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Oral Irritant Effects of Nicotine: Psychophysical Evidence for Decreased Sensation Following Repeated Application and Lack of Cross-desensitization to Capsaicin

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Abstract

Psychophysical methods were used to assess changes in the intensity of irritant sensations elicited by repeated application of capsaicin and nicotine delivered unilaterally to the tongue of human subjects. Whereas capsaicin (0.5 or 3 p.p.m.; repeated at 1 min intervals over 10 min) evoked progressively stronger ratings of irritation (sensitization), there was a significant decrement in irritation ratings (desensitization) to repeated application of nicotine (0.1%). A two-alternative forced-choice (2-AFC) procedure was additionally used to test for self- and cross-desensitization. After the subjects had received either repeated capsaicin or nicotine, a rest period ensued followed by the 2-AFC procedure. Either capsaicin or nicotine was delivered bilaterally to the tongue and subjects were asked to choose which side yielded a stronger sensation. Following capsaicin pretreatment, subjects reported that capsaicin evoked a stronger sensation on the previously untreated side (capsaicin self-desensitization). Similar self-desensitization was observed with nicotine. Furthermore, nicotine evoked a significantly weaker sensation on the side of the tongue pretreated with capsaicin (cross-desensitization). In contrast, capsaicin did not consistently evoke a weaker sensation on the nicotine-pretreated side, indicating an absence of cross-desensitization. These results are discussed in terms of physiological mechanisms that might underlie the contrasting sensory effects of nicotine versus capsaicin. **Chem. Senses 22: 483–492, 1997.**

Introduction

A variety of naturally occurring chemicals, such as capsaicin from red chili peppers, piperine from black pepper, and nicotine from tobacco, cause irritation when delivered to the oral mucosa (for recent review, see Green and Lawless, 1991). Capsaicin in low concentration (3 p.p.m.) evokes a burning sensation which increases when delivered repeatedly at 1 min intervals (sensitization), but then decreases markedly following a 10–15 min rest period (self-desensitization) (Green, 1989, 1991b, 1996; Karrer and Bartoshuk, 1991; reviewed in Holzer, 1991). It is interesting

that following desensitization of the oral (Green, 1991a; Gilmore and Green, 1993) or nasal (Geppetti *et al.*, 1993) mucosa by capsaicin, irritant sensations evoked by other chemicals such as cinnamic aldehyde, NaCl at concentrations above 1 M, or citric acid were also reduced (cross-desensitization). These results suggest that oral irritation from some agents may be mediated by a population of capsaicin-sensitive trigeminal polymodal nociceptors innervating the oral mucosa.

Nicotine is also well known to evoke a burning pain sensation when applied to skin (Keele and Armstrong, 1964) or to the oral (Jarvik and Assil, 1988), nasal (Hummel *et al.*, 1992; Greiff *et al.*, 1993) or ocular (MacIver and Tanelian, 1993) mucosa. The oral irritation from nicotine appears to act via nicotinic cholinergic receptors (Jansco *et al.*, 1961; Jarvik and Assil, 1988) presumably residing on nociceptor terminals (Steen and Reeh, 1993). In the present study, we wished to investigate further the irritant effect of nicotine delivered sequentially to the oral cavity, and its possible interactions with capsaicin. We have modified a paradigm used by Green (1989) in which subjects gave magnitude ratings of irritation elicited by capsaicin delivered sequentially to the surface of the tongue bilaterally. In the present study, nicotine or capsaicin was delivered to only one side of the tongue and subjects rated the magnitude of irritation using a visual analog scale (VAS). The unilateral application allowed us to employ a two-alternative forced choice (2-AFC) to test for desensitization. That is, following sequential unilateral application of one of the chemicals and an ensuing rest period, the same chemical was then applied to both sides of the tongue at once and subjects had to choose which side yielded the stronger sensation. We have used this method to verify the sensitizing and self-desensitizing effects of capsaicin, to determine if nicotine has similar effects, and to test if these chemicals exhibit cross-desensitization with one another.

