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RESEARCH ARTICLE

Nociceptive Modulation by Inactivation of TRPA1 and TRPV1 Channels

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ABSTRACT

Background: Pain and itch are unpleasant sensations specifying actual or potential tissue damage. Pain is a necessary alert signal that sets off protective responses, while itch mainly provokes the desire to scratch. Painful or itching stimulation applied to the peripheral receptors evokes a cascade of nociceptive signaling in ascending somatosensory pathways. Transient receptor potential (TRPA1 and TRPV1) channels are involved in pain and itch sensations, making them attractive targets for therapeutics.

Aims: We aimed to investigate pungent nociceptive substances, cinnamon aldehyde (CA), allyl isothiocyanate (AITC, the main compound of mustard oil), and capsaicin (CAPS) from chili peppers, which elicit thermal hyperalgesia and mechanical allodynia in mice, and studied the potential roles of TRP channels in pain modulation.

Methods: We measured nociceptive thermal paw withdrawal latencies (Hargreaves test) and mechanical thresholds (electronic von Frey test) bilaterally in mice at various time points following intraplantar applications of AITC, CA, and CAPS, which produced thermal hyperalgesia and mechanical allodynia.

Results: When pretreated with the TRPA1 antagonist (HC-030031), we found a significant reduction of these pain behavior responses to AITC and CA applications. Pretreatment with the TRPV1 antagonist (capsazepine) produced significant attenuation of thermal hyperalgesia and mechanical allodynia evoked by two doses of CAPS. We thus showed that noxious chemical irritants eliciting thermal hyperalgesia and mechanical allodynia are mediated via the activation of TRPA1 and TRPV1 cation channels.

Conclusion: The discovery of TRP channel antagonists, particularly dual antagonists that simultaneously target both TRPA1/TRPV1 channels, represents a new and promising therapeutic approach for pain treatment.

Keywords: allodynia, antinociception, hyperalgesia, mechanical withdrawal, thermal withdrawal, pain.

Introduction

Pain and itch (pruritus) are unpleasant sensations that indicate actual or potential tissue damage. Pain is a necessary alert signal that sets off protective responses, while itch mainly provokes the desire to scratch. Both are multifaceted or multidimensional experiences. However, pain and itch usually perform an important biological function for the body in normal states. For pruritus, the general discourse mainly deals with histaminergic and nonhistaminergic itch[1-3].

In the normal state, painful or itching stimulation applied to the peripheral receptors evokes a cascade of nociceptive signaling in ascending somatosensory pathways, which culminate in a distributed multidimensional brain representation that is required to localize, interpret, modulate, and appropriately respond to the stimulus[4].

It is well known that most cellular functions are regulated by the influx of different ions across the cell membrane through selective or nonselective channels. These channel gene families demarcate their important role in mammalian physiology. The transient receptor potential (TRP) channel superfamily is distinct in that these channels are nonselective for cations (mainly for sodium and calcium) and involved in a diverse array of biological functions, making them attractive targets for therapeutics[5-7].

TRP channels regulate many functions, among them sensory perception, pain and itch, immunity, digestion, cancer, development, urological, cardiovascular, and nervous systems[6]. Moreover, these channels are widely distributed in various tissue membranes, and mutations in TRP channels are observed in several different diseases, such as hereditary diseases and many other TRP channelopathies[8].

TRP ankyrin 1 (TRPA1) is a calcium-permeable channel that is co-expressed on a subpopulation of TRPV1-expressing nociceptive nerve fibers' terminals and distal endings of mechanosensitive C-fibers, contributing to the transduction of noxious signals[9,10]. This channel is considered to detect non-histaminergic itch and cold hypersensitivity (noxious cold). Various irritant chemicals and endogenous products of tissue injury activate TRPA1. Among them are pungent compounds in some spices such as cinnamon, mustard oil, wasabi, as well as chloroquine, acrolein, formalin, cannabinoids, and various reactive oxygen, nitrogen, and carbonyl species[11-12].

TRP vanilloid 1 (TRPV1) is an ion channel activated by capsaicin and heat, and is a prominent nociceptor in C-fiber sensory neurons involved in pain and itch pathways. It has become the first family member with a postulated and subsequently verified link to pain and itch, with increasing evidence that considers this protein receptor as a hub for nociceptive (algesic) and pruriceptive (pruritogenic) signals. Like TRPA1, TRPV1 is a nonselective cation channel with high calcium permeability that is activated by endogenous factors such as acids (pH < 6), temperature (> 42°C), and by natural vanilloids capsaicin (CAPS) and resiniferatoxin (RFT). Upon stimulation, receptor activation leads to excitation of primary sensory neurons and ultimately central perception of harmful signals such as pain, itching, burning, and stinging sensations. Its pivotal role in pain and itch pathways has attracted much attention as a therapeutic target[13-14].

We have recently discovered a significant thermal hyperalgesia and mechanical allodynia induced by histamine as well as the non-histaminergic pruritogens chloroquine (CQ), bovine adrenal medulla peptide (BAM8-22), and the tethered peptide Ser-Leu-Ile-Gly-Arg-Leu (SLIGRL). Histamine-evoked thermal hyperalgesia and mechanical allodynia were prevented by pretreatment with a TRPV1 channel antagonist (AMG-517) but not a TRPA1 antagonist (HC-030031), indicating that histaminergic hyperalgesia and allodynia require TRPV1 but not TRPA1. In contrast, thermal hyperalgesia and mechanical allodynia induced by CQ, BAM8-22, and SLIGRL were significantly attenuated or prevented by the TRPA1 antagonist, implying a critical role for the TRPA1 channel[15].

