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Regional Specificity of Chlorhexidine Effects on Taste Perception

Ruchi Grover[1,2] and Marion E. Frank[3]

[1]Department of Biology, University of Hartford, CT 06117, USA, [2]New York University College of Dentistry, New York, NY 10010, USA and [3]Center for Neurosciences, Department of Oral Health and Diagnostic Sciences, School of Dental Medicine, University of Connecticut Health Center, Farmington, CT 06030-1715, USA

Correspondence to be sent to: Marion E. Frank, Center for Neurosciences, Department of Oral Health and Diagnostic Sciences, School of Dental Medicine, University of Connecticut Health Center, Farmington, CT 06030-1715, USA. e-mail: mfrank@neuron.uchc.edu

Abstract

Chlorhexidine (CHX) gluconate, a bitter bis-biguanide antiseptic, reduces the intensity of the salty taste of NaCl and bitter taste of quinine in humans. This study addresses regional specificity of CHX's effects on taste. Perceptual intensity and quality were measured for separate taste bud containing oral loci innervated either by afferent fibers of cranial nerve (CN) VII or CN IX. Measurements were obtained following three 1-min oral rinses with either 1.34 mM CHX or water, the control rinse. CHX rinse reduced the intensity of NaCl more at the tongue tip and palate than at posterior oral sites. Thus, fungiform and palatal salt-taste receptors may differ from salt-taste receptors of the foliate and circumvallate taste papillae. The intensity of quinine·HCl was reduced equally by CHX at all sites tested but was frequently tasteless on the less sensitive anterior sites, suggesting quinine receptor diversity. In rodents, a portion of NaCl-taste receptors in the receptive field of CN VII is sensitive to the epithelial Na^+ channel blocker amiloride and a portion is amiloride insensitive; all CN IX receptors are amiloride insensitive. The current results are the first to suggest that there may also be distinct, regionally specific populations of NaCl-taste receptors in humans.

Key words: bitter taste, facial nerve, glossopharyngeal nerve, palatal taste, salt taste, taste papillae

Introduction

Chlorhexidine (CHX) (Figure 1) is a bis-cationic biguanide antiseptic, with strong cationic properties at physiological pH (Al-Tannir and Goodman 1994; Gilbert and Moore 2005). CHX rinse reduces the intensity of the salty taste of NaCl and the bitter taste of quinine but has little effect on the sweet taste of sucrose or sour taste of acid (Frank et al. 2001).

Specific receptor-signaling pathways and ion channels are involved in transduction for each taste quality. Ion channels are implicated in sour and salty tastes but taste responses to sweet and bitter compounds are likely initiated by G-protein–coupled receptors (GPCR) followed by GPCR α-gustducin cascades (He et al. 2002; Margolskee 2002; Chandrashekar et al. 2006). Therefore, it would seem that CHX would affect salty and bitter taste by independent mechanisms. But, salty and bitter tastes may interact. The bitter taste of quinine is suppressed in mixtures with NaCl in humans and rodents (Nowlis and Frank 1981; Breslin and Beauchamp 1995, 1997; Frank et al. 2003), and after self-adaptation (Smith and van der Klaauw 1995), the salty taste of weaker concentrations of NaCl and water are transformed to ''bitter–sour'' (Bartoshuk et al. 1964; McBurney and Shick 1971; Bartoshuk 1974).

The chorda tympani (CT), a branch of the facial nerve (cranial nerve [CN] VII), innervates taste buds in the fungiform papillae on the tip of the tongue. The greater superficial petrosal (GSP), also a CN VII branch, innervates taste buds on the palate. The lingual branch of the glossopharyngeal (GL) nerve (CN IX) innervates taste buds in the foliate and circumvallate papillae on the back of the tongue. In rodents, the CT and GL supply distinctive information to the brain (Hettinger and Frank 1992).

Rodent CN VII and CN IX afferent taste neurons are associated with disparate stimulus chemistries and distinct behavioral taste responses (Frank 1991; St John and Spector 1998; Frank 2000; King et al. 2000). For example, there are 2 physiological types of NaCl-responsive taste nerve fibers: the amiloride sensitive and amiloride insensitive; both are found in the facial but all GL NaCl-responsive taste afferents are insensitive to amiloride (Ninomiya and Funakoshi 1988; Hettinger and Frank 1990; Formaker and Hill 1991; Kitada et al. 1998).

