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Taste Suppression Following Lingual Capsaicin Pre-treatment in Humans

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Abstract

The effect of oral capsaicin on taste sensations in humans was reinvestigated with attention to methodological issues raised in previous studies, including the mode of presentation and temperature of the tastant stimulus, as well as the sensitizing and desensitizing properties of capsaicin. One-half of the dorsal anterior tongue was pre-treated with capsaicin, followed by bilateral tastant application (sucrose, NaCl, quinine, monosodium glutamate and citric acid). Subjects indicated on which side the taste intensity was greater in a two-alternative, forced-choice procedure and also rated taste intensity independently on each side of the tongue. Each of the five tastants was tested sequentially, with reapplication of capsaicin between trials in order to maintain a constant level of burn. Four experiments were conducted: (i) a high concentration (33 p.p.m.) (109 μM) capsaicin effect on taste intensity elicited by high tastant concentrations; (ii) a high concentration capsaicin effect on taste intensity elicited by low tastant concentrations; (iii) a low concentration (1.5 p.p.m.) (4.9 μM) capsaicin effect on taste intensity elicited by low tastant concentrations; and (iv) validation of the method for localizing taste by pre-treating one side of the tongue with Gymnema sylvestre, followed by bilateral application of sucrose. In the first experiment, a significant proportion of the subjects chose the non-treated side in the two-alternative, forced-choice procedure and assigned significantly higher ratings to that side for sucrose-induced sweetness, quinine-induced bitterness and glutamate-induced umami sensations. Salty and sour sensations were not different between sides. A 15 min break was imposed in order to allow the capsaicin burn to disappear and desensitization to set in, followed by reapplication of the tastant test solutions. There were no bilateral differences in the intensity of the sensations elicited by any of the five tastants. Similar results were obtained in experiments 2 and 3. In the fourth experiment, all 15 subjects tested chose the side not treated with Gymnema sylvestre as having a stronger sweet taste and assigned significantly higher ratings to that side, thereby validating the method for taste localization. These results indicate that oral capsaicin reduces certain but not all taste sensations and are discussed in terms of possible physiological and cognitive interactions.

Introduction

Capsaicin, which is the pungent principle of chilli peppers, elicits an irritant sensation by binding to 'capsaicin' (VR-1) receptors that are also gated by noxious heat and acidic stimuli (Caterina *et al*., 1997; Tominaga *et al*., 1998). VR-1 receptors are expressed in the endings of trigeminal nociceptors that innervate the oral mucosa (Sostman and Simon, 1991; Bryant and Moore, 1995; Liu and Simon, 1996; Liu *et al*., 2000), afferent fibres of which project to the brainstem trigeminal complex (Carstens *et al*., 1995, 1998). Chemicals eliciting taste, on the other hand, evoke activity in taste receptor cells, which in turn activate primary gustatory neurons of the chorda tympani, glossopharyngeal and/or vagus nerves that project to the rostral aspect of the nucleus of the solitary tract (Hamilton and Norgren, 1984; Scott *et al*., 1986; Travers *et al*., 1986; McPheeters *et al*., 1990; Nakamura and Norgren, 1991; Travers and Norgren, 1995; Di Lorenzo and Lemon, 2000; Smith *et al*., 2000). Although the trigeminal and gustatory pathways are anatomically distinct, the significant overlap of receptive fields and the convergence of lingual somatosensory and gustatory information within the brainstem (Ogawa *et al*., 1984, 1988; Hayama *et al*., 1985; Sweazey and Bradley, 1988; Travers and Norgren, 1995), thalamus (Nomura and Ogawa, 1985; Pritchard *et al*., 1986, 1989; Lenz *et al*., 1997) and cortex (Yamamoto, 1984; Cerf-Ducastel *et al*., 2001) provide substrates for possible interactions.

Anecdotal evidence suggests that oral irritants may alter the perceptions of taste elicited by various foods. Infrequent consumers of spicy food often complain that irritant chemicals make it difficult to taste food, whereas frequent consumers suggest that taste sensations are enhanced by the presence of these same chemicals (Lawless *et al*., 1985). In controlled psychophysical studies, pre-treating the oral cavity with capsaicin had no reported effect on the recognition thresholds of gustatory stimuli (Szolcsányi, 1977). In other studies the perception of suprathreshold taste

sensations appeared to be modified (Lawless and Stevens, 1984; Lawless *et al*., 1985; Cowart, 1987; Prescott *et al*., 1993; Prescott and Stevenson, 1995; Stevenson and Prescott, 1997). There is a general consensus that capsaicin reduces perceived sweet intensity. However, its effect on other taste qualities varies. Indeed, it appears that the method of capsaicin/tastant delivery (pre-treatment versus mixture) influences the degree to which any effects are observed (Cowart, 1987).

Several methodological concerns are worthy of special attention. Repeated application of capsaicin at short inter-stimulus intervals results in an increase in the perceived burn intensity (Stevens and Lawless, 1987; Cliff and Green, 1996; Dessirier *et al*., 1997; Prescott and Swain-Campbell, 2000), a phenomenon now termed sensitization. In contrast, if capsaicin is reapplied following a longer inter-stimulus interval (>2.5–5 min), the perceived irritation is markedly reduced, resulting in a state of desensitization (Szolcsányi, 1977; Geppetti *et al*., 1993; Cliff and Green, 1996; Green, 1998; Green and Rentmeister-Bryant, 1998; Prescott and Swain-Campbell, 2000), which can eventually be overcome by reapplying the capsaicin at shorter inter-stimulus intervals (stimulus-induced recovery) (Green, 1998; Green and Rentmeister-Bryant, 1998). It is therefore important to control for the inherent sensitizing and desensitizing properties of capsaicin when assessing its effect on taste.

Second, cooling can reduce or eliminate the irritant effects elicited by capsaicin. An earlier study found a modest decrement in the burn intensity of capsaicin solutions presented at 21°C as compared to 37°C (Prescott *et al*., 1993). Interestingly, varying the temperature did not modulate the effects of capsaicin on taste when the irritant was presented in a mixture with sucrose or NaCl. However, as indicated by those authors, this lack of effect may have been due to temporal factors associated with how the cooling was administered (i.e. administration of a cooled capsaicin–tastant mixture versus cooling a pre-existing burn).

The present study was undertaken in order to re-evaluate the effect of pre-treating the lingual epithelium with capsaicin while controlling for any possible confounding effects of capsaicin sensitization/desensitization or cooling by tastant administration. Moreover, we employed the very sensitive half-tongue, two-alternative, forced-choice protocol coupled with bilateral intensity ratings that we have used previously for studying oral irritation (Dessirier *et al*., 1998, 1999; Simons *et al*., 1999).

Materials and methods

General procedures

Subjects

A total of 107 healthy volunteers aged from 18 to 63 years volunteered to participate in the experiments. All were either students or staff at the University of California, Davis. The subjects were divided into three approximately equal groups, with a fourth group of 15 subjects used in experiment 4. Each group participated in a single experiment (experiment 1, 2, 3 or 4) (see below) lasting ~45 min (except for experiment 4 which lasted ~10 min). The subjects were asked to refrain from eating, drinking or smoking for at least 1 h prior to their scheduled session (12 subjects reported being smokers). Moreover, the subjects were asked to avoid eating anything spicy for at least 2 days prior to the experiment and these criteria were verified by interview. The University of California, Davis Human Subjects Review committee approved the experimental protocol.