We have presently addressed the question of whether pungent nociceptive substances, including cinnamon aldehyde (CA), allyl isothiocyanate (AITC, the main compound of mustard oil), and capsaicin (CAPS) from chili peppers, elicit thermal hyperalgesia and mechanical allodynia in mice. We have additionally studied the potential roles of TRP channels in pain modulation. In particular, we investigated modulation in mediating hyperalgesia and allodynia evoked by CA and AITC via the TRPA1 channel, as well as via the TRPV1 channel in nociception evoked by CAPS using the TRPA1 antagonist (HC-030031) and TRPV1 antagonist capsazepine (CAPSAZ), respectively.

Materials and Methods

ANIMALS. The experiments were conducted in male C57BL/6J mice purchased from Jackson Laboratories (Bar Harbor, ME, USA), with a body weight of < 50 g. A breeding colony has been established at our laboratory vivarium in Tbilisi. The animals were kept under standard housing conditions (22±2 ℃, 65% humidity, lights from 6:00 a.m. to 8:00 p.m.), and fed a standard dry diet; water was freely available. All experiments are performed using protocols approved by the local Bioethics Committee of Beritashvili Exp BMC (BCA-17/23). All behavioral tests were performed from 10 AM to 3 PM in the light cycle. All mice are acclimated for 30 to 60 min to their testing environment two days before behavioral tests. In vivo experiments were carried out according to European Union guidelines (EU Directive application 2010/63/EU) and the guidelines of the International Association for the Study of Pain (IASP) regarding investigations of experimental pain in conscious animals[16]. Every attempt was made to follow ARRIVE guidelines (https://arriveguidelines.org/).

CHEMICALS. CA at a concentration of 20%, AITC at a dose of 15%, and TRPA1 antagonist (HC-030013) were purchased from Sigma-Aldrich Chemicals (St. Louis, MO, USA). The TRPV1 channel agonist CAPS (0.2% and 0.4%) and its antagonist capsazepine (CAPSAZ) were purchased from Hello Bio Inc. (Princeton, NJ, USA). HC-030031 was dissolved in 30 µL 1% DMSO and saline to obtain a final dose of 100 µg/30 µL; CAPSAZ was dissolved in 30 µL 1% DMSO and saline to obtain a final dose of 5 µg/30 µL, consistent with our previous studies[15,17-20].

TREATMENT PROTOCOLS. Each chemical was applied to the plantar surface of the hind paw of mice (C57BL/6J), separated by at least 5-7 days. We used the plantar glabrous skin route to assess thermal hyperalgesia and mechanical allodynia on the plantar hindpaw, consistent with previous studies[15]. The same volume of vehicle (1% DMSO in isotonic saline) was applied in the same manner, separately as a control. Mice were divided into groups with n = 6 per group. Each group received two supra-plantar applications in a volume of 2 µL, of either saline or one of the three nociceptive agents (CA, AITC), and two doses of CAPS, requiring 24 mice per substance. Successive applications were separated by at least 7 days. Following the application, the mouse was tested bilaterally in either the thermal withdrawal (Hargreaves) test or the mechanical paw withdrawal (electronic von Frey) test using a counterbalanced design. Immediately following the applications, we observed that many mice exhibited biting and licking behaviors directed at the applied hindpaw.

In the next set of experiments, the effects of pretreatment with TRPA1 antagonist HC-030031or the TRPV1 antagonist CAPSAZ, applied at the same plantar surface, were tested to study the latency of thermal or threshold of mechanical withdrawals elicited by supraplantar application of each of the three nociceptive substances. The mice were again divided into groups of 6. Groups of mice received application of either HC-030031 or CAPSAZ, and then, 15 minutes later, one of the three drugs: CA and AITC, or CAPS, respectively. This procedure was done twice for each mouse, once for either the Hargreaves or the von Frey test, and again at least 7 days later for the other test.

BEHAVIORAL TESTS. Before formal testing, the baselines were assessed for mice in the experimental and control groups in thermal and mechanical withdrawal tests, averaging multiple (three times) baseline measurements for the left and right hind paws, with 5-minute intervals between tests. Behavioral tests were conducted starting immediately after plantar application of irritants or antagonists. In prior studies, each irritant chemical tested elicits pain-related wiping behavior. The thermal and mechanical paw withdrawal tests were conducted during this period out to 120 min post-injection

Thermal paw withdrawal (Hargreaves) test. Mice were first habituated to stand on a glass surface heated to 30°C within a Plexiglas enclosure, over three separate daily sessions. A light beam (Plantar Test 390, IITC, Woodland Hills, CA, USA) was focused onto the plantar surface of one hind paw through the glass plate from below, and the latency from onset of the light to brisk withdrawal of the stimulated paw was measured. The other hind paw was similarly tested 30-60 seconds later. The mouse was then held gently, and one hind paw received a plantar application of chemicals or vehicle. The mouse was then placed back onto the glass plate, and withdrawal latencies of both paws were measured at 5, 15, 30, 45, 60, and 120 min

post-application. Reductions in latency were considered to reflect thermal hyperalgesia.