Materials and methods

Subjects

Subjects were drawn from a pool of 59 (22 males, 37 females, age 18–42 years) students and staff at the University of California at Davis who voluntarily participated in the study. All subjects signed an informed consent form approved by the UC Davis Human Subjects Review committee which explained the general nature of the study, but subjects were naive as to its specific goals. All were non-smokers and were asked not to eat spicy food for 3 days prior to testing. Each subject participated in only one individual session of data collection per day, which typically lasted <30 min. Some subjects returned to participate in subsequent sessions; individual sessions were conducted a minimum of 3 days apart.

Stimulus application

From a stock solution of 1% capsaicin (vanillyl nonamide; 98–100%, Sigma Chemical Co., St Louis, MO) in 80% ethanol (from 100% punctilious; Quantum, Los Angeles, CA), 0.5–10 p.p.m. capsaicin solutions were made by diluting with distilled water. A 15 µl aliquot of the diluted capsaicin was pipetted onto small (78.5 mm^2) and 35 µl onto large (176.7 mm^2) circular filter papers (Whatmann, Maidstone, UK, product # 1001-0105 and 1001-0155). To avoid any effect of ethanol, the filter papers were air-dried before application. Nicotine (free base, 98–100%, Sigma) was diluted to 0.1 or 0.12% in distilled water, and was pipetted in the same volumes onto the small or large filter papers just prior to application. Subjects reported the nicotine and capsaicin to be tasteless at these concentrations.

Sequential stimulation procedure

In all subjects, the larger size filter paper containing either capsaicin (experiments 1, 3) or nicotine (experiments 2, 3) was applied with forceps onto one side of the dorsal surface of the tongue. After application the mouth was closed. The side of stimulation was counterbalanced across subjects. At various intervals following application of the filter paper (see Table 1), the subject was asked to rate the intensity of the irritant sensation using a VAS (Price *et al.*, 1983) which was modified to have the descriptors 'no sensation' at the lower end and 'most intense irritation imaginable' at the upper end. The rating was quantified in terms of centimeters read from a 0–15 cm ruler on the back of the VAS. We have prior experience using the VAS (Douglass *et al.*, 1992). Thirty seconds after stimulus onset, the filter paper was removed. Thirty seconds later, a fresh filter paper was applied to the same area of the tongue. Thus, a new filter paper was applied at successive 1 min intervals for a total of 10 repetitions.

Because capsaicin stimulation increases salivary flow (Duner-Engstrom *et al.*, 1986), a suction device (Saliva Ejector, 6″ clear; Sullivan Dental Products Inc., Sacramento,

Table 1 Experimental details; specific details are provided for each experiment (see text for explanation)

Experiment	No. of subjects	Sequential stim. (10×)	Time of rating(s)	Rest (min)	Bilateral stim. (1×)	2-AFC (min)
1a: Cap self-desens.	20	cap 3 p.p.m.	25	10	cap 3 p.p.m.	5
1b: Cap-nic cross desens.	20	cap 3 p.p.m.	25, 40, 55	10	nic 0.1%	5
2a: nic self-desens.	14	nic 0.1%	40, 55	5	nic 0.1%	5
2b: nic-cap cross-desens.	20	nic 0.1%	40, 55	5	cap 10 p.p.m.	5
3a: cap-nic cross-desens.	10	cap 0.5 p.p.m.	25	10	nic 0.12%	3
3b: nic-cap cross-desens.	20	nic 0.12%	25	5	cap 0.5 p.p.m.	3

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 10 s period prior to giving VAS or 2-AFC responses. This avoided a possible influence of cooling caused by opening the mouth.

