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Thermosensitive transient receptor potential (TRP) channel agonists and their role in mechanical, thermal and nociceptive sensations as assessed using animal models

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Abstract

Introduction—The present paper summarizes research using animal models to investigate the roles of thermosensitive transient receptor potential (TRP) channels in somatosensory functions including touch, temperature and pain. We present new data assessing the effects of eugenol and carvacrol, agonists of the warmth-sensitive TRPV3, on thermal, mechanical and pain sensitivity in rats.

Methods—Thermal sensitivity was assessed using a thermal preference test, which measured the amount of time the animal occupied one of two adjacent thermoelectric plates set at different temperatures. Pain sensitivity was assessed as an increase in latency of hindpaw withdrawal away from a noxious thermal stimulus directed to the plantar hindpaw (Hargreaves test). Mechanical sensitivity was assessed by measuring the force exerted by an electronic von Frey filament pressed against the plantar surface that elicited withdrawal.

Results—Topical application of eugenol and carvacrol did not significantly affect thermal preference, although there was a trend toward avoidance of the hotter surface in a 30 vs. 45°C preference test for rats treated with 1 or 10% eugenol and carvacrol. Both eugenol and carvacrol induced a concentration-dependent increase in thermal withdrawal latency (analgesia), with no significant effect on mechanosensitivity.

Conclusions—The analgesic effect of eugenol and carvacrol is consistent with previous studies. The tendency for these chemicals to increase the avoidance of warmer temperatures suggests a possible role for TRPV3 in warmth detection, also consistent with previous studies. Additional roles of other thermosensitive TRP channels (TRPM8 TRPV1, TRPV2, TRPV4, TRPM3, TRPM8, TRPA1, TRPC5) in touch, temperature and pain are reviewed.

Keywords

eugenol; carvacrol; TRPV3; TRPV1; TRPA1; TRPM8; pain; temperature; touch

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Compliance with Ethical Standards. The authors declare no conflicts of interest.

Introduction

Since the mid 20th century, many substantial research attempts have been made to elucidate the mechanisms of pain using animal models (Mogil, 2009; Mogil et al., 2009). Within the last two decades, thermosensitive transient receptor potential (TRP) channels have been shown to play an important role in somatosensation, including the transduction and encoding of thermal, mechanical and chemical stimuli (Cortright, et al, 2007; Levine, Alessandri-Haber, 2007; Myers, Julius, 2007; Patapoutian et al, 2003; Stucky et al, 2009). TRP channel agonists elicit irritation (i.e., burning, pricking/stinging, numbing) in human subjects. TRP channels expressed on sensory nerve fibers are thought to contribute to the chemical sensibility of the skin and mucous membranes, also known as chemesthesis. Chemesthetic sensations are evoked when chemicals open channels expressed by sensory nerve fibers involved in nociception, temperature or mechanical sensation. Within six of the subfamilies (TRPV, TRPM, TRPC, TRPA, TRPP), eight TRP channels exhibit sensitivity to changes in environmental temperature, osmotic/mechanical pressure, as well as chemical ligands. These chemicals include many commonly used food spices such as capsaicin from chili peppers, menthol from mint, mustard oil, cinnamaldehyde from cinnamon, piperine from black pepper, eugenol from cloves, carvacrol from oregano, and many others (Caterina, Julius, 2001; Peier et al., 2002; Bandell et al., 2004; Bautista et al., 2005; McNamara et al., 2005; Willis, 2009). In this paper we summarize the properties of the currently known thermo TRP channels (TRPV1, TRPV2, TRPV3, TRPV4, TRPM3, TRPM8, TRPA1, TRPC5), effects of their agonists on somatosensation (pain, temperature, touch), and the potential underlying neural mechanisms. Particular emphasis is placed on our recent studies of the TRPV3 agonists, eugenol and carvacrol on thermal and mechanical stimulation in rats.

Materials and Methods

Animals

Adult male Sprague Dawley rats (~200–400 g, Simonsen laboratories) were housed in pairs and given rodent chow and water ad libitum. Behavioral studies were conducted at approximately the same time each day to reduce circadian effects, in a quiet room with the temperature maintained constant at 22–24° by thermostat. The study protocols were approved by the UC Davis Animal Care and Use Committee.

Chemical Application

Eugenol and carvacrol (Sigma,-Aldrich, St. Louis, MO) were emulsified in 10% ethanol and 1% Tween-80 (Fisher Scientific, Fair Lawn, NJ) at concentrations of 0.1%, 1.0%, 10%, or 30%. Eugenol or carvacrol was topically applied by cotton tip applicator to one (for thermal and mechanical paw withdrawal tests) or both ventral hindpaws (for thermal preference testing) allowed to dry for 2 min, and the paw(s) was (were) wiped dry prior to placing the animal in to the test arena. Eugenol and carvacrol were also intradermally injected in separate groups of animals into the ventral plantar surface in a volume of 10 μl using a 30.5 G hypodermic needle connected to a Hamilton microsyringe.

Thermal preference test

The apparatuses and protocols for behavioral testing were the same as those employed by Klein et al., 2010, and are described briefly here. The rats were habituated to the test arena with both thermoelectric plates set at 30°C, for a minimum of three successive daily exposures. Vehicle-treated rats exhibited no preference for either surface when they were both set at 30°C, indicating an absence of positional preference. Preference testing was done by setting one plate at 30°C and the other plate at a higher temperature in 5°C increments, using a counterbalanced design to avoid order effects and possible lighting inconsistencies within the room. Eugenol, carvacrol or vehicle was topically applied bilaterally as described above. The rats were placed onto one of the plates in a matched block design alternating initial rat position and temperature on each plate. Animals were videotaped from above for at least 20 min, and the time the animal spent on each plate was monitored off-line and recorded. At least two days intervened between successive tests when using the same rat.