Mechanical paw withdrawal threshold (von Frey) test. Mice were first habituated to standing on the mash stand surface. For formal testing, baseline withdrawals were assessed using an Electronic von Frey Esthesiometer (2390, IITC, CA, USA) filament that was pressed against the ventral paw from below. This device samples and holds force (g) at the moment that the hind paw is withdrawn away from the filament. Each paw was tested for baseline mechanical withdrawals at least three times, with at least 5 min elapsing between successive measurements of a given paw. The mouse then received a unilateral plantar application and was placed back onto the mesh stand surface. Mechanical paw withdrawals were measured at the same post-application times as above for thermal paw withdrawals. The same groups of mice were used for thermal and mechanical withdrawal tests, with a minimum of 7 days in between successive tests to avoid possible carryover effects of stimuli.

STATISTICAL ANALYSIS. All data from behavioral tests were subjected to one-way analysis of variance (ANOVA) or repeated measures analysis of variance (rMANOVA) and then were compared between chemicals and vehicle treatment groups, or irritants and antagonists injected groups by paired *t*-test (Dunnett or Tukey-Kramer multiple comparison tests). The data are expressed as mean ± s.e.m. Statistical significance is acknowledged if $P < 0.05$. The statistical software utilized was InStat 3.05 (GraphPad Software, Inc., San Diego, CA, USA).

Results

ALLYL ISOTHIOCYANATE. In the first set of experiments, we found that plantar application of AITC resulted in a significant reduction in the ipsilateral thermal paw withdrawal latency (Hargreaves test) compared to vehicle control, returning to the base control level at 120 min (Figure 1A).

Statistical analyses by rMANOVA reveal significant differences between AITC-treated and vehicle-treated hindpaws for the thermal latency test ($F = 29.328$, $P < 0.0001$) and mechanical threshold test ($F = 43.557$, $P < 0.0001$). For the mechanical paw withdrawal threshold (von Frey test), similar significant differences were observed after 15 min post-AITC application ($p < 0.001$ for each) except at 120 min, showing recovery (Figure 1C). In the contralateral (AITC untreated) paw, there was a tendency toward a weaker mirror image effect of thermal hyperalgesia and mechanical allodynia induced by AITC (Figure 1B, D).

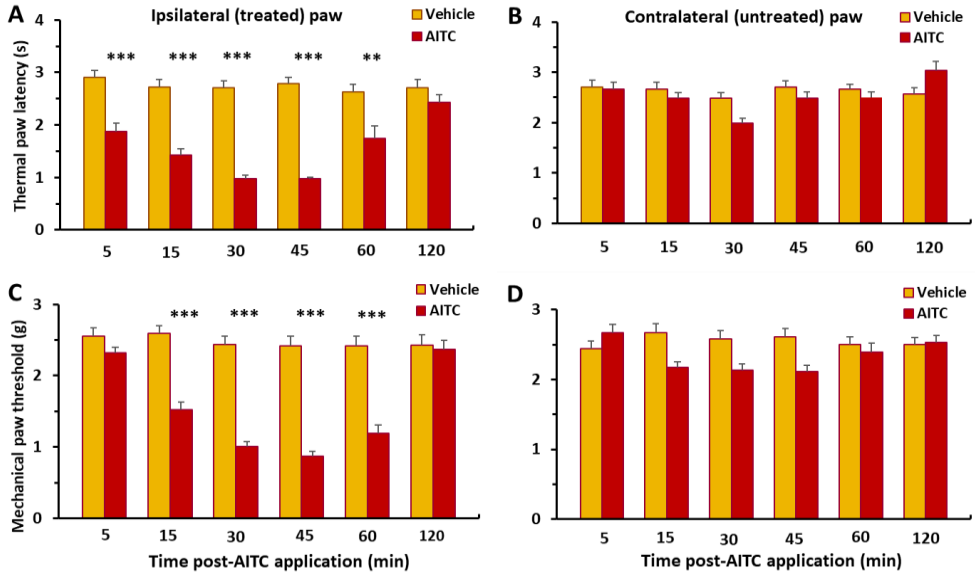

Figure 1. Plantar application on the ipsilateral paw of AITC results in a significant decrease of the thermal paw latency (A) and mechanical paw threshold (C) ($P < 0.001$) (test paw, red bars; control paw, yellow bars), i.e., development of thermal hyperalgesia and mechanical allodynia, respectively. Similar trends of weaker mirror image effects are observed for the contralateral paw (B, D). n = 6 mice/group. Note: here and subsequent figures, P values - * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Concerning the comparison between the ipsilateral, AITC-treated paw and contralateral (untreated) paw, statistical analyses revealed significant differences for the thermal test (rMANOVA, $F = 27.115$, $P < 0.0001$) and mechanical test (rMANOVA, $F = 42.669$, $P < 0.0001$) in the 60-minute post-application period. In particular, these differences were highest at the 45-minute point

post-AITC application for the thermal test (t = 11.783, P < 0.001) and mechanical test (t = 13.011, P < 0.001), respectively (Figure 2A and 2B). Here, we also observed weaker mirror-image effects of thermal hyperalgesia and mechanical allodynia in the contralateral paw (yellow lines) induced by AITC applied to the ipsilateral paw.