Two-alternative forced choice (2-AFC) procedure

After the 10 sequential stimulus applications, the subjects rested for 10 min following capsaicin (Green, 1989), or for 5 min following nicotine, since pilot experiments indicated that nicotine had a short-lasting desensitizing effect. During the waiting period the subjects sat quietly without speaking. Then two small size filter papers either containing the same chemical as applied previously (self-desensitization) or a different chemical (cross-desensitization) were applied simultaneously onto both sides of the tongue at a location corresponding to the one where the larger filter papers were applied earlier (see Figure 2, inset above). After application, the mouth was closed. Subjects were then asked to choose which side of the tongue gave rise to a stronger sensation (2-AFC; Green and Swets, 1966). This initial 2-AFC rating provided the data shown in Figure 2. The 2-AFC testing procedure was continued for 5 min in experiments 1 and 2 and was shortened to 3 min in experiment 3. During this period, subjects were instructed to indicate any time that the side of stronger sensation changed, and the time of such occurrence was noted. From this, the total time that each side of the tongue yielded a stronger sensation was determined for the entire period. In experiment 1, capsaicin filter papers remained on the tongue for 5 min. In experiment 2 with nicotine, the filter papers were removed after 30 s. In experiment 3, both the capsaicin and nicotine filter papers remained on the tongue for the entire 3 min period of 2-AFC testing. The various procedural changes for each experiment are listed in Table 1.

Experiment 1: capsaicin

1a. Sensitization and self-desensitization

In these experiments we sought to reproduce the finding (Green, 1989) that sequential application of capsaicin leads to successively increasing intensity ratings (sensitization), and to decreased ratings following a rest period (self-desensitization).

Capsaicin (3 p.p.m.) was successively applied in 10 subjects, and ratings were taken at 25 s following stimulus onset. After a 10 min rest period, self-desensitization was tested using the 2-AFC procedure (Table 1).

1b. Sensitization and nicotine cross-desensitization

Capsaicin (3 p.p.m.) was again applied successively (see Table 1). After a 10 min rest period, nicotine (0.1%) was applied onto both sides of the tongue and the 2-AFC procedure was performed. Of the 20 subjects tested, three had performed in experiment 1a.

Experiment 2: nicotine

In these experiments, we tested if sequential application of nicotine also gives rise to increasing ratings of irritation like capsaicin, and if nicotine exhibits self-desensitization and cross-desensitization to capsaicin.

2a. Sequential stimulation and self-desensitization

Nicotine (0.1%) was applied sequentially as above, and after a 5 min resting period self-desensitization was tested using the 2-AFC procedure as before (Table 1). Of the 14 subjects tested, nine had participated in experiment 1a.

2b. Sequential stimulation and cross-desensitization with capsaicin

Nicotine (0.1%) was applied sequentially as before, and after a 5 min rest period capsaicin (10 p.p.m.) was applied and the 2-AFC procedure was conducted as before (Table 1). Thirteen of the 14 subjects tested in experiment 2a (including nine who had participated in experiment 1a) were tested, and an additional seven subjects were added (total: 20 subjects) to increase the number of observations for the 2-AFC test.

Experiment 3: nicotine and capsaicin cross-desensitization

This experiment was performed to replicate our findings of experiments 1 and 2 using concentrations of capsaicin and nicotine that were matched more closely in terms of the intensity of the irritant sensation. In experiments 1 and 2, the capsaicin concentrations were chosen with a view to confirming earlier studies (e.g. Green, 1989; Karrer and Bartoshuk, 1991), and yielded higher ratings compared with 0.1% nicotine. In experiment 3 we used the highest nicotine concentration allowed by FDA regulations (0.12%) and a lower capsaicin concentration (0.5 p.p.m.) to achieve approximately matched sensory ratings.

3a. Sequential stimulation with capsaicin and cross-desensitization with nicotine

With 10 naive subjects capsaicin (0.5 p.p.m.) was applied sequentially as described above, and after a 10 min rest period nicotine (0.12%) was applied and the 2-AFC procedure carried out with the following changes. The period of 2-AFC testing was shortened to 3 min since the earlier experiments indicated that the cross-desensitization effect wore off by this time. Also, for the initial 2-AFC test, subjects were asked if they really detected a difference or if they chose randomly. In addition, they were asked to report when any spontaneous laterality change in sensory intensity occurred, or when they could no longer detect any laterality difference. When the latter occurred, subjects were nonetheless asked to choose which side yielded a stronger sensation as required by 2-AFC.