Thermal Paw Withdrawal (Hargreaves) Test

Rats were first habituated to stand on a glass surface heated to 30°C in individual Plexiglas enclosures for one hour for a minimum of three successive daily exposures. Before chemical injection/application, baseline latencies for paw withdrawals evoked by radiant thermal stimulation were measured using a light beam (Plantar Test 390, IITC, Woodland Hills CA) focused onto the ventral plantar surface of the hind paw. Latencies from onset of the light exposure to paw withdrawal/flinching of the stimulated paw were measured on each hindpaw. A 20 sec cut-off was used if no paw movement occurred to prevent tissue damage. The latencies for the treated (i.e. ipsilateral) and untreated (i.e. contralateral) paw were measured at 3, 15, 30, 45, 60 and 120 min post-injection/application to one hindpaw.

Von Frey mechanical paw withdrawal threshold

Rats were first habituated over 3 successive days to stand on a wire mesh screen surface. Baseline mechanical withdrawal thresholds were assessed using an electronic von Frey filament (1601C, IITC) that was pressed against the ventral plantar surface. The force in grams was recorded at the moment that the hind paw was withdrawn away from the instrument. Mechanical paw withdrawal thresholds were measured at the same post-application times as noted above for thermal paw withdrawals for vehicle, eugenol and carvacrol treated animals.

Statistical Analysis

For thermal preference testing, the percent time spent on the 30°C plate for each animal was subjected to a one-way ANOVA with post-hoc LSD tests comparing each treatment group. Thermal and mechanical withdrawal responses were normalized to baseline averages and subjected to two-way repeated measures analysis of variance (ANOVA) using SPSS 9.0 software (SPSS, Chicago IL). Multiple comparisons were done post-hoc using Least Significant Difference (LSD) tests. A 95% confidence interval was used and the error reported is the standard error of the mean.

Results

TRPV1

The first cloned thermo TRP channel, TRPV1, is expressed by sensory (i.e., dorsal root ganglion [DRG] and trigeminal ganglion [TG]) neurons and is activated by capsaicin, piperine, zingerone (found in ginger), anandamide, prostaglandins, bradykinin, and the capsaicin analogs olvanil and resiniferatoxin. Since TRPV1 is activated by both capsaicin and temperatures above 43°C (Caterina et al., 1997; Caterina and Julius, 2001), it appears that TRPV1 is responsible for the pungent burning irritation and extreme heat sensation elicited by chili peppers. Capsaicin, piperine, and zingerone all elicited irritant sensations of burning quality when applied to the lingual surface in humans (Green, 1993; Dessirier et al., 1999). Intraplantar administration of capsaicin evoked hindpaw licking and biting in rats and mice (Klein et al., 2011; Sakurada et al., 1992). Capsaicin, olvanil and piperine all evoked ocular wiping after corneal exposure in mice (Ursu et al., 2010; Karai et al., 2004). Intraplantar injection of anandamide, prostaglandins and bradykinin all evoked nocifensive behaviors (e.g. paw lifting, flinching and licking) in rats (Hong and Abbott, 1994; Potenzieri et al., 2009). Together these studies indicate that TRPV1 is highly conserved among mammals and its activation is accompanied by nociceptive sensations.

In humans, intracutaneous injection of capsaicin induced primary heat hyperalgesia and primary and secondary mechanical allodynia (Simone et al., 1987; LaMotte et al., 1991). In electrophysiological experiments, capsaicin sensitized C-fiber nociceptors (Baumann et al., 1991) and responses of primate spinothalamic tract neurons (Simone et al., 1991). In contrast, capsaicin did not affect cold pain in human skin (Simone et al., 1987; Simone & Ochoa, 1991) or tongue (Albin et al., 2008), which is consistent with previous studies showing no deficits in cold-evoked behavioral responses in TRPV1 knockout mice.

TRPV1 knockout mice exhibited reduced responses to strongly noxious (>50°C) thermal stimuli and to capsaicin exposure (Caterina et al., 2000). In rodents, intraplantar injection of capsaicin induced a concentration-dependent heat hyperalgesia and mechanical allodynia (Gilchrist et al., 1996). Capsaicin also produced a facial heat hyperalgesia and mechanical allodynia in rats as assessed using an operant response model (Neubert et al., 2006). Prostaglandins, which are released during inflammation or following thermal/chemical injury, also induced heat hyperalgesia after intraplantar administration; these effects were absent in TRPV1 knockout animals (Moriyama et al., 2005). Unlike most other TRPV1 agonists, anadamide delivered intrathecally did not appear to promote hyperalgesia as assessed using a radiant paw withdrawal test (Horvarth et al., 2008). The thermal hyperalgesia seen with TRPV1 agonists may occur at least partly at a peripheral site on sensory nerve endings expressing TRPV1, since capsaicin enhanced the responses of DRG cells to heat (Guenther et al., 1999).

TRPV2

TRPV2 is expressed in medium to large, myelinated TG and DRG cells (Caterina et al., 1999; Ichikawa and Sugimoto, 2000) as well as other tissues including central nervous system (Lewinter et al., 2004), intestine (Kashiba et al., 2004), pancreas (Hisanaga et al.,

2009) and muscle cells (Muraki et al., 2003). TRPV2 is sensitive to Δ-9-tetrahydrocannabinol (THC), cannabidiol and probenecid (Qin et al., 2008; Bang et al., 2007). Although systemic and local injections of THC into the hindpaw of male or female rats induced analgesia lasting at least one week post treatment with complete Freund's adjuvant (CFA) (Craft et al., 2013), this was most likely due to activation of cannabinoid CB2 receptors rather than TRPV2. Probenecid, a highly selective TRPV2 agonist, was first shown to evoke nociceptive behaviors (e.g., licking and flinching) after intradermal injection, but only after an inflammatory mediator such as CFA or carageenan was administered first (Bang et al., 2007). This suggests that TRPV2 plays a role in nociception after tissue injury but not under normal conditions.