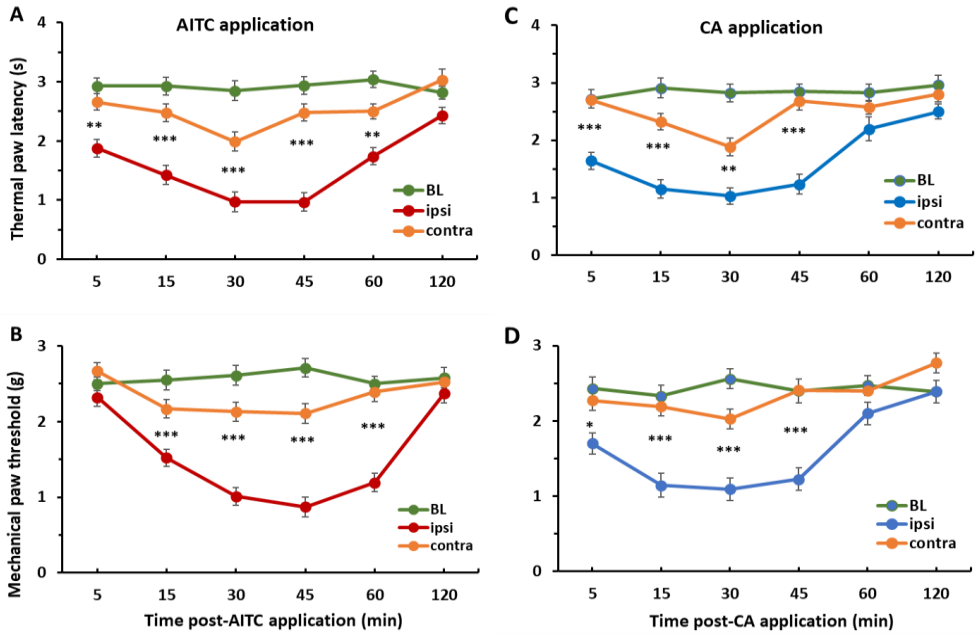

Figure 2. Plantar application of AITC on the ipsilateral paw resulted in a significant decrease of the thermal paw latency (red line) compared to the contralateral, untreated paw (yellow line) (A) and of the mechanical paw threshold (B). The same effects were observed for CA application on the ipsilateral paw (blue line) compared to the contralateral paw (yellow line) for the thermal test (C) and mechanical test (D), respectively. Following the AITC and CA application, we observed weak mirror-image effects of hyperalgesia and allodynia. Asterisks indicate significant differences between ipsi- and contralateral paws. Note: green lines indicate baseline values of intact untreated mice for both paws, sample size n = 12.

Furthermore, the TRPA1 channel antagonist HC-030031 applied to the same ipsilateral hindpaw 15 min prior AITC application attenuated thermal hyperalgesia and mechanical allodynia produced by AITC for the thermal paw withdrawal test (F = 1.568, P = 0.1166, not significant) and mechanical paw withdrawal test (F = 0.5920, P = 0.8324, not significant), respectively (Figure 3A and 3B). Thus, differences between the values of both pain behavior parameters for the ipsilateral, treated paw and contralateral, untreated paw are not significant.

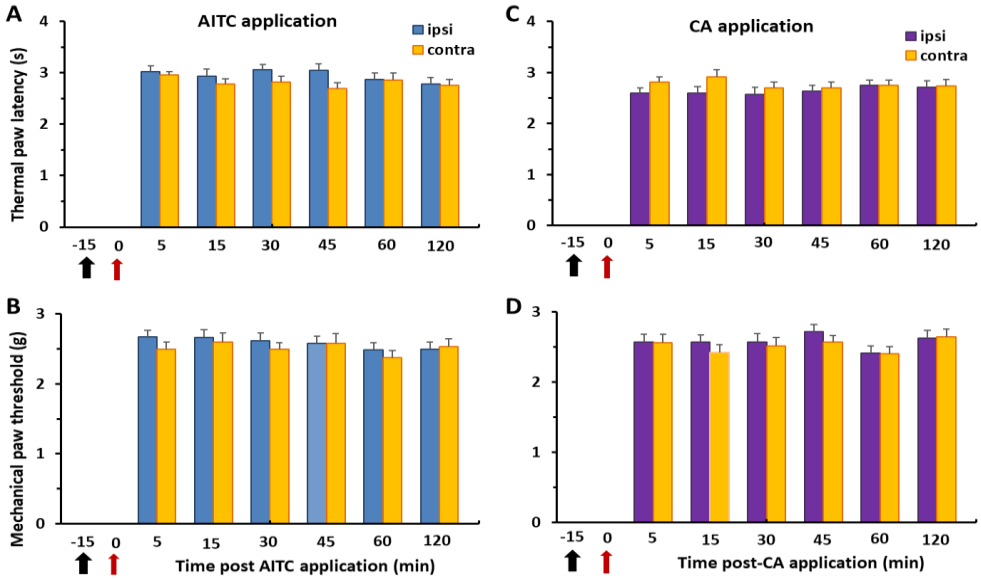

Figure 3. Pretreatment with the TRPA1 antagonist HC-030031 in the ipsilateral chemicals-treated paw (blue bars) attenuates hyperalgesia and allodynia compared to the contralateral (non-treated) paw (yellow bars) for both the thermal test (A) and mechanical test (B). The same effects were observed for CA application on the ipsilateral paw (purple bars) compared to the contralateral paw (yellow bars) for the thermal test (C) and mechanical test (D), respectively. Note: the bold black arrows indicate the time of injection of HC-030031, the thin red arrow indicates the time of injection of AITC or CA.

