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Oral Irritant Properties of Piperine and Nicotine: Psychophysical Evidence for Asymmetrical Desensitization Effects

Jean-Marc Dessirier[1–3], Nancy Nguyen[1], Jean-Marc Sieffermann[3], E. Carstens[1] and Michael O'Mahony[2]

[1]Section of Neurobiology, Physiology and Behavior, [2]Department of Food Science and Technology, University of California—Davis, Davis, CA, USA and [3]Département de Science de l'Aliment, ENSIA (Ecole Nationale Supérieure des Industries Agro-alimentaires), Massy, France

Correspondence to be sent to: Professor E. Carstens, Section of Neurobiology, Physiology and Behavior, University of California—Davis, Davis, CA 95616, USA. e-mail: eecarstens@ucdavis.edu

Abstract

Using a bipolar rating scale, human subjects rated the intensity of irritation sensation evoked by repeated application of piperine (75 p.p.m.) or nicotine (0.12%) to one side of the dorsal surface of the tongue. The intensity of irritation elicited by repeated application of piperine significantly increased, while irritation elicited by repeated nicotine significantly decreased. We additionally tested if nicotine or piperine desensitized the tongue. After either piperine or nicotine was repeatedly applied to one side of the tongue, a 5 or 10 min rest period ensued, followed by re-application of piperine or nicotine to both sides of the tongue. Subjects were asked to choose which side of the tongue gave rise to a stronger irritation in a two-alternative forced choice (2-AFC) paradigm. In addition, they gave separate ratings of the intensity of irritation on the two sides of the tongue. When piperine was applied bilaterally after unilateral pretreatment with piperine and a 10 min rest period, subjects consistently chose the non-pretreated side to yield stronger irritation and assigned significantly higher ratings to that side, indicative of piperine self-desensitization. A similar self-desensitization effect was found when bilateral application of nicotine followed unilateral treatment with nicotine and a 5 min rest period. Unilateral treatment with piperine also reduced nicotine-evoked irritation on the pretreated side (cross-desensitization), but treatment with nicotine did not affect piperine-evoked irritation. This asymmetrical cross-desensitization pattern is similar to that observed between capsaicin and nicotine and constitutes an additional similarity between piperine and capsaicin.

Introduction

Piperine, the pungent principle in black pepper, belongs to a family of capsaicin-like compounds that produce a burning, stinging/pricking, and sometimes painful sensation when placed on the tongue of human subjects. Capsaicin, the pungent principle in red peppers, and piperine are thought to bind to 'vanilloid' (capsaicin) receptors one subtype of which (VR1) was cloned recently (Caterina *et al.*, 1997; Tominaga *et al.*, 1998). Although piperine and capsaicin are reported to elicit qualitatively and quantitatively different sensations in various areas of the oropharyngeal region (Lawless and Stevens, 1990; Rentmeister-Bryant and Green, 1997), the two compounds share several properties. Recent electrophysiological studies of trigeminal ganglion neurons have shown that inward currents can be evoked by both capsaicin and piperine, and that both currents are potentiated in a low pH environment (Martenson *et al.*, 1994, 1997). Depolarization of trigeminal ganglion neurons by both capsaicin and piperine is blocked by the vanilloid receptor antagonist, capsazepine (Liu and Simon, 1996c; Martenson *et al.*, 1997). However, the specificity of capsazepine for vanilloid receptors has recently been challenged, since capsazepine also reduced nicotine-evoked depolarization of trigeminal ganglion neurons (Liu and Simon, 1997). Finally, piperine-evoked irritation on the human tongue is reduced after prior piperine application followed by a rest period (self-desensitization or tachyphylaxis), and piperine and capsaicin also exhibit mutual cross-desensitization effects as well as mutual 'stimulus-induced recovery', a phenomenon by which desensitization can be overcome by recurrent stimulus application (Green, 1996).

Capsaicin-evoked irritation has been shown to increase with repeated application, a phenomenon called sensitization (Green, 1989, 1991c). In an earlier study, Stevens and Lawless (1987) showed that piperine-evoked irritation sensation increased when applied a second time. One aim of the present study was to extend these earlier studies to determine whether repeated application of piperine produced a consistent sensitization similar to that observed with capsaicin. This was accomplished using a scaling procedure that was designed to be sensitive for comparing

successive intensities, and reducing 'end effects'. Also, we previously showed that irritation elicited by application of nicotine, the major irritant chemical in tobacco, was reduced by prior application of capsaicin followed by a rest period, but that capsaicin-evoked irritation was not reduced by prior nicotine desensitization (Dessirier *et al.*, 1997). To further compare the sensory properties of capsaicin and piperine, we investigated whether a similar asymmetrical cross-desensitization pattern would be found between piperine and nicotine. An abstract of this work has appeared previously (Dessirier *et al.*, 1998b).

Materials and methods

Subjects

Twenty healthy individuals (11 males, 9 females, age 19–27 years), who were students and staff at the University of California at Davis, volunteered to participate in the study. None were smokers and all refrained from eating or drinking at least 1 h prior to each experimental session. In addition, subjects were asked not to eat spicy food for 2 days prior to testing. To verify this, subjects were asked to answer questions regarding their food intake over the last 48 h.

Chemical stimuli and application procedure

All chemicals were purchased from Sigma Chemical Co. (St Louis, MO) unless otherwise specified. A 1% w/v piperine stock solution (98–100% pure) was made up in 95% ethanol. A 75 p.p.m. (0.26 mM) piperine solution was made by diluting the stock solution with 95% ethanol. Fifteen microliters of this solution was pipetted onto small (78.5 mm^2) and 40 µl onto large (176.7 mm^2) filter paper disks (Whatmann, Maidstone, UK). To avoid any stimulating effect of ethanol, the filter papers were air-dried then soaked with the same volumes of distilled water immediately prior to application. Nicotine (free base, 98–100%) was diluted to 0.12% w/v (7.4 mM) in distilled water, and was pipetted in the same volumes onto the small or large filter papers just prior to application.