TRPV2 is of interest within the pain field because of its response to intense levels of noxious heat (~52°C) (Caterina et al., 1999). Since TRPV2 is expressed by myelinated fibers, it has been postulated that TRPV2 mediates the first (bright, pricking) pain sensation conducted by myelinated heat-sensitive nociceptors (Price and Dubner, 1977; Leffler et al., 2007). DRG cells expressing either TRPV2 or TRPV1/TRPV2 rapidly sensitized to repeated noxious heating (Rau et al., 2007). Conversely, neurons lacking TRPV1 and TRPV2 have been reported to exhibit normal responses to noxious heating (Woodbury et al., 2004). Unlike capsaicin, which rapidly induces mechanical allodynia and thermal heat hyperalgesia after intrathecal or intraplantar application, probenecid only enhanced mechanical hypersensitivity (Petitjean et al., 2014). Therefore it is postulated that TRPV2 may have a large role in mechanotranduction, rather than in processing nociceptive heat information. This is consistent with observations that many TRPV2 positive neurons are not heat sensitive and are expressed by either Aδ high-threshold mechanoreceptors or Aβ rapidly adapting low threshold fibers (Lawson et al., 2008). However, it has been shown using assays of acute thermal (e.g., tail immersion), mechanical (e.g., von Frey mechanical threshold), or chemical nociception (e.g., formalin, capsaicin eye wipes), or in models of chronic pain (e.g., CFA, spinal nerve ligation), that nocifensive behaviors were all normal in TRPV2 knockout mice (Park et al., 2011). The role of TRPV2 in processing sensory information in naïve and chronic pain conditions will need to be further studied.

TRPV3

TRPV3 is found in the central nervous system (Smith et al., 2002) and skin keratinocytes (Chung et al., 2004; Peier et al., 2002) as well as in sensory neurons (Xu et al., 2002). TRPV3 activity can be initiated or potentiated by endogenous ligands including ATP, arachidonic acid or protein kinase C (Hu et al., 2006), which makes TRPV3 a good target candidate for inflammatory pain. TRPV3 is also activated by many aromatic compounds found in commonly used spices. These compounds include the monoterpenoids such as eugenol, carvacrol, and thymol (found in thyme) (Xu et al., 2006; Vogt-Eisele et al., 2007). TRPV3 also responds to innocuous warming (>33°C) especially within the physiological range of 36–38°C (Xu et al., 2002). Farnesyl pyrophosphate (FPP) was recently reported to selectively activate TRPV3 in transfected HEK293 cells. When applied intradermally into the hindpaws, FPP evoked nociceptive behaviors (i.e. paw lifting and licking)(Bang et al., 2010). Eugenol and carvacrol activate highly overlapping populations of primary and

secondary trigeminal neurons responsive to other TRP channel agonists, including capsaicin and cinnamaldehyde (Klein et al., 2014).

Mice lacking TRPV3 were originally reported to have deficits in detecting temperatures in the warm and noxious (50–52°C) range, as assessed using thermal gradient and tail immersion tests, respectively (Moqrich et al., 2005). This suggests roles for TRPV3 in innocuous warmth sensation and potentially heat-induced pain. The role of TRPV3 in murine heat sensation has been debated and appears to be background strain dependent (Huang et al., 2011). An alternative explanation could involve a synergy between TRPV3 and another heat sensitive ion channel, perhaps TRPV1. TRPV1/TRPV3 double knockout mice exhibited a larger deficit in sensitivity to heat in the 48–50°C range compared to single TRPV1 or TRPV3 knockout animals, as assessed by hot plate, tail immersion and thermal gradient tests (Marics et al., 2014). In behavioral tests, FPP enhanced carrageenan-evoked nociceptive behaviors and lowered paw withdrawal thresholds in the radiant heat test in mice (Bang et al., 2010). Eugenol and carvacrol enhanced sensations of innocuous warmth and heat pain on the human tongue (Klein et al., 2013) and enhanced TG/DRG and trigeminal subnucleus caudalis (Vc) neuronal responsiveness to innocuous warming and noxious heat (Klein et al., 2014). Camphor, another TRPV3 agonist, weakly enhanced warmth sensation when applied on human skin (Green, 1990), consistent with the rodent studies.

To date, there have been few animal studies of the behavioral effects of TRPV3 agonists on thermal and mechanical sensitivity. We therefore investigated the effects of two TRPV3 agonists, eugenol and carvacrol, on thermal and mechanical sensitivity in adult rats. To assess possible effects of these agents on thermosensitivity, we used a thermal preference test. For this test, rats received topical application of eugenol or carvacrol (1, 10%) to the ventral hindpaws and were allowed to move freely between two adjacent thermoelectric plates, one set at 30°C and the other set at 35, 40, 45 or 50°C, on separate testing days. We measured the relative amount of time the rat spent on each plate. We hypothesized that if eugenol and carvacrol sufficiently enhanced warmth, temperatures at the upper end of the thermoneutral zone might be perceived to be unpleasantly hot and thus avoided. Data are shown in Fig. 1. Overall, there was no significant effect of chemical (i.e. eugenol or carvacrol) or concentration (1% or 10%) on the relative percent time spent on either thermoelectric plate for any temperature difference ($p>.05$, ANOVA). Temperatures of 35 and 40°C were not significantly avoided by any treatment group (Fig. 1A, B). However, there was a tendency for eugenol- and carvacrol-treated rats to avoid the warmer plate in the 30 vs. 45°C preference test more, compared to vehicle-treated rats (Fig. 1C), although this did not reach statistical significance ([$F(1, 75) = 0.235$, $p = 0.63$]). In the 30 vs. 50°C preference test, vehicle-treated rats avoided the hotter temperature >95% of the time (Fig. 1D), creating a ceiling effect that may have obscured any further effects of eugenol or carvacrol treatment.

