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Mustard oil enhances spinal neuronal responses to noxious heat but not cooling

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Abstract

The TRPA1 agonist mustard oil (allyl isothiocyanate= AITC) induces heat hyperalgesia and mechanical allodynia in human skin and sensitizes rat spinal wide dynamic range (WDR) neuronal responses to noxious skin heating. We presently used electrophysiological methods to investigate if AITC affects the responsiveness of individual spinal WDR neurons to intense skin cooling. Recordings were made from cold-sensitive WDR neurons in lamina I and deeper dorsal horn; 21/23 also responded to noxious skin heating. Topical application of AITC excited 8/18 units and significantly enhanced their responses to noxious heat while not significantly affecting responses to the cold stimulus. Vehicle (mineral oil) had no effect on thermal responses. The data confirm a role for the TRPA1 agonist AITC in enhancing heat nociception without significantly affecting cold sensitivity.

Keywords

Spinal cord; noxious heat; skin cooling; rat; dorsal horn; nociception; TRPA1; mustard oil

INTRODUCTION

Mustard oil (allyl isothiocyanate=AITC) is the pungent chemical in mustard and wasabi that imparts the characteristic burning sensation of these spices. AITC is an agonist of the thermosensitive TRP (transient receptor potential) ion channel TRPA1 that was initially implicated in sensing cold temperatures below 18°C [22]. TRPA1 is also activated by other irritant chemicals including cinnamic aldehyde (CA), bradykinin, allicin from garlic, and formalin [2,8,17,22]. AITC has long been recognized to produce hyperalgesia and allodynia [11] and to sensitize C-fiber nociceptors [7], and more recently was shown to sensitize spinal and trigeminal dorsal horn neuronal responses to noxious heat [3,14,16,18,21]. In contrast, the role of TRPA1 in cold pain has been controversial. TRPA1 was initially reported to respond to intense cooling [22] although this was not confirmed [2,8]. Studies using TRPA1 knockout mice report that they exhibit normal sensitivity to cold [2], or show a partial [12] or severe [9] deficit in cold pain sensitivity, and cultured nodose ganglion neurons from TRPA1 knockout mice exhibited reduced cold sensitivity [5]. The human data are also mixed with reports that

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application of CA on the skin induces cold hypoalgesia [19] whereas lingual application of AITC or CA induces a weak, brief cold hyperalgesia [1]. In the present study we have addressed a role for TRPA1 in cold sensing, by investigating if cutaneous application of AITC affects responses of spinal wide dynamic range (WDR)-type neurons to skin cooling. We chose to investigate WDR neurons based on evidence that they are sufficient for the transmission of pain [15] and that they are well-suited to encode pain intensity [13].

METHODS

Experiments were conducted using 15 adult male Sprague-Dawley rats (wt. 370-590 g) under a protocol approved by the UC Davis Institutional Animal Care and Use Committee. Anesthesia was induced with sodium pentobarbital ($65 \text{ mg} \cdot \text{kg}^{-1} \text{ ip}$) and maintained by constant infusion of pentobarbital via a jugular vein catheter at a rate sufficient to maintain areflexia for the duration of the experiment ($10\text{--}20 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{hr}^{-1}$). Oxygen was delivered via a tracheal cannula. A laminectomy exposed the lumbar spinal cord for single-unit recording as detailed previously [18]. Briefly, a tungsten microelectrode (FHC, Bowdoin, ME) was driven into the dorsal horn to record extracellular single-unit activity. We specifically searched for mechanoresponsive units that additionally responded to cooling. Cold sensitivity was assessed using a Peltier thermode (0.5" diameter; NTE-2A, Physitemp, Clifton, NJ) programmed to deliver cold and heat stimuli to the cutaneous mechanical receptive field. The skin-thermode interface temperature was monitored using a thermocouple (IT-21, Physitemp) connected to a BAT-12 (Physitemp) thermometer and was displayed along with single-unit activity and EKG using a Powerlab interface (ADInstruments, Colorado Springs CO). The cold stimulus was a decrease from the adapting temperature of 32°C to 0°C over 75 sec, followed by rewarming to 32°C . This was followed 3 min later by a noxious heat stimulus (32°C to 45°C over 40 sec). Only cold-responsive units were studied further. They were tested for mechanical sensitivity using a series of von Frey hairs (0.07 g, 1.5 g, 12.0 g, and 126.0 g, duration 1s), air puff, cotton wisp (1 Hz), touch, and pinch (duration 3s) applied to the receptive field. All of the present units were classified as wide dynamic range (WDR), based on their thermal sensitivity and incrementally increasing responses to graded mechanical stimuli. After this initial classification, the cold and heat stimulus sequence was reapplied, followed by topical application of either mineral oil (vehicle control) or AITC ($5 \mu\text{l}$ in mineral oil) to the hind paw receptive field 2 min after the heat stimulus. For chemical application, the Peltier thermode was withdrawn from the skin. The thermode was mounted on a micromanipulator to allow precise repositioning of the thermode surface at the same skin site for post-chemical thermal testing. The cold-heat stimulus sequence was delivered again 10 min post-AITC (or mineral oil).