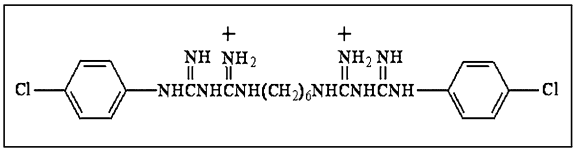

Figure 1 Structure of chlorhexidine in one of its possible bis-cationic forms.

CHX reversibly and partially blocks perception of salty and bitter tastes when measured by whole-mouth sampling, which detects the functioning of all taste bud fields simultaneously (Gent et al. 1986). The ''Spatial Taste Test'' (Bartoshuk 1989) used by the University of Connecticut's Taste and Smell Clinic, a test that measures responses originating from separate oral regions (Figure 2), was employed to determine whether CHX effects are localized to specific taste bud fields.

Materials and methods

Subjects

Twelve subjects (9 women, 3 men), aged 18–40 years (overall mean = 28.3, standard deviation = 5.88), nonsmokers reporting no taste disorders, participated in this study. Subjects were recruited from the students and staff of University of Connecticut Health Center (UCHC). The UCHC Institutional Review Board approved the study protocol. Testing took place in a room within the Dental General Clinical Research Center clinic. All subjects gave informed consent before participating.

Treatment rinses, stimuli, and presentation

Three 1-min whole-mouth treatment rinses, paced one after another with 20-s pauses, with 1.34 mM chlorhexidine (CHX) gluconate, the concentration in the mouth rinse Peridex (treatment rinse), or water (control rinse) were used. Subjects were told to ''hold and swish'' in their mouths each 5-mL treatment or control rinse sample for the duration of the rinse to ensure that all taste bud containing oral regions would be well bathed. After the 3 rinses, the subjects thoroughly rinsed with water and waited for 5 min before the regional taste tests with the test stimuli were initiated. Subjects participated in one session, lasting less than 1 h, in which the water rinse condition was followed by the CHX rinse condition, with a rest period intervening. The test stimuli were 1 M NaCl, 32 mM citric acid, 1 M sucrose, and 1 mM quinine·HCl, compounds that represent the ''salty,'' ''sour,'' ''sweet,'' and ''bitter'' taste qualities, respectively.

Psychophysical methods

Subjects named the quality of each stimulus using the words ''salty,'' ''sour,'' ''sweet,'' ''bitter,'' or ''tasteless'' and rated the intensity of each stimulus on a 0–9 point, fixed-interval scale (0 = tasteless, 3 = weak, 5 = medium, 7 = strong, and 9 = very strong). Stimuli were presented in accordance with the Spatial Taste Test (Bartoshuk 1989). Sterile Q-tip, cotton swabs, were used to apply taste solutions to front and rear regions of the tongue and on the palate. Each solution used was deposited sequentially in pairs to the left and right side. The tongue tip was stimulated first, followed by the posterolateral sides and back of the tongue, and the palate was last. Different taste papillae are present in the 4 regions: fungiform, foliate, vallate, and palatal. Once spatial testing with the first solution was complete, the subjects were asked to swallow a small amount of the solution. The swallow test permits comparison of stimulation of localized areas with stimulation of all sensitive regions including the throat (Bartoshuk 1989), which was not treated with CHX; thus, swallow results were not included in data analysis. The 4 solutions were presented to each subject in random order.

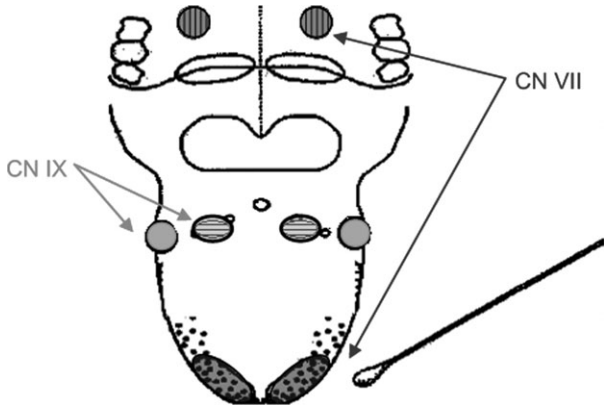

Figure 2 Diagram of tongue and palate showing regions stimulated in the Spatial Taste Test. Solutions were deposited on a palatal area between soft and hard palate (circles, vertical stripe), foliate papillae (circles, filled), circumvallate papillae (ovals, horizontal stripe), and fungiform papillae (tongue tip, large ovals) (Figure modified from Bartoshuk [1989]).