Chemical stimuli

The oral irritant capsaicin (Sigma, St Louis, MO) was used at a concentration eliciting a burn of moderate intensity (109 μM) in experiments 1 and 2 or of low intensity (4.9 μM) in experiment 3. KCl (Mallinkrodt, Paris, KY) was used at a concentration of 250 mM (experiments 1 and 2) or 100 mM (experiment 3) as a distractor/control (see below). A series of five tastant test solutions (sucrose, NaCl, citric acid, quinine and monosodium glutamate) that were approximately matched in intensity were used in each experiment. The tastant concentrations are given for each experiment under the section on specific procedures. All chemicals were of reagent grade and dissolved in distilled water except for capsaicin, which was dissolved initially as a stock solution of 3.3 mM in a solution containing 50% ethanol. Working capsaicin concentrations contained 1.65% and 0.075% ethanol, which is well below the level needed for eliciting activity in rat trigeminal nocieptive neurons (Carstens *et al*., 1998).

Experiment 4 was a control experiment for ensuring that the subjects could detect lateral differences in taste intensity. The chemicals used and their concentrations are given in the section on specific procedures.

Stimulus application

Prior to the start of each experiment, the subjects familiarized themselves with the quality and intensity of each of the tastant test solutions. All subjects reported that the tastant solutions were suprathreshold. Capsaicin's effect on taste perception was then tested using the same half-tongue, two-alternative, forced-choice procedure we have used previously (Dessirier *et al*., 1998, 1999; Simons *et al*., 1999). Capsaicin was applied unilaterally to the anterior half of the dorsal lingual surface using a cotton-tipped applicator. The side of the tongue receiving capsaicin was counterbalanced across the subjects. It has been shown previously that subjects often have the expectation that spices decrease perceived taste (Stevenson and Prescott, 1997). In order to counter this potential bias, we applied KCl (250 or 100 mM) in a similar manner to the opposite side of the tongue as a distractor/control. The subjects were instructed that two spicy chemicals were going to be painted on their tongues, one of which had a pungent quality but no taste (capsaicin)

and the other of which had a taste but no pungency (KCl). Following this, the subjects were instructed to rinse with warm water (37°C) in order to remove any residual capsaicin and KCl from their tongues. It also had the effect of eliminating taste adaptation effects that might have been elicited by the KCl. Immediately after the rinse, the subjects were required to attend to the burning sensation produced by the capsaicin and rate the intensity using a 0–10 point intensity scale (0 = no sensation and 10 = strongest sensation imaginable). The subjects were then given a small plastic cup containing 5 ml of one of the five tastant test solutions warmed to 37°C, instructed to place the solution in the anterior half of the buccal cavity and choose the side of their tongues having the strongest taste sensation. In addition, the subjects were asked to provide bilateral ratings of taste intensity using the same 0–10 point intensity scale. In an effort to prevent the subjects from combining sensations of taste and irritation, they were asked to ignore the burning sensation and focus exclusively on the taste of the tastant solution. After expectoration of the tastant test solution, the subjects rinsed their mouths with warmed distilled water in order to remove the tastant and avoid cross-adaptation effects in subsequent taste stimuli and rated the intensity of the residual burn left on their tongues. If the intensity rating decreased by >2 points from its original intensity, capsaicin and KCl were reapplied until the level of irritation on the capsaicin-treated side was once again equal to its original level. This procedure was followed by a rinse, as before, in order to remove any residual KCl taste that may have confounded the results. The subjects were then given the second tastant test solution and the procedure was repeated. This procedure was repeated successively for each of the five tastant solutions. The order of presentation of the tastants was randomized across the subjects.

In order to evaluate the effect of capsaicin desensitization on taste perception, the subjects were asked to rinse three times with distilled water and wait a minimum of 15 min or until the burn from the previous capsaicin application(s) had completely dissipated. When the rest period concluded, sequential application of the tastant series was repeated in the absence of any oral irritation, with the subjects performing the two-alternative, forced-choice procedure and giving bilateral intensity ratings as described above. The tastant test solutions were presented in a randomized order and the subjects rinsed with warmed (37°C) distilled water between tastants. At the conclusion of the session, capsaicin desensitization was verified as follows. Two filter papers (1 cm diameter) (Whatman International Ltd, Maidstone, UK) were saturated with 20 μl of the same capsaicin solution applied previously to the tongue and placed with forceps onto each side of the tongue in the areas previously treated with capsaicin or KCl. After ~10 s during which the mouth was held closed, the filter papers were removed and, in a two-alternative, forced-choice procedure, the subjects chose the side of the tongue having the stronger burning sensation.

The procedure used for stimulus application in experiment 4 is given in the section on specific procedures.

Data analysis

A binomial analysis was used for determining whether a significant majority of the subjects chose the control (KCl-pre-treated) side of the tongue as having a stronger taste sensation. In addition, a d' analysis (Ennis, 1993) was performed in order to measure the strength and significance of the effect using a previously used method (Bi and O'Mahony, 1995). Significant differences in the mean taste intensity ratings for each side of the tongue were assessed using Student's paired *t*-tests with a Bonferroni correction. One-way analysis of variance (ANOVA) was used for determining whether significant differences existed in the mean level of irritation experienced by the subjects during each of the tastant evaluations. All data are presented as means ± SE. A significance level of $P < 0.05$ was taken as significant for the binomial analysis and the ANOVA, whereas a significance level of $P < 0.01$ was used in the Bonferroni-corrected *t*-tests.

Specific procedures

Experiment 1

Thirty-one subjects (11 male and 20 female) ranging from 18 to 43 years in age participated in experiment 1. This experiment tested the effect of unilateral application of the high capsaicin concentration (109 μM, with 250 mM KCl applied to the opposite side) on taste that was elicited by high concentrations of each tastant: sucrose (300 mM) (Mallinkrodt, Paris, KY), NaCl (300 mM) (Fisher Scientific, Fair Lawn, NJ), citric acid (5.6 mM) (Mallinkrodt, Paris, KY), quinine HCl (0.1 mM) (BDH Chemicals, Poole, UK) and monosodium glutamate (30 mM) (Sigma, St Louis, MO). All aspects of the experiment were carried out as described under the section on general procedures.

Experiment 2

Thirty-two subjects (eight male and 24 female) ranging from 18 to 42 years in age participated in experiment 2. This experiment tested the effect of unilateral application of the high capsaicin concentration (109 μM capsaicin, with 250 mM KCl applied to the opposite side) on taste that was elicited by the low tastant concentrations (50 mM sucrose, 40 mM NaCl, 0.93 mM citric acid, 0.01 mM quinine HCl and 10 mM monosodium glutamate). All other aspects of the experiment were identical to those described in the section on general procedures.