Recordings sites were marked by electrolytic lesion, and the spinal cord was post-fixed in 10% buffered formalin. Sections of the spinal cord were cut on a freezing microtome and examined under the light microscope to locate lesion sites [18].

Action potentials were stored and analyzed using Chart 5 (AD Instruments) and custom software [6] to generate peristimulus-time histograms (PSTHs; 1 sec bins). During cooling, action potentials were counted over the initial 30 sec period when most of the evoked responses occurred. During heating, the onset of firing was delayed so that we counted action potentials over a 60-sec period in order to capture the evoked responses. Neuronal activity was corrected by subtracting spontaneous activity for an equivalent period preceding stimulus onset. Corrected thermal responses were summed over 30- or 60-sec intervals and compared using a repeated-measures ANOVA with post hoc least significant difference (LSD) tests. Responses to AITC were analyzed with paired t-tests. The thermal response threshold was the temperature at which the unit first exhibited a detectable (>2 -fold) increase in frequency during application of the cooling or heating stimulus. A $p < 0.05$ was considered significant.

RESULTS

The 23 cold-sensitive units recorded were all WDR type and most (21/23) also responded to noxious heat. Units were located in lamina I and deeper dorsal horn with no apparent difference between AITC-responsive and unresponsive units (Fig. 1, inset; Fig. 2B, inset). A typical example is shown in Fig. 1. This lamina I unit responded weakly to cooling but robustly to noxious heat and topical application of AITC. After AITC the response to noxious heat was markedly larger while the response to cooling appeared slightly larger.

Sixteen units were tested with AITC, 5 with mineral oil, and 2 with both. Fig. 2A shows an averaged PSTH of the 7 units tested with mineral oil, and Fig. 3 shows in graphic format that mineral oil had no significant effect on neuronal responses to cooling (Fig. 3A, left-hand bars; pre-mineral oil: 40.7 ± 29.1 [SE]; post: 66.6 ± 29.7) or noxious heat (Fig. 3B, left-hand bars; pre-mineral oil: 612 ± 117.8 ; post: 552.3 ± 117.4) ($p > 0.1$ for both).

Eight of 18 units (44%) were directly excited by topical AITC. An individual example is shown in Fig. 1, and Fig. 2B shows the averaged response of all 18 units in PSTH format. There was a numeric, but statistically insignificant, increase in the mean response to cold 10 min following AITC (Fig. 3A, right-hand bars; pre-AITC: 66.8 ± 13.1 ; post-AITC: 106.2 ± 22.9 ; $n=18$; $p=0.085$). This was associated with a non-significant 3.7°C decrease in threshold post-AITC (pre-AITC: $23.1^\circ\text{C} \pm 2.3$; post: $26.8^\circ\text{C} \pm 1.9$; $p > 0.1$). The averaged response to noxious heat was significantly greater following AITC (Fig. 3B, right-hand bars; pre-AITC: 502.0 ± 135.7 ; post-AITC: 630.2 ± 147 ; $n=16$; $p < 0.05$). This was associated with a non-significant 1°C decrease in threshold (pre-AITC: $41.2^\circ\text{C} \pm 0.6$; post: $40.2^\circ\text{C} \pm 1$; $p > 0.1$). A similar pattern of results is seen for AITC-responsive and —unresponsive units displayed separately in Fig. 2C and D, respectively.

