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Research report

Thermal and nociceptive sensations from menthol and their suppression by dynamic contact

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Abstract

It was recently found that cooling the skin to temperatures as mild as 25–30 °C can induce nociceptive sensations (burning, stinging or pricking) that are strongly suppressed by dynamic contact between the thermode and skin (*contact suppression*). Here we investigated whether nociceptive sensations produced by menthol can be similarly suppressed. In the first experiment subjects rated the intensity of cold and burning/stinging/pricking sensations before and after application of 10% *l*-menthol to the forearm. Ratings were compared at resting skin temperature (≈33 °C) and at 28, 24, or 20 °C during static or dynamic contact cooling via a Peltier thermode. At resting skin temperature, menthol produced cold and nociceptive sensations, both of which were suppressed by dynamic contact. When the skin was cooled during static contact, menthol increased nociceptive sensations but not cold sensations; when the skin was cooled during dynamic contact, cold sensations were again unchanged while nociceptive sensations were suppressed. A second experiment tested whether contact suppression of menthol's cold and nociceptive sensations at resting skin temperature was caused by slight deviations of thermode temperature above skin temperature. The results showed that suppression occurred even when the thermode was slightly cooler (−0.5 °C) than the skin. These findings support other evidence that the menthol-sensitive channel, TRPM8, plays a role in cold nociception, and raise new questions about how dynamic tactile stimulation may modify perception of nonpainful cold stimulation.

Keywords: Menthol; Cold; Nociception; Touch; Inhibition; Psychophysics

1. Introduction

Cooling the skin to temperatures above the cold pain threshold has been assumed to produce only sensations of cold. Recent evidence [1] indicates that sensations of burning, stinging, or pricking can be evoked at temperatures as mild as 25–31 °C when cooling occurs after a thermode is already in contact with the skin (i.e., static contact cooling). However, these sensations, which were termed *innocuous cold nociception* (ICN), can be greatly reduced when cooling occurs by touching an already cold thermode to the skin (i.e., dynamic contact cooling). Additional experiments indicated that the reduction in ICN during dynamic contact cooling was most likely caused by tactile stimulation produced as the thermode touched the skin [1,2].

The occurrence of ICN provides evidence that mild cooling stimulates the nociceptive system as well as the cold system. This finding raises questions about the longstanding assumption that painful and nonpainful cold are mediated by afferent fibers that have very different sensitivities to cold. Although examples can be found in the literature of C- or A-δ fibers that respond to noxious cold yet have thresholds above 25 °C [3–5], such fibers have not been considered important for perception of nonpainful cold. Consistent with this view, the nonspecific cation channel TRPM8 [6–8], which is sensitive to menthol and has a threshold to cooling ≤28 °C, has been designated as a cold receptor. But menthol can induce nociceptive sensations of burning, stinging or pricking as well as cold sensations [9–12], and a recent study demonstrated that topically applied menthol can induce cold hyperalgesia [13]. In addition, there is evidence that some fibers that express TRPM8 project in the nociceptive system [14], and TRPM8 has been reported to be co-expressed in rat dorsal root

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ganglion (DRG) neurons with the heat, irritant- and pH-sensitive channel, TRPV1 [15,16]. Although other studies have failed to find co-expression of TRPM8 and TRPV1, the weight of the current evidence points to TRPM8 as the receptor most likely to mediate ICN.

Accordingly, the primary objective of the present study was to determine whether nociceptive sensations produced by menthol could be suppressed by dynamic contact. The approach was to measure the intensity of nociceptive sensations produced by topically applied menthol under conditions of static and dynamic contact at both resting skin temperature (RST) and during active cooling. Suppression of menthol's nociceptive sensations by dynamic contact would imply that menthol stimulates the same class of fibers that mediate ICN, and that these fibers are a type of nociceptor that responds to mild cooling. The results of two experiments supported this hypothesis. After finding in the first experiment that at RST dynamic contact suppressed menthol's cold sensations as well as its nociceptive sensations, we ran a second experiment to rule out the possibility that suppression of cold might have resulted from systematic miss-adjustments of thermode temperature rather than from dynamic mechanical contact.

2. Methods

2.1. Subjects

Thirty-nine subjects (21 females and 18 males) served in experiment 1, and 25 (14 females and 11 males) served in experiment 2. All were self-reported healthy individuals between the ages of 18 and 45 years with no history of nerve injury or neuropathology.