Data analysis

Intensity ratings

Effects of CHX on averaged left and right side replicate taste intensity ratings at various locations were evaluated using repeated measures analysis of variance. The within-subject factors were rinse (water, CHX), compound (NaCl, sucrose, citric acid, quinine·HCl), nerve (CN VII, CN IX), and within-nerve receptive field taste bud sites (fungiform/palatal, foliate/circumvallate). For all analyses, an a priori level of 0.05 was adopted. Post hoc comparisons used Newman–Keuls tests and paired *t*-tests, with α adjusted with Bonferroni corrections.

Quality identification

In order to compare quality responses for water and CHX rinses, each subject's quality responses to a stimulus were converted to the frequency of correct responses.

For example, a response of salty to NaCl was coded as 1 (correct) and any other response to NaCl was coded as 0 (for sweet, sour, bitter, or tasteless). Correct responses were salty for NaCl, sweet for sucrose, sour for citric acid, and bitter for quinine·HCl. Paired *t*-tests of water versus CHX values for each compound were used to establish significance; Bonferroni-adjusted $P = 0.0125$.

Results

Regional taste, stimulus intensity

Controls

Ratings after water rinsing for the different compounds were higher for posterior sites innervated by CN IX than anterior sites innervated by CN VII (Figure 3, Table 1). Across all stimulation sites, 1 M NaCl was rated stronger than 1 M sucrose; but NaCl and 32 mM citric acid or 1 mM quinine were rated equally intense.

Chlorhexidine rinse: sites combined

Averaged across separate stimulation sites, CHX reduced taste intensity ratings differentially across the 4 compounds (Figure 4, Table 2). Post hoc comparisons verified reductions for the quinine·HCl and NaCl ($P = 0.0001$) and no significant change for the citric acid and sucrose intensities. The results are identical to those previously obtained with whole-mouth stimulation (Frank et al. 2001).

Chlorhexidine rinse: NaCl and quinine

CHX reduced intensity ratings for NaCl differentially across stimulation sites associated with CN VII and CN IX (Figure 5, Table 3). Post hoc comparisons of the NaCl intensity reductions at CN VII and CN IX sites verified that CHX reduced the intensity of NaCl more at the fungiform and palatal stimulation sites than at the vallate and foliate sites ($t_{11} = 2.93$, $P = 0.01$). The differences in control and CHX rinse intensity ratings for NaCl were larger at CN VII stimulation sites, -2.67 ± 0.42, than they were at CN IX sites, -1.46 ± 0.33. Control and CHX rinse intensity rating differences for quinine·HCl were -3.48 ± 0.50 and -3.43 ± 0.27, respectively, for CN VII and CN IX sites.

Percent of control NaCl and quinine perceptual intensity remaining following CHX rinse was influenced by the higher

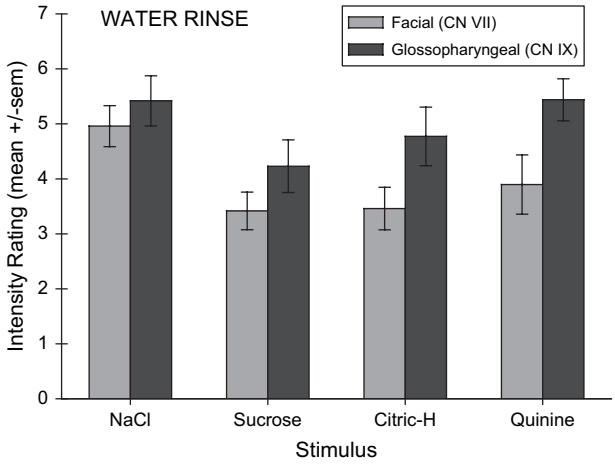

Figure 3 Mean [±standard error] intensity ratings for the 4 compounds: NaCl, sucrose, citric acid, and quinine·HCl after water rinses at separate sites innervated by CN VII or CN IX. Ratings were higher at CN IX sites than at CN VII sites.