Throughout the experiment, a suction device (Saliva Ejector, 6″ clear, Sullivan Dental Products Inc., Sacramento, CA) was placed in the mouth to remove saliva. This freed the subject from having to swallow or spit, and avoided spreading the chemical solution across the tongue. Subjects were instructed to use the suction device at any time, except for the 15 s period prior to giving intensity ratings or 2-AFC (two-alternative forced choice) responses. This avoided a possible influence of cooling caused by opening the mouth.

Experimental design

Control of matching intensities evoked by piperine and nicotine

All subjects participated in an initial session to verify that the concentration selected for piperine yielded an intensity of irritation that was approximately equivalent to that of 0.12% nicotine. The piperine concentration used, 75 p.p.m., was selected on the basis of prior pilot tests using a different group of subjects. It was necessary to check for an approximate match in intensity in a separate session because the intensity rating scale used in later sessions was 'relative' (all intensity ratings always started at '1'; see below) and thus would not provide this information. To test that the concentrations were approximately matched in intensity, one large filter paper disk soaked with 40 µl of the 75 p.p.m. piperine solution was placed onto one side of the dorsal anterior surface of the tongue and another, soaked with 40 µl of the 0.12% nicotine solution, was simultaneously placed onto the other side. The sides receiving piperine or nicotine were counterbalanced across subjects. After 30 s, subjects performed a 2-AFC test in which they were asked to report which side of the tongue yielded a stronger sensation. Subjects then rated each side separately for irritation intensity using a category scale with two labels, 0 (no sensation) at one end and 10 (intense irritation) at the other end. To assess dynamic changes in intensity over time, 2-AFC and rating tasks were repeated at 30 s intervals for a total period of 2 min. The filter papers were left on the tongue for the entire 2 min period.

Sequential stimulation and desensitization

After the initial session to verify that the intensities of piperine and nicotine were matched on average across subjects, four additional experimental sessions followed, each separated by at least 2 days. Each session consisted of two parts. In the first part, the larger size filter paper disks containing 40 µl of either 75 p.p.m. piperine or 0.12% nicotine were applied with forceps to one side of the anterior dorsal surface of the tongue. Thirty seconds after stimulus onset, the filter paper was removed. After a further 30 s a fresh filter paper containing the same stimulus (piperine or nicotine) was applied to the same area of the tongue, then removed 30 s later. This was repeated 10 times. Thus, a new filter paper was applied for 30 s at successive 1 min intervals for a total of 10 repetitions. For each of the 10 filter paper applications, subjects were asked to provide a rating of the perceived oral irritation 25 s (filter paper on) and 55 s (filter paper off) after stimulus onset using a bipolar category scale (see below).

After the 10 unilateral sequential stimulus applications, the subjects rested for 10 min following piperine or 5 min following nicotine. The 5 min rest period for nicotine was selected on the basis of our previous findings (Dessirier *et al.*, 1997) that nicotine self-desensitization is reliably detected 5 min after stimulation, but might start to decay within 10 min. During the waiting period the subjects sat quietly without speaking. Then two small-sized filter papers containing either the same chemical as applied previously (self-desensitization) or a different chemical (cross-desensitization) were applied simultaneously with two forceps onto the treated side and the corresponding site on the