Experiment 3

Twenty-nine subjects (nine male and 20 female), 18–57 years old, participated in experiment 3. This experiment tested the effect of the low capsaicin concentration (4.9 μM, with 100 mM KCl applied to the opposite side) on taste that

was elicited by low tastant concentrations (50 mM sucrose, 40 mM NaCl, 0.93 mM citric acid, 0.01 mM quinine HCl and 10 mM monosodium glutamate). All other procedures were identical to those described in the section on general procedures.

Experiment 4: validation of the method for taste

This experiment was conducted in order to show that the present half-tongue, two-alternative, forced-choice procedure can detect lateral differences in taste intensity. Fifteen healthy volunteers (25–63 years of age) were tested. Gymnema sylvestre (5% in dH_2O) (Natrol, Inc., Chatsworth, CA) was painted onto one-half of the dorsal lingual surface using a cotton-tipped applicator. Quinine HCl (10 mM) approximately matched in terms of bitter taste intensity was painted onto the opposite side as a control. The subjects rinsed their mouths with distilled water and were asked to sip 5 ml of a 300 mM sucrose solution into the anterior portion of their mouth and assess the sweetness on both sides of the tongue. As in the other experiments, the subjects indicated which side of the tongue had the strongest sweet sensation in the two-alternative, forced-choice procedure. In addition, the subjects gave bilateral intensity ratings for the sweetness using a 0–10 scale (0 = no sensation and 10 = strongest sensation imaginable).

Results

Experiment 1

One goal of this experiment was to ensure that the level of irritation experienced during each of the five tastant evaluations was maintained at a constant level. In this regard, the mean intensity of the irritation induced by 109 μM capsaicin did not differ significantly ($P = 0.976$) when assessed prior to the application of each of the five tastants. However, capsaicin pre-treatment differentially affected the perceived taste intensities elicited by the higher tastant concentrations (Figure 1). A significant majority of the subjects chose the control side of the tongue as having the stronger taste sensation (26 out of 31) ($P < 0.001$) for sucrose-induced sweetness, giving a significant d' value of 1.4 ($P < 0.001$). Consistent with this, significantly lower intensity ratings were assigned to the capsaicin-treated side as compared to the control side (4.2 ± 0.4 versus 5.6 ± 0.3) ($P < 0.001$) (Figure 1A). A significant majority of the subjects also chose the control side of the tongue as having the stronger bitter taste for quinine-induced bitterness (22 out of 31) ($P = 0.028$) (equivalent to a d' value of 0.78). However, the bilateral intensity ratings were not significantly different although there was a trend towards higher ratings on the control side (3.4 ± 0.4 versus 4.0 ± 0.4) ($P = 0.079$) (Figure 1B). Our interpretation is that the subjects reliably perceived an intensity difference in the two-alternative, forced-choice procedure, but that this difference was too small to detect by the scaling method. In contrast to sweetness and bitterness the subjects did not consistently choose the control side as having a stronger intensity for NaCl-induced saltiness (18 out of 31) ($P = 0.473$) (equivalent to a d' value of 0.29) ($P = 0.473$), citric acid-induced sourness (13 out of 31) ($P = 0.537$) (equivalent to a d' value of 0.29) ($P = 0.537$) and monosodium glutamate-induced umami (18 out of 31) ($P = 0.473$) (equivalent to a d' value of 0.29) ($P = 0.473$) in the two-alternative, forced-choice procedure, nor was there a significant bilateral intensity difference for saltiness (4.7 ± 0.4 versus 4.9 ± 0.4 respectively) ($P = 0.691$) (Figure 1C), sourness (3.5 ± 0.4 versus 3.3 ± 0.4 respectively) ($P = 0.531$) (Figure 1D) or umami (4.3 ± 0.3 versus 4.7 ± 0.4 respectively) ($P = 0.353$) (Figure 1E).

After the capsaicin burn had dissipated and desensitization had set in, the subjects sampled the five tastant test solutions again. During this period the subjects did not consistently choose either side of the tongue as having the stronger taste sensation in the two-alternative, forced-choice procedure, nor were there any significant laterality differences in the intensity ratings for any of the tastants (Figure 2A–E). Desensitization by the prior capsaicin applications was verified. One hundred percent of the subjects chose the control side as having the stronger irritant sensation when capsaicin was reapplied at the end of the session (31 out of 31) ($P < 0.001$) and gave significantly higher ratings to the control side as compared to the desensitized side (5.8 versus 2.8) ($P < 0.001$).

Experiment 2

As predicted by Weber's Law, we hypothesized that the taste suppression effect of capsaicin would be more obvious if the intensity of the various tastant test solutions were minimized. Therefore, the concentrations of the tastant test solutions in experiment 2 were just above recognition thresholds. As in experiment 1, the level of perceived irritation remained constant during each of the five tastant evaluation periods ($P = 0.644$). Moreover, as with the higher tastant concentrations, a significant majority of the subjects chose the control side of the tongue as having the stronger taste sensation for sucrose-induced sweetness (28 out of 32) ($P < 0.001$) and quinine-induced bitterness (26 out of 32) ($P < 0.001$). The equivalent d' values (1.63 and 1.25 respectively) were also significant ($P < 0.001$ respectively). In addition, a significant majority of the subjects chose the control side as having a stronger monosodium glutamate-induced umami taste (24 out of 32) ($P = 0.008$) giving a significant ($P = 0.008$) d' value of 0.95. In accordance with these findings, significant differences in the bilateral intensity ratings were found for sweetness (3.3 ± 0.3 versus 4.6 ± 0.4 respectively) ($P = 0.001$) (Figure 3A) and bitterness (3.3 ± 0.4 versus 4.3 ± 0.4 respectively) ($P = 0.009$) (Figure 3B). Although approaching significance, the bilateral intensity ratings for monosodium glutamate-induced umami were not statistically different (3.6 ± 0.3 versus 4.6 ± 0.3 respectively) ($P = 0.015$) (Figure 3E) again suggesting that

