkage metric (Euclidian distance) yielded the same two clusters. The two clusters agreed very closely, although not perfectly, with the two groups initially determined by the criterion of greater than 'weak' bitterness. Note that the three subjects (3, 6, 24) for whom no linkage is shown rated bitterness as zero for every irritant on every trial and thus are actually nontasters.

Differences in irritant bitter perception between the two groups were summarized by calculating the log-mean of the peak bitterness ratings on the first trial across the respective subjects. Figure 2 indicates that bitterness was perceived in a virtually all-or-nothing manner. Based on group membership, we therefore classified subjects as either irritant bitter-tasters (iBTs) or irritant non-tasters (iNTs). Of the 39 subjects tested, 20 (51.3%) were classified as iBTs.

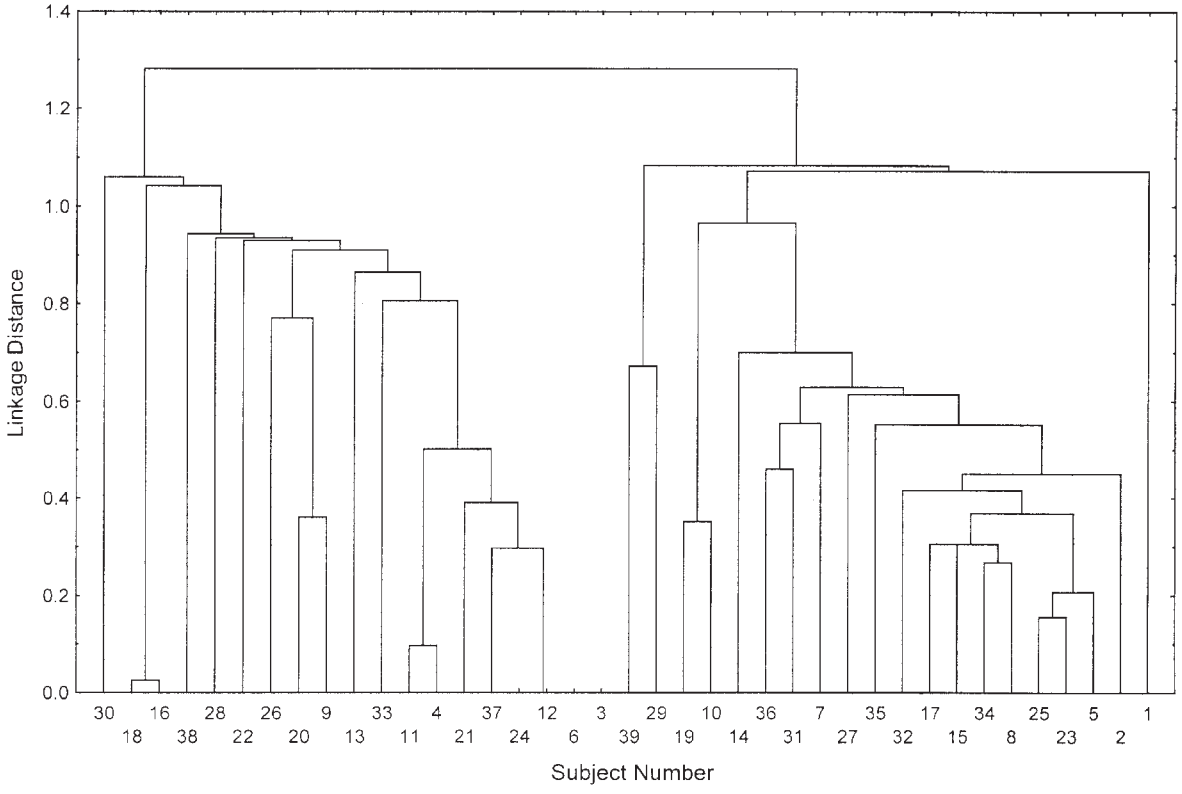

Figure 1 Cluster analysis results for peak perceived bitterness ratings of capsaicin, piperine and zingerone. The two distinct clusters indicate that the subjects fell into to groups, those who did and those who did not consistently report bitterness during exposure to the three irritant stimuli.