CINNAMON ALDEHYDE. In the second set of experiments carried out with the second TRPA1 channel agonist CA, we found similar effects of thermal hyperalgesia and mechanical allodynia in the ipsilateral, CA-treated hindpaw. Figure 4 shows significant reductions of thermal paw latency (Figure 4A) and mechanical threshold (Figure 4B) compare to vehicle control groups (rMANOVA, F = 23.748, P < 0.0001) with a maximum effect at the 30 min time point post-CA application for paw withdrawal latency (t = 9.735, P < 0.001) and mechanical threshold (t = 8.314, P < 0.001), respectively. For the contralateral paw, we again observed weaker mirror image effects for both thermal latency (Figure 4C) and mechanical threshold (Figure 4D), respectively.

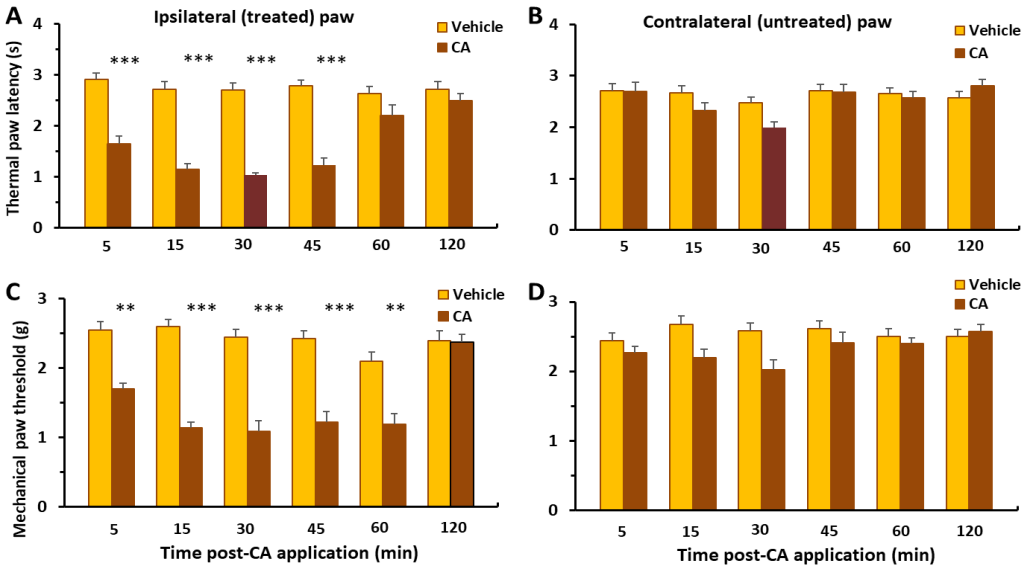

Figure 4. Plantar application of CA onto the ipsilateral paw results in significant decreases of the thermal paw latency (A) and mechanical paw threshold (C) (P < 0.001) (test paw, brown bars; control paw, yellow bars), i.e., development of thermal hyperalgesia and mechanical allodynia, respectively. Similar but weaker mirror image effects were observed for the contralateral paw (B, D).

Statistical comparisons between the ipsilateral, CA-treated paw and contralateral (non-treated) paw, revealed significant differences for the thermal latency test (F = 20.439, P < 0.0001) and mechanical threshold test (F = 22.457, P < 0.0001). These differences between ipsi- and contralateral paws were highest at 45 min post-CA application for the thermal test (t = 9.762, P < 0.001) and for the mechanical test (t = 10.078, P < 0.001), respectively (blue lines) (Figure 2C and 2D). We also observed weaker mirror image effects of thermal hyperalgesia and mechanical allodynia in the contralateral paw (yellow lines) induced by CA applied to the ipsilateral paw.

Moreover, pretreatment with the TRPA1 antagonist HC-030031 in the ipsilateral CA-treated paw (Figure 3) (purple bars) prevented thermal hyperalgesia (F = 0.8136, P = 0.6265, not significant) and mechanical allodynia (F = 0.8897, P = 0.5525, not significant) compared to contralateral (non-treated) paw (yellow bars) for both thermal (Figure 3C) and mechanical tests (Figure 3D).

CAPSAICIN. In the third set of experiments, plantar application of two doses of CAPS (0.2% and 0.4%) resulted in thermal hyperalgesia (ANOVA, F = 44.584, P < 0.0001) that persisted out to 45 minutes (Figure 5A). For thermal withdrawals (Hargreaves test), both CAPS-treated groups of mice were significantly different from the vehicle-treated group. For the higher dose of CAPS, differences were significant at 5 min (t =16.246, P < 0.001), 15 min (t = 17.722, P < 0.001), 30 min (t = 19.353, P < 0.001), 45 min (t = 14.354, P < 0.001), and 60 min (t = 6.408, P < 0.01), but not at 120 min (P > 0.05) post-CAPS application. Interestingly, statistically significant differences were observed between the two dose groups of mice within the first 45 minutes (Figure 5A). There were mirror-image effects of hyperalgesia on the contralateral (untreated) hindpaw, with significant differences for the higher concentration of CAPS at 5-45 minutes post-CAPS application compared with the vehicle group (P < 0.001) (Figure 5B).

