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Human and Animal Physiology

TRPV1 and TRPA1 Channels are Involved in Pain Sensations

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(Presented by Academy Member Tengiz Zaalishvili)

Pain sensation signalizes the organism of potentially dangerous stimuli and is associated with defensive motor reactions. Recent findings support the notion that capsaicin-evoked pain requires the presence of the transient receptor potential vanilloid 1 (TRPV1) channel in peripheral nociceptors while most agonists such as cinnamon aldehyde and mustard oil require the TRPA1 channel. In the present paper, we measured nociceptive thermal paw withdrawal latencies and mechanical thresholds bilaterally in rats at various time points following intraplantar injection of capsaicin, cinnamaldehyde, or allyl isothiocyanate (a principal compound of mustard oil) producing thermal hyperalgesia and mechanical allodynia. When pretreated with the TRPV1 antagonist (AMG-517) we found a significant reduction of these pain behavior responses. In the second session, pretreatment with the TRPA1 antagonist (HC-030031) produced a significant attenuation of thermal hyperalgesia and mechanical allodynia evoked by cinnamaldehyde and allyl isothiocyanate. Thus, we showed that noxious chemical irritants eliciting thermal hyperalgesia and mechanical allodynia are mediated via the activation of TRPV1 and TRPA1 cation channels. *© 2021 Bull. Georg. Natl. Acad. Sci.*

Allodynia, antinociception, hyperalgesia, Hargreaves' test, von Frey's test, pain

Understanding the neurobiology of pain is of high interest due to the enormous burden of pain for patients and society. There have been very few major breakthroughs leading to effective interventions for acute and chronic pain in recent times. Indeed, most available treatments have been in use for decades and provide limited long-term effects. Side effects, drug interactions and drug abuse present a major challenge for successful pain management [1-3].

A large body of data, human and animal model observations, distinguish between acute and chronic pain: while acute pain is commonly observed as a sign of tissue insult or of disease-related tissue injury, chronic pain is now conceptualized as a pathological state. It should be emphasized that the strict temporal cut-off (3 months) between acute and chronic pain remains uncertain, and it is perhaps dependent on the details of specific clinical conditions [1].

The Transient Receptor Potential (TRP) channel superfamily is comprised of a large group of cation-permeable channels, which display an extraordinary diversity of roles in sensory signaling and are involved in a plethora of animal behaviors. These channels are activated through a wide variety of mechanisms and participate in virtually every sensory modality. In particular, TRPs are critical for sensing the external environment, functioning in vision, thermosensation, olfaction, taste, mechano-, and hygro-sensations, pain and itch. Consequently, these channels have a profound impact on animal behavior and survival mechanisms in challenging environments [4-11].

We have recently clearly shown that natural chemical substances such as cinnamaldehyde (CA) [12], allyl isothiocyanate (AITC) (a principal compound of mustard oil), capsaicin (CAPS) [13], and menthol [14] affected the sensitivity to heat, innocuous and noxious cold, and mechanical stimuli in male rats. These results indicated that TRPA1 and TRPV1 channels are clearly involved in pain reactions, and the TRPA1 agonists AITC and CA enhanced the heat pain sensitivity, possibly by indirectly modulating TRPV1 channels co-expressed in nociceptors with TRPA1 [6, 7, 9, 13, 15, 16]. Overall, our previous data supported the role of thermosensitive TRPA1 and TRPV1 channels in pain modulation and showed that these two thermoreceptor channels are in a synergistic and/or conditional relationship with noxious heat and cold cutaneous stimulation [8-11,13].

In the presented paper, we further report that TRPV1 channel antagonist AMG-517 and the TRPA1 channel antagonist HC-030031 attenuated thermal hyperalgesia and mechanical allodynia induced by their agonists, CAPS (for TRPV1), and AITC and CA (for TRPA1).