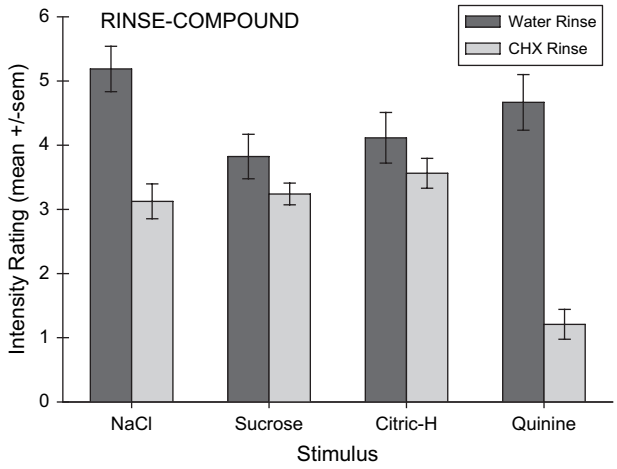

Figure 4 Mean [±standard error] intensity ratings for NaCl, sucrose, citric acid, and quinine·HCl for separate stimulation sites combined. Ratings for CHX rinse were significantly lower than for water rinse for NaCl and quinine·HCl not for sucrose and citric acid.

Table 1 Taste intensity after water rinses

Source	Degrees of freedom (factor, error)	*F*	*P* value
Nerve	1, 11	15.23	0.002
Compound	3, 33	3.23	0.03
All others			Not significant

Nerve, compound, and site were the 3 within-subject factors.

Table 2 Chlorhexidine effect on taste intensity

Source	Degrees of freedom (factor, error)	*F*	*P* value
Rinse	1, 11	86.72	<0.00001
Compound	3, 33	4.45	0.01
Nerve	1, 11	12.39	0.0001
Rinse × compound	3, 30	6.55	<0.00001
All others			Not significant

Rinse, compound, nerve, and site were the 4 within-subject factors.

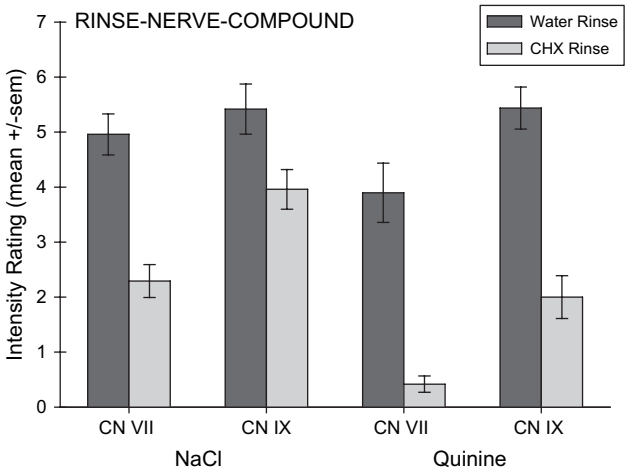

Figure 5 CHX reduced mean [±standard error] intensity of NaCl more at CN VII: fungiform and palatal sites, than CN IX: circumvallate and foliate. There is no evidence for a differential effect of CHX on quinine·HCl intensity ratings across sites.

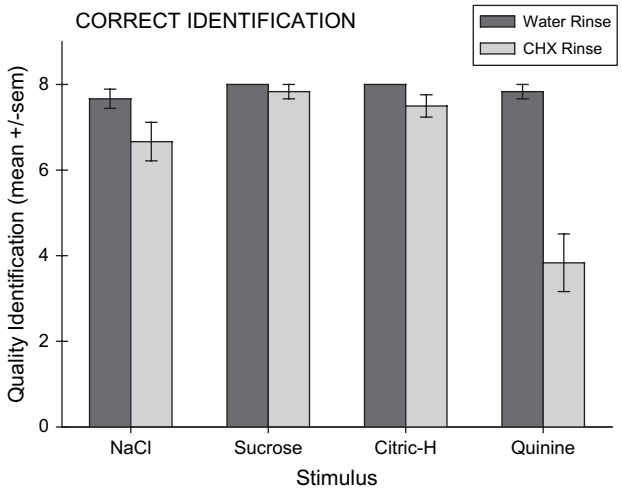

Figure 6 Mean [±standard error] number correct of 8 total identifications for NaCl, sucrose, citric acid, and quinine·HCl with water and CHX rinses. Quinine·HCl (bitter) identification was significantly reduced by CHX rinse.