Similarly, for mechanical withdrawals of the ipsilateral (treated) hindpaw (von Frey test), the CAPS-treated groups were significantly different from the vehicle group (ANOVA, F = 58.673, P < 0.0001), indicating clear allodynia, especially for

the high CAPS concentration (Figure 5C). In particular, these differences between vehicle and high CAPS dose were significant in 5 min (t = 18.408, P < 0.001), 15 min (t = 20.469, P < 0.001), 30 min (t = 19.966, P < 0.001), 45 min (t = 17.261, P < 0.001), and 60 min (t = 10.885, P < 0.05) post-CAPS application. For the contralateral (untreated) hindpaw, there were weaker mirror-image allodynia effects, especially for the high dose of CAPS (Figure 5D).

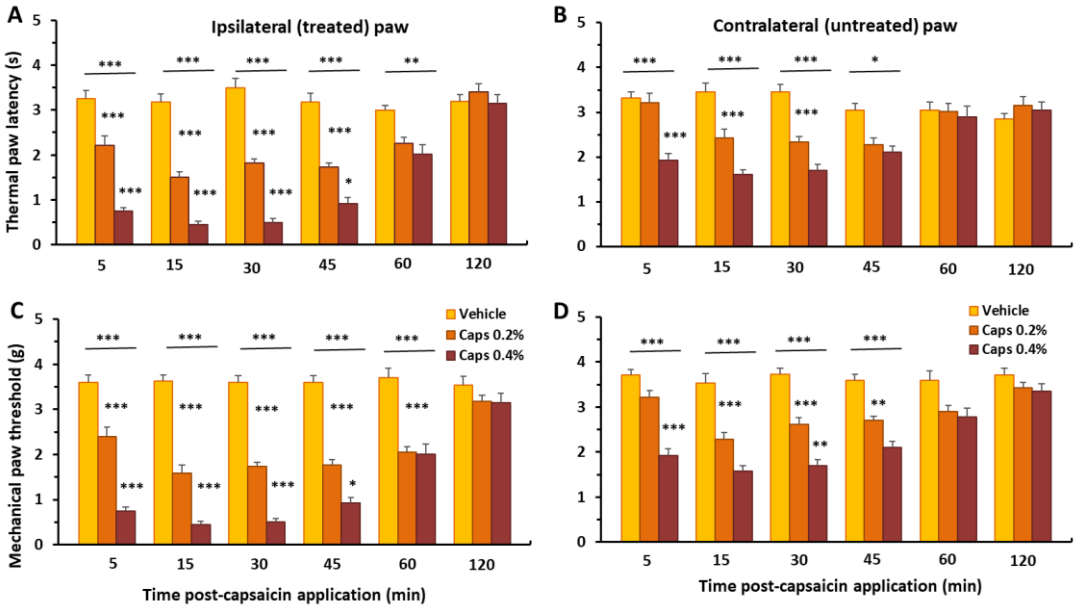

Figure 5. Plantar application onto the ipsilateral paw of two doses of CAPS resulted in significant dose-dependent decreases of the thermal paw latency (A) and mechanical paw threshold (C) (P < 0.001) (test paw, light and dark brown bars; control paw, yellow bars), i.e., development of thermal hyperalgesia and mechanical allodynia, respectively. Similar but weaker mirror image effects were observed for the contralateral paw (B, D). Note: asterisks on horizontal lines indicate significant differences between the vehicle and high doses of CAPS (0.4%). Other asterisks indicate significant differences between groups of vehicle and low doses of CAPS and between the two, low doses and high doses of CAPS.

Concerning the comparison between the ipsilateral, CAPS-treated paw and the contralateral (untreated) paw, statistical analyses revealed significant differences for the thermal test (for 0.2% CAPS, rMANOVA, F = 26.61, P < 0.0001; for 0.4% CAPS, rMANOVA, F = 47.56, P < 0.0001). These differences are greatest for the high CAPS concentration at 5 min (t = 8.269, P < 0.001) and at 30 min (t = 8.058, P < 0.001) of post-CAPS applications (Figure 6A). Similar results were obtained for the mechanical test (for 0.2% CAPS, rMANOVA, F = 19.602, P < 0.0001; for 0.4% CAPS, rMANOVA, F = 47.051, P < 0.0001). Differences between contralateral and ipsilateral (0.4% CAPS) paw were highest at 5 min (t = 8.263, P < 0.001), 30 min (t = 8.245, P < 0.001), and 45 min (t = 8.015, P < 0.001) of post-CAPS applications (Figure 6B). Here, we also observed weak mirror-image effects of thermal hyperalgesia and mechanical allodynia in the contralateral (untreated) paw (yellow lines).

