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Oral Irritation by Mustard Oil: Self-desensitization and Cross-desensitization with Capsaicin

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Abstract

We investigated the temporal pattern of oral irritation elicited by sequential application of mustard oil (allyl-isothiocyanate), and whether it exhibits self-desensitization and cross-desensitization with capsaicin. Mustard oil (0.125%, 40 μl) was sequentially applied to one side of the tongue at 1 min intervals, and subjects rated the intensity of the irritant sensation elicited by each stimulus. Ratings successively declined across trials, indicating desensitization. In contrast, sequential application of capsaicin (10 ppm) elicited irritation that increased in intensity across trials (sensitization). To test for self-desensitization by mustard oil, a 10 min hiatus was imposed following the series of unilateral mustard oil stimuli, after which mustard oil was applied to both sides of the tongue. In a two-alternative forced-choice paradigm, subjects chose which side had stronger irritation and also independently rated the irritant intensity on each side. A significant majority of subjects chose the side not previously receiving mustard oil as more intense, and assigned significantly higher intensity ratings to that side, indicating self-desensitization. In two additional sessions, the same paradigm was used to show mustard oil cross-desensitization of irritation elicited by capsaicin, and capsaicin cross-desensitization of irritation from mustard oil. In a final session, sequential application of mustard oil at faster (20 s) intervals initially evoked a sensitizing pattern followed by desensitization. The temporal patterns of oral irritation exhibited by mustard oil, and its reciprocal cross-desensitization with capsaicin, are similar to those of menthol and nicotine.

Key words: mustard oil (allyl isothiocyanate), oral irritation, tongue, psychophysics, trigeminal chemoreception

Introduction

Mustard oil (allyl isothiocyanate) is a pungent chemical that imparts the oral irritant sensation of mustard. Mustard oil applied to the skin elicits a burning pain sensation, followed by development of sensitization, which is characterized by hyperalgesia (increased pain to a normally painful stimulus) and allodynia (pain elicited by a non-painful stimulus) (Koltzenburg *et al.*, 1992). It is not known if mustard oil induces sensitization in the oral cavity. Repeated oral application of some irritants, such as capsaicin from chili peppers, at 1 min interstimulus intervals elicits a progressive rise in the intensity of oral irritation that is also called sensitization (Green, 1989; Green and Rentmeister-Bryant, 1998). However, following a hiatus of >3.5 min, re-application of capsaicin induces much weaker irritation due to the development of desensitization (Green, 1989). Repetitive application of other irritants such as nicotine, menthol or zingerone, induces irritation which declines in intensity across trials, a phenomenon also called desensitization (Cliff and Green, 1994; Prescott and Stevenson, 1996; Dessirier *et al.*, 1999, 2001a). However, application of menthol at a shorter interstimulus interval (20 s) initially elicits a sensitizing pattern of irritation, followed in later trials by desensitization (Dessirier *et al.*, 2001a). One aim of the present study was to determine if repetitive application of mustard oil at either short (20 s) or longer (1 min) interstimulus intervals elicits a sensitization or desensitization in the intensity of oral irritation, in comparison with capsaicin.

Pretreating the oral cavity with capsaicin not only reduces the intensity of irritation elicited by subsequent capsaicin (self-desensitization), but also that evoked by other irritants (cross-desensitization) (Green, 1991). The second aim of this study was to determine if mustard oil exhibits self-desensitization and cross-desensitization with capsaicin-evoked oral irritation.

Methods

This study was approved by the UC Davis Human Subjects Review Committee. Twenty-eight subjects (18 F, 10 M; 19–51 years of age) participated in four experimental

sessions that were counterbalanced across subjects. All were students or staff at the university. Subjects were requested not to consume spicy food for 3 days prior to each experimental session, as verified by *post hoc* interview.

The chemical stimuli used were mustard oil (allyl isothiocyanate; 0.125% in dH_2O; Fluka, St Louis, MO) and capsaicin (10 ppm = 3.3 μM; dissolved in dH_2O from a 1% stock solution in 80% ethanol; Sigma Chemical Co., St Louis, MO). The mustard oil and capsaicin concentrations were approximately matched for sensory intensity as described previously (Dessirier *et al.*, 1997, 1999, 2001b). Briefly, laboratory personnel were tested in a two-alternative forced-choice (2-AFC) procedure in which one filter paper with mustard oil and another with capsaicin were placed onto opposite sides of the tongue. Subjects stated which side had a stronger irritancy. That the mustard oil and capsaicin treated sides were selected in approximately equal numbers verified that the two chemicals were approximately matched in intensity, as confirmed in the Results (see Figure 2A,B, open bars).