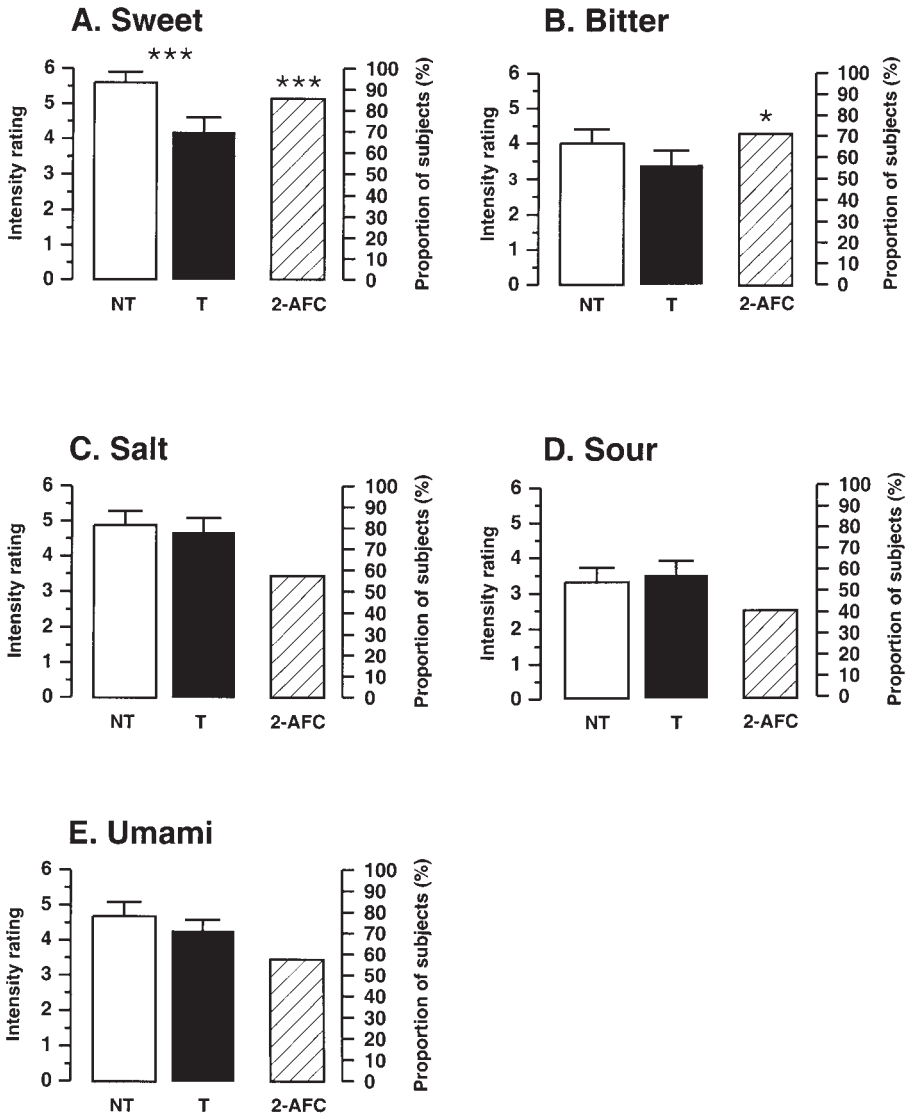

Figure 1 Effect of the irritation elicited by the high capsaicin concentration on the perceived intensity of taste elicited by high tastant concentrations (experiment 1). Each panel shows a set of bar graphs. The left-hand pair plots the ratings of taste intensity on the non-treated (NT) (hollow bar) and capsaicin-treated (T) (solid bar) sides of the tongue. The cross-hatched bar on the right-hand side shows the proportion of subjects choosing the non-treated side as having a stronger taste intensity in the two-alternative, forced-choice procedure. **(A)** Capsaicin pre-treatment significantly suppressed the intensity of sucrose-induced (300 mM) sweetness. **(B)** Capsaicin pre-treatment significantly suppressed the intensity of quinine-induced (0.1 mM) bitterness. Capsaicin-induced irritation had no significant effect on the perceived intensity of **(C)** NaCl-induced (300 mM), **(D)** citric acid-induced (0.56 mM) or **(E)** glutamate-induced (30 mM) saltiness, sourness or umami. The error bars indicate SEs. $^{***}P < 0.001$ (filled bars): a significant difference between the capsaicin-treated and control sides. $^{***}P < 0.001$ and $^{*}P < 0.05$ (cross-hatched bars): a significant majority of subjects chose the non-treated side.

data derived from paired comparisons are more sensitive to small discriminatory differences than those obtained from scaling. The subjects did not consistently choose either side as having the stronger taste for NaCl-induced saltiness (20 out of 32) ($P = 0.215$) or citric acid-induced sourness (19 out of 32) ($P = 0.377$), nor were the bilateral intensity ratings significantly different for saltiness (2.9 ± 0.4 versus 3.4 ± 0.4 respectively) ($P = 0.081$) (Figure 3C) or sourness (3.8 ± 0.4 versus 4.2 ± 0.3 respectively) ($P = 0.531$) (Figure 3D).

When the subjects were retested after the capsaicin burn had subsided they did not consistently choose either side the tongue in the two-alternative, forced-choice procedure, nor were there any significant laterality differences in the intensity ratings for any of the tastants (Figure 4A–E). As in experiment 1, all of the subjects (32 out of 32) ($P < 0.001$) were desensitized to capsaicin as determined in the two-alternative, forced-choice procedure and gave significantly ($P < 0.001$) lower ratings to the capsaicin pre-treated side (2.2) as opposed to the control side (5.9).

