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Lingual Heat and Cold Sensitivity Following Exposure to Capsaicin or Menthol

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Introduction

Capsaicin and menthol are the most extensively studied of all chemesthetic agents. Capsaicin is best known as a nociceptive stimulus and menthol as an artificial cooling agent, but both have a combination of thermal and nociceptive properties. Capsaicin can affect perception of nonpainful warmth and heat as well as burning pain and menthol can induce burning and stinging as well as cold. However, few studies have investigated capsaicin's effects on temperature perception (Green, 1986b) and only recently have psychophysical reports of irritation from menthol (Green, 1992; Dessirier *et al.*, 2001) been substantiated by evidence that it stimulates nociceptors as well as low-threshold cold fibers (Okazawa *et al.*, 2004; Wasner *et al.*, 2004).

The identification of separate transient receptor protein (TRP) gated channels sensitive to capsaicin and menthol has further increased interest in these chemicals. TRPV1 is sensitive to capsaicin, heat and protons (Caterina *et al.*, 1999) and has been localized in taste papillae as well as other oral tissue (Ishida *et al.*, 2002; Kido *et al.*, 2003). TRPM8 is sensitive to menthol (McKemy *et al.*, 2002; Peier *et al.*, 2002) and is assumed to be the primary receptor for innocuous cold. However, its high threshold (<30°C) and recent evidence that it is sometimes co-expressed with TRPV1 (Okazawa *et al.*, 2004) leaves open the possibility that TRPM8 is expressed on some nociceptors as well.

Psychophysical studies of the effects of menthol and capsaicin on temperature perception have yielded a complicated array of interactions. In the first study of menthol's oral thermal effects (Green, 1985), brief (5 s) exposures to L-menthol in water enhanced perceived warmth but failed to enhance perceived cold. However, pre-exposure to L-menthol not only led to the expected enhancement of cooling but also to a suppression of warmth. Two later studies replicated the suppressive effect of warmth on the lip (Green, 1986a) and forearm (Green, 1992). On the other hand, capsaicin has been shown to enhance warmth during transient oral exposures (Green, 1986b) and to suppress warmth following repeated applications to the skin that are sufficient to induce self-desensitization (Simone and Ochoa, 1991). What was unclear in all of these studies was whether the reduced heat perception from the two chemicals resulted from effects on warm receptors, nociceptors, or both.

Experimental design

The present study was intended to answer this question by measuring the effects of menthol and capsaicin pre-treatments on nociceptive (i.e. burning, stinging) sensations as well as warmth and cold. Also of interest was any possible effect of both chemicals on nociceptive sensations during cooling. To establish baseline intensity ratings, subjects (n = 20 for menthol, n = 41 for capsaicin) first rated the perceived thermal (warm, cold) and nociceptive (burning, stinging) intensity of separate blocks of cold (30–10°C in 5° steps) and warm (36–44°C in 2° steps) stimuli. The thermal stimuli were presented in pseudo-random order using a computer-controlled 0.64 cm^2 Peltier thermoelectric module (ΔT = ±1.5°/s) specially constructed for oral testing. Intensity ratings were made using the Labeled Magnitude Scale displayed on a computer monitor. After a short break, subjects received 10 L-menthol (experiment 1) or 10 capsaicin (experiment 2) stimuli in one of two concentrations (3.2 and 10 μM menthol; 300 and 900 μM capsaicin). The chemicals were applied to the tongue tip via cotton-tipped swaps at the rate of one per minute.

Thermal perception was measured again beginning 15 min after the final capsaicin stimulus and either 5 or 15 min after menthol application. Two different post-stimulation delays were used for menthol because previous research (Green, 1986a) had indicated that its post-excitatory effects might change within this timeframe.

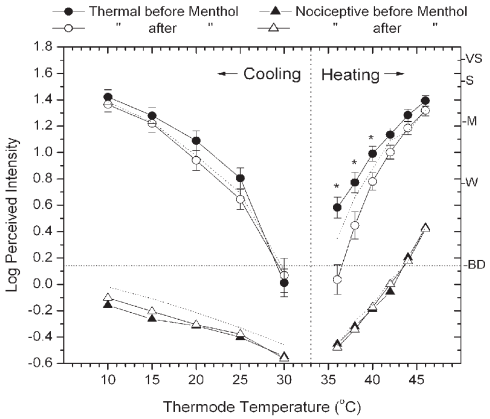

Figure 1 Log perceived intensity of temperature (circles) and burning stinging (triangles) sensations before (filled symbols) and 15 min after (open symbols) 10 exposures to l-menthol on the tongue tip. The dashed data lines show the results for tests conducted 5 min after menthol exposure. Asterisks above data points indicate significant differences (Tukey test, P < 0.05) between the baseline and 15 min post-exposure condition. The dotted vertical line indicates approximate thermal neutrality and the dotted horizontal line indicates the level of 'barely detectable' sensations. Letters on the right y-axis represent intensity descriptors on the rating scale: BD, barely detectable; W, weak; M, moderate; S, strong; VS, very strong. Error bars denote the standard error of the mean.