For unilateral sequential stimulation, a filter paper disc (1.5 cm diameter, 176.7 mm^2; Whatmann, Maidstone, UK) soaked with 40 μl of the mustard oil or capsaicin was placed, with the aid of forceps, onto one side of the anterior dorsal surface of the subject's tongue. The mustard oil was pipetted onto the filter paper just prior to lingual application. The capsaicin filter papers were prepared 2–3 days prior to the experimental session by pipetting the capsaicin onto the filter paper and then air-drying them to allow evaporation of the ethanol vehicle. They were then rewetted with 40 μl dH_2O just prior to lingual application. For bilateral stimulus application, two smaller filter paper discs (1 cm diameter, 78.5 mm^2; Whatmann) soaked with 20 μl of mustard oil or capsaicin were placed onto each side of the dorsal tongue surface in a location within the region corresponding to that covered by the larger filter paper stimulus.

For sequential application of mustard oil at 1 min intervals, the first filter paper was applied to one side of the dorsal tongue (side of application was counterbalanced across subjects) and left on for 15 s, after which it was removed. Subjects were asked to rate the intensity of the irritant sensation 10 s after stimulus application. The second stimulus was applied 1 min after the onset of the first stimulus. The procedure was repeated 10 times. For sequential application of capsaicin, the same procedure was followed except that ratings were given 20 s after stimulus application, with the filter paper removed 5 s later. The 10 s difference in timing of mustard oil vs capsaicin ratings was necessary because mustard oil-evoked irritation peaked and subsided more rapidly compared to the irritation elicited by capsaicin. After each application of filter paper subjects held the tongue inside the mouth to avoid any spurious effects of cooling. Subjects were trained to hold the tongue still and not to touch it against the hard palate or cheek so as to avoid spread of the stimulus. Subjects additionally had the choice to use a dental suction device to remove saliva from the mouth in between filter paper applications.

For sequential application of mustard oil at the shorter, 20 s interval, the first stimulus was applied, the rating given 10 s later, the filter paper removed 5 s after that, and the next filter paper was applied 5 s after that (20 s after the onset of the first stimulus). This procedure was repeated 20 times.

Sequential ratings of irritant intensity were made using a bipolar category scale (Dessirier *et al.*, 1999, 2000a,b, 2001a,b). The scale was centered at '1' and numbers increased to the left and right up to 15 on each side. Ten such scales were printed on a single sheet presented to the subject [see Figure 1 of Dessirier *et al.* (Dessirier *et al.*, 1999)]. Extensions (16–30, or higher) were available to attach at either end of the scale if needed; the scale was open-ended to reduce or eliminate end effects. No assumptions were made about the nature of the numerical estimates produced (e.g. ratio scale), nor do we claim that the numbers represent absolute values. In this respect, scores were treated as relative measures. Subjects had access to their prior ratings so as to reduce errors of memory (Kim and O'Mahony, 1998). Our rationale is that subjects can judge whether a stimulus-evoked sensation was stronger or weaker than a preceding one, but may give a numeric rating that does not correspond to the perceived change in intensity if they cannot remember the previous number.

Subjects were instructed to rate the first stimulus as having an intensity of 1. Subsequent ratings were made relative to the initial rating. If the intensity was higher, subjects were instructed to mark the number to the right of 1 corresponding to the relative increase in intensity. If the intensity was lower, subjects marked a corresponding number to the left of 1. Subjects thus had continuous access to all prior ratings. For data analysis, the 1 was transformed to 0. Each number to the left of 0 was reduced by 1 and made negative, while each number to the right of 0 was increased by 1 and remained positive. Rating data were subjected to analysis of variance (ANOVA) followed by *post hoc* Least Significant Difference (LSD) tests to determine if ratings increased or decreased across trials. Significance was assumed at the $P < 0.05$ level.

Following each sequential stimulus series, a 10 min rest period was imposed during which the subject sat quietly with the mouth closed except during suctioning of saliva. After the rest period, subjects were tested for self- or cross-desensitization.

Either mustard oil or capsaicin (20 μl; 1 cm diameter filter paper) was applied bilaterally to the dorsal tongue surface within the corresponding region that had received the prior stimulus. In a two-alternative forced choice (2-AFC) paradigm, subjects were asked to state which side of the tongue had a stronger irritant sensation. In addition, they rated the irritant intensity independently on each side of the tongue using a 0–10 category scale with 0 = no sensation and 10 = most intense irritation imaginable. The rationale

for using separate rating scales for the bilateral vs sequential ratings is provided in our prior publications (Dessirier *et al.*, 1999, 2001b).