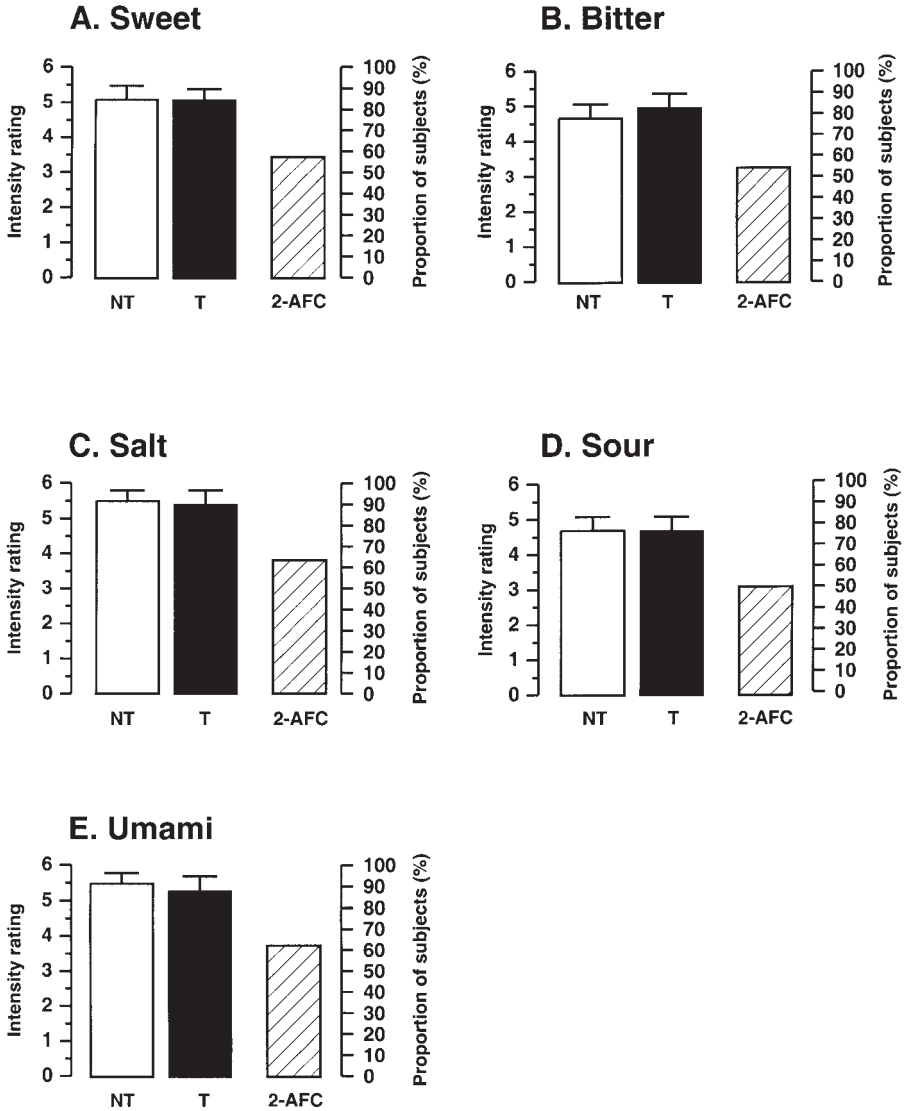

Figure 2 Absence of an effect of capsaicin desensitization on taste intensity (high tastant concentrations). The graphs show the bilateral intensity ratings and two-alternative, forced-choice procedure data (the same subjects and tastants as in Figure 1) when tested after the capsaicin burn had subsided. There were no differences in the perceived intensity of **(A)** sweetness, **(B)** bitterness, **(C)** saltiness, **(D)** sourness or **(E)** umami. The format is the same as in Figure 1.

Experiment 3

In order to minimize any potential confounding effects of masking, a lower concentration of capsaicin (4.9 μM), which elicited a very weak burning sensation, was used in conjunction with low tastant concentrations. The level of perceived irritation remained constant during each of the five tastant evaluation periods ($P = 0.936$). As in experiments 1 and 2, a significant majority of the subjects chose the control side of the tongue as having the stronger sensation of sucrose-induced sweetness (22 out of 29) ($P = 0.008$) and monosodium glutamate-induced umami (21 out of 29) ($P = 0.024$). The corresponding d' values (0.990 and 0.840 respectively) were also significant ($P = 0.008$ and 0.024 respectively). The intensity ratings for sucrose-induced sweetness were consistent as significantly higher ratings were given to the untreated side of the tongue (3.1 ± 0.3 versus 2.3 ± 0.4) ($P = 0.011$) (Figure 5A). The intensity difference for monosodium glutamate-induced umami failed to reach a significant level (2.8 ± 0.3 versus 2.2 ± 0.3) ($P = 0.048$) (Figure 5E), although there was a trend for higher ratings on the untreated side. Neither side of the tongue was consistently selected as having the stronger sensation for all the other tastant test solutions, nor were there any differences in the intensity ratings assigned to each side (Figure 5B–D).

The subjects did not consistently choose either side of the tongue as having the stronger taste sensation during the period of capsaicin desensitization, nor were there any significant laterality differences in the intensity ratings for

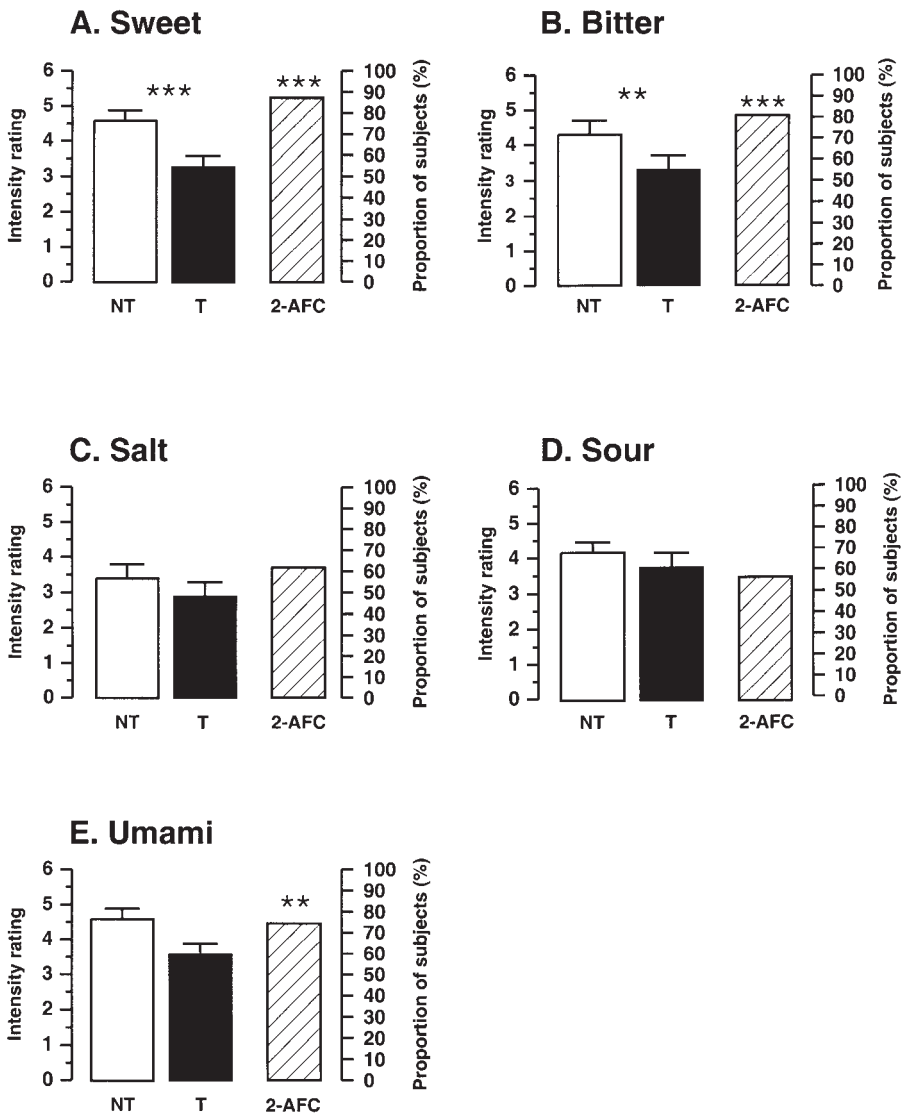

Figure 3 Effect of the irritation elicited by the high capsaicin concentration on the perceived intensity of taste elicited by low tastant concentrations (experiment 2). Capsaicin (109 μM) pre-treatment significantly suppressed the intensity of **(A)** sucrose-induced (50 mM) sweetness, **(B)** quinine-induced (0.03 mM) bitterness and **(E)** monosodium glutamate-induced (10 mM) umami. Capsaicin-induced irritation had no effect on the perceived intensity of **(D)** citric acid-induced (0.1 mM) sourness or **(C)** NaCl-induced (40 mM) saltiness. The format is the same as in Figure 1. $^{***}P < 0.001$ and $^{**}P < 0.01$ (filled bars). $^{***}P < 0.001$ and $^{**}P < 0.01$ (hatched bars).

any of the tastants (Figure 6A–E). Capsaicin desensitization was verified in 86% of the subjects that chose the control side as having the stronger irritant sensation when capsaicin was reapplied at the end of the session (25 out of 29) ($P < 0.001$). As expected, the mean burn rating for the control side of the tongue was significantly higher than the mean rating for the capsaicin-desensitized side (2.4 versus 4.1 respectively) ($P < 0.001$). The data from the four subjects not desensitized were excluded from this analysis.