3b. Sequential stimulation with nicotine and cross-desensitization with capsaicin

This experiment used the same 10 subjects from experiment 3a to allow comparison of results with capsaicin and nicotine in an identical subject population. An additional 10 naive subjects were added to increase the number of observations in the 2-AFC procedure. Nicotine (0.12%) was applied sequentially as before, and after a 5 min rest period capsaicin (0.5 p.p.m.) was applied and the 2-AFC procedure carried out as above (Table 1).

Statistical analyses

For sequential stimulation, a two-factor (application trial, subjects) or three-factor (application trial, time of rating, and subject) analysis of variance (ANOVA) was performed. For the 2-AFC data, a binomial test was performed. The mean total time that each side of the tongue yielded a stronger sensation across the period of 2-AFC testing was compared using a Student's t-test. For all tests the significance level was taken as $P < 0.05$.

Results

Experiment 1: capsaicin

1a. Capsaicin sensitization and self-desensitization

Our results confirm a sensitization by capsaicin as reported earlier (Green, 1989) (Figure 1A). Mean ratings of perceived intensity of irritation increased across trials of capsaicin application. This is illustrated in Figure 1A. There was a significant main effect of application trial ($P < 0.001$ for both experiment 1a, $n = 10$ subjects, and 1b, $n = 20$ subjects; ANOVA), indicating a significant increase in ratings from the first to 10th trial. This was confirmed by the observation that ratings increased across trials in a significant majority (18/20) of subjects ($P < 0.001$, binomial). There was also a significant main effect of time of rating ($P < 0.001$, ANOVA); a Fisher's LSD test revealed significant differences among all three times of rating (Figure 1A).

Results with 2-AFC testing confirm self-desensitization by capsaicin (Green, 1989). A significant majority of subjects (9/10; $P = 0.02$, binomial) initially reported a stronger irritant sensation on the previously unstimulated side of the tongue following bilateral testing with capsaicin (Figure 2, first hatched bar). During the 5 min period of 2-AFC testing, the total time that the irritation was perceived to be stronger on the previously unstimulated side was significantly greater compared with the other

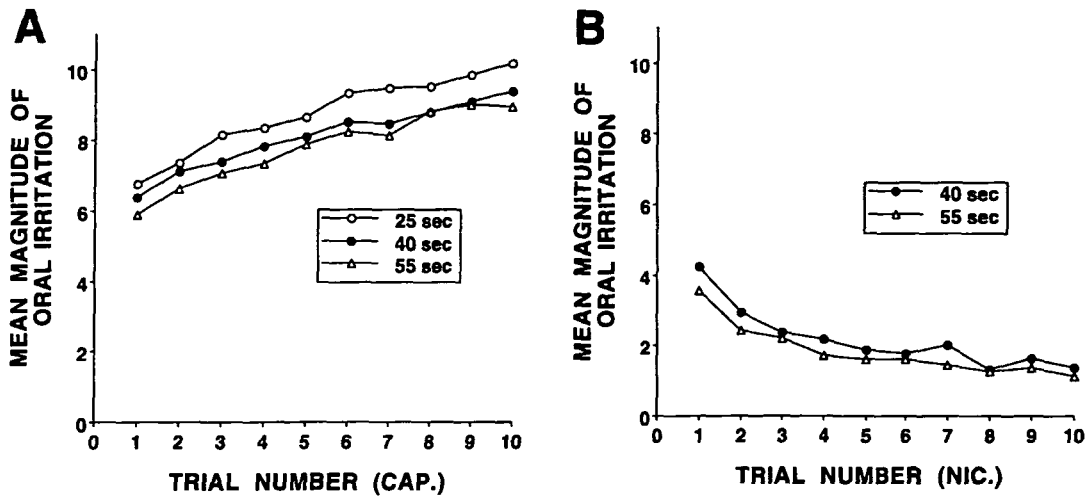

Figure 1 Different effects of sequential stimulation with capsaicin or nicotine. **(A)** Sensitization in sequential ratings of irritation intensity by capsaicin. Graph plots mean ratings of perceived irritation (n = 20 subjects) versus the trial of capsaicin application (1 min intervals). Three ratings were given at 25, 40 and 55 s after each application. **(B)** Decrement in sequential ratings of irritation intensity by nicotine. Graph as in (A) for ratings (n = 20 subjects) across trials. Ratings were given at 40 and 55 s after each application.