Since eugenol and carvacrol also enhanced responses of primary and second-order sensory neurons to noxious heat (Klein et al., 2014), we additionally tested the effect of these agents in the nociceptive thermal paw withdrawal (Hargreaves) test. We observed a significant, concentration-dependent increase in paw withdrawal latency (analgesia) ipsilateral to the

side of topical hindpaw application of eugenol compared to baseline immediately after application (30%: 196%, 10%: 168%, 1%: 153%, 0.1%: 145%; Fig. 2A). This was accompanied by a similar analgesic effect on the contralateral paw at the highest concentration (30%: 156%, Fig. 2B). Topical application of 30% eugenol had the greatest effect, being significantly different compared to 0.1% eugenol on the ipsilateral paw and compared to all other concentrations of eugenol on the contralateral paws (Figure 2A). The similar ipsilateral and contralateral analgesic effects observed on the paws after eugenol application are reminiscent of the bilateral effects observed with topical menthol application (Klein et al., 2010). Intraplantar injection of eugenol across the same concentration range had no significant effect on thermal paw withdrawal latencies for the ipsilateral (injected) or contralateral paw ($p>0.05$, repeated measures ANOVA, data not shown).

A similar albeit weaker analgesic effect was observed with carvacrol. There was a significant difference between the 30% and 10% carvacrol treatment groups (Fig. 2C; $p<0.05$, repeated measures ANOVA). Similar to the effect of topical application of eugenol, there was a contralateral analgesic effect of carvacrol whereby the 30% carvacrol group exhibited a significantly ($p<0.05$, repeated measures ANOVA) longer withdrawal latency for the contralateral hindpaw compared to all other carvacrol or vehicle groups (Fig. 2D). Also similar to the effects observed with eugenol, intraplantar injection of carvacrol had no significant effect on ipsilateral or contralateral mechanical paw withdrawal latencies ($p>0.05$, repeated measures ANOVA, data not shown). The putative selective TRPV3 agonist, FPP, also did not elicit signs of pain or hyperalgesia (Bang et al., 2010). Overall, the ipsilateral and contralateral analgesic effects of unilateral topical application of eugenol and carvacrol are similar to effects reported previously with topical hindpaw application of menthol (Klein et al., 2010). These bilateral analgesic effects might reflect (a) a systemic action mediated by entry of eugenol and carvacrol transdermally into the bloodstream, (b) a segmental secondary hyperalgesic effect crossing the midline, or (c) a counter-irritant effect mediated via activation of supraspinal descending antinociceptive pathways.

It is noteworthy that analgesia was observed following topical application, but not intraplantar injection, of eugenol and carvacrol. We speculate that when applied topically, eugenol and carvacrol readily accessed skin keratinocytes and/or superficial sensory nerve endings to exert their analgesic effect. The mechanism is unclear, but may involve a local anesthetic action via inhibition of voltage-gated sodium channels in sensory nerve endings (Park et al., 2006; 2009). Any role for TRPV3 in this analgesic effect is also unclear. TRPV3 is highly expressed in keratinocytes and is also expressed in sensory neurons (Peier et al., 2002; Smith et al., 2002; Xu et al., 2002; Chung et al., 2004; Moqrich et al., 2005). Consistent with this, eugenol and carvacrol activated 7–30% and FPP activated 2–5% of TG and DRG neurons (Klein et al., 2014). Eugenol and carvacrol elicited irritation that is presumably mediated by direct excitation of nerve endings expressing TRPV3, TRPA1 and/or TRPV1, possibly followed by a delayed local anesthetic effect to result in thermal analgesia. In contrast, intraplantar injection of eugenol and carvacrol did not significantly affect paw withdrawals. Conceivably, intraplantar injection deposited the agents more deeply in the skin, where they could not readily access the more superficially located keratinocytes and/or nerve endings.

Topical or intraplantar hindpaw application of neither eugenol nor carvacrol up to 30% significantly affected mechanical sensitivity, as assessed by hindpaw withdrawal thresholds elicited by innocuous mechanical stimulation using an electronic von Frey filament delivered to the plantar surface ($p>0.05$, repeated measures ANOVA, data not shown).

Citral (3,7-dimethyl-2,6-octadienal) is a fragrant terpene compound found in lemongrass and citrus fruit. Similarly to eugenol and carvacrol, citral is an agonist of TRPV3, and it is also a partial agonist of TRPM8, TRPV1 and TRPA1 (Stotz et al., 2008). Citral was also found to reduce formalin induced pain, mechanical hyperalgesia after nerve injury, and responses evoked by i.t. administration of substance P and TNFα (Nishijima et al., 2014). The former effects were reversed by i.p. administration of ketanserin, a $5HT_{2A}$ antagonist. It remains unknown if the thermal analgesia seen with eugenol and carvacrol (and other terpenoid agonists of TRPV3) is also dependent on inhibition of TRPV1/TRPA1 (Stotz et al., 2008) or serotonergic systems.

TRPV4

TRPV4 is activated by a variety of endogenous stimuli including heat, stretch and chemical mediators, but not all stimuli utilize the same intracellular pathways (Vriens et al., 2004). TRPV4 is another candidate target for pain relief since it is activated by inflammatory mediators such as arachidonic acid and anandamide (Watanabe et al., 2003). TRPV4 is co-expressed in dorsal root ganglia with substance P and CGRP, and paw swelling after injection with formalin was greatly reduced after either siRNA knockdown of TRPV4 or in TRPV4 knockout animals (Vergnolle et al., 2010). TRPV4 knockout animals also showed a dramatic reduction in acetic acid writhing 10 min after application (Suzuki et al., 2003). These data suggest that TRPV4 plays an important role in regulating inflammatory responses through the immune system, in addition to modulating nociception.