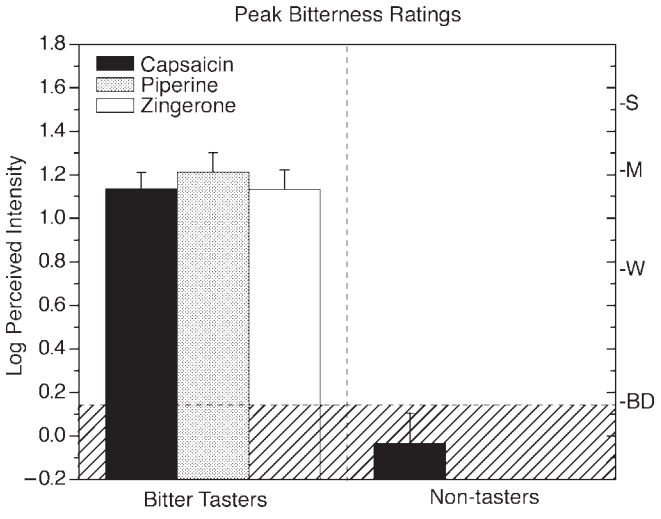

Figure 2 Log means of perceived intensity of bitterness for the two groups identified by cluster analysis. Letters on the right y-axis represent semantic labels of the Labeled Magnitude Scale (BD = barely detectable; W = weak; M =moderate; S = strong; VS = very strong). The hatched area in this and subsequent graphs indicates mean intensity ratings below 'barely detectable' on the LMS and thus near the threshold for detection. Vertical bars represent standard errors of the means (SEM).

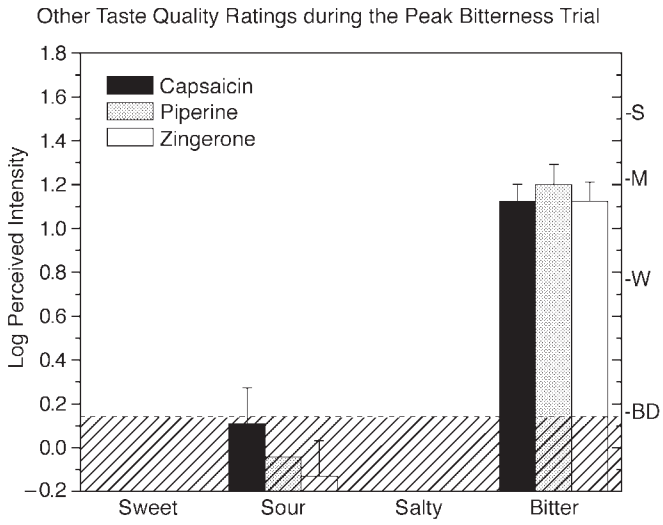

Figure 3 Log means of perceived taste intensity for bitter tasters during the trial in which each subject experienced peak bitterness from the irritant. Nearly all subjects reported peak bitterness on the first or second rating after the stimulus was applied. Bitterness is clearly the only quality reliably reported. Vertical bars represent SEMs.

Quality specificity of irritant tastes

To rule out the possibility that iBTs exhibited a general response bias toward reporting taste qualities of all kinds in response to irritants, for each subject we calculated the mean taste quality profiles at the time at which peak bitterness was reported. Figure 3 shows definitively that bitterness was the only quality reliably reported to be more than 'barely detectable' for all three stimuli.

Intensity and time-course of irritant bitterness versus burn

Figure 4 illustrates that on average, the perceived intensity of burning and stinging was similar whether or not subjects reported bitterness. This was confirmed by a three factor (Stimulus × Time × Group) repeated measures ANOVA, which indicated that there was no significant main effect of Group, nor was there an interaction between Time and Group. Thus neither the intensity or decay in burning sensation was associated with the ability to taste bitterness. The same ANOVA did, however, reveal a significant interaction between Time and Stimulus [$F(12,444) = 15.120$, $P < 0.0001$], confirming as others have reported (Lawless, 1984; Stevens and Lawless, 1986; Prescott and Stevenson, 1996), that burning sensations decay at different rates for the three stimuli (Figure 5). Tukey's HSD post-hoc tests confirmed that the interaction was driven primarily by piperine, whose burning sensation decayed more slowly than that of the other two stimuli. However, Tukey tests also confirmed that the zingerone burn dropped-off more rapidly than the piperine burn during the first 2 min after exposure.