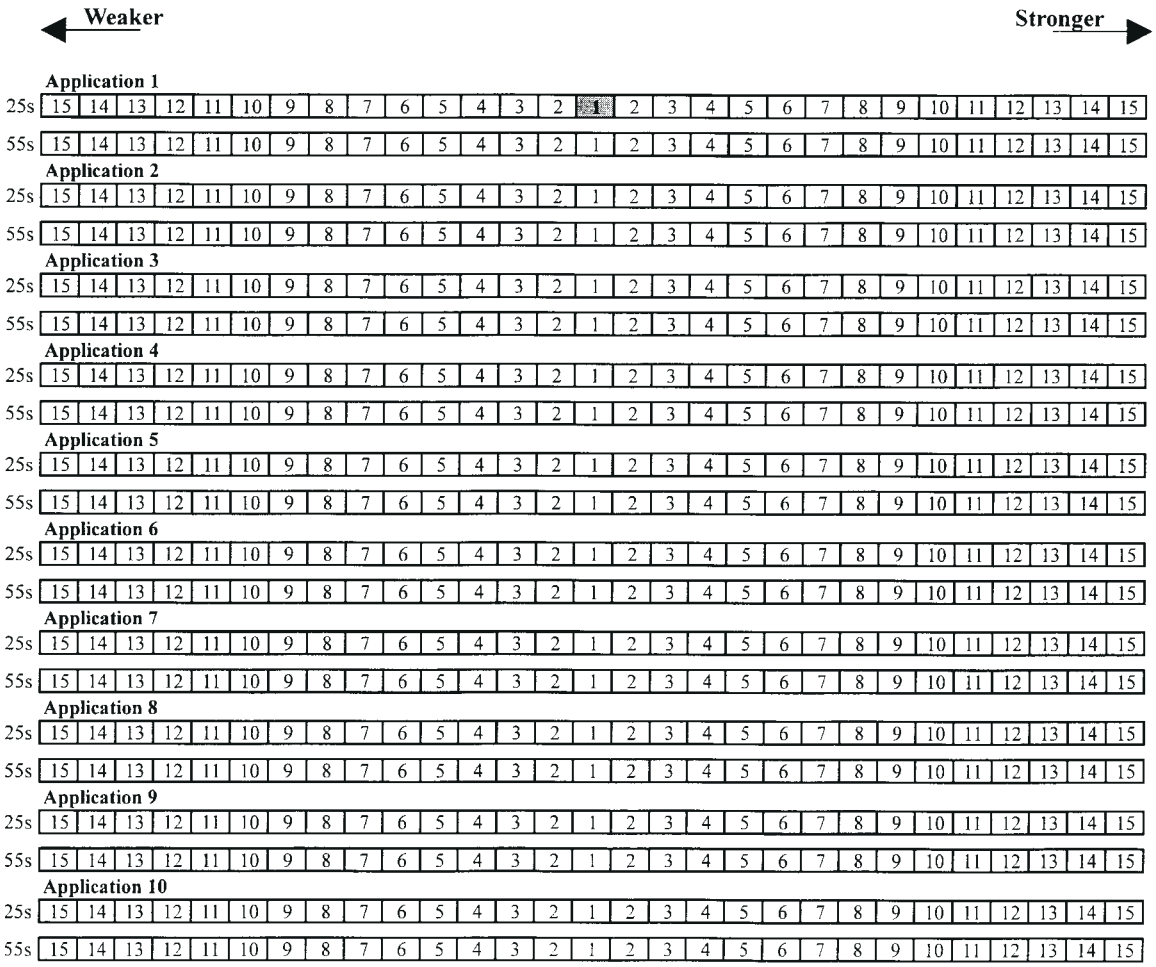

Figure 1 Sample of the bipolar category scale used by subjects to rate the intensity of piperine or nicotine irritation during 10 repeated applications on one side of the tongue.

contralateral side. Subjects then performed a 2-AFC test (Green and Swets, 1966), see below.

After the matching session, subjects participated in four further sessions consisting of two sessions with unilateral sequential application of piperine, followed by bilateral piperine or nicotine stimulation ('pip–pip' or 'pip–nic' sessions respectively), and two sessions with unilateral nicotine stimulations followed by bilateral application of nicotine or piperine ('nic–nic' or 'nic–pip' sessions respectively).

Rating procedures

Bipolar scale

During formal testing, when stimuli were repeatedly applied to one side of the tongue, subjects rated the intensity of the chemically evoked sensation using a bipolar category scale (Figure 1). This consisted of a set of 10 pairs of category scales. Each pair corresponded to one application of the filter papers and, within each pair, one scale was used for ratings given 25 s after stimulus application and another for the rating 55 s after stimulus application. All the scales were printed on a single piece of paper to allow subjects to compare intensity ratings during the experiment. This approach has been shown to reduce errors of estimation (Kim and O'Mahony, 1998). Each scale started with a category labeled '1' in the center, with categories ranging from 2 to 15 toward the right, and from 2 to 15 toward the left, away from the center. The words 'stronger' and 'weaker' appeared on the right and left sides, respectively, above the scale. The intensity of the irritant sensation that the subject perceived 25 s after the initial filter paper application was automatically designated as a category rating of '1' in the center of the first scale. The value '1' was used rather than zero which might have erroneously been interpreted as corresponding to an absence of sensation. The second rating, made 55 s after application of the initial filter paper, was assigned a higher number toward the right if the sensation was stronger, or a higher number toward the left if the sensation was weaker. Subsequent ratings were

made in this manner for each of 10 stimulus applications. Subjects circled the appropriate category number on the scale themselves.

In addition, to avoid 'cut-off' effects at the ends of the scale, extensions with categories raging from 16 to 30 and even more extreme unnumbered categories, were provided at both ends of the scale on separate sheets of paper. Subjects were instructed to use those extensions if they ever needed more than 15 categories when rating the intensity of the sensation.

Two-alternative forced choice (2-AFC)

Thirty seconds after the bilateral application of either piperine or nicotine, subjects were asked to choose which side of the tongue gave rise to a stronger sensation—2-AFC (Green and Swets, 1966). The choice of the 2-AFC was to ensure enough sensitivity to detect differences between the two sides of the tongue should they be small enough to be confusable. This also enabled d' values to be computed to measure the strength of the effect. However, should the intensities on both sides of the tongue be perfectly discriminable, ratio scale measures like d' tend to infinity and are thus inappropriate. Thus, subjects also gave ratings of the perceived irritation intensity of each side separately using the unipolar category scale with 0 (no sensation) at one end and 10 (intense irritation) at the other end, as in the first 'matching' session. Here, the 0–10 unipolar scale was used rather than the bipolar scale because the goal was to compare intensities on the two sides of the tongue rather than to record relative increases or decreases in intensity during repeated stimulation. The 2-AFC and rating tasks were performed at 30 s intervals for a total time of 2 min following the initial application. The filter papers were left on the tongue for the entire 2 min period.

Statistical analysis

The sequential stimulation data were analyzed using analysis of variance (ANOVA) and LSD (Fisher's least significant difference) *post hoc* tests. To perform the ANOVA, the ratings were transformed first by allocating a positive sign to the scores corresponding to categories on the right-hand side of the scale (sensations stronger compared to the initial sensation) and a negative sign to the number corresponding to categories on the left-hand side of the scale (sensations weaker than the initial sensation). Then, the scale was renumbered to avoid distortion, by restoring zero to the center of the scale rather than unity.

The self- and cross-desensitization part of the experiment was analyzed using a d' analysis (Ennis, 1993; Bi *et al.*, 1995) with an additional binomial analysis for the 2-AFC results and Student's *t*-test for the intensity ratings. For all significant differences reported, $P < 0.05$.

Fifteen subjects completed the five sessions, while five extra subjects performed only the initial matching session and the 'cross-desensitization' sessions where piperine was first applied unilaterally 10 times followed by a bilateral stimulation of nicotine ('pip–nic') and where nicotine was first applied 10 times followed by a bilateral stimulation of piperine ('nic–pip').

Results

Approximate matching of intensities evoked by piperine and nicotine

Initial intensities of piperine and nicotine were not rated as significantly different, judging from the 2-AFC results in which 12 of 20 subjects indicated that the side stimulated by 75 p.p.m. piperine gave rise to a stronger sensation (measured 30 s after stimulus application), while the remainder chose the side stimulated by 0.12% nicotine (binomial, $P = 0.5$). In addition, the mean intensity ratings for piperine (4.6) and nicotine (4.1) were not significantly different (*t*-test, $P = 0.41$). However, there was considerable between-subject variability.