Table 3 Chlorhexidine effects on (A) NaCl and (B) quinine intensity

Source	Degrees of freedom (factor, error)	*F*	*P* value
(A) NaCl			
Rinse	1, 11	42.73	<0.0001
Nerve	1, 11	8.95	0.01
Rinse × nerve	1, 11	8.57	0.01
All others			Not significant
(B) Quinine			
Rinse	1, 11	119.34	<0.0001
Nerve	1, 11	34.78	0.0001
Rinse × nerve	1, 11	0.001	0.93
All others			Not significant

Rinse, nerve, and site were the 3 within-subject factors for each analysis of variance.

control ratings at CN IX than CN VII stimulation sites (Figure 3). For NaCl, 74.4 ± 4.4% and 48.2 ± 6.8% of control NaCl intensity remained for CN IX and VII sites, respectively (P = 0.001), after CHX rinse. For quinine, 38.8 ± 5.6% and 9.3 ± 3.5% of control quinine intensity remained for IX and VII sites, respectively (P = 0.001), after CHX rinse; the CN VII value is consistent with the frequent labeling of quinine as tasteless at CN VII sites after CHX rinse noted below.

Regional taste, stimulus quality

Compared with control water rinse, CHX rinse reduced identification of quinine as bitter (P < 0.0001) (Figure 6). Out of a possible 8 correct identifications, the mean number of quinine bitter identifications was 7.83 ± 0.17 for water and 3.83 ± 0.67 for CHX. The mean number of NaCl salty identifications averaged 7.67 ± 0.22 for water and 6.67 ±0.44 for CHX (P = 0.08). CHX did not affect sweet or sour identifications of sucrose and citric acid.

Quality labels chosen for quinine·HCl showed that quinine frequently was tasteless with CHX rinse: 98% of quality identifications were bitter for water rinse and 48% were bitter after CHX treatment; the remaining were tasteless for either rinse condition. Tasteless labels were chosen for 75% of fungiform and palatal, but 30% of foliate and circumvallate testing sites (χ^2 = 9.7, P = 0.01). In all, 98% of NaCl quality identifications were salty after water rinse; but NaCl was rarely tasteless with CHX rinse: just 2 subjects found NaCl, on the palate, tasteless. With CHX rinsing, NaCl developed a sour (bitter) off-taste, primarily (10 of 12 incidents) at fungiform sites (χ^2 = 4.0, P = 0.05).

Discussion

Regional versus whole-mouth testing

At each stimulation site, CHX specifically blocked responses to NaCl and quinine; responses to sucrose or citric acid were not significantly affected. Thus, regional results at each separate site were qualitatively consistent with our published results with whole-mouth taste testing (Frank et al. 2001). However, summed across the separate stimulation sites, CHX rinse reduced the intensity of 1 mM quinine more than 1 M NaCl, ~75% and ~40%, respectively ($F_{1,11}$ = 8.97, P = 0.01); CHX had reduced whole-mouth intensity of 0.1 M NaCl and 0.1 mM quinine each by ~75% (Frank et al. 2001). The CHX rinsing encompassed the entire oral cavity in both the present regional and earlier whole-mouth studies.

Thus, quantitative differences are due to the distinct stimulus deliveries and/or intensities rather than differential CHX rinse efficacy.

Although the restricted regions tested likely sampled the taste receptor diversity available to whole-mouth testing, swabbing local sites activated the receptors in posterior lingual papillary trenches more than the receptors distributed over anterior tongue and palatal surfaces. Stimulation of many more fungiform and palatal receptors, where CHX had a big effect on NaCl, may explain the more equal intensity reductions for NaCl and quinine with whole-mouth tests. The strong regional stimulus concentrations would more likely irritate the lingual epithelium, yet chemesthesis occurs in CN VII– and CN IX–receptive fields (Rentmeister-Bryant and Green 1997; Bandell et al. 2007). Interestingly, 500 mM NaCl appeared to elicit distinct taste quality patterns at CN VII and CN IX sites (Green and Scullery 2003). In rodents, at least 1.0 M NaCl is required to elicit responses in lingual nerve (CN V) and trigeminal brain-stem neurons (Sostman and Simon 1991; Sudo et al. 2003).