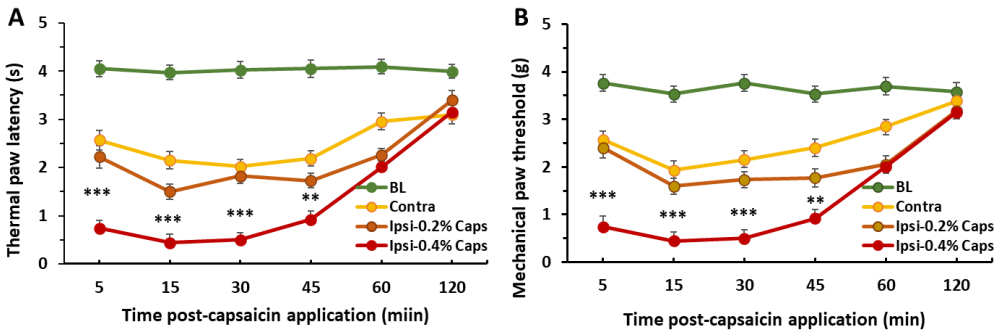

Figure 6. Plantar applications on the ipsilateral paw of two CAPS concentrations result in a significant decrease of the thermal paw withdrawal latency (brawn and red lines) compared to the contralateral, untreated paw (yellow line) (A), as well as a reduction of the mechanical paw threshold (B). Significant mirror-image effects were also observed between the contralateral (untreated) paw (yellow lines in A, B) and the baseline group (BL) (green lines) (P < 0.001). Note: here, contralateral (untreated) paw values express average data of 0.2% and 0.4% CAPS applied to ipsilateral (treated) paws. Contralateral group values express the average data of both paws and both doses of CAPS, sample size n = 24. Asterisks indicate significant differences between the two CAPS concentrations. Note: green lines indicate baseline values of intact untreated mice for both paws, sample size n = 24.

Finally, we found that the TRPV1 channel antagonist capsazepine (CAPSAZ) applied to the same ipsilateral hindpaw 15 min before CAPS application attenuated thermal hyperalgesia and mechanical allodynia produced by both CAPS concentrations. Reductions were not statistically significant for the thermal paw withdrawal test (0.2% CAPS, rMANOVA, F = 1.443, P = 0.1625, not significant; 0.4% CAPS, rMANOVA, F = 0.7064, P = 0.7305, not significant), and for the mechanical paw withdrawal test (0.2% CAPS, F = 1.306, P = 0.2289, not significant; 0.4% CAPS, rMANOVA, F = 0.7599, P = 0.679, not significant), respectively. These data show that there are no significant differences between the values of both doses of CAPS for thermal (Figure 7A, B) and mechanical pain behavior indices (Figure C, D) for the ipsilateral (treated) paw and the contralateral (untreated) paw.

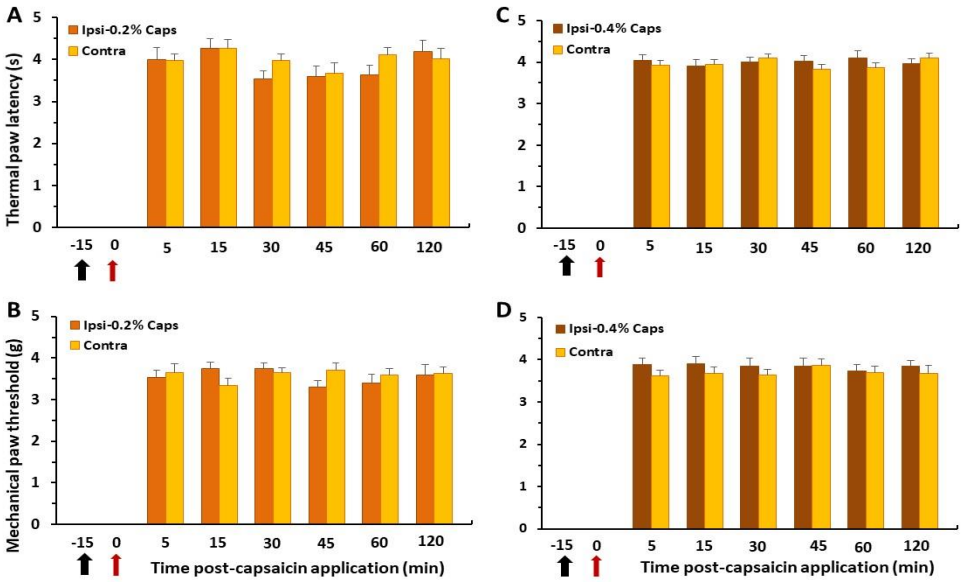

Figure 7. Pretreatment with the TRPV1 antagonist CAPSAZ prevents thermal hyperalgesia and mechanical allodynia elicited by two doses of CAPS. There were no significant effects of application of two different doses of CAPS on thermal paw withdrawal latencies (A, C) or mechanical withdrawal thresholds (B, D). CAPSAZ in the ipsilateral two (0.2% and 0.4%) concentrations of capsaicin-treated paw (light and dark brown bars) attenuates hyperalgesia and allodynia compared to the contralateral (untreated) paw (yellow bars) for both thermal test (A, C) and mechanical (B, D) test. The bold black arrow indicates the time of injection of capsazepine, and the thin red arrow indicates the time of injection of capsaicin.

Discussion

Following plantar application of AITC, CA, and CAPS, there was a decrease in the latency of the paw withdrawal reflex to thermal stimuli and a reduction in mechanically-evoked paw withdrawal, thereby indicating thermal hyperalgesia and mechanical allodynia. The thermal hyperalgesia and mechanical allodynia induced by CA and AITC were significantly attenuated or prevented by the TRPA1 antagonist (HC-030013), implying a critical role for the TRPA1 channel. On the contrary, CAPS-evoked thermal hyperalgesia and mechanical allodynia were completely prevented by pretreatment with a TRPV1 antagonist, capsazepine, indicating the requirement of a TRPV1 channel.