Results

Because separate repeated-measures analyses of variance (ANOVAs) performed on data from the menthol and capsaicin experiments indicated that there was no effect of concentration on the thermal effects of either chemical, the data from both concentrations are combined in Figures 1 and 2.

Figure 1 shows that the principle effect of pre-exposure to menthol was a significant reduction in warmth intensity that diminished as temperature rose [condition × temperature interaction; $F(10,190)$ = 6.61, $P < 0.0005$]. There was no effect on nociceptive sensations and

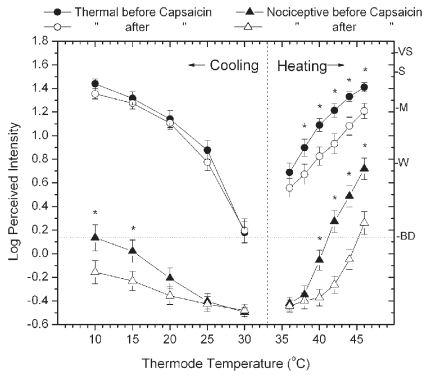

Figure 2 As Figure 1, except the treatment stimulus was capsaicin and there was no 5 min post-treatment condition.

a trend toward lower cold ratings at the lowest four temperatures was not significant.

The results for capsaicin were more complex (Figure 2). First, during heating capsaicin pre-treatment reduced heat intensity as well as burning and stinging. The reduction in heat intensity showed an opposite trend to the effect of menthol, with a larger effect at higher temperatures [$F(5,200) = 2.5$, $P < 0.05$]. Nociceptive sensations were also strongly suppressed as temperature increased [$F(5,200) = 14.4$, $P < 0.0001$]. Cold sensation was unaffected despite a significant decrease in burning and stinging at the coldest temperatures [$F(4,160) = 7.3$, $P < 0.0001$].

Discussion

Capsaicin and menthol have very different post-excitatory effects on temperature perception and thermal nociception. Capsaicin's more complex effects reflect its ability to desensitize CPNs (Buck and Burks, 1986; Holzer, 1991) and, apparently, low-threshold warm fibers. Nociceptive sensations from both heating and cooling were reduced over the temperature range served by CPNs (<25 and >40°C). However, the weakness of the nociceptive sensation and the absence of a change in cold sensation (Figure 2) suggests that CPNs do not contribute substantially to perception of nonpainful cold and the lesser desensitization of heat at higher temperatures than at moderate temperatures is consistent with recent evidence that noxious heat sensitivity is unaffected in TRPV1 knockout mice (Woodbury *et al.*, 2004). Evidence of warm fiber desensitization comes from the significantly lower warmth ratings at 38°C, a temperature at which burning and stinging was less than barely detectable and was unaffected by capsaicin treatment. Simone and Ochoa (1991) had previously found equivocal evidence of warm fiber desensitization.

The absence of a desensitizing effect of menthol on cold is not surprising in view of previous work and implies that menthol does not readily desensitize TRPM8 to cold. However, earlier reports of self- and cross-desensitization of irritation by menthol (Cliff and Green, 1994; Green and McAuliffe, 2000; Dessirier *et al.*, 2001) indicates that menthol must stimulate and desensitize an as yet undiscovered receptor that is expressed on nociceptors (Okazawa *et al.*, 2004). In addition, prior evidence of transient enhancement of warmth by menthol (Green, 1985) together with the reduced ratings of warmth and heat >36°C raises the possibility that menthol stimulates and then desensitizes TRPV3 and/or TRPV4, two receptor-gated cation channels that are believed to act as warm receptors (Guler *et al.*, 2002; Smith *et al.*, 2002; Xu *et al.*, 2002). Studies of menthol's effects on warm fibers and on the these two TRP channels in particular, would therefore be useful.

Acknowledgements

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