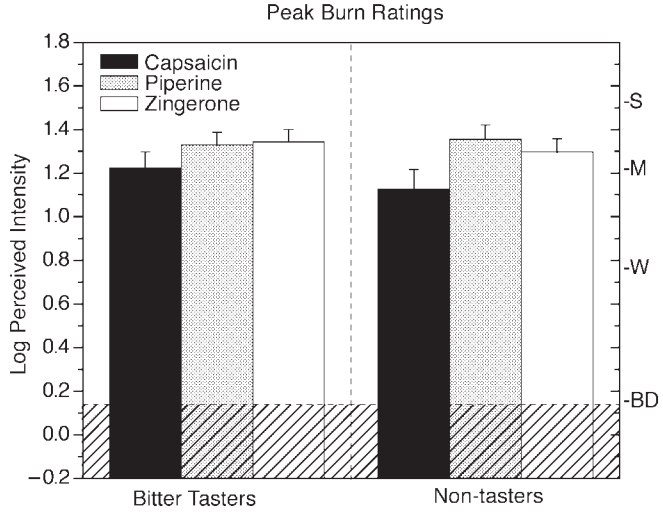

Figure 4 Same as Figure 2, but for perceived burning or stinging from the three test stimuli. Vertical bars represent SEMs.

Figure 6 shows that bitterness ratings dropped to 'barely detectable' on the LMS ~75, 120 and 180 s after exposure to zingerone, capsaicin and piperine, respectively. A two-way repeated measures ANOVA conducted on the data from iBTs showed a significant interaction between Time and Stimulus [$F(12,228) = 3.3081$, $P = 0.00019$], which confirmed that the decay rates were reliably different. The temporal pattern was generally consistent with that of burning sensations, although bitterness tended to decline faster, particularly for zingerone.

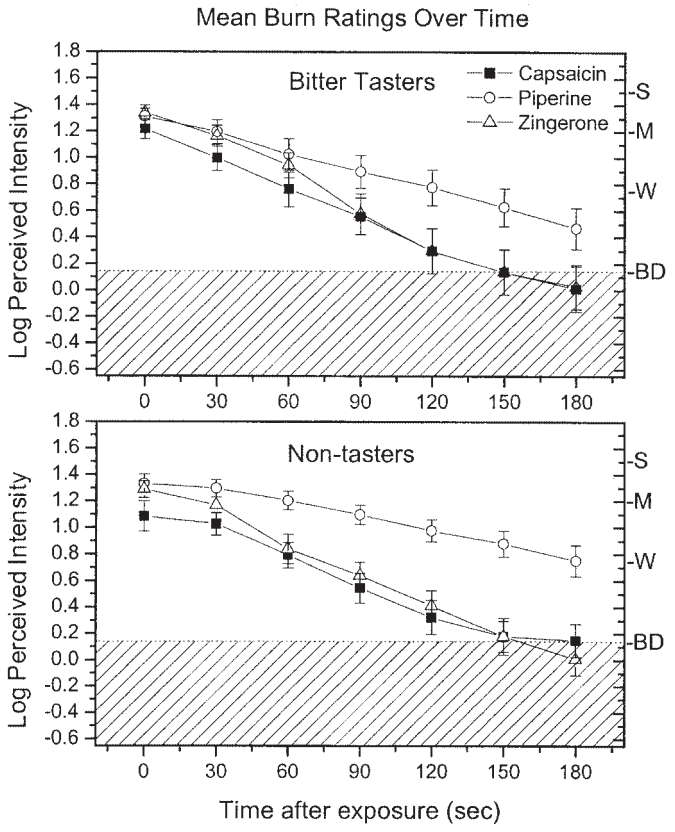

Figure 5 The decay in perceived burn over time is shown for each irritant for the first 3 min after exposure. Vertical bars represent SEMs.