Piperine sensitization

As observed earlier for capsaicin (Green, 1989, 1991c, 1993; Dessirier *et al.*, 1997), when piperine was applied repeatedly at 1 min intervals, the average perceived irritation intensity increased significantly from the first application to the tenth for ratings at both 25 and 55 s after stimulus onset [separate ANOVA for 25 and 55 s with subjects and trials as factors, $P < 0.001$ for both 'pip–pip' ($n = 15$) and 'pip–nic' ($n = 20$) sessions]. The average ratings for the 15 subjects who performed both sessions are presented in Figure 2A.

Sensitization was observed in that a significant majority of subjects rated the irritation intensity for the 10th application significantly higher than for the first application (12/15, binomial, $P = 0.035$ in the 'pip–pip' condition and 15/20, binomial, $P = 0.041$, in the 'pip–nic' condition for both the 25 and 55 s ratings).

In addition, there was a significant effect of time of rating (ANOVA with subjects, trials, sessions and time of rating as factors, $P < 0.001$); ratings at 55 s were significantly lower than at 25 s (Figure 2A). Furthermore, a significant subject-by-trial interaction indicated that all subjects did not experience sensitization in the same fashion. Indeed, although sensitization was significant on average across subjects, a visual inspection of individual ratings revealed large inter-individual differences. This occurred in both the pip–pip and pip–nic conditions; the former is illustrated in Figure 3A.

Sensitization was further analyzed by computing Spearman's ρ coefficient, in order to determine how many subjects experienced a significantly monotonic increase in rating from the first application to the tenth. Spearman's ρ was significant in 10/15 subjects in the 'pip–pip' session and 10/20 subjects in the 'pip–nic' session. In addition, among the 15 subjects who performed in both sessions, five subjects

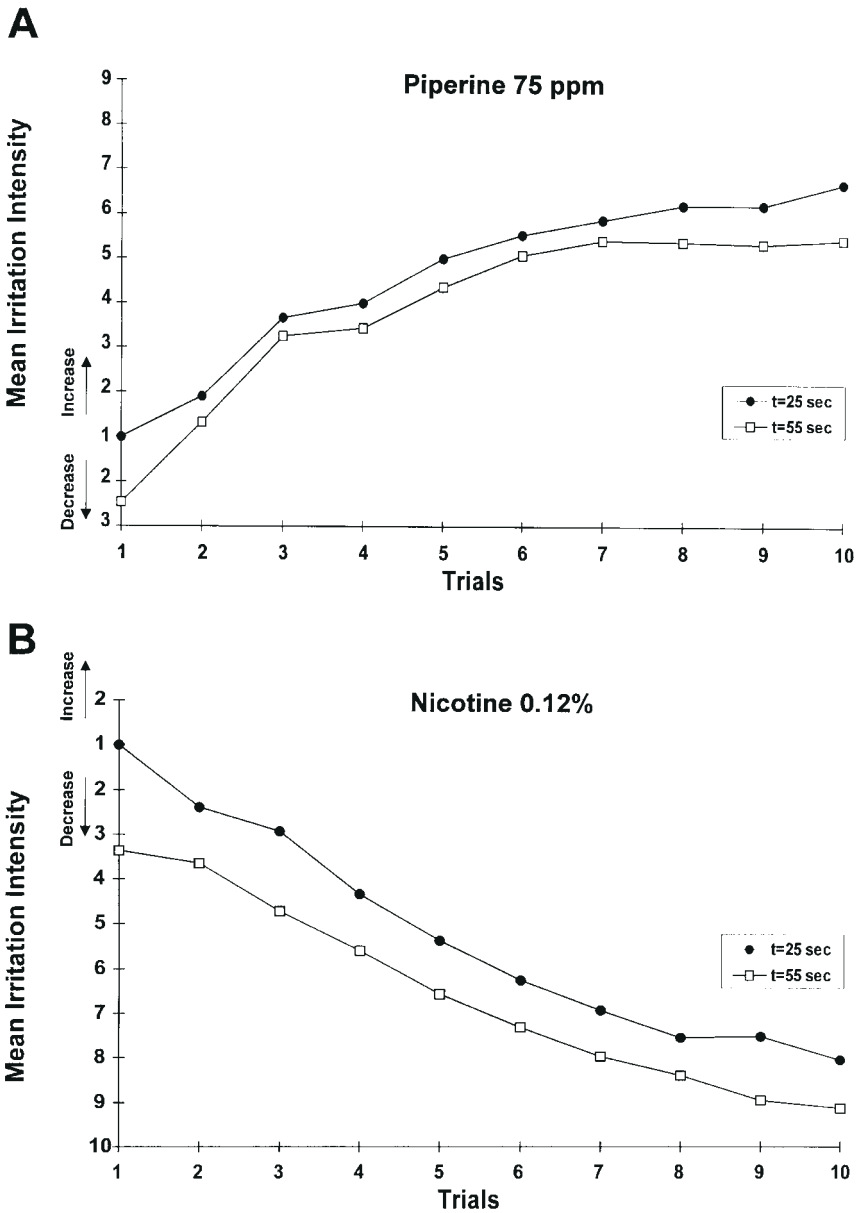

Figure 2 **(A)** Piperine sensitization. Graph plots mean irritation intensity vs trials of piperine (75 p.p.m.) application, given 25 and 55 s following stimulus onset (n = 15 subjects over two sessions). **(B)** Nicotine desensitization. Graph as in (A) plotting mean irritation intensity versus trial of nicotine (0.12%) application (n = 15 subjects).

obtained a significant ρ in one session but not the other, indicating a lack of consistency between sessions.

Piperine self-desensitization

When piperine was applied bilaterally following its repeated unilateral application and the 10 min rest period, the previously non-treated side was chosen as having a stronger sensation in the 2-AFC test by a significant majority of subjects (14/15, binomial P = 0.002; Figure 4A) yielding a significant group d' value of 2.12 (P = 0.0026). This proportion remained significant over the 2 min testing period. In addition, mean intensity ratings for the two sides of the tongue were significantly different (1.07 versus 2.8, t-test P = 0.002; Figure 4A). This difference remained significant throughout the 2 min testing period. This was interpreted as piperine self-desensitization. Such a self-desensitization effect has been observed for many other irritants, including capsaicin (Green, 1989), menthol (Cliff and Green, 1996), zingerone (Prescott and Stevenson, 1996a, 1996b), and nicotine (Dessirier *et al.*, 1997).