TRPV4 is also associated with innocuous warmth sensitivity in sensory neurons and keratinocytes. TRPV4 is responsive in the innocuous warming range, with a peak response at approx. 34°C (Güler et al., 2002; Watanabe et al., 2002). Knockout mice lacking TRPV4 exhibited decreased sensitivity to warming as assessed by thermal gradient (Lee et al., 2005). TRPV4 deficient mice exhibited reduced thermal nociception in carageenan-inflamed skin but not in naïve skin (Todaka et al., 2004). A more recent study has shown that TRPV3/TRPV4 double knockout mice did not have a deficit in warmth sensitivity, but exhibited reductions in nocifensive reflexes as assessed by thermal paw withdrawal and tail immersion tests (Huang et al., 2011).

Although the role of TRPV4 in heat sensation is unclear, there is much evidence to support a role for TRPV4 in detecting osmotic changes and mechanical stimuli (Mizuno et al., 2003; Liedtke et al., 2005). Compared to littermate controls, food-deprived TRPV4 knockout animals exhibited hyperosmotic blood and increased systemic osmotic pressure following challenge with systemic (i.p.) injection of hyperosmotic saline (Liedtke and Freidman, 2003). TRPV4 knockout animals also showed a reduced sensitivity to noxious pressure stimulation of the tail, while retaining normal thermal and low-threshold mechanical thresholds (Suzuki et al., 2003). The disruption of mechanosensitivity in TRPV4 deficient animals may be state-dependent, becoming more apparent under inflammatory pain

conditions. An inflammatory soup injected into the hindpaw of mice typically induced mechanical hyperalgesia, which was absent in TRPV4 knockout animals (Alessandri-Haber et al., 2006). Proteases are well-known mediators of inflammatory pain, and their established presence upon tissue injury in turn sensitizes primary afferent nerve fibers. TRPV4 is sensitized by protease activated receptor agonists to induce mechanical hyperalgesia in mice, an effect that is lost in TRPV4 knockout animals (Grant et al., 2006). Thus, TRPV4 may play an important role in sensitization of mechanical nociception.

TRPM3

TRPM3 is expressed in a variety of tissues, including small diameter sensory neurons (Vriens et al., 2011), the central nervous system (Hoffmann et al., 2010; Zaumudio-Bulcock et al., 2011) and pancreas (Thiel et al., 2013). TRPM3 is activated by steroids, including pregnenolone sulfate (Wagner et al., 2008). Intraplantar injection of pregnenolone sulfate induced nocifensive behaviors (Vriens et al., 2011) and dose-dependently increased nociceptive flexor responses in mice (Ueda et al., 2001). These nocifensive behaviors were abolished in TRPM3−/− knockout mice but not in TRPV1 or TRPA1 knockout animals (Vriens et al., 2011). TRPM3 positive neurons responded to heat (30–45°C), and most of these were capsaicin sensitive. Heat responses of TRPM3 transfected HEK293T cells were also potentiated by pregnenolone. Not surprisingly, TRPM3 knockout mice have reduced sensitivity to noxious heat, and surprisingly, noxious cold also (Vriens et al., 2011). More studies will need to be done in order to determine the role of TRPM3 under inflammatory and neuropathic pain conditions.

TRPM8

TRPM8 is found in sensory neurons, including both TG and DRG cells (Madrid et al., 2006; Reid et al., 2002). TRPM8 agonists include compounds that induce a cooling sensation when applied intraorally or topically, including menthol, linalool (from lavender oil and rosewood), geraniol (from bergamont and coriander) and the synthetic agonist icilin (Behrendt et al., 2004). Menthol induced oral irritation at high concentrations in human subjects (Cliff and Green, 1994; Klein et al., 2011). In rodents, menthol induced eye wiping behavior when delivered in high doses to the cornea (Robbins et al., 2012). Intraperitoneal injection of icilin induced nocifensive behaviors including “wet dog shakes” and writhing (Wei et al., 1976). Although TRPM8 agonists have a pleasant aroma and are used for many commercial purposes, in high doses they are capable of evoking nocifensive behaviors.

TRPM8 is activated by temperatures between 21 – 26°C in heterologous systems (McKemy et al., 2002; Nealen et al., 2003; Peier et al., 2002). Mice lacking TRPM8 exhibited decreased sensitivity to cold surfaces that are normally avoided, as assessed by temperature preference and acetone-evoked flinch tests (Bautista et al., 2007; Colburn et al., 2007; Dhaka et al., 2007). Cold avoidance and icilin sensitivity were also reduced or absent in TRPM8 conditionally ablated mice, even though these animals appeared to have normal heat and mechanical pain sensitivity (Knowlton et al., 2013). In agreement with these findings, global knockout mice lacking both TRPM8 and TRPA1 were no different than knockout mice lacking only TRPM8 in their avoidance of innocuous and noxious cold stimuli (Knowlton et al., 2010). High concentrations of topically applied menthol (40%) enhanced

cold pain in human skin (Hatem et al., 2006; Namer et al., 2005; Neddermeyer et al., 2008; Wasner et al., 2008) and oral mucosa (Green 1992; Albin et al., 2008) while inhibiting warmth sensitivity (Green, 1986). Menthol also activated a large proportion of cold sensitive TG neurons (Thut et al., 2003) and enhanced responses of cold-sensitive superficial neurons in trigeminal subnucleus caudalis (Vc) (Zanotto et al., 2007). In a temperature preference test, low menthol concentrations (<1%) significantly increased avoidance of the 15°C and 20°C surfaces versus a 30°C surface, reflecting increased sensitivity to cold (Klein et al., 2010). In rats, icilin enhanced orofacial cold avoidance (Rossi et al., 2006). Menthol interacts with TRPM8 to enhance cooling-evoked gating (Malkia et al., 2007; Rohacs et al., 2005; Voets et al., 2004) and enhanced responses of sensory neurons to cooling (Morenilla et al., 2014), properties that may explain enhanced cold sensitivity following topical application of menthol.