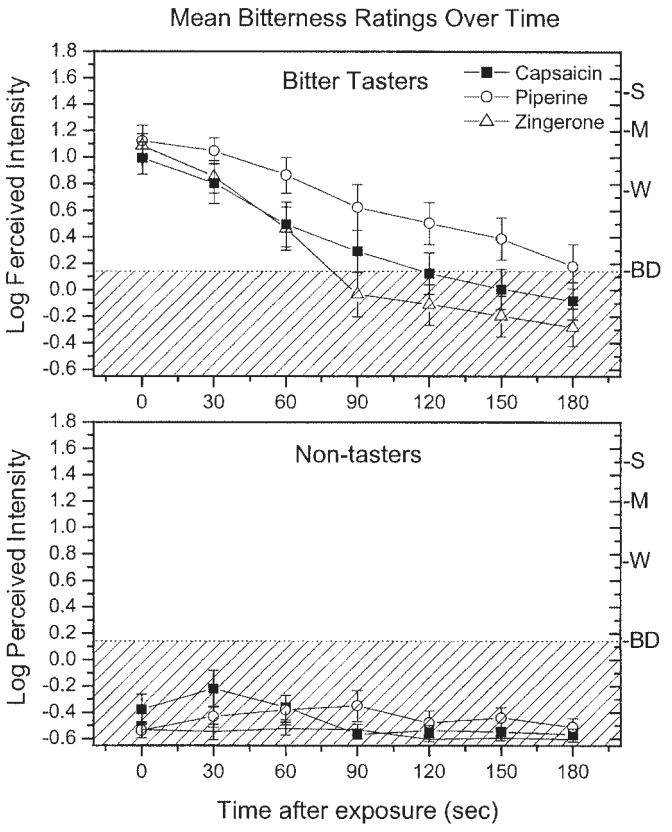

Figure 6 Same as Figure 5, except for perceived bitterness intensity. Vertical bars represent SEMs.

Prototypical taste responsiveness

Although Figure 4 shows that iBTs did not exhibit a response bias toward reporting taste qualities for irritants, it was possible that on the circumvallate papillae iBTs were more responsive to bitter tastants of all kinds. A three-way repeated measures ANOVA conducted on the five qualities for the four prototypical stimuli revealed a main effect of Group [$F(1,37) = 8.9094$, $P = 0.005$], indicating that iBTs tended to rate the intensity of the taste stimuli higher than iNTs (Figure 7). In addition, because there was no significant interaction between Group and Stimulus, it cannot be concluded that iBTs were more responsive to the bitterness of quinine than to the tastes of the other three stimuli. An apparent tendency for iBTs to report more side-tastes than did iNTs (e.g. sourness for NaCl) was not supported by the ANOVA, as there was no significant three-way interaction among Stimulus, Sensation Quality and Group.

Discussion

The results of the present study support previous evidence (Green and Hayes, 2003; Green and Schullery, 2003) that capsaicin can elicit a bitter taste on the back of the tongue in some but not all individuals. Although the results from two earlier studies (Lawless, 1984; Lawless and Stevens, 1988) included evidence that capsaicin (and piperine) might have 'side bands' of taste, uncertainties about the quality, magnitude and reliability of the tastes caused them to be overlooked. The present results further show that the capsaicin congeners piperine and zingerone evoke bitterness on the CV papillae in the same individuals who perceive bitterness from capsaicin, and that those individuals tend to rate the taste of prototypical tastants higher as well.

The possible role of VR1

Given that subjects who tasted bitterness from one irritant tended to taste bitterness from all three, it seems likely that capsaicin, piperine and zingerone act through a common taste transduction mechanism. Green and Schullery (2003) have speculated that capsaicin bitterness might involve either a receptor-gated channel or a nonspecific biophysical effect on the taste cell membrane. A nonspecific effect, such as depolarization of taste cells or afferent neurons via disruption of the lipid membrane (Feigin *et al.*, 1995), can probably be ruled out because of the large individual differences that have been found and because capsaicin evoked no other tastes besides bitterness (Green and Schullery, 2003). The combination of taste specificity and individual differences could be more easily explained by differences in the availability of a specific sensory receptor. One possibility suggested previously (Green and Schullery, 2003) is that a subset of taste cells that respond to bitter-tasting stimuli also