Materials and Methods

Animals. Behavioral studies were conducted on adult albino male rats that were singly housed with free access to rodent chow and water. The Beritashvili Experimental BMC Animal Care and Use Committee approved the study protocol. Every effort was made to minimize both the number of animals used and their suffering. Throughout the experiments, animals were treated in accordance with the Guidelines for the Care and Use Mammals in Neuroscience and Behavioral Research [17]. Guidelines of the International Association for the Study of Pain with regard to animal experimentation were followed throughout [18].

Drug injections: CAPS at concentrations of 0.1, 0.3, or 0.5%, AITC at doses 5, 10, 15%, and CA (5, 10, 20%) (Sigma-Aldrich, St. Louis, Missouri, USA), or vehicle control (Tween 80, Fisher Scientific, Pittsburg, PA, USA) were injected intraplantarly to one hindpaw using a 30G needle connected by PE 50 tubing to a Hamilton micro-syringe. In a second set of experiments, the effect of intraplantar pretreatment with two different doses of the TRPV1 antagonist AMG-517 (10, 20 μg) or two doses of the TRPA1 antagonist HC-030031 (50, 100 μg) on thermal or mechanical withdrawals elicited by intraplantar injection of three doses of each irritants were tested. Groups of rats received one of two doses of AMG-517 in a volume of 50 μL injected intraplantar, followed 15 min later by one of three doses of capsaicin also injected intraplantar. In separate groups, rats similarly received one of two doses of HC-030031 (50μl), followed 15 min later by one of three doses of CA or AITC. This procedure was done twice for each rat, once for either the Hargreaves or the von Frey test. Different animal groups were used for the experiments and they were tested with one concentration of irritant chemicals, antagonists or vehicle and not repeatedly used. Six mice were used for each group.

Behavioral tests. Before formal testing, the baselines were assessed for rats 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 min intervals between tests. Behavioral tests were conducted starting immediately after intraplantar injection 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. Rats first were habituated to stand on a glass surface heated to 30ºC within a Plexiglas's enclosure, over three separate daily sessions. A light beam (Plantar Test 390, IITC, Woodland Hills, CA, USA) was focus 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 sec later. The rat was then held gently and one hind paw received an intra-plantar injection of chemicals or vehicle. The rat then was placed back onto the glass plate and withdrawal latencies of both paws were measured at 5, 15, 30, 45, 60 and 120 min post-injection. Reductions in latency were considered to reflect thermal hyperalgesia.

Mechanical paw withdrawal threshold (von Frey) test. Rats 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 was 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 rat then received a unilateral intra-plantar injection (see above) and was placed back onto the mash stand surface. Mechanical paw withdrawals were measured at the same post-injection 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 repeated measures of 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

Capsaicin. Intraplantar injection of CAPS resulted in thermal hyperalgesia and mechanical allodynia compare to the control group ($P<0.0001$; rMANOVA) that persisted beyond 2 hours (Fig. 1). For thermal withdrawals (Hargreaves test), the CAPS-treated groups of rats were all significantly different from the vehicle-treated group ($P<0.001$), but not from each other (Fig. 1A). There were some mirror-image effects on the contralateral hindpaw, which were not significantly different among all concentrations, but were significantly different for higher concentrations of CAPS compared with the vehicle group ($P<0.01$) (Fig. 1B).

For mechanical withdrawals (von Frey test), the CAPS-treated groups were significantly different from the vehicle group indicating allodynia ($P<0.001$) (Fig. 1C), but not from each other in the ipsilateral (treated) hindpaw. For the contralateral hindpaw, there were some mirror-image effects, especially for the 0.3 and 0,5% CAPS doses ($P<0.01$; Fig. 1D).