Topical application of menthol to the ventral hindpaw evoked a dose-dependent heat analgesia (Klein et al., 2010), consistent with previous human psychophysical studies showing menthol suppression of heat pain (Albin et al., 2008; Green, 1986; 2005; Klein et al., 2010) and capsaicin irritancy (Green and McAuliffe, 2000). Menthol suppressed nocifensive responses to noxious cold, (−5°C), as assessed by cold plate test. Menthol had a biphasic effect on innocuous cold sensitivity assessed using a two-temperature preference test, increasing cold avoidance at low concentrations and reducing cold avoidance at high concentrations (Klein et al., 2010). Similarly, high (>10%) menthol concentrations significantly decreased avoidance of 15°C and 20°C surfaces compared to a 30°C surface (Klein et al., 2010), indicating reduced aversion to the colder surface consistent with cold hypoalgesia (i.e. analgesia). The mechanism underlying menthol's analgesic effect on thermal nociception is not known, but might involve inhibition of TRPA1 or T-type calcium currents, expressed on nociceptive nerve fiber endings in the periphery (Karashima et al 2007; Macpherson et al., 2006; Swandulla et al., 1987). Menthol also activates cold receptors that may result in central inhibition of nociceptive spinal and trigeminal neurons. Menthol might additionally engage descending inhibition of spinal nociceptive neurons, consistent with the contralateral analgesic effect of menthol seen in dorsal horn neurons (Klein et al., 2012).

TRPC5

TRPC5 is an additional cold sensitive ion channel which is predominantly expressed in the central nervous system (Okada et al., 1998; Phillip et al., 1998) as well as kidney and dorsal root ganglion (Inada et al., 2006). TRPC5 activity is also potentiated by cooling (<30°C), which does not inactivate at persistently colder temperatures, unlike TRPM8 (Zimmermann et al., 2011). TRPC5 is reportedly activated by lysophospholipids (LPLs), especially lysophosphatidylcholine (LPC) and lysophosphatidylinositol (Flemming et al., 2005). Intrathecal injection of LPC evokes mechanical and thermal hyperalgesia in mice (Inoue et al., 2008). On a similar note, LPCs are known to induce demyelination and mechanical hyperalgesia in rats (Banghoo et al., 2007) and promote inflammatory skin responses in humans (Ryborg et al., 2000), perhaps through a TRPC5-dependent mechanism. It remains to be seen if other LPLs activate TRPC5 and evoke nocifensive behaviors and/or promote

cold allodynia in rodents. It also remains to be seen if TRPC5 represents another molecular target for the relief of cold hyperalgesia seen in certain peripheral nervous system diseases.

TRPA1

TRPA1 is a another ligand-gated ion channel in sensory neurons that is activated by many inflammatory mediators and pungent chemicals such as allyl isothiocynate (AITC), cinnamaldehyde (CA), formalin, acrolein, and bradykinin (Bautista et al., 2006, McNamara et al., 2007). Topical application of CA elicited a burning sensation (Namer et al., 2005) and CA and AITC induced heat hyperalgesia in human subjects (Albin et al., 2008; Namer et al., 2005; Prescott et al., 2000; Simons et al., 2003). Intraplantar formalin injection in rats elicited prolonged spontaneous paw flinching, lifting, and licking which are indicative of inflammatory pain (Chen et al., 1999). Orofacial injections of formalin or carageenan also induced nocifensive behaviors such as cheek/face rubbing in rodents (Roboisson and Dallel, 2004; Moilanen et al., 2012). Animals deficient in TRPA1 exhibited a remarkable reduction in formalin- and carageenan- induced nocifensive behaviors (McPhearson et al., 2007; Moilanen et al., 2012) indicating that TRPA1 is important for processing inflammatory pain.

Consistent with the heat hyperalgesia observed in humans, intraplantar CA (Tsagereli et al., 2009) and topical/intraplantar AITC (Takechi et al., 2013; Weng et al., 2012) induced heat hyperalgesia in rodents. Formalin also induced a delayed heat hyperalgesia in mice (Karim et al., 2006). TRPA1 is highly co-expressed with TRPV1 in sensory neurons (Story et al., 2003; Kobayashi et al., 2005). Thus, TRPA1 agonists might induce heat hyperalgesia via sensitization of cutaneous nociceptor nerve ending that co-express TRPV1. Alternatively, TRPA1 agonists might act indirectly via intradermal release of inflammatory mediators to reduce the thermal threshold of TRPV1 or other heat sensitive ion channels (Chuang et al., 2001; Suguira et al., 2002). Lastly, these agonists may additionally trigger central sensitization (Urban et al., 1991), which could explain the reduction in contralateral paw withdrawal latency after unilateral topical application of CA (Tsagereli et al., 2009).

TRPA1 was first reported to be activated by noxious cold temperatures (<18°C) (Story et al., 2003; Kwan et al., 2006; Karashima et al., 2009; del Camino et al., 2013) but this has been disputed (Jordt et al., 2004, Bautista et al., 2006). As previously mentioned, TRPA1/TRPM8 double-knockout animals did not exhibit any greater deficits in behavioral responses to cold temperatures than TRPM8 knockout mice, arguing against a role for TRPA1 in cold detection (Knowlton et al., 2010). However TRPV1 ablated (and consequently TRPA1 ablated) mice showed greater aversion to cold temperatures compared to control mice (Pogorzala et al., 2013), suggesting that TRPV1- and TRPA1-expressing sensory neurons do contribute to cold sensitivity. Consistent with this, intraplantar injection of CA in rats resulted in enhanced cold avoidance as assessed by thermal preference test (Tsagarli et al., 2009). Both CA and AITC significantly lowered withdrawal thresholds in cold plate tests (−5 to +5°C) indicative of cold hyperalgesia (Tsagareli et al., 2009; Nozadze et al., 2014). However, neither AITC nor CA significantly affected responses of rat wide dynamic range or nociceptive specific spinal cord dorsal horn neurons to cooling of hindpaw skin (Merrill et al., 2008; Sawyer et al., 2009). Moreover, both AITC and CA induced a brief cold hyperalgesia on the tongue in human subjects (Albin et al., 2008). Additional explanations

for the discrepancies in the involvement of TRPA1 in thermal and mechanical pain include physiological factors such as stress, cellular damage or infection (da Costa et al., 2010; Moilanen et al., 2012; Meseguer et al., 2014).