(capsaicin-desensitized) side (means: 4.6 versus 0.4 min; $P < 0.001$, t-test).

1b. Cross-desensitization with nicotine

Sequential application of capsaicin also led to a cross-desensitization of the irritant effect of nicotine. Following bilateral testing with nicotine, a significant majority of subjects (18/20, $P < 0.001$, binomial) reported the side not previously stimulated with capsaicin to yield a stronger irritant sensation (Figure 2, second hatched bar). After nicotine application, the total time that the previously unstimulated side was reported to yield a stronger sensation was significantly larger compared with the capsaicin-desensitized side (means: 4.5 versus 0.5 min; $P < 0.001$, t-test).

Experiment 2: nicotine

2a. Sequential stimulation and self-desensitization

In contrast to the sensitizing effect of capsaicin, sequential application of nicotine led to a progressive reduction (desensitization) in the intensity of irritation (Figure 1B). This was observed in experiment 2a with 14 subjects and again in experiment 2b with 20 subjects (13 of whom had participated in experiment 2a). The main effect of application trial was significant ($P < 0.001$, ANOVA), indicating that mean ratings decreased significantly from the first to 10th trial. This was the case in a significant majority (19/20) of subjects ($P < 0.001$, binomial). The main effect of time of rating was significant ($P < 0.001$, ANOVA). For the 13 subjects participating in experiments 2a and 2b, the results were reproducible. This was verified in a four-factor ANOVA (subject, application trial, time of rating, and session as factors) for which the effect of session was not significant.

Sequential application of nicotine also resulted in an apparent self-desensitization. All (14/14) subjects reported a stronger irritant sensation on the previously unstimulated side of the tongue when nicotine was tested bilaterally after the rest period (Figure 2, first solid bar); this was statistically significant ($P < 0.001$, binomial). Furthermore, the total time that subjects reported a stronger sensation on the previously unstimulated side was significantly greater compared with the other side (mean 4.8 versus 0.2 min; $P < 0.001$, t-test).

2b. Lack of cross-desensitization with capsaicin

In contrast to the cross-desensitization of nicotine by capsaicin, the results do not support cross-desensitization of capsaicin by nicotine. Following bilateral testing with capsaicin, only six of 13 subjects used in experiment 2a (46%) indicated a stronger sensation on the side that did not receive prior nicotine. To confirm this, seven additional subjects were tested and 11 of 20 of the total subject sample initially reported the previously unstimulated side to yield a stronger irritant sensation (Figure 2, second solid bar), while nine reported the opposite ($P > 0.8$, binomial). The