Piperine cross-desensitization to nicotine

When nicotine was applied bilaterally following repeated unilateral application of piperine and the 10 min rest

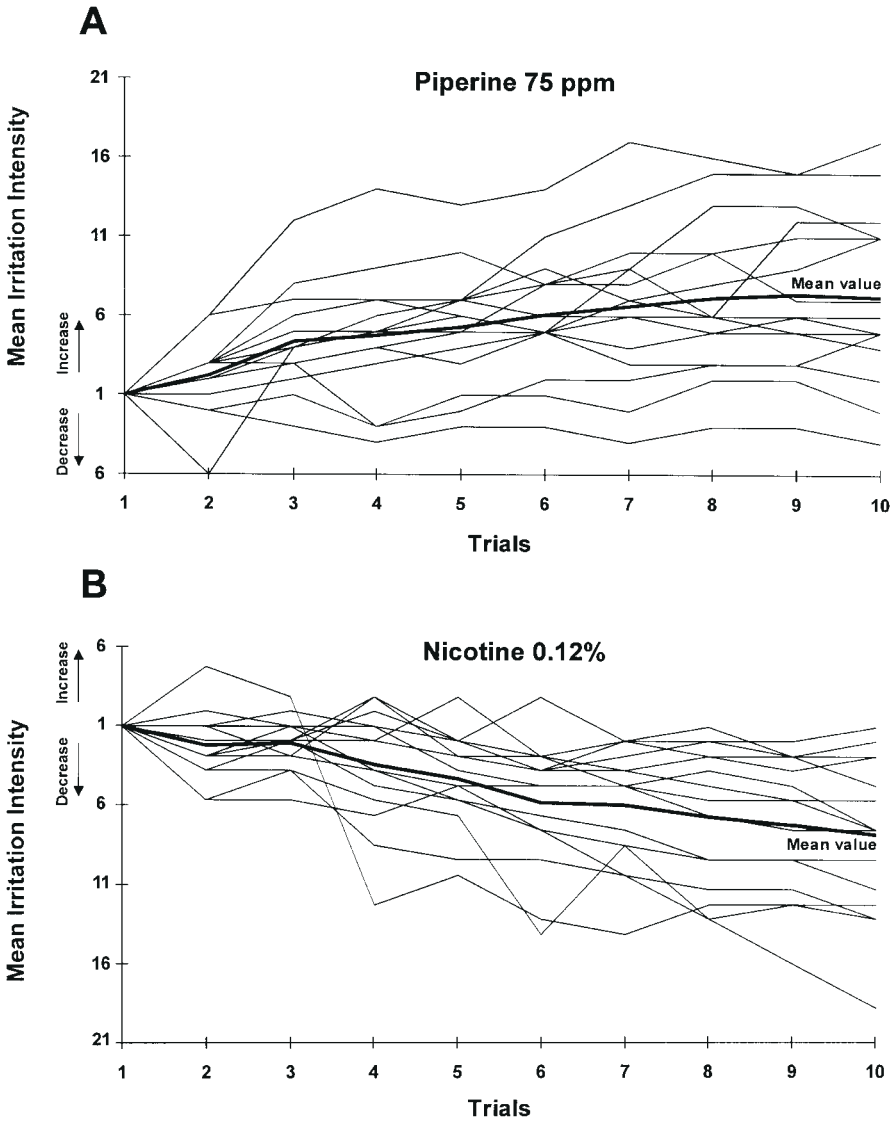

Figure 3 **(A)** Piperine sensitization: individual data. Graph plots individual (thin lines) and mean (thick line) irritation intensity ratings at 25 s following stimulus onset versus trial of piperine (75 p.p.m.) application (n = 15 subjects). **(B)** Nicotine desensitization: individual data. Format as in (A) (n = 15 subjects).

period, the previously non-treated side was again chosen as having a stronger sensation by a significant majority of subjects (18/20, binomial $P < 0.001$; Figure 4B), corresponding to a significant group d' value of 1.81 ($P < 0.001$). This proportion remained significant throughout the 2 min testing period. In addition, the mean intensity ratings for the two sides of the tongue were significantly different (1.95 versus 3.65, t-test $P < 0.001$; Figure 4B). This difference remained significant throughout the 2 min testing period. This was interpreted as piperine cross-desensitization of nicotine irritation. A similar cross-desensitization effect of capsaicin to nicotine has been observed (Dessirier *et al.*, 1997) and thus represents another similarity between capsaicin and piperine.

Nicotine desensitization

When nicotine was applied repeatedly at 1 min intervals, the perceived irritation decreased significantly from the first application to the tenth for both the 25 and 55 s ratings [ANOVA with subjects and trials as factors, $P < 0.001$ for both 'nic–nic' (n = 15) and 'nic–pip' (n = 20) sessions]. The average ratings for the 15 subjects who performed both sessions are presented in Figure 2B. Desensitization was observed in a significant majority of subjects who rated the irritation intensity for the 10th application significantly lower than for the first application (for the 25 s ratings, 14/15, binomial, P = 0.002 in the 'nic–nic' condition and 18/20, binomial, $P < 0.001$, in the 'nic–pip' condition; for the 55 s ratings, 14/15, binomial P = 0.002 in the 'nic–nic'