Since its discovery, TRPA1 has been implicated in mechanotransduction pathways (Corey et al., 2004; Kwan et al., 2009). Slowly adapting currents evoked by mechanical pressure stimuli applied to the membrane of DRG cells were absent in a population of small diameter neurons after either TRPA1 deletion or antagonism (Vilceanu and Stucky, 2010). A separate study indicated that TRPA1 function is essential for intermediately adapting currents in DRG cells; these are the most abundant type of currents present in nociceptors (Brierly et al., 2011). Pharmacological blockade of TRPA1 decreased formalin- and mechanically-evoked responses in C fibers (Kerstein et al., 2009.) These data imply that TRPA1 is essential for determining the duration and/or intensity of sensory neuronal responses to mechanical stimulation, factors that are crucial for encoding mechanical pain. Topical application of AITC or CA induced mechanical allodynia in humans (Koltzenburg et al., 1992; Namer et al., 2005). Using rodent models, intraplantar injection of TRPA1 agonists reduced mechanical paw withdrawal thresholds (Trevisani, et al., 2007; Chen et al., 1999). The TRPA1 antagonists HC-030031 and AP18 both attenuated mechanical hyperalgesia in inflammatory (e.g., CFA) and nerve injury models (Eid et al., 2008; Petrus et al., 2007). Thus, TRPA1 may contribute to mechanical hyperalgesia in addition to acute mechanical pain.

Conclusions and Implications

At least eight (and possibly more) thermosensitive ion channels contribute to thermoreception, mechanoreception and nociception across a wide range of environmental stimuli. Future studies will continue to investigate the biophysical mechanisms of TRP channel gating by thermal, chemical and mechanical stimuli (Latorre et al., 2007). The role of thermosensitive TRP channels in pain sensation is of particular interest (Julius, 2013), since TRP channel agonists/antagonists present interesting new targets for the development of novel analgesics to treat chronic pain (Nilius et al., 2007; Patapoutian et al., 2009; Brenderson et al., 2013).

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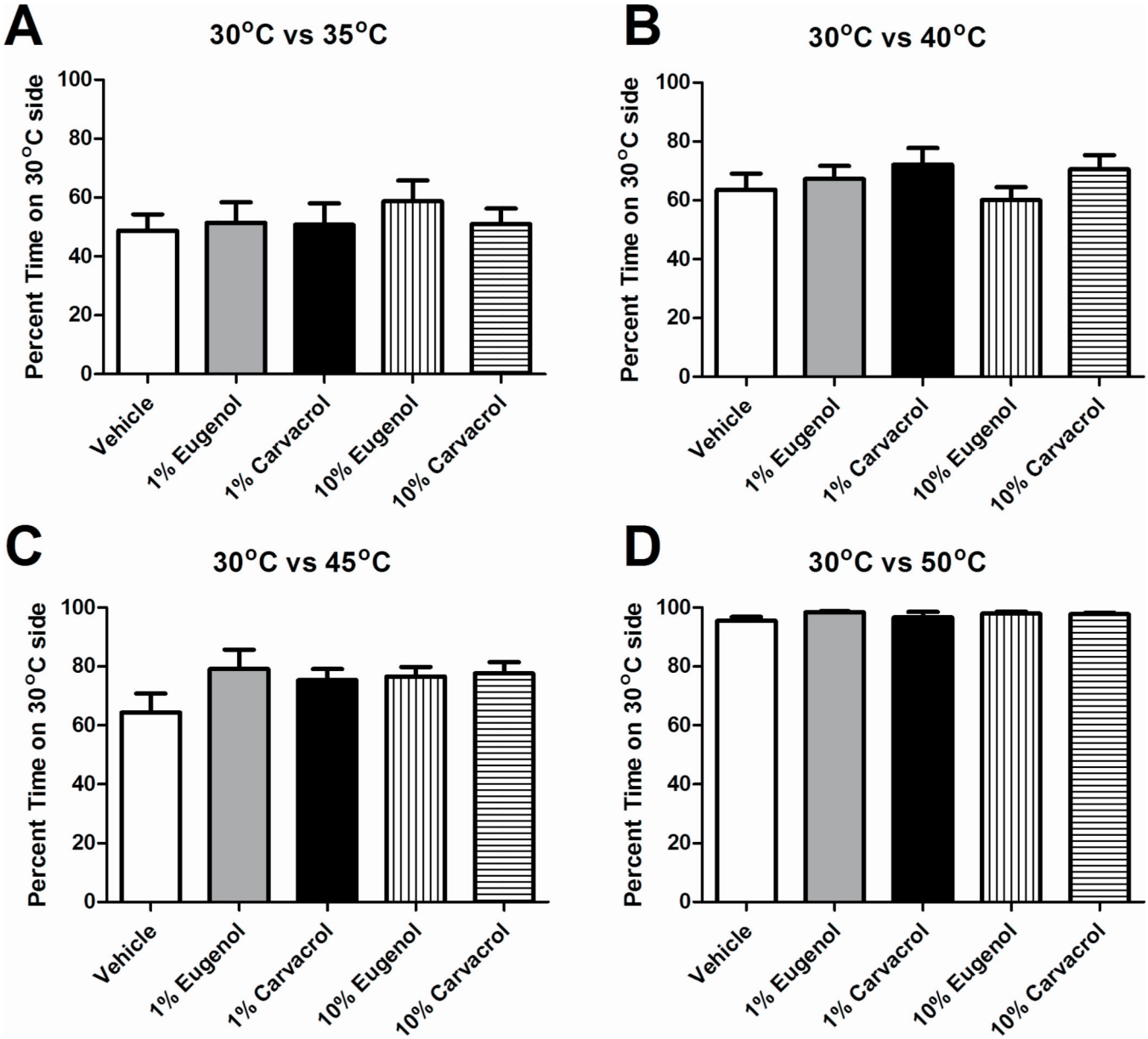

Figure 1.