Experiment 4: validation of the method for taste

Fifteen out of 15 subjects ($P < 0.001$) chose the side of the tongue not receiving Gymnema sylvestre as being sweeter when evaluating the sweetness of a 300 mM sucrose solution. In addition, the ratings of sweetness were significantly higher ($P < 0.001$) on the quinine-treated side of the tongue as compared to those on the Gymnema sylvestre-treated side (4.1 ± 0.5 versus 1.8 ± 0.4 respectively).

Discussion

The results obtained in the present study confirm the suppressive effects of capsaicin on certain gustatory qualities, as reported previously (Lawless and Stevens, 1984; Lawless *et al*., 1985; Cowart, 1987; Prescott *et al*., 1993; Prescott and Stevenson, 1995; Stevenson and Prescott, 1997). Whereas most prior studies have reported a suppressive effect on sucrose-induced sweetness, the effects on other taste qualities were inconsistent. These findings are discussed in terms

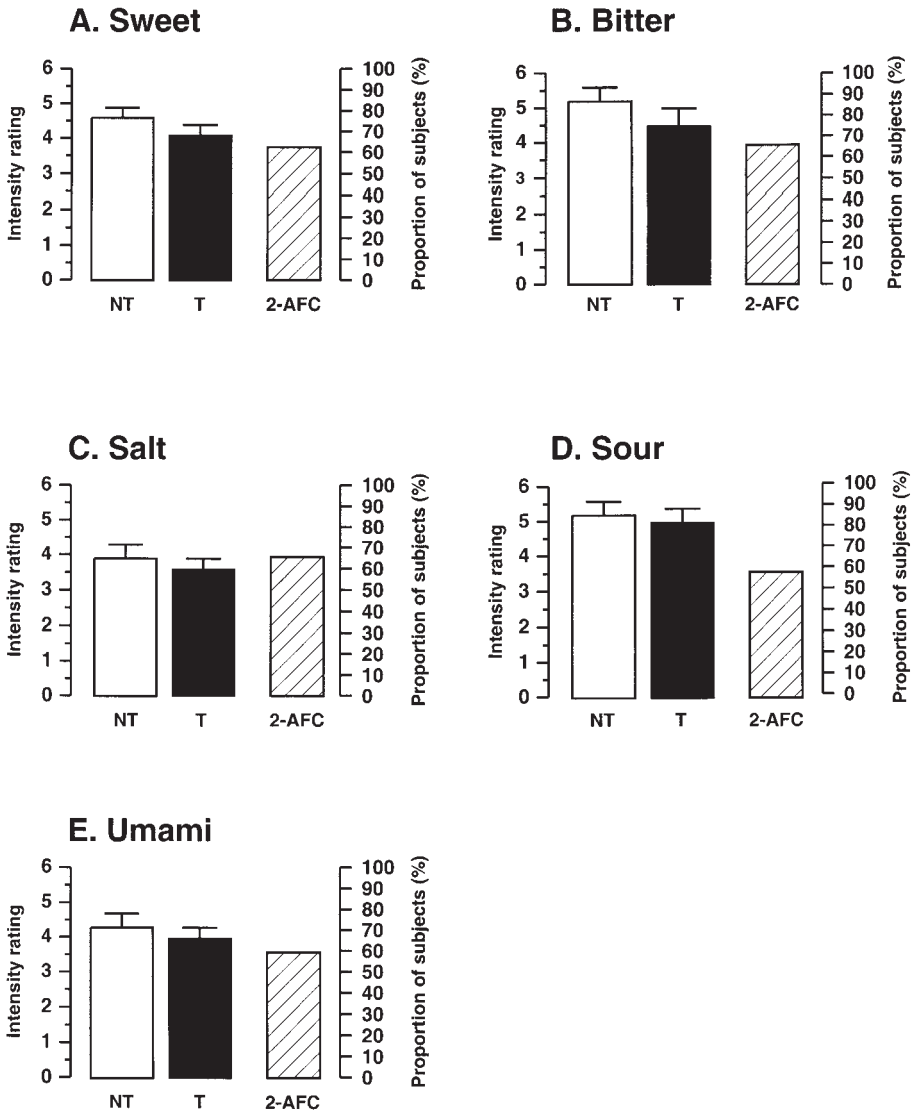

Figure 4 Absence of an effect of capsaicin desensitization on taste intensity (low tastant concentrations). The graphs show the bilateral intensity ratings and two-alternative, forced-choice procedure data (the same subjects and tastants as in Figure 3) when tested after the capsaicin burn had subsided. There were no differences in the perceived intensity of **(A)** sweetness, **(B)** bitterness, **(C)** saltiness, **(D)** sourness or **(E)** umami. The format is the same as in Figure 1.

of the methodology as well as underlying psychological and physiological mechanisms.

Methodological issues

The present study employed a novel half-tongue, two-alternative, forced-choice procedure in which the subjects were asked to compare taste sensations bilaterally following unilateral capsaicin application. This requires that normal subjects are capable of localizing tastes within the oral cavity. Clinical evidence indicates that patients with unilateral damage to the chorda tympani may be unaware of the resultant taste deficit (Kveton and Bartoshuk, 1994). Moreover, normal subjects with unilateral local anaesthesia of the chorda tympani mislocalize tastes to the anaesthetized side (Lehman *et al*., 1995; Yanagisawa *et al*., 1998), possibly with the aid of tactile cues ('tactile capture') (Todrank and Bartoshuk, 1991). However, recent psychophysical data have indicated that normal subjects are capable of localizing tastes on the tongue under controlled conditions (Delwiche *et al*., 2000; Shikata *et al*., 2000; McMahon *et al*., 2001). In order to ensure that our method was appropriate for assessing laterality differences in taste intensity, we made the subjects rate the intensity of sweetness elicited by sucrose after one side of the tongue had been treated with Gymnema sylvestre, which blocks sweet taste transduction (Warren *et al*., 1969). The other control side was pre-treated with quinine matched approximately to the bitterness of the Gymnema sylvestre. That all of the subjects chose the control side as having a stronger sweet sensation supports our contention that the