Results

When applied to the tongue at an interstimulus interval (ISI) of 1 min, mustard oil elicited a burning sensation that decreased significantly ($F_{9,243} = 8.6$; $P < 0.001$) in intensity across trials (Figure 1, open circles). *Post hoc* LSD tests indicated that by the fifth mustard oil application, the intensity of the perceived burning sensation was significantly ($P < 0.001$) lower than that elicited by the first application. Ten minutes after the end of the unilateral series of mustard oil stimuli, we tested for self-desensitization by applying mustard oil bilaterally. Indicative of self-desensitization, a significant majority of subjects (27/28; $P < 0.001$) chose the previously untreated side of the tongue as having the stronger irritant sensation (Figure 2A, 2-AFC, hatched bar) and assigned significantly ($P < 0.001$) higher ratings to that side of the tongue (Figure 2A, NT, open bar) as compared with the treated side (Figure 2A, T, filled bar). Cross-desensitization to capsaicin was also tested. When capsaicin was applied bilaterally following unilateral mustard oil application, a significant (26/28; $P < 0.001$) majority of subjects chose the previously untreated side as having a stronger irritant sensation (Figure 2B, 2-AFC, hatched bar). Subjects also assigned significantly ($P < 0.001$) higher intensity ratings to the untreated side (Figure 2B, NT, open bar) compared with the treated side (Figure 2B, T, filled bar).

In contrast to mustard oil, capsaicin elicited a burning sensation that increased significantly ($F_{9,243} = 10.9$; $P < 0.001$) in intensity across trials when applied at an ISI of 1 min (Figure 1; filled triangles). *Post hoc* LSD tests indicated that the perceived irritant sensation elicited by the fifth capsaicin application was significantly ($P < 0.001$) higher than that elicited by the first. The ability of capsaicin to cross-desensitize mustard oil irritation was tested 10 min after the end of the unilateral capsaicin series. Following bilateral mustard oil application, a significant majority (25/28; $P < 0.001$) of subjects chose the untreated side as having the stronger burning sensation (Figure 2C, 2-AFC, hatched bar) and similarly gave significantly ($P < 0.001$) higher intensity ratings to this side (Figure 2C, NT, open bar) as compared with the side previously treated with capsaicin (Figure 2C, T, filled bar).

To further compare the magnitudes of self- and cross-desensitization, d' values can be calculated from both 2-AFC and rating data (Ennis, 1993; Bi *et al.*, 1995). Calculated d' values are provided in Table 1, which also includes data for capsaicin (5 ppm) from a prior study (Dessirier *et al.*, 2001b).

When mustard oil was applied unilaterally to the tongue

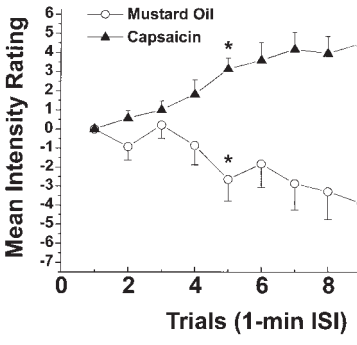

Figure 1. Desensitization by mustard oil and sensitization by capsaicin. Graph plots mean ratings of irritant intensity across 10 successive presentations of mustard oil (open circle) or capsaicin (filled triangle) at 1 min ISI. Capsaicin and mustard oil irritancy ratings were significantly higher and lower, respectively, at the fifth trial compared with trial 1 (*).