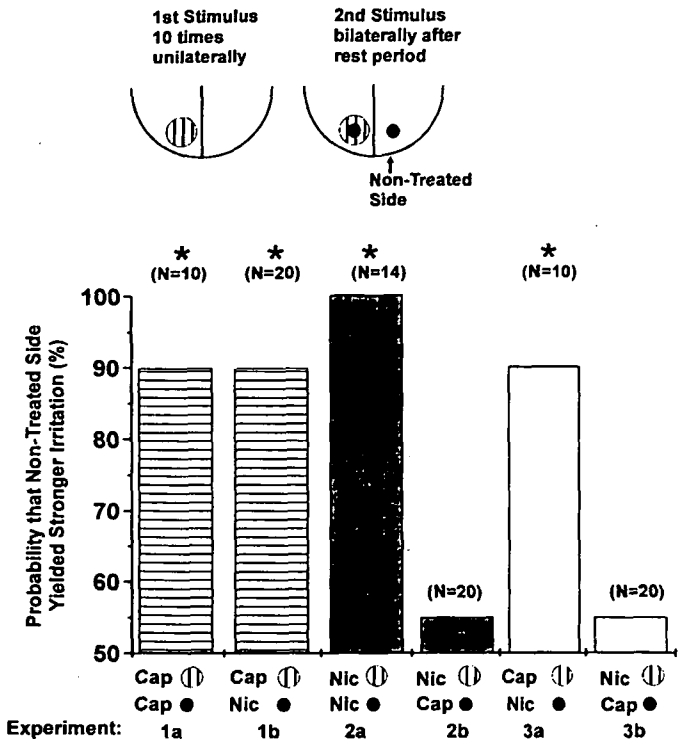

Figure 2 Cross-desensitization studies using 2-AFC. Bar graph plots the probability that subjects reported the non-pretreated side of the tongue to yield a stronger sensation of irritation, under six different experimental conditions. Experiment 1 (hatched bars): capsaicin followed by capsaicin (1a) or nicotine (1b). Experiment 2 (filled bars): nicotine followed by nicotine (2a) or capsaicin (2b). Experiment 3 (open bars): capsaicin followed by nicotine (3a) and nicotine followed by capsaicin (3b). Upper diagrams of the tongue illustrate the 2-AFC procedure. First, either capsaicin or nicotine was delivered sequentially to one side of the tongue (hatched circle in upper left diagram) 10 times at 1 min intervals. After a rest period, the second test stimulus (capsaicin or nicotine) was applied bilaterally at sites corresponding to the previously stimulated area (filled circles in upper right diagram). Subjects were asked to report which side of the tongue gave rise to a more intense sensation. Therefore, in the graph 100% means that all subjects reported the non-pretreated side to yield a stronger sensation, while 50% indicates that subjects chose the untreated and treated sides with equal probability.

average total time that either side of the tongue was judged to yield a stronger irritant sensation was virtually identical.

Experiment 3: nicotine and capsaicin cross-desensitization

This last experiment was carried out for four reasons: (i) to retest capsaicin and nicotine at concentrations that were better matched for sensory intensity; (ii) to check that a lower (0.5 p.p.m.) capsaicin concentration cross-desensitized irritation produced by nicotine; (iii) to confirm that nicotine does not cross-desensitize irritation evoked by capsaicin at this low concentration; and (iv) to compare effects of capsaicin and nicotine in an identical subject sample.

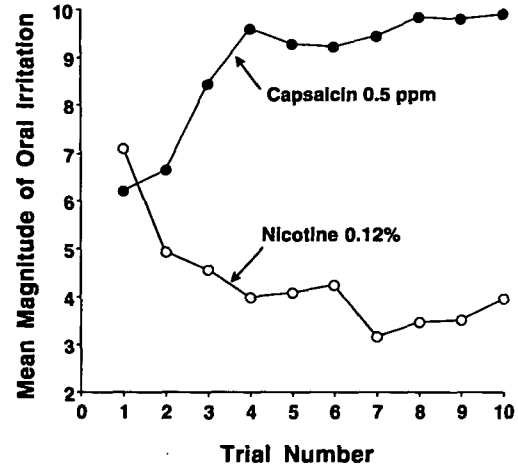

Figure 3 Differential responses to repeated application of nicotine and capsaicin at concentrations matched for intensity. Graph as in Figure 1 plotting mean ratings for each of 10 sequential applications of 0.12% nicotine (O) and 0.5 p.p.m. capsaicin (●).

3a. Sequential stimulation with capsaicin and cross-desensitization with nicotine

Sensitization was again demonstrated with 0.5 p.p.m. capsaicin (Figure 3). The main effect of application trial was significant ($P < 0.001$, ANOVA), and increasing ratings across trials were obtained in a significant majority (19/20) of subjects ($P < 0.001$, binomial).

The 2-AFC procedure revealed that 0.5 p.p.m. capsaicin led to cross-desensitization of the irritant effect of 0.12% nicotine. A significant majority of subjects (9/10, $P = 0.02$, binomial) initially reported a stronger sensation on the previously unstimulated side following bilateral testing with nicotine (Figure 2, first open bar). In addition, the total time that subjects reported a stronger sensation on the previously unstimulated side was significantly greater (mean 2.3 versus 0.6 min; $P = 0.01$, t-test).