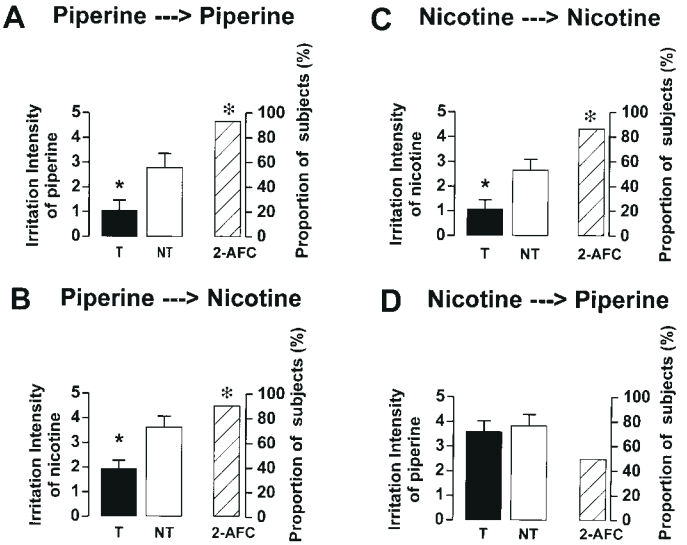

Figure 4 In each panel, the pair of bars to the left indicate intensity ratings on the treated (T, filled bar) and non-treated (NT, open bar) sides of the tongue, respectively. The hatched bar to the right indicates the proportion of subjects who chose the non-treated side to yield a stronger sensation in the 2-AFC test. **(A)** Effect of piperine pretreatment on irritation evoked by piperine (piperine self-desensitization). **(B)** Effect of piperine pretreatment on irritation evoked by nicotine (piperine cross-desensitization of nicotine). **(C)** Effect of nicotine pretreatment on irritation evoked by nicotine (nicotine self-desensitization). **(D)** Effect of nicotine pretreatment on irritation evoked by piperine (lack of nicotine cross-desensitization of piperine). Error bars: SE. Asterisks over filled bars indicate significant difference between T and NT ($P < 0.05$, *t*-test). Stars over hatched bars indicate that a significant majority of subjects chose non-treated side ($P < 0.05$, binomial test).

condition and 20/20, binomial $P < 0.001$ in the 'nic–pip' condition). This confirms earlier results (Dessirier *et al.*, 1997).

As with piperine, there was a significant difference between the ratings at 25 and 55 s (ANOVA with subjects, trial and time of rating as factors, $P < 0.001$), the ratings at 55 s being lower (Figure 2B). A significant subject-by-trial interaction indicated that the desensitization pattern varied among subjects. This was also confirmed by visual inspection of the individual data for both nic–nic and nic–pip conditions; the former is illustrated in Figure 3B.

To further demonstrate the monotonicity of the decrease, Spearman's ρ was calculated and found significant for 10/15 subjects in the 'nic–nic' session and 11/20 subjects in the 'nic–pip' session. Among the 15 subjects who performed both sessions, the subjects who obtained a non-significant ρ in one session were not the same as those having a non-significant ρ in the other. This indicated, as with piperine, a lack of consistency between sessions.

Nicotine self-desensitization

Thirty seconds after bilateral application of nicotine following its unilateral repeated application and the 5 min rest period, the previously non-treated side was chosen as yielding a stronger sensation in the 2-AFC test by a significant majority of subjects (13/15, binomial $P = 0.007$; Figure 4C), yielding a significant group d' value of 1.57 ($P = 0.0066$). This proportion remained constant throughout the 2 min testing period. In addition, the mean intensity ratings for the two sides of the tongue were significantly different (1.07 versus 2.67, *t*-test $P < 0.001$; Figure 4C). Although the magnitude of the difference decreased over the 2 min testing period, it remained significant. This was interpreted as nicotine self-desensitization, and confirmed earlier results (Dessirier *et al.*, 1997).

Lack of nicotine cross-desensitization to piperine

When piperine was applied following pretreatment with nicotine and the ensuing rest period, only 10 of the 20 subjects chose the non-treated side as having a stronger sensation (binomial, $P > 0.82$; Figure 4D) and the group d' value was zero indicating no difference between the two sides of the tongue. The proportion choosing the non-treated side remained close to 50% throughout the 2 min testing period. Moreover, the mean ratings for the two sides of the tongue were not significantly different (3.6 versus 3.85, *t*-test $P = 0.62$; Figure 4D). The difference remained non-significant over the 2 min testing period. This was interpreted as a lack of cross-desensitization of nicotine on piperine irritation. A similar lack of cross-desensitization of nicotine on capsaicin was previously observed (Dessirier *et al.*, 1997).

Discussion

Similarities between piperine and capsaicin: sensitization

The present study established that repeated application of piperine on the tongue led to an increase in irritation intensity similar to that observed for capsaicin (Green, 1989; 1991c, 1993) and also NaCl and KCl, two other chemicals that are irritants in the molar range (Green and Gelhard, 1989). Similar repeated applications of piperine on the tongue were previously carried out by Green (1996), but did not lead to a significant increase in intensity rating. This, along with the present results, reflects the high individual variability observed for sensitization. Capsaicin sensitization has also been found to be highly variable across subjects (Cliff and Green, 1996; Prescott, 1998). From Green's work (Green, 1996; Green and Rentmeister-Bryant, 1998) it seems possible that part of the 'sensitization' observed in some studies might be a 'stimulus-induced recovery' from prior capsaicin desensitization, possibly from dietary food. It is thus possible that the difference between subjects was partly due to differences in prior capsaicin consumption, since piperine was shown to exhibit 'cross-stimulus induced recovery' following capsaicin desensitization (Green, 1996). In this study, subjects were asked to refrain from consuming 'spicy food' at least 48 h prior to testing and successive experimental sessions were separated by at least 48 h.

However, capsaicin desensitization was shown to last for periods of several days (Karrer and Bartoshuk, 1991) and subjects who previously ate spicy food might have had a lingering desensitization.

Yet, prior dietary experience might not be the only variable responsible for the observed individual difference; other variables such as intensity of the applied stimulus might have an effect. Future research will address these topics.

Similarities between piperine and capsaicin: desensitization

Following the repeated applications and a 10 min rest period, the piperine-evoked irritation was reduced on the piperine-pretreated side of the tongue. This is consistent with self-desensitization, a phenomenon also observed with capsaicin as well as most irritant chemicals studied so far (Cliff and Green, 1996; Prescott and Stevenson, 1996a, 1996b; Dessirier *et al.*, 1997). Also, as with capsaicin, nicotine-evoked irritation was also decreased by prior application of piperine (cross-desensitization).