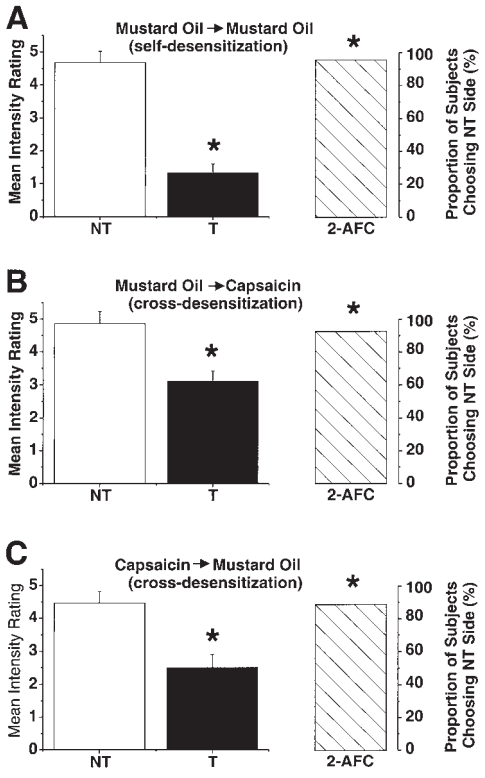

Figure 2. Mustard oil self-desensitization and reciprocal cross-desensitization with capsaicin. **(A)** The hatched bar graph to the right plots the percentage of subjects choosing the side that had not previously received mustard oil (non-treated = NT) as having stronger irritation in the 2-AFC. Mustard oil was applied bilaterally 10 min after the end of the unilateral series of mustard oil stimuli. *Significant majority chose the NT side ($P < 0.001$). Left-hand bars plot mean intensity ratings for the non-treated (NT; open bar) and mustard oil-treated (T; filled bar) sides of the tongue. *Significant difference between NT and T ($P < 0.001$). **(B)** Graphs as in (A) showing mustard oil cross-desensitization of capsaicin-evoked irritation. Capsaicin was applied bilaterally 10 min after the end of the unilateral series of mustard oil stimuli. **(C)** Graphs as in (A) showing capsaicin cross-desensitization of mustard oil-evoked irritation. Mustard oil was applied bilaterally 10 min after the end of the unilateral series of capsaicin stimuli.

Table 1. Listed are *d′* values calculated from 2-AFC and rating data (Ennis, 1993; Bi *et al*., 1995). All *d′* values were significant at $P < 0.001$

	2-AFC	Rating
Mustard oil self-desensitization	2.55	2.131
Mustard oil cross-desensitization to capsaicin	2.07	0.958
Capsaicin cross-desensitization to mustard oil	1.76	1.164
Capsaicin (5 ppm) self-desensitization[a]	>2.25	1.2

[a]Data from Dessirier *et al*. (2001b).

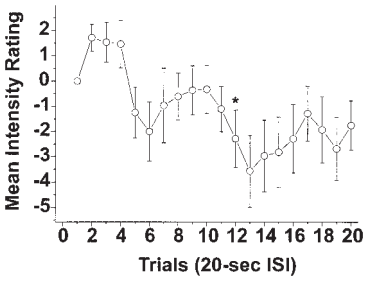

Figure 3. Sensitization followed by desensitization to sequential unilateral application of mustard oil at a faster (20 s) ISI. Ratings tended to increase over the first three trials and then decreased significantly by trial 12 (*).

at the faster ISI of 20 s, a different pattern of sensation emerged where an initial sensitization was followed by desensitization (Figure 3); the effect of trial was found to be significant ($F_{19,513} = 3.6$; $P < 0.001$). Irritancy ratings tended to increase over the initial three trials and then decreased significantly (LSD; $P < 0.05$) by the twelfth application.

Discussion

When applied at the relatively long ISI of 1 min, mustard oil elicited a desensitizing pattern of oral irritation such that intensity ratings of irritancy progressively decreased across trials. In contrast, capsaicin elicited a sensitizing pattern of oral irritation when applied at the same ISI. When mustard oil was delivered at the more rapid interval of 20 s, the intensity ratings for irritation initially increased before decreasing, indicating different time courses for the sensitizing and desensitizing properties of this irritant. Furthermore, mustard oil exhibited self-desensitization and a reciprocal cross-desensitization with capsaicin. These properties of mustard oil are discussed further below in comparison with other oral irritant chemicals and possible cellular mechanisms.