To examine the time course of cross-desensitization, the 3 min 2-AFC test period was broken into three 1 min segments. During the initial segment, nine of 10 subjects reported a stronger sensation on the previously unstimulated side for a significantly greater total amount of time (mean 0.9 versus 0.1 min, $P = 0.003$, t-test). A similar result was obtained during the second 1 min segment (mean 0.78 versus 0.22 min, $P = 0.03$, t-test). However, during the last 1 min segment the difference was no longer significant (7/10 subjects chose the unstimulated side; $P = 0.17$, binomial; mean time 0.73 versus 0.27 min, $P = 0.14$, t-test). This indicates that the cross-desensitization began to wear off toward the end of the 3 min 2-AFC test period. This is supported by the mean time of occurrence of a 'no difference'

judgement, which was 1.51 min (although two subjects continued to rate one side as consistently more intense for the entire 3 min period).

3b. Sequential stimulation with nicotine and lack of cross-desensitization to capsaicin

In confirmation of experiment 2a, sequential application of nicotine led to a decrease in the perceived intensity of irritation (Figure 3). The main effect of application trial was significant ($P < 0.001$ ANOVA), and decreasing ratings across trials were obtained in a significant majority (17/20) of subjects ($P = 0.003$, binomial).

Data from the 2-AFC procedure were again consistent with an absence of cross-desensitization of nicotine to capsaicin. For the 10 subjects participating in experiment 3a, five reported a stronger sensation on the nicotine-pretreated side, and five on the untreated side, immediately after capsaicin was delivered bilaterally. This was verified in a larger population with 10 additional naive subjects. Eleven of the 20 subjects (55%) reported that the side of the tongue not previously exposed to nicotine yielded a stronger sensation immediately after capsaicin was delivered bilaterally (Figure 2, second open bar); this was not significant ($P = 0.50$, binomial). This ratio remained unchanged 45 s later, arguing against the possibility that the sensation required time to develop. The total time that subjects reported a stronger sensation on the previously unstimulated side was not significantly greater during the entire 3 min 2-AFC test period (mean 1.86 versus 1.14 min, $P > 0.8$, t-test), or during any of the 1 min segments. Finally, the mean time to a 'no difference' judgement was 0.9 min, and only one subject reported a consistent difference over the entire 3 min test period.

Discussion

The present results confirm that sequential intra-oral stimulation with capsaicin elicits progressively stronger irritant sensations (sensitization). In contrast, repeated application of nicotine evokes progressively weaker irritant sensations which we will refer to as desensitization. In addition, an asymmetric cross-desensitization effect was observed whereby capsaicin reduced the perceived intensity of irritation evoked by nicotine, but not *vice versa*. These findings are discussed in terms of methodology and possible physiological mechanisms.

Sequential sensitization and self-desensitization by capsaicin

The present data confirm the sensitizing and self-desensitizing effects of capsaicin reported by Green (1989). We presume that capsaicin and nicotine, which were judged to be tasteless at the concentrations used (see also Lawless and Stevens, 1990), activated trigeminal nociceptors in the oral cavity, although activation of gustatory receptors cannot be ruled out. Capsaicin binds specific molecular capsaicin ('vanilloid') receptors in the nociceptor terminal (Szallasi *et al.*, 1994; reviewed in Szallasi, 1994) to evoke a depolarization (Wood *et al.*, 1988; Cholewinski *et al.*, 1993; Liu and Simon, 1994, 1996b; Chard *et al.*, 1995). The means by which capsaicin can sensitize yet also desensitize nociceptors is not known; desensitization appears to require Ca^{2+} influx (Cholewinski *et al.*, 1993; Chard *et al.*, 1995; Liu and Simon, 1996b). Recent studies indicate that the relationship between capsaicin sensitization and desensitization is complex, since desensitization (i) is masked or delayed in the presence of capsaicin; (ii) only manifests itself after capsaicin is removed (Green, 1989, 1991b) even if a non-self-desensitizing irritant such as zingerone is present (Green, 1993); and (iii) can be overcome by recurrent capsaicin application (Green, 1996).