This demonstrates that, like capsaicin which was shown to cross-desensitize to most if not all other irritant chemicals (Green, 1991a; Gilmore and Green, 1993; Dessirier *et al.*, 1997), piperine exhibits cross-desensitization. The ability of capsaicin to reduce irritation intensity evoked by other irritant compounds in humans may relate to a generalized Ca^{2+}-dependent reduction in excitability of the trigeminal nerve fiber (Cholewinski *et al.*, 1993; Liu and Simon, 1996b). Thus, piperine, which possibly acts via a subtype of the vanilloid receptor (Liu and Simon, 1996c), might affect the excitability of lingual nociceptors to nicotine in a similar fashion, provided that some cells respond to both compounds as supported by electrophysiological studies of trigeminal ganglion cells (Liu and Simon, 1996a).

In contrast with capsaicin and piperine, the repeated application of nicotine on the tongue led to a decrease in irritation intensity lasting at least 5 min after the end of stimulation, which confirmed earlier results (Dessirier *et al.*, 1997). A similar decrease in irritation intensity lasting several minutes after the stimulation had ceased was also reported for other irritant compounds such as zingerone (Prescott and Stevenson, 1996a, 1996b), and menthol (Cliff and Green, 1994, 1996). In addition, as was found for capsaicin, piperine-evoked irritation was not affected by prior nicotine desensitization. This supports the hypothesis that nicotine's shorter lasting (5–10 min) self-desensitization might reflect desensitization of the molecular nicotinic receptors, decreasing the excitability of lingual nociceptors to nicotine but not to other irritant compounds such as capsaicin or piperine that act via separate molecular receptors (Dessirier *et al.*, 1998a). However, our results showing a lack of cross-desensitization of nicotine to piperine (present study) or capsaicin (Dessirier *et al.*, 1997) were obtained using a fairly weak nicotine solution of 0.12%. It is possible that an effect might be found if a stronger nicotine solution were used. This should be investigated further in animal studies, 0.12% over 10 trials being the highest nicotine dose allowed for our studies by FDA regulations.

Psychophysical methodology

One of our primary goals was to establish whether sensations increased or decreased upon repeated application of piperine and nicotine. In using the bipolar category scale, no assumptions were made about the nature of the numerical estimates produced (for example, ratio scale), nor did we make any claims that these estimates might represent an absolute set of values. In this respect, scores were treated as relative measures. The issue of relative vs absolute interpretation of scaling data remains controversial (Zwislocki and Goodman, 1980; Mellers, 1983), but with the minimal assumptions made in this paper it becomes a moot point. Further, when using the bipolar scale, subjects were able to view their past ratings to minimize errors. By an 'error' is meant that the subject gives a lower score to a more intense yet easily discriminable stimulus. Such errors are often caused by forgetting past scores, so that viewing them has been demonstrated to reduce this type of memory error (Kim and O'Mahony, 1998). Finally, the use of extensions aimed to prevent 'cut-off' effects due to lack of space between the previous rating and the end-point of the scale, and thus allowed subjects to express the increase or decrease of the sensation freely. Indeed, the average ratings did not appear to show overt plateaux across stimulus trials (Figure 2; $t = 25$ s) suggesting that this may have been the case.

The 2-AFC discrimination task between the two sides of the tongue has been used in previous work (Dessirier *et al.*, 1997, 1998a) and constitutes the best combination of sensitivity and power available. It is the most sensitive compared to any other method because the subject can directly compare the sensations on each side of the tongue, thereby minimizing interference by sequence or memory effects (Rousseau and O'Mahony, 1997; Dessirier and O'Mahony, 1999). Its statistical power stems from the cognitive strategy used by subjects during testing (O'Mahony *et al.*, 1994). Accordingly, this method will detect very slight differences between the two sides of the tongue and thus small desensitization effects. In addition, the phenomenon of counter-irritation, by which a painful stimulus on one side of the body tends to suppress another painful stimulus of milder intensity on the other side of the body (Green, 1991b), would enhance any difference between the two sides of the tongue and thus help the discrimination.

Acknowledgements

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References

Bi, J., Ennis, D.M. and **O'Mahony, M.** (1995) *How to estimate and use the variance of* d' *from difference tests.* J. Sens. Stud., 12, 87–104.

Caterina, M.J., Schumacher, M.A., Tominaga, M., Rosen, T.A., Levine, J.D. and **Julius, D.** (1997) *The capsaicin receptor: a heat-activated ion channel in the pain pathway.* Nature, 389, 816–824.

Cholewinski, A., Burgess, G.M. and **Bevan, S.** (1993) *The role of calcium in capsaicin-induced desensitization in rat cultured dorsal root ganglion neurons*. Neuroscience, 55, 1015–1023.

Cliff, M.A. and **Green, B.G.** (1994) *Sensory irritation and coolness produced by menthol: evidence for selective desensitization of irritation*. Physiol. Behav., 56, 1021–1029.

Cliff, M.A. and **Green, B.G.** (1996) *Sensitization and desensitization to capsaicin and menthol in the oral cavity: interactions and individual differences*. Physiol. Behav., 59, 487–494.

Dessirier, J.-M. and **O'Mahony, M.** (1999) *Comparison of* d' *values for the 2-AFC (paired comparison) and 3-AFC discrimination methods: Thurstonian models, sequential sensitivity analysis and power*. Food Qual. Pref., 10, 51–58.

Dessirier, J.M., O'Mahony, M. and **Carstens, E.** (1997) *Oral irritant effects of nicotine: psychophysical evidence for decreased sensation following repeated application and lack of cross-desensitization to capsaicin*. Chem. Senses, 22, 483–492.

Dessirier, J.M., O'Mahony, M., Sieffermann, J.M. and **Carstens, E.** (1998a) *Mecamylamine inhibits nicotine but not capsaicin irritation on the tongue: psychophysical evidence that nicotine and capsaicin activate separate molecular receptors*. Neurosci. Lett., 240, 65–68.