Sensitization and self-desensitization of mustard oil irritation

The desensitizing pattern of oral irritation elicited by sequentially delivered mustard oil (Figure 1) confirms a recent report that mustard oil vapor delivered to the nasal epithelium elicits a burning sensation that exhibits desensitization when reapplied at long ISIs of 3–4 min (Brand and Jacquot, 2002). The desensitizing pattern observed with mustard oil is very similar to that observed when nicotine (Dessirier *et al*., 1997), menthol (Cliff and Green, 1994; Dessirier *et al*., 2001) or zingerone (Prescott and Stevenson, 1996) is sequentially applied at 1 min ISI. When mustard oil was applied at a faster (20 s) ISI, there was an initial pattern of sensitization followed by desensitization (Figure 3) very similar to the biphasic pattern of oral irritation observed with rapid sequential application of menthol (Dessirier *et al*., 2001), and consistent with the recent report that reapplication of mustard oil vapor to the nasal mucosa at a relatively short ISI (<2 min) elicited increased irritancy ratings (Brand and Jacquot, 2002). These findings suggest that mustard oil (and menthol) initially excite trigeminal nociceptors to elicit irritation and at the same time engage neural mechanisms for both sensitization and desensitization (Green, 1996; Carstens *et al*., 2002). The desensitizing pattern observed with mustard oil differs from that of capsaicin-evoked irritation in which sensitization predominates at short ISIs (Figure 1), and a longer hiatus (>3.5 min) is required in order for desensitization to overcome sensitization (Stevens and Lawless, 1987; Green, 1989; Dessirier *et al*., 1997; Green and Rentmeister-Bryant, 1998; Prescott, 1999).

Inspection of Figure 3 reveals an apparent variation in irritancy ratings for mustard oil at the fast (20 s) ISI with a period of ~6 min. It may be speculated that this represents a periodic variation in sensitizing (e.g. release of local inflammatory mediators) vs desensitizing processes.

Cross-desensitization

In addition to mustard oil self-desensitization, we presently observed a reciprocal cross-desensitization between mustard oil- and capsaicin-evoked oral irritation. Capsaicin cross-desensitization of oral irritation elicited by other irritant chemicals has been previously reported (Green, 1991) while the present report is, to our knowledge, the first showing mustard oil cross-desensitization. Capsaicin excites cutaneous nociceptors via the molecular VR-1 receptor (Caterina *et al*., 1997). Capsaicin cross-desensitization to other irritants has been shown in trigeminal ganglion cells (Liu and Simon, 1996a). If capsaicin and mustard oil excite the same trigeminal nociceptive nerve endings in the oral mucosa, then cross-desensitization might be explained if capsaicin reduces the excitability of these nociceptors to subsequent stimulation with mustard oil. Such a reduction in cellular excitability might be mediated by capsaicin acting through VR-1 receptors to induce Ca^{2+} influx into the fiber ending which would, in turn, engage intracellular pathways. Self-desensitization of capsaicin-evoked inward currents is abolished by removal of Ca^{2+} (Liu and Simon, 1996b) or by inhibition of calcineurin, a Ca^{2+}-dependent phosphatase (Docherty *et al*., 1996; Piper *et al*., 1999). One potential outcome is reduced excitability of voltage-gated Na^{+}

channels (Su *et al*., 1999; Liu *et al*., 2001), which would render the fibers less sensitive to subsequent stimuli. Cross-desensitization by mustard oil is more difficult to explain, given the current paucity of information regarding its cellular effects. It is not known if mustard oil interacts with a specialized molecular receptor analogous to VR-1 for capsaicin or the cold-menthol receptor (CMR-1) for menthol (McKemy *et al*., 2002), or if it excites nerve endings via a non-specific action on the plasma membrane (Kress and Reeh, 1996). In any event, the excitatory action of mustard oil presumably engages cellular mechanisms that render the mucosal nociceptors less sensitive to subsequent capsaicin stimulation.

The relative magnitudes of cross-desensitizing effects can be expressed as d' values that can be calculated from 2-AFC data and bilateral intensity ratings (Ennis, 1993; Bi *et al*., 1995); these values are provided in Table 1. Although we presently did not assess capsaicin self-desensitization, we used comparable data from a previous study employing the same bias-free 2-AFC procedure and using a lower (5 ppm) capsaicin concentration (Dessirier *et al*., 2001b). The d' values from both 2-AFC and rating data from that study are give in Table 1, and we assume that the higher (10 ppm) capsaicin concentration used in the present study would have resulted in equivalent or stronger self-desensitization. Comparison of d' values indicates that the magnitude of capsaicin self-desensitization was similar to (or slightly greater than) that of capsaicin cross-desensitization of mustard oil (Table 1). This supports the argument that mustard oil primarily excited capsaicin-sensitive nociceptors, although the slightly greater d' values for self- vs cross-desensitization suggests that mustard oil may have also excited a small population of capsaicin-insensitive fibers. Conversely, the degree of mustard oil self-desensitization was greater than mustard oil cross-desensitization of capsaicin, implying that capsaicin activated mustard oil-insensitive nociceptors.