Sequential desensitization and self-desensitization by nicotine

Using the same testing procedure, we found that ratings of nicotine irritation decreased, rather than increased, across trials (desensitization) and that this effect persisted for at least 10 min (self-desensitization). Cliff and Green (1994, 1996) similarly reported decreasing ratings of irritation evoked by intra-oral menthol across trials (at 1 or 5 min interstimulus intervals), consistent with desensitization. Sub-populations of cutaneous (Brown and Gray, 1948; Douglas and Gray, 1953; Douglas and Ritchie, 1960; Fjallbrant and Iggo, 1961; Steen and Reeh, 1993) and lingual receptors (Wang *et al.*, 1993), as well as trigeminal ganglion neurons (Sucher *et al.*, 1990; Liu *et al.*, 1993), are sensitive to nicotine and other cholinergic agents via a 'neuronal' nicotinic receptor (Sucher *et al.*, 1990; Liu *et al.*, 1993; Wang *et al.*, 1993; for recent reviews, see Ochoa and McNamee, 1990; Deneris *et al.*, 1991; Sargent, 1993). The role of neuronal nicotinic receptors is supported by previous (Jarvik and Assil, 1988) work, as well as current work in our laboratory, showing that nicotine irritation is reduced by the ganglionic blocker, mecamylamine.

Cross-desensitization

The present data indicate an asymmetric pattern of cross-desensitization since capsaicin reduced the perceived intensity of irritation elicited by nicotine, but not *vice versa*. That pretreatment with capsaicin reduces the intensity of irritation evoked by nicotine provides a psychophysical confirmation of early animal studies (Jansco *et al.*, 1961). It was recently reported that capsaicin similarly reduces the perceived intensity of irritation elicited by menthol, but that menthol does not reduce the perceived intensity of capsaicin-evoked irritation (Cliff and Green, 1996). It has been argued that the ability of capsaicin to reduce the intensity of irritation elicited by other chemicals is evidence favoring the idea that a common population of capsaicin-sensitive trigeminal nociceptive fibers conveys sensations of oral irritation (Green, 1991). If so, then the present data would suggest that the terminal membrane of trigeminal fibers in the oral cavity should express molecular receptors for both capsaicin and nicotine. This is supported by a recent study showing that a fraction of trigeminal ganglion neurons are depolarized by both capsaicin and nicotine; other subsets responded only to one or the other of these chemicals or to neither (Liu and Simon, 1996a). The present data showing a lack of effect of nicotine pretreatment on the perceived intensity of capsaicin-evoked irritation might be explained if nicotine irritation were mediated by its activation of trigeminal fibers possessing only nicotinic molecular receptors. However, the effect of capsaicin pretreatment to reduce the perceived intensity of nicotine-evoked irritation would seem to require that both capsaicin and nicotine activate a common set of trigeminal fibers. Capsaicin pretreatment would decrease the response of such fibers to subsequent capsaicin and nicotine, whereas nicotine pretreatment would decrease their response to subsequent nicotine but not capsaicin. The cellular mechanisms underlying such a non-reciprocal cross-desensitization are not known and warrant further electrophysiological investigation.

2-AFC and desensitization/cross-desensitization

Our use of 2-AFC with half-tongue stimuli to investigate oral irritation is novel, although a 2-AFC method has been employed recently to assess sensations evoked by chemical irritants versus solvent when both were applied simultaneously to the face (Green and Bluth, 1995). From a theoretical viewpoint, 2-AFC has the advantage of greater sensitivity to small differences in sensation compared with scaling methods, and constitutes one of the most powerful discrimination methods available (O'Mahony, 1992).

A potential confound associated with bilateral stimulus application is that the larger of two simultaneously presented noxious stimuli reduces irritation from the weaker stimulus (Green, 1991c). However, such a 'counterirritation' effect would enhance any differences in perceived intensity of simultaneously presented irritants, and this difference should arguably be detected more readily using the present 2-AFC method. A drawback of the 2-AFC procedure as presently used is that it assesses if there is a bilateral difference in the intensity of sensation, but not the magnitude of the difference. The relative magnitude of self-desensitization effects by capsaicin and nicotine therefore cannot be compared. The feasibility of combining a magnitude rating procedure with 2-AFC to evaluate differences in the magnitude of sensations on the two sides of the tongue is currently being examined.

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