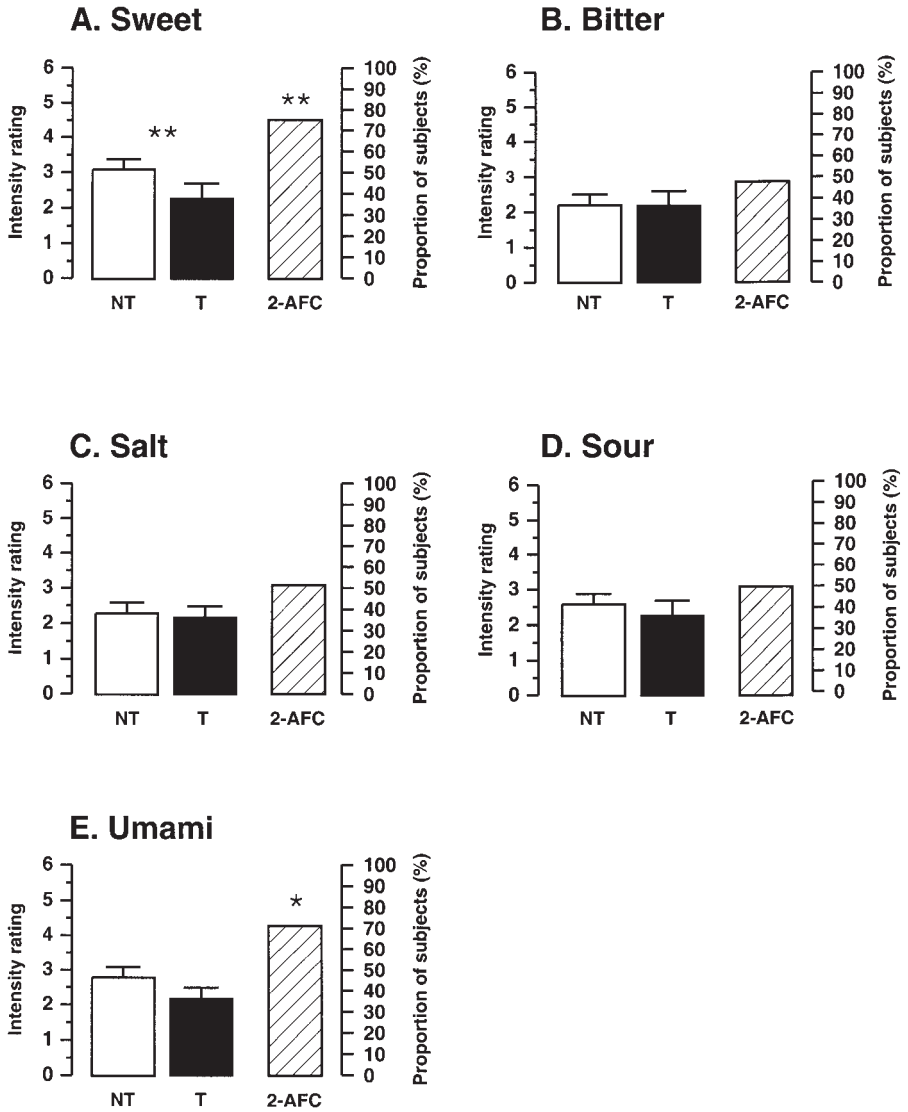

Figure 5 Effect of the irritation elicited by the low (4.9 μM) capsaicin concentration on the perceived intensity of taste elicited by low tastant concentrations (experiment 3). Capsaicin pre-treatment significantly suppressed the intensity of **(A)** sucrose-induced (50 mM) sweetness and **(E)** glutamate-induced (10 mM) umami. There was no effect on the perceived intensity of **(B)** quinine-induced (0.01 mM) bitterness, **(C)** NaCl-induced (40 mM) saltiness or **(D)** citric acid-induced (0.1 mM) sourness. The format is the same as in Figure 1. $^{**}P < 0.01$ (filled and hatched bars). $^{*}P < 0.05$ (hatched bar).

half-tongue, two-alternative, forced-choice method is appropriate for assessing bilateral taste intensity differences.

As noted in the Introduction, we attempted to control for a variety of methodological nuances that may underlie some of the reported differences regarding the effect of oral capsaicin on taste, including the mode of stimulus presentation, the temperature of the stimuli at presentation and the presence of capsaicin sensitization and/or desensitization. Since our pilot studies indicated that the capsaicin effects were likely to be small, we maximized the probability of discriminating small differences by using a paradigm in which we pre-treated the dorsal lingual surface with capsaicin followed by tastant rinses, as opposed to making the subjects assess the taste intensity of a mixture containing both the irritant and the tastant. In a direct comparison of these two paradigms, the former method was shown to result in a greater apparent reduction in taste intensities (Cowart, 1987). All solutions were presented at 37°C in order to obviate any effect of temperature. In addition, capsaicin was reapplied as necessary in order to maintain a constant level of burn throughout each tastant evaluation. Finally, we employed a strategy using difference tests coupled with intensity ratings. Difference tests are more sensitive in detecting small differences in sensory intensity compared to scaling procedures alone (Kim *et al*., 1998), the latter having their sensitivity reduced by boundary variance. Another advantage of our present strategy is that the subjects compared the treated and untreated sides of the tongue simultaneously rather than sequentially as is done in traditional 'sip and spit' techniques. This allowed the subjects to