DISCUSSION

The present results confirm recent reports that AITC enhances noxious heat-evoked responses of lumbar WDR and trigeminal subnucleus caudalis (Vc) neurons in rats and mice [3,14,16, 18,21], and extends them by showing a lack of significant effect of AITC on responses to intense cooling although there was a trend toward enhancement. These findings are consistent with our prior observation that another TRPA1 agonist, CA, did not significantly affect cold-evoked responses of WDR neurons while significantly enhancing their responses to noxious heat [18].

Consistent with previous reports, AITC directly excited nearly half of the presently-recorded WDR neurons in rats [18,24], and significantly enhanced their responses to noxious heat. Heat-evoked responses were enhanced regardless of whether the unit was directly excited by AITC or not [18]. The present data are consistent with the effects of topically applied AITC or CA to induce burning pain and to produce thermal hyperalgesia [1,11,19]. Since AITC acts at TRPA1 [8], its ability to enhance heat nociception may involve sensitization of thermal gating of TRPV1 which is co-expressed in primary sensory neurons expressing TRPA1 [10,22]. Alternatively, AITC may induce the intradermal release of inflammatory mediators that lower the heat threshold of TRPV1 [4,23]. The possibility of central sensitization of thermal nociception by TRPA1 agonists was refuted by our recent demonstration that topical application of AITC or CA did not enhance WDR neuronal responses to peripheral nerve electrical stimulation [18].

AITC was presently observed to have no significant effect on WDR responses to cooling. Although some units exhibited increased responses to cooling post-AITC, this effect was less pronounced compared to AITC enhancement of heat-evoked responses. Previous human studies have reported mixed effects of TRPA1 agonists on cold pain sensitivity. Namer et al.

[19] reported that CA produced cold hypoalgesia on forearm skin, while we reported that both AITC and CA induced a brief cold hyperalgesia on the tongue [1]. We also observed mixed effects of TRPA1 agonists on lingual cold-evoked responses of rat Vc neurons; a lower concentration of CA enhanced cold-evoked responses, while a higher CA concentration had a biphasic effect, with an initial reduction followed by later enhancement of cold-evoked responses [25]. In a different study both AITC and CA reduced cold-evoked responses of rat Vc neurons [3]. In recent behavioral experiments we did not observe any significant effect of CA on cold sensitivity as assessed in thermal preference and cold-plate tests (authors' unpublished observations). We therefore conclude that AITC does not have a consistent effect on cutaneous sensitivity to cooling. This implies that if TRPA1 plays a role in detection of intense cold stimuli in the temperature range below 18°C, TRPA1 agonists including AITC and CA do not exert a consistent effect on TRPA1 thermosensitivity.

Acknowledgments

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Fig. 1. Peristimulus-time histogram (PSTH, bins: 1 sec) shows increased firing during application of the following stimuli, from left to right: cooling, noxious heat, topical AITC, and cooling and heating again. Thermode: time of replacement of Peltier thermode on skin. Traces above show recordings of thermode-skin interface temperature. Inset shows recording site in lamina I.

Fig. 2.

Averaged responses of cold-sensitive dorsal horn units. A: mean PSTH of 7 units to sequential application of cold, noxious heat, mineral oil (AITC vehicle control), thermode replacement, and cold and heat stimuli repeated (arrows). Gray error bars: SEM. B: PSTH as in A for all units tested with AITC. *: significantly different compared to mean response to noxious heat prior to AITC ($p < 0.05$, paired t-test). Inset shows histologically recovered recording sites, compiled on representative section of L4 (from atlas of Paxinos & Watson [20]). Open circles: AITC-responsive units; filled circles: units not directly activated by AITC. C, D: PSTHs separately showing mean responses of AITC-responsive (C) and AITC-unresponsive (D) subsets of units from the combined population shown in (B).

Fig. 3.

Summary of effects of vehicle (mineral oil) and AITC on neuronal responses to cooling and noxious heat. A: Left-hand bars plots mean cooling-evoked responses of 7 units before (white bar) and after (black bar) application of mineral oil (vehicle). Right-hand bars plot mean cooling-evoked responses of 18 other units before (striped bar) and after (gray bar) application of AITC. Error bars: SEM. B: graph as in A plotting mean noxious heat-evoked responses before and after mineral oil (left) or AITC (right). *: significantly different ($p < 0.05$).