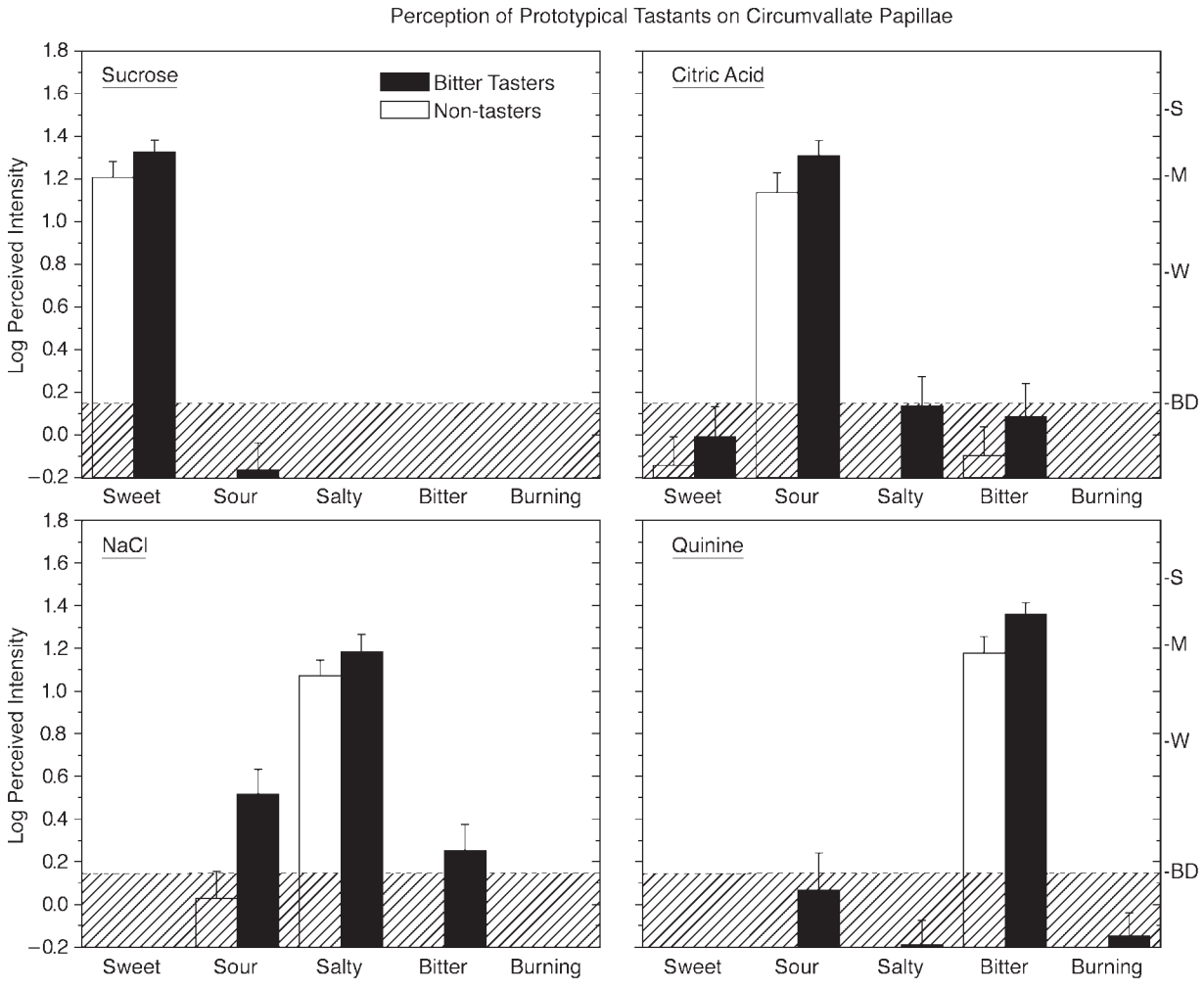

Figure 7 Log means of perceived taste intensity of the four prototypical tastants for bitter-tasters and non-tasters. Taste sensations induced by the stimuli were rated significantly higher overall for bitter-tasters than for non-tasters. Vertical bars represent SEMs.

possess the irritant receptor VR1 (Szallasi, 1994; Caterina *et al.*, 1997). Although evidence of VR1 has been reported in CV papillae of rats (Liu and Simon, 2001), other studies have failed to find VR1-immunoreactivity in rat taste papillae or taste cells (Ishida *et al.*, 2002; Kido *et al.*, 2003), and there is as yet no evidence that VR1 is present in human CV or fungiform taste cells. In addition, our recent finding that the bitterness of capsaicin was not desensitized while its burning sensations were (Green and Hayes, 2003) argues against a role of VR1 in irritant bitterness. A more definitive psychophysical test for involvement of VR1 would be to pre-treat the tongue with a VR1 antagonist, such as capsazepine or ruthenium red (Amann and Lembeck, 1989; Maggi *et al.*, 1993; McIntyre *et al.*, 2001) prior to stimulation with capsaicin, piperine, or zingerone. Unfortunately, such a test is not possible because none of the known antagonists are approved for use in humans.