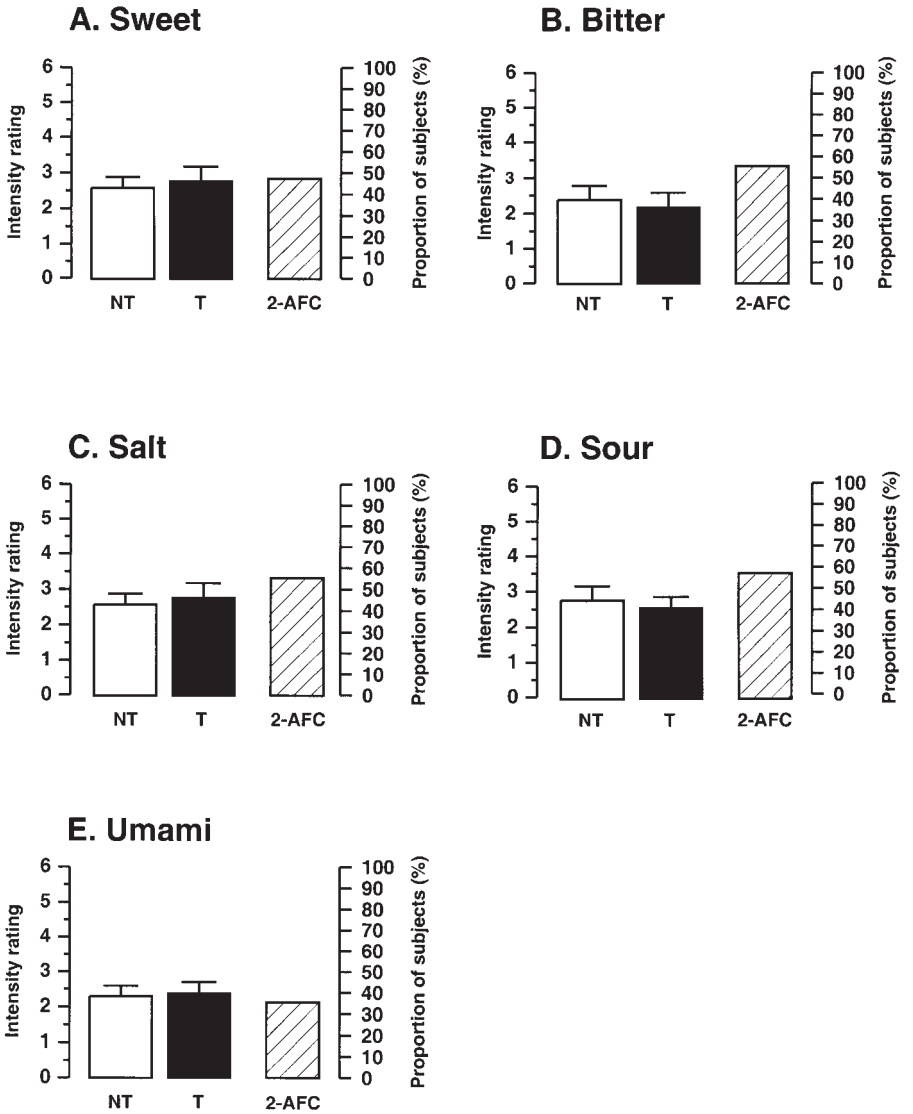

Figure 6 Absence of an effect of desensitization by the low capsaicin concentration on taste intensity (low tastant concentrations). The graphs show the bilateral intensity ratings and two-alternative, forced-choice procedure data (the same subjects and tastants as in Figure 5). There was no effect on the perceived intensity of **(A)** sweetness, **(B)** bitterness, **(C)** saltiness, **(D)** sourness or **(E)** umami. The format is the same as in Figure 1.

make direct sensory comparisons that were devoid of any confounding memory effects.

We found that sucrose-induced sweetness and quinine-induced bitterness were suppressed when the tongue was pre-treated with a relatively high capsaicin concentration. Interestingly, the magnitude of the suppression of the sweetness and bitterness was larger, with significant suppression of umami in the two-alternative, forced-choice procedure when the tastant concentrations were lowered to near-threshold levels (experiment 2). This result is consistent with Weber's Law which predicts that, for a given absolute sensory intensity difference between the two sides of the tongue, the fractional difference is greater (and, hence, more easily detectable) for low than high baseline intensities. The inhibitory effect of capsaicin on sweetness and bitterness is consistent with previous findings showing a modest (Lawless and Stevens, 1984) to substantial reduction in sweetness following a capsaicin rinse (Lawless *et al.*, 1985; Prescott *et al.*, 1993; Prescott and Stevenson, 1995; Stevenson and Prescott, 1997) as well as a reduction in bitterness ratings following either a capsaicin (Lawless, 1984; Cowart, 1987) or piperine (Lawless and Stevens, 1984) pre-rinse. The capsaicin suppression (in the two-alternative, forced-choice procedure) of umami taste at the low monosodium glutamate concentration was unique to the present report.

If a hiatus of >2.5–5 min is imposed following an oral capsaicin stimulus subsequent application of capsaicin elicits a burn of substantially reduced intensity, a phenomenon that is called desensitization (Szolcsányi, 1977; Green, 1989, 1998; Geppetti *et al.*, 1993; Cliff and Green, 1996;

Green and Rentmeister-Bryant, 1998; Prescott and Swain-Campbell, 2000). Previous studies have shown that quinine- and propylthiouracil-induced bitterness (Karrer and Bartoshuk, 1995) as well saltiness and sourness sensations induced by high NaCl or citric acid concentrations (Gilmore and Green, 1993) are reduced when given >15 min after pre-treatment with capsaicin. In the present study we did not find bilateral intensity differences for any tastant when it was applied during the period of capsaicin desensitization after the burn had subsided. However, our data are not inconsistent with previous studies. In an earlier study (Gilmore and Green, 1993), capsaicin at a concentration (10 p.p.m.) intermediate between those used in the present study (1.5 and 33 p.p.m.) had no effect on the salty or sour sensations evoked by low or moderate concentrations of NaCl or citric acid similar to those used in this study. In another earlier study (Karrer and Bartoshuk, 1995) desensitization by an intermediate concentration of capsaicin (10 p.p.m.) had no effect on taste intensity, consistent with our present findings. Capsaicin suppression of taste was only observed following desensitization by a much higher (100 p.p.m.) concentration of capsaicin (Karrer and Bartoshuk, 1995). While this probably accounts for the different results with capsaicin desensitization, there were also substantial methodological differences. The latter study (Karrer and Bartoshuk, 1995) tracked the effects of capsaicin on taste over a period of days, requiring the subjects to remember prior intensity ratings. This differs markedly from the present study in which the subjects rated the tastants ~15 min following capsaicin application in a two-alternative, forced-choice paradigm that did not require memory of prior ratings.

Mechanisms of differential taste suppression

The differential effects of capsaicin on various taste qualities may have cognitive and/or physiological underpinnings. A potential cognitive effect might result from the dominant burning sensation drawing attention away from the gustatory sensations, thereby resulting in lower perceived intensity scores. A strong argument against this is our observation that only sweet, bitter and umami taste qualities were affected while salty and sour taste qualities were not. Moreover, capsaicin suppression of sweetness but not strawberry flavour was demonstrated in a direct test of this hypothesis (Prescott and Stevenson, 1995), thereby suggesting that distraction does not adequately describe capsaicin's effect on gustatory responses.

Peripherally capsaicin application can lead to localized areas of oedema of the tongue by its documented ability to elicit plasma extravasation (Bryant and Moore, 1995; Holzer, 1998). Localized oedema could lead to closure of the taste pore thereby making it difficult for tastant molecules to access their receptors. However, such a non-specific mechanism should apply to all tastes and not just sweet, bitter and umami tates. Neural mechanisms may also contribute to the selective suppressive effect of capsaicin. Activation of the lingual nerve electrically (Wang *et al*., 1995) or chemically with capsaicin (Osada *et al*., 1997) led to a reduction in NaCl-evoked responses recorded in the rat chorda tympani. A peripheral mechanism was proposed for explaining these findings whereby substance P released from nociceptive terminals of the lingual nerve alters the sensitivity of taste receptor cells (Wang *et al*., 1995; Osada *et al*., 1997) which express substance P (NK-1) receptors (Chang *et al*., 1996). Capsaicin was recently found to activate second messenger systems that inhibit voltage-gated Na^+ ion channels in trigeminal ganglion cells (Liu *et al*., 2001), as well as voltage-gated K^+ channels in taste receptor cells (S.A. Simon, personal communication). Given the present findings that capsaicin selectively affected sweet, bitter and umami tastes, it is enticing to speculate that capsaicin might affect G-protein-coupled transduction mechanisms that have been demonstrated for these tastants (Gilbertson *et al*., 2000) while ionotropic transduction mechanisms for bitter (quinine) and glutamate tastants (Gilbertson *et al*., 2000) might contribute to residual taste sensitivity.

Finally, a central neural effect is also possible whereby capsaicin activation of the trigeminal system modulates gustatory processing at some stage in the taste pathway such as the NTS, thalamus or cortex. Capsaicin modulation of taste could conceivably involve both peripheral and central neural interactions.

In summary, the present study showed a significant suppression of sweetness, bitterness and umami sensations following pre-treatment of the lingual epithelium with capsaicin. The suppression apparently requires the presence of an active irritant sensation, as the suppressive effect was not observed after the burn dissipated. Additional studies are needed in order to delineate the mechanisms responsible for the observed suppressive effect.

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