Modeling the temporal dynamics of oral irritation

McBurney and colleagues (McBurney *et al*., 1997, 2001; Balaban *et al*., 1999) have developed a theoretical model to explain temporal changes in the intensity of capsaicin-evoked oral irritation. Capsaicin (100 ppm) was applied by filter paper at 1 min intervals to the tongue and subjects provided ratings of the intensity of irritation after the first minute and at 3 min intervals thereafter for 34 min. Overall, intensity ratings increased to a plateau after ~15–20 min and tended to decline thereafter. However, there were individual differences, with some subjects' ratings increasing to a plateau ('tonic' pattern), while others showed an initial increase followed by a decline ('phasic' pattern), and still others continued to rise ('rising' pattern). The model takes account of the different patterns by including a low-pass filter with a 7 min time constant (tonic component or level detector), a high-pass filter with 15 min time constant (phasic component or change detector), and a double integrator (rising component), respectively. By adjusting the gain of the different components, the model successfully fit the different temporal patterns as well as adaptation of capsaicin irritation across days (McBurney *et al*., 1997) and cross-adaptation between zingerone and vanilloids (Affeltranger *et al*., 2002).

We assume that the components of the McBurney and Balaban model should ultimately have underlying cellular mechanisms. We propose that sensitization is mediated largely by spatial recruitment of nociceptors as the irritant chemical diffuses through epithelial tissue, and that desensitization involves cellular process leading to reduced excitability of the nociceptor nerve endings (see above). These two processes may occur simultaneously. Capsaicin has limited diffusion through the epithelium so that spatial recruitment occurs over a longer time course. During this time, desensitization also starts to build up. The net perception is the difference between the two processes. During sensitization spatial recruitment is greater than desensitization, whereas both processes are in equilibrium when the sensation reaches a plateau. When the maximal degree of spatial recruitment is reached (the rate of diffusion equals the rate of clearance of the irritant chemical), overall irritation ratings should start to decline due to continued desensitization; this pattern was observed by McBurney *et al*. (McBurney *et al*., 1997). In contrast, mustard oil diffuses more readily through epithelial tissue, such that maximal spatial recruitment of nociceptors may already be achieved after the first application. The overall decline in irritant ratings thereafter can be attributed to progressive desensitization of the nociceptors. Conceivably, spatial recruitment and physiological desensitization of nerve endings may represent the cellular bases for the 'tonic' and 'phasic' processes of the model proposed by McBurney and colleagues.

Comparison with hairy skin

Most prior psychophysical studies investigating the irritant properties of mustard oil have been conducted on hairy skin (LaMotte *et al*., 1982; Margerl *et al*., 1990; Handwerker *et al*., 1991; Koltzenburg *et al*., 1992). When applied to the skin, mustard oil evoked a burning sensation and induced primary and secondary hyperalgesia (Koltzenburg *et al*., 1992) that may be due to peripheral and central sensitization, respectively (Torebjörk *et al*., 1992). In human microneurography experiments, sensations of pain evoked by cutaneous mustard oil application correlated with activity in C-fiber polymodal nociceptors (Handwerker *et al*., 1991). The recruitment of myelinated nociceptive fibers may also contribute to hyperalgesia (Treede *et al*., 1995). The development of hyperalgesia following cutaneous mustard oil application is at odds with our observation of self-desensitization in the oral cavity, suggesting important differences in the chemosensory properties of these two tissues that deserve further investigation. One obvious

difference is the greater accessibility of irritant chemicals to nociceptive endings in lingual and nasal epithelia as compared with skin.

In animal studies, mustard oil injected under the skin (Handwerker *et al*., 1987; Lippe *et al*., 1993a,b; Dux *et al*., 1996) or into the temporomandibular joint (Haas *et al*., 1992; Bereiter and Benetti, 1996; Yu *et al*., 1996; Fiorentino *et al*., 1999) has been used extensively to study the neuroinflammatory properties of this chemical. The inflammatory response evoked by mustard oil was unaffected by pretreatment with histamine H1 and 5-HT receptor antagonists as well as ruthenium red, a non-specific VR-1 channel blocker (Inoue *et al*., 1997), suggesting that neither mast cell degranulation nor VR-1 are important components in mustard oil sensitization. Similarly, tachykinin NK2 and NK3 antagonists were not effective in reducing mustard oil-induced inflammation (Inoue *et al*., 1997). However, pre-treating the skin with the NK1 antagonist SR 140 333 completely blocked the neurogenic skin inflammation in mouse skin (Inoue *et al*., 1997), suggesting that substance-P is an important mediator of the mustard oil-induced inflammatory response.

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