2.2. Stimuli

Thermal stimuli were delivered via a 16-channel thermode composed of independently controllable, 8 mm × 8 mm Peltier thermoelectric modules arranged in a 4 × 4 matrix with 2-mm separations between neighboring modules. The 16 modules were bonded with thermally conductive epoxy to a water-circulated heat sink [2,17]. Skin–thermode interface temperature was monitored by a 40-ga type-T thermocouple recessed into the face plate of each module. Baseline temperature, target temperature (28, 24, and 20 °C), rate of temperature change (5 °C/s), and dwell time (5 s) were controlled with LabView software. The thermode was fixed to a floor mounted positioning device that stood next to a modified dental chair in which the subject sat with his or her right forearm resting on a 4-in. thick foam pad. A lockable ball joint on the positioning device enabled manual placement of the thermode flush against the surface of the subject's forearm.

The chemical stimulus was 10% *l*-menthol (Pfalz & Bauer, Waterbury, CT) dissolved in 95% ethanol and applied for 15 min to the volar surface of the forearm on a saturated filter paper square that was equal in size (16 cm^2) to the 16-channel thermode. The filter paper was occluded by a wide strip of parafilm draped across the forearm and weighted at both ends to keep the filter paper flat against the forearm. After the parafilm and filter paper were removed, the forearm was wrapped in a single layer of cellophane (Saran Wrap™, S.C. Johnson) to prevent evaporative cooling from residual menthol and ethanol, and to avoid transference of residual menthol to the thermode during thermal testing. Skin temperature was monitored via a digital thermometer connected to a 40-ga thermocouple wire positioned underneath the cellophane at a point a 2–3 cm from the distal edge of the menthol-treated skin.

2.3. General procedure

Both experiments included a practice session for new subjects who had not participated in thermal perception experiments in this laboratory. The practice consisted of two brief (10–15 min) exercises designed to train individuals to use the Labeled Magnitude Scale (LMS; [18,19]) to rate the intensity of thermal sensations. The LMS is a "category-ratio" scale [20] in which labeled intensity descriptors (e.g., "weak", "strong") are spaced according to their empirically derived semantic magnitudes. The scale is bounded at the bottom by "no sensation" and at the top by "strongest imaginable sensation of any kind", and subjects enter their ratings by using a computer mouse to move an arrow to the appropriate location on the scale. Subjects were first asked to imagine 16 commonly experienced thermal sensations (e.g., washing hands in cold tap water; walking barefoot on hot pavement) and rate their intensity. This exercise served both to familiarize subjects with the scale and to encourage use of the broadest possible perceptual context. Subjects then received a series of 11 practice thermal stimuli (ranging from 18 to 42 °C) presented on two rows of the thermode array as it rested on the right forearm. Warm and cold stimuli were presented alternately across trials on separate pairs of rows of the thermode with an inter-trial interval of 30 s. Subjects had three tasks per trial: to rate thermal sensation intensity (cool–cold, warm–hot), nociceptive sensation intensity (burning, stinging or pricking), and to indicate the specific sensations they had perceived by clicking on one or more descriptors displayed on the computer screen: nothing, cool, cold, warm, hot, burning, stinging/pricking, aching, and painful. The instructions were to choose as many words as necessary to describe each sensation fully, and to click on "nothing" if no sensation was felt.

2.4. Experiment 1: static versus dynamic contact with and without cooling

This experiment investigated the effect of dynamic mechanical contact on sensations produced by menthol at RST and during cooling to 28, 24 and 20 °C. Two conditions of thermal stimulation, static contact and dynamic contact, were tested before and after menthol application in separate sessions (see Fig. 1).

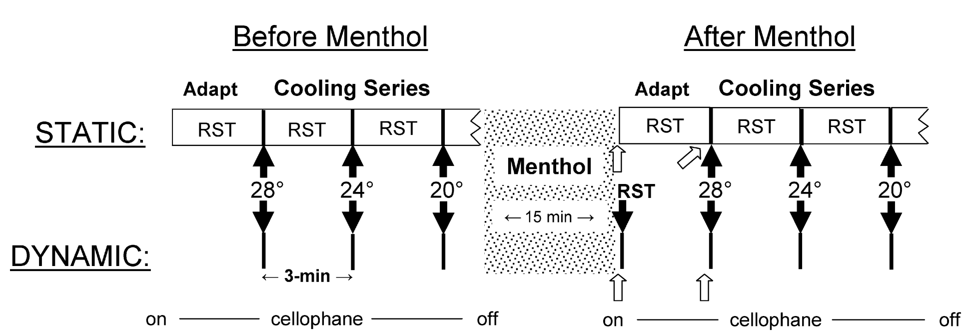

Fig. 1. Shown is a diagram of the procedure used in experiment 1. Rectangular boxes in the static condition indicate periods when the thermode was in contact with the skin (RST, resting skin temperature). Thick vertical lines in both conditions indicate bouts of 5-s thermal stimulation at the specified temperatures. Solid black arrows indicate intensity ratings made in response to dynamic contact and/or thermal stimulation; open arrows indicate baseline intensity ratings at RST prior to dynamic contact or thermal stimulation.