T2Rs and irritant bitterness

An alternative explanation for irritant bitterness is that it occurs via stimulation of one or more types of the family of T2R receptors that have been implicated in bitter taste transduction (Matsunami *et al.*, 2000). Members of this receptor family are differentially expressed on the front and back of the tongue in rats (Adler *et al.*, 2000; Chandrashekar *et al.*, 2000) and have been identified in human circumvallate papillae (Ueda *et al.*, 2001; Bufe *et al.*, 2002). Because there are at least 28 different types of human T2Rs (Conte *et al.*, 2002), it is impossible at present to speculate about which one might be responsible for capsaicin bitterness. To date, specific bitter-taste ligands have been identified for only two T2Rs: hT2R4 responds to 6-*n*-propyl-2-thiouracil (PROP) and denatonium benzoate (Chandrashekar *et al.*, 2000) and hT2R16 responds to salicin and certain other beta-glucopyranosides (Bufe *et al.*, 2002). Tentatively, hT2R4 can be eliminated as the primary mediator of capsaicin bitterness

because we previously found no significant correlation between bitterness ratings for capsaicin and PROP (Green and Hayes, 2003). Whether hT2R16 may play a role could be determined by measuring the correlation between the sensitivity to beta-glucopyanosides and capsaicin bitterness psychophysically. Alternatively, because so few ligand-T2R pairings have so far been identified, including irritants in the battery of stimuli used to screen functionally expressed T2Rs may be a more fruitful approach to determining if one or more receptors from this family is involved in capsaicin bitterness.

Individual differences

Because only ~50% of subjects reported bitterness, it is reasonable to hypothesize that just a small subset of bitter-encoding taste cells possess the necessary receptor or channel, and that the number of these taste cells varies across individuals. Differences in the number of capsaicin-sensitive taste cells could result from differential rates of genetic expression of the relevant receptor itself and/or differences in the number of taste cells overall. There is at present no way to know whether the relevant receptor is expressed differentially across people; there is, however, ample evidence of individual differences in the number of taste papillae and taste cells. On the front of the tongue, studies have linked differences in fungiform papillae density with differences in perceived taste intensity, although the reports are mixed with regard to taste quality and stimulus specificity (Miller and Reedy, 1990; Bartoshuk *et al.*, 1994; Delwiche *et al.*, 2001; Doty *et al.*, 2001). No such linkage has been established between taste intensity and either number of papillae or number of taste buds within the CV region, but cadaver studies have provided ample evidence for individual variation of both kinds. Miller and Bartoshuk (1991) reported that the number of CV taste papillae varied from three to 13, and estimates of the number of taste buds from two earlier studies ranged from 240 ± 125 (Arey *et al.*, 1935) to between 12 and 624 (Mochizuki, 1937). Such wide variation in taste papillae and taste buds and, hence, in the number of taste cells and receptors, would have a disproportionate impact on perception of tastants that stimulate a relatively small number of receptors. That is, individuals who have fewer taste receptors overall might have too few of a particular subtype to taste a particular stimulus.

Perhaps this is the case for gustatory sensitivity to capsaicin and its congeners. The tendency for iBTs to give higher taste ratings than nBTs in response to prototypical gustatory stimuli (as much as 68 % higher for quinine) is consistent with the possibility that these individuals possess a higher gustatory innervation density in the CV papillae. The alternative possibility that the difference in ratings was due to a response bias (e.g. idiosyncratic use of the LMS) is contradicted by the similarity in burn/sting ratings for the two groups. The comparability of the burn/sting ratings further suggests that any inter-individual differences in innervation density are specific to the gustatory component of the glossopharyngeal nerve, which is the sole sensory nerve innervating the CV papillae. In any case, whether variation in gustatory innervation density or some other factor underlies the difference in general taste perception, the fact that only about half of our subjects reported bitterness from the irritants suggests that only a small fraction of bitter receptors are sensitive to capsaicin.

Summary and implications

The present data indicate that in the CV region of the tongue, capsaicin and two of its congeners, piperine and zingerone, are tasted as bitter by ~50% of individuals. The same individuals did not exhibit differences in responsiveness to the sensory irritancy of the same stimuli, suggesting that the transduction mechanisms for the taste and chemesthetic components of this class of irritants are independent. We therefore hypothesize that CV taste cells that encode bitterness express at least one receptor, perhaps belonging to the T2R family, that can be stimulated by capsaicin. In addition, because iBTs also reported slightly but significantly stronger tastes from all four prototypical taste stimuli, we propose that those individuals may have a higher gustatory innervation density in the CV papillae. A full understanding of the cellular mechanism responsible for the bitterness of capsaicin and similar irritants will require inclusion of these irritants as potential ligands in studies of bitter taste transduction, and in particular, T2R bitter receptors.

Acknowledgement

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