These results confirm our previous findings that TRP channel agonists CA, AITC, and CAPS, but not menthol, elicit thermal hyperalgesia and mechanical allodynia expressed in withdrawal pain behavior in rats [18]. Concerning menthol in our previous experiments, its application to the rat's plantar surface resulted in hypoalgesia, thereby showing antinociceptive effects[21].

Recently, we have found thermal hyperalgesia and mechanical allodynia induced by non-histaminergic pruriceptor (itch receptors) agonists, chloroquine (CQ), BAM8-22, and SLIGRL in mice that were significantly attenuated by the TRPA1 antagonist (HC-030013). Histamine also elicited thermal hyperalgesia and mechanical allodynia, which, however, was reduced by the TRPV1 channel antagonist (AMG-517) but not the TRPA1 antagonist, implying a critical role for TRPV1 but not TRPA1 in histaminergic itch[15,20]. These data confirm the important role of TRP channels in pain and itch sensations and their involvement in processes of hyperalgesia and allodynia.

In recent years, due to the key role of TRP protein channels in pain and itch signaling, the development of clinically useful antagonists for these target receptor modulators has intensified. In particular, TRPA1 and TRPV1 offer attractive targets to combat pain and itch sensations. A wide range of experimental data using animal models suggests the therapeutic potential of TRPA1 antagonists, including HC-030031, A-967079, AMG0902, and GRC-17536, among others. Recent findings have revealed that GDC-0334 possesses better target engagement in human subjects[22].

Another novel selective antagonist of TRPA1 ion channels, LY3526318, was orally administered to patients with knee osteoarthritis, chronic low back pain, and diabetic peripheral neuropathy. It had a safety profile in the phase 1 clinical trials that was acceptable for further clinical development[23].

The TRPV1 agonists, MRD-652 and olvanil (NE19550), exhibited promising results in animal models of pain; however, their clinical manifestations are yet to be studied[24]. Resiniferatoxin, a capsaicin analog, is undergoing phase 1 and 2 clinical trials, and the outcomes have revealed promising results[25]. Animal model studies employing the TRPV1 antagonist GRC-6211 suggested therapeutic potency in suppressing chronically inflamed bladder hyperactivity, although clinical studies of their implication in human subjects remain to be established. Antagonist like MK-2295 was withdrawn owing to the burn injuries, and AMG-517 due to febrile reactions[22].

Thus, to date, no TRPV1 antagonists have been reported for clinical use. Furthermore, numerous endogenous compounds released under inflammatory conditions have been described to activate TRPA1, suggesting a role for this channel in thermal nociception, pathological pain, and itch[22]. On the other hand, a novel TRPV1 receptor antagonist (ACD440 gel) recently demonstrated significant analgesic effects when applied topically to healthy subjects. These data support further clinical development of this antagonist, which has potential as a new treatment for painful conditions affecting the skin, such as chronic peripheral neuropathic pain, without any local or systemic side effects[26].

It is well known that histamine G-protein-coupled receptors H1R and H4R, as well as TRPV1 channels, are important for the signal transduction of histaminergic itch in mice. However, it has recently been shown in vivo and in vitro experiments that, in addition to TRPV1, TRPA1 channel inhibition reduces H4R-induced itch, but not H1R-induced itch, emphasizing TRPA1 channel's important role for histamine-induced itch transmission in mice[27].

In this regard, the South Korean group realized an interesting idea targeting both TRPA1 and TRPV1 simultaneously with dual antagonists, which offers a promising approach to pain relief. They investigated a series of hybrid analogs of TRPA1 and TRPV1 channel antagonists to discover novel therapeutic agents for pain. This group synthesized a novel dual TRPA1/TRPV1 antagonist (Compound 50) that demonstrated dose-dependent analgesic activity in the formalin test in mice, inhibiting pain behavior completely at a dose of 100 mg/kg. This discovery, thus, characterizes a novel dual TRPA1/TRPV1 antagonist, highlighting its therapeutic potential for pain management[28].

Finally, it has recently been suggested that soft drugs targeting cutaneous TRP channels may represent the next generation of therapeutic drugs for the treatment of acute pain and itch disorders (dermatitis, psoriasis, and others) and chemotherapy-induced peripheral neuropathy[14].

Conclusions

The current study confirms that chemical irritants (AITC, CA, and CAPS) elicited thermal hyperalgesia and mechanical allodynia via the activation of TRP channels. This hyperalgesia and allodynia were attenuated by the TRPA1 channel antagonist HC-030031 and the TRPV1 channel antagonist capsazepine. Our findings indicate that these thermo- and mechanosensitive ion channels are capable of signaling temperature and mechanical changes across the range normally encountered in the environment.

Progress in the development of TRP channel antagonists will largely be driven by clinical trials of potential drugs, leading to the creation of more effective and efficient treatments for multiple pain pathologies. Therefore, the discovery of dual antagonists that simultaneously target both TRPA1 and TRPV1 channels will represent a new and promising therapeutic approach for pain treatment.

Conflict of Interest Statement:

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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