CHX and the salty taste of NaCl

NaCl taste is thought to require epithelial ionic transport and, in rodents, the passage of Na^+ through epithelial channels (ENaC) in the plasma membrane of taste bud receptor cells specialized for Na^+-specific taste (Ninomiya and Funakoshi 1988; Hettinger and Frank 1990; Hill et al. 1990; Margolskee 2002). In humans, saltiness of all salts tested has been reduced by CHX rinses (Breslin and Tharp 2001; Frank et al. 2001). Post-CHX, whole-mouth, salty identifications of 100 mM NaCl (Frank et al. 2001) and saltiness intensity ratings for 300 mM NaCl dropped by ~50% (Breslin and Tharp 2001). CHX, once bound to oral tissue surfaces, may disrupt ionic transport to impair salt-taste perception.

CHX treatment did not produce NaCl taste blindness, as can anterior tongue NaCl self-adaptation (Smith and van der Klaauw 1995), in either the CN VII– or CN IX–receptive field. We define "taste blindness" as a complete loss of taste, that is, a zero intensity rating and a tasteless quality. But, as with whole-mouth testing (Frank et al. 2001; Gent et al. 2002), misidentifications of the quality of NaCl suggest an "off-taste" develops in CN VII sites, a taste likely contributing to the remaining NaCl intensity after CHX rinses. Notably, NaCl solutions weaker than ambient NaCl salivary levels are bitter–sour to people, and after NaCl self-adaptation has reduced saltiness to zero, the water rinse tastes bitter (Bartoshuk 1974).

Treatment with the ENaC blocker, amiloride, also fails to produce NaCl taste blindness but changes the taste quality of NaCl in rodents (Hill et al. 1990); NaCl no longer has a Na^+/Li^+-specific taste but tastes similar to quinine-like KCl (Frank and Nowlis 1989). Tasteless to rodents (Markison and Spector 1995), amiloride is very bitter to humans (Breslin and Beauchamp 1995) and modestly reduces the perceptual intensity of 1 M NaCl without affecting its saltiness (Ossebaard and Smith 1995, 1996; Smith and Ossebaard 1995). Clearly, ion channels involved in rodent and human salt-taste reception differ.

In the current study, the perceptual intensity 1 M NaCl was reduced more at CN VII sites than at CN IX sites after CHX rinse, suggesting that humans, like rodents, may have several populations of salt-taste receptors that distribute differentially across the receptive fields of 2 CNs. Salt-taste receptors blocked by amiloride in rodents are located primarily, if not exclusively, in the receptive fields CN VII: fungiform papillae and palate (Ninomiya and Funakoshi 1988; Hettinger and Frank 1990; Formaker and Hill 1991; Kitada et al. 1998; Sollars and Hill 1998; Lundy and Contreras 1999). Amiloride sensitivity is observed in a set of Na^+/Li^+-specific CT and GSP facial nerve afferents, and the change in taste of NaCl after amiloride treatment is associated with reduced activity in these specialist peripheral neurons (Frank 2000). Electrolyte generalist peripheral neurons in CN VII and CN IX, which also respond to NaCl, are unaffected by amiloride (Ninomiya and Funakoshi 1988; Hettinger and Frank 1990).

Human salty-taste stimulus chemistry neither matches responses of Na^+-specific, rodent facial-nerve afferents nor rodent behavior toward salts, which show greater Na^+ specificity than human salty taste. Human salty taste more closely resembles salt sensitivities of a subset of rodent electrolyte generalist neurons (Hettinger and Frank 1992), which have been subdivided into distinct categories, all of which respond to NaCl and other non-Na^+/Li^+ electrolytes: one group responds to NaCl, KCl, and quinine·HCl and another to NaCl and citric acid (Lundy and Contreras 1999; Breza et al. 2006, 2007). Humans, like rodents, may have multiple populations of electrolyte receptors that distribute differentially across regions innervated by CN VII and CN IX.

The off-taste of NaCl may be accounted for by the mix of human NaCl receptors, likely including human orthologues of those receptors tied to rodent electrolyte generalist neurons (Breza et al. 2007). Human electrolyte generalist neurons, which remain active after CHX treatment, may generate the sour taste blocked by amiloride treatment (Ossebaard and Smith 1996). Peripheral interactions, perhaps among taste bud cells endowed with multiple Na^+-detecting receptors, may be involved in suppression of quinine responses by Na^+ salts in mixtures in human (Breslin and Beauchamp 1995, 1997). In rodents, perceptual suppression of quinine by NaCl (Frank et al. 2003) is mirrored in the CT nerve (Rehnberg et al. 1992; Formaker and Frank 1996).