Dessirier, J.-M., O'Mahony, M., Sieffermann, J.-M. and **Carstens, E.** (1998b) *Oral irritant properties of piperine and nicotine: psychophysical evidence for asymmetrical desensitization effects*. Chem. Senses 23, 561.

Ennis, D.M. (1993) *The power of sensory discrimination methods.* J. Sens. Stud., 8, 353–370.

Gilmore, M.M. and **Green, B.G.** (1993) *Sensory irritation and taste produced by NaCl and citric acid: effects of capsaicin desensitization*. Chem. Senses, 18, 257–272.

Green, B.G. (1989) *Capsaicin sensitization and desensitization on the tongue produced by brief exposures to a low concentration*. Neurosci. Lett., 107, 173–178.

Green, B.G. (1991a) *Capsaicin cross-desensitization on the tongue: psychophysical evidence that oral chemical irritation is mediated by more than one sensory pathway*. Chem. Senses, 16, 675–689.

Green, B.G. (1991b) *Interactions between chemical and thermal cutaneous stimuli: inhibition (counterirritation) and integration*. Somatosens. Mot. Res., 8, 301–312.

Green, B.G. (1991c) *Temporal characteristics of capsaicin sensitization and desensitization on the tongue*. Physiol. Behav., 49, 501–505.

Green, B.G. (1993) *Evidence that removal of capsaicin accelerates desensitization on the tongue*. Neurosci. Lett., 150, 44–8.

Green, B.G. (1996) *Rapid recovery from capsaicin desensitization during recurrent stimulation*. Pain, 68, 245–253.

Green, B.G. and **Gelhard, B.** (1989) *Salt as an oral irritant.* Chem. Senses, 14, 259–271.

Green, B.G. and **Rentmeister-Bryant, H.** (1998) *Temporal characteristics of capsaicin desensitization and stimulus-induced recovery in the oral cavity*. Physiol. Behav., 65, 141–149.

Green, D.M. and **Swets, J.A.** (1966) Signal Detection Theory and Psychophysics. John Wiley, New York.

Karrer, T. and **Bartoshuk, L.** (1991) *Capsaicin desensitization and recovery on the human tongue.* Physiol. Behav., 49, 757–764.

Kim, K.-O. and **O'Mahony, M.** (1998) *A new approach to category scales of intensity I. Traditional versus rank-rating*. J. Sens. Stud., 13, 241–249.

Lawless, H.T. and **Stevens, D.A.** (1990) *Differences between and interactions of oral irritants: neurophysiological and perceptual differences*. In Green, B.G., Mason, J.R. and Kare, M.R. (eds), Chemical Senses. Vol. 2: Irritation. Dekker, New York, pp. 197–211.

Liu, L. and **Simon, S.A.** (1996a) *Capsaicin and nicotine both activate a subset of rat trigeminal ganglion neurons*. Am. J. Physiol., 270, C1807–C1814.

Liu, L. and **Simon, S.A.** (1996b) *Capsaicin-induced currents with distinct desensitization and* Ca^{2+} *dependence in rat trigeminal ganglion cells*. J. Neurophysiol., 75, 1503–1514.

Liu, L. and **Simon, S.A.** (1996c) *Similarities and differences in the currents activated by capsaicin, piperine, and zingerone in rat trigeminal ganglion cells*. J. Neurophysiol., 76, 1858–1569.

Liu, L. and **Simon, S.A.** (1997) *Capsazepine, a vanilloid receptor antagonist, inhibits nicotinic acetylcholine receptors in rat trigeminal ganglia*. Neurosci. Lett., 228, 29–32.

Martenson, M.E., Ingram, S.L. and **Baumann, T.K.** (1994) *Potentiation of rabbit trigeminal responses to capsaicin in a low pH environment.* Brain Res., 651, 143–147.

Martenson, M.E., Arguelles, J.H. and **Baumann, T.K.** (1997) *Enhancement of rat trigeminal ganglion neuron responses to piperine in a low-pH environment and block by capsazepine*. Brain Res., 761, 71–76.

Mellers, B.A. (1983) *Evidence against 'absolute' scaling.* Percept. Psychophys., 33, 523–526.

O'Mahony, M., Masuoka, S. and **Ishii, R.** (1994) *A theoretical note on difference tests: models, paradoxes and cognitive strategies.* J. Sensory Studies, 9, 247–272.

Prescott, J. (1998*) The generalizability of capsaicin sensitization and desensitization*. Chem. Senses, 23, 619.

Prescott, J. and **Stevenson, R.J.** (1996a) *Desensitization to oral zingerone irritation: effects of stimulus parameters.* Physiol. Behav., 60, 1473–1480.

Prescott, J. and **Stevenson, R.J.** (1996b) *Psychophysical responses to single and multiple presentations of the oral irritant zingerone: relationship to frequency of chili consumption*. Physiol. Behav., 60, 617–624.

Rentmeister-Bryant, H. and **Green, B.G.** (1997) *Perceived irritation during ingestion of capsaicin or piperine: comparison of trigeminal and non-trigeminal areas*. Chem. Senses, 22, 257–266.

Rousseau, B. and **O'Mahony, M.** (1997) *Sensory difference tests: Thurstonian and SSA predictions for flavored yogurts*. J. Sens. Stud., 12, 127–146.

Stevens, D.A. and **Lawless, H.T.** (1987) *Enhancement of responses to sequential presentation of oral chemical irritants*. Physiol. Behav., 39, 63–65.

Tominaga, M., Caterina, M.J., Malmberg, A.B., Rosen, T.A., Gilbert, H., Skinner, K., Raumann, B.E., Basbaum, A.I. and **Julius, D.** (1998) *The cloned capsaicin receptor integrates multiple pain-producing stimuli.* Neuron, 21, 531–543.

Zwislocki, J.J. and **Goodman, D.A.** (1980) *Absolute scaling of sensory magnitudes: a validation.* Percept. Psychophys., 28, 28–38.

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