Eugenol and carvacrol effects on thermal preference behavior. A: Graph plots the mean percent time that rats occupied each of two adjacent thermoelectric plates, one set at 30 and the other set at 35°C. Rats received topical application to both ventral hindpaws of vehicle, or a low (1%) or high (10%) concentration of eugenol or carvacrol. B: As in A for 30 vs 40°C temperature difference. C. As in A for 30 vs 45°C temperature difference. D. As in A for 30 vs 50°C temperature difference. Error bars: SEM; n=16/group. There were no significant differences between vehicle and eugenol or carvacrol treated animals for any temperature difference ($p>0.05$, ANOVA).

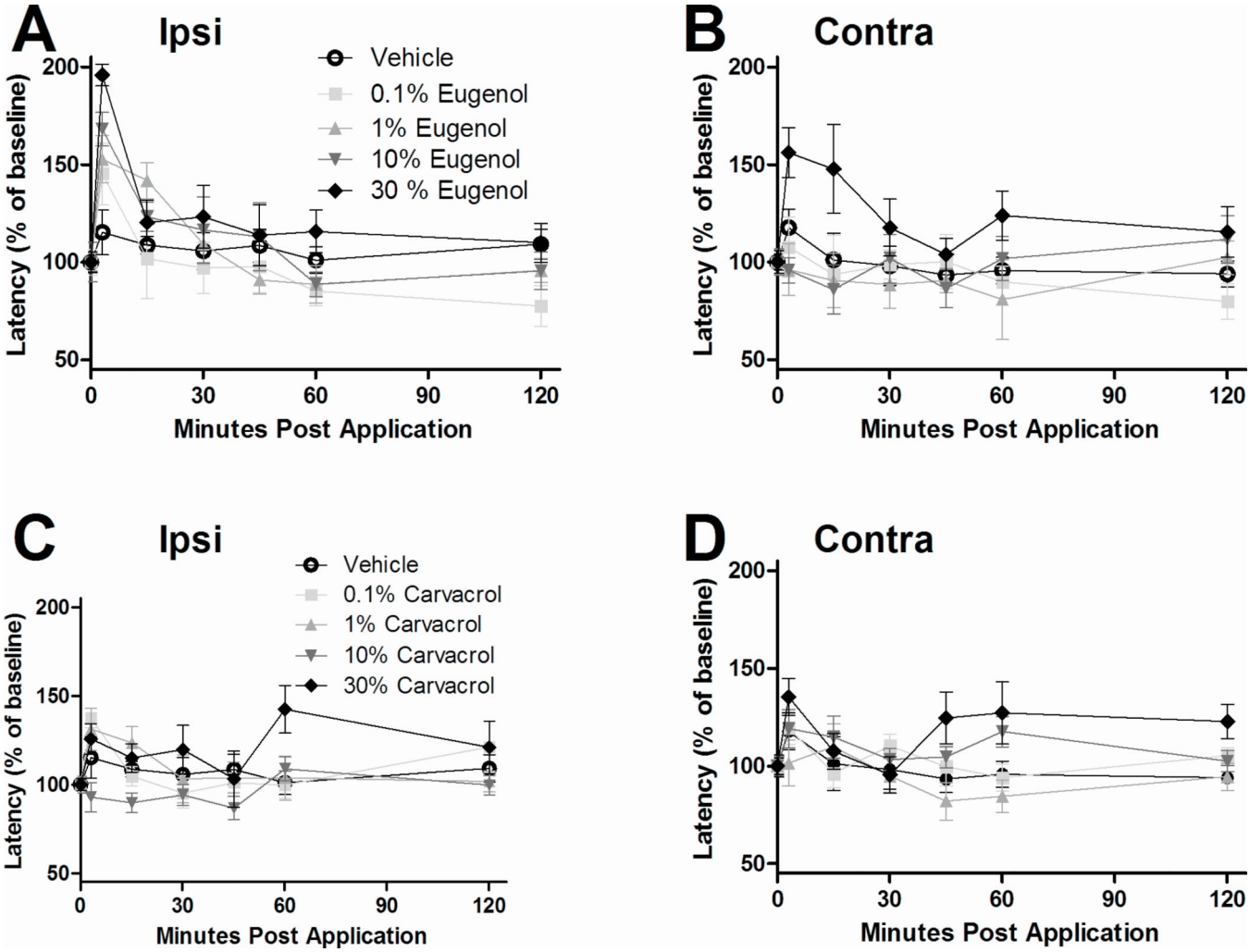

Figure 2.

Concentration-dependent analgesic effects of topically-applied eugenol and carvacrol on thermal hindpaw withdrawal latency (Hargreaves test). A: Eugenol, ipsilateral hindpaw. Eugenol treatment resulted in a concentration-dependent increase in withdrawal latency (analgesia). Thirty percent eugenol was significantly different from 0.1% eugenol ($p<0.05$, repeated-measures ANOVA). B: Eugenol, contralateral hindpaw. The 30% eugenol treatment was different from all other concentrations ($p<0.05$, repeated-measures ANOVA). C: Carvacrol, ipsilateral hindpaw. The 30% carvacrol treatment group was significantly different from 10% carvacrol group ($p<0.05$, repeated-measures ANOVA). Error bars: SEM; n=8/group. D: Carvacrol, contralateral hindpaw, The 30% carvacrol treatment group was significantly different from all other concentrations ($p<0.05$, repeated-measures ANOVA). Error bars: SEM; n=8/all groups.