CHX and the bitter taste of quinine

Bitter taste is thought to depend on multiple GPCR localized within the plasma membrane of taste bud receptor cells specialized for the bitter taste (Chandrashekar et al. 2000; He et al. 2002; Chandrashekar et al. 2006). Bitter ligands that interact with distinct GPCR may fail to perceptually

cross-adapt and/or vary in susceptibility to Na^+-induced bitter suppression (Keast and Breslin 2002). In humans, the intensity and identification as bitter of bitter ionic and nonionic stimuli, including quinine, bitter salts with monovalent cations, urea, and sucrose octaacetate (SOA), are reduced by CHX. Only tastes of bitter salts with divalent cations like $CaCl_2$ and $MgSO_4$, are known to be unaffected by CHX (Breslin and Tharp 2001; Frank et al. 2001). Divalent stimulus cations may be able to displace CHX (Slee and Tanzer 1979). CHX, which tastes very bitter to people, could impair bitter taste by occupying polygenic sites on GPCR coding for bitter (Nelson et al. 2005), an impairment that would persist due to the strong affinity of CHX for oral surfaces (Rölla et al. 1970), termed substantivity (Al-Tannir and Goodman 1994) and generate a recurrent cross-adaptation. Yet, reduced human bitter intensity following CHX rinse and cross-adaptation do not necessarily match; for example, CHX obtunds the tastes of quinine and urea, which do not cross-adapt (McBurney et al. 1972; Breslin and Tharp 2001; Keast and Breslin 2002).

Quinine's taste intensity and identification of its bitter quality were both greatly reduced after CHX treatment and, unlike NaCl, the change in choice of quality labels was more or less exclusively toward tasteless. Misidentifications of quinine as other than bitter after CHX treatment were also rare with whole-mouth testing (Frank et al. 2001). CHX reduced the bitter taste of quinine·HCl by equal amounts at sites innervated by CN VII and CN IX. However, quinine was labeled tasteless after CHX treatment 75% of the time at CN VII sites but was infrequently tasteless and more intense post-CHX at CN IX than CN VII sites ($t_{11,1} = 4.36$, $P = 0.001$). Thus, CHX treatment produced quinine taste blindness, as has been reported for quinine self-adaptation with anterior tongue stimulation (Keast and Breslin 2002), only in CN VII–receptive fields.

Distinct quinine receptors for quinine would be consistent with the existence of rodent quinine-sensitive electrolyte generalist and CN IX quinine specialist neurons (Frank 1991; Lundy and Contreras 1998). However, if human orthologues exist for several rodent quinine-sensing receptors, we have no evidence that quinine elicits more than a unitary bitter quality. Labels chosen for quinine were either bitter or tasteless, never sour, salty, or sweet. However, bitter may have remained the best choice for quinine among the possible labels even after CHX rinse; and alternate labels may allow subjects to distinguish between tastes of quinine remaining in the treated CN IX field from quinine in the untreated CN VII field (Yang and Lawless 2005). Rodents do not perceive all stimuli that are bitter to people alike. Nonionic bitter-tasting compounds like SOA and cycloheximide, which primarily activate CN IX sites (Frank 1991; Geran and Travers 2006), have an aversive quality that is distinct from the quality of bitter salts, such as quinine hydrochloride and $MgSO_4$, which activate CN VII and CN IX sites (Frank et al. 2004; Hettinger et al. 2007).

Conclusions

Humans may have less specialized oral taste fields than rodents (Pritchard and Norgren 2004). Perceptions activated by either the facial or GL nerve encompass sweet, salty, sour, and bitter taste qualities. In rodents, taste quality perception depends especially on CN VII, with CN IX more important for gustatory reflexes (St John and Spector 1998; King et al. 2000). The dulling or elimination of quinine's taste by CHX without quality change, as well as CHX's failure to block sweet and sour, support the independence of human taste qualities (Breslin and Tharp 2001; Frank et al. 2001). However, like rodents as well as other primates (Sato et al. 1975; Hellekant, Danilova, et al. 1997; Hellekant, Ninomiya, et al. 1997), humans may have several types of NaCl-detecting peripheral taste systems that distribute differentially to CN VII and CN IX fields, are associated with distinct taste qualities, and can be distinguished by sensitivity to different guanidinium-containing inhibitors of epithelial ion transport (Breslin and Tharp 2001; Frank et al. 2001).

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