Pretreatment with the TRPV1 channel antagonist AMG-517 significantly reduced the latencies of thermal paw withdrawal reflex and

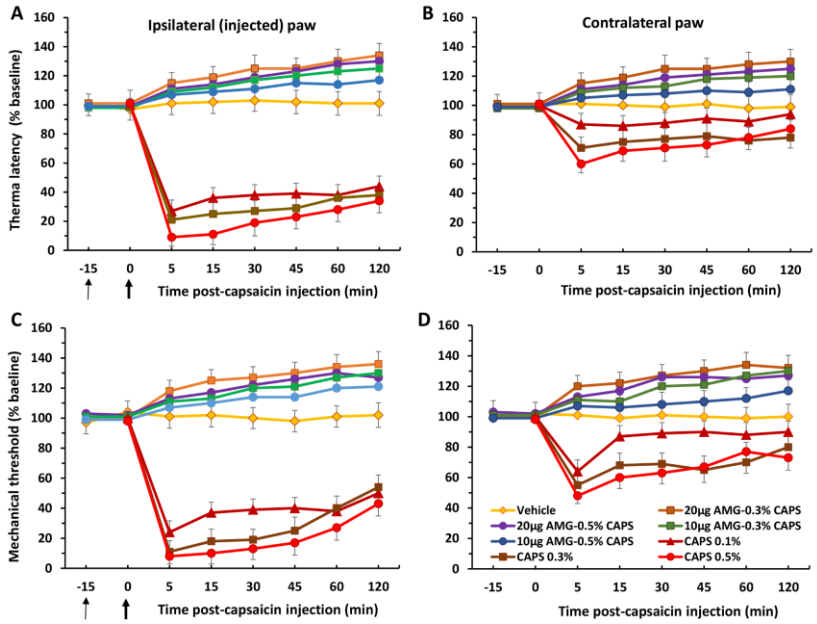

Fig. 1. Intraplantar injection of capsaicin results in significant decrease of the thermal paw latency (A) and mechanical paw threshold (C), i.e., develops thermal hyperalgesia and mechanical allodynia, respectively. However, pretreatment with TRPV1 antagonist AMG-517 reduces these hyperalgesia and allodynia. There is observed similar week effects for the contralateral paw (B, D). The thin black arrow indicates the time of injection of AMG-517 and the bold arrow indicates the time of injection of capsaicin. BL - pre-injection baseline.

mechanical paw withdrawal threshold, i.e. completely prevented CAPS-evoked activation of TRPV1 (Fig. 1A, C). However, we did observe similar effects for the contralateral paw showing also prevented the mirror image of hyperalgesia and allodynia (Fig. 1B, D).

Cinnamon Aldehyde. In the second set of experiments, we tested if pre-treatment with the TRPA1 channel antagonist HC-030031 attenuated hyperalgesia produced by CA. Each of the 8 groups of rats received an intraplantar injection of the vehicle in one hindpaw to establish baseline responses. Three days later, 3 groups of rats were injected with different doses of CA in the same hindpaw showing strong thermal hyperalgesia and mechanical allodynia ($P<0.0001$, rMANOVA) (Fig. 2A, C). Four other groups of mice prior to injection of CA pretreated with the TRPA1 channel antagonist HC-030031.

Intraplantar pretreatment with the TRPA1 channel antagonist HC-030031 at two concentrations (50 and 100 µg/50 µL) attenuated thermal hyperalgesia produced by CA injected in the same hindpaw. For the thermal paw withdrawal test (ipsilateral paw), there were significant differences between HC-030031 pretreatment groups and CA-only groups ($p<0.001$) (Fig. 2A). The weaker mirror image thermal hyperalgesic effects were also prevented by pretreatment with HC-030031 (Fig. 2B).

In the mechanical paw withdrawal (von Frey) test, significant differences were similarly observed ($p<0.001$) (Fig. 2C). The weaker mirror image mechanical allodynia effects were also reduced by pretreatment with HC-030031 (Fig. 2D).

Allyl Isothiocyanate. Application of AITC resulted in a significant dose-dependent reduction in the ipsilateral thermal paw withdrawal latency. Figure 3A shows the mean withdrawal latencies of the injected paw vs. time relative to injection of vehicle or AITC at each concentration tested. There was a dose-dependent reduction in the latency, with the 15% AITC concentration being significantly different from vehicle and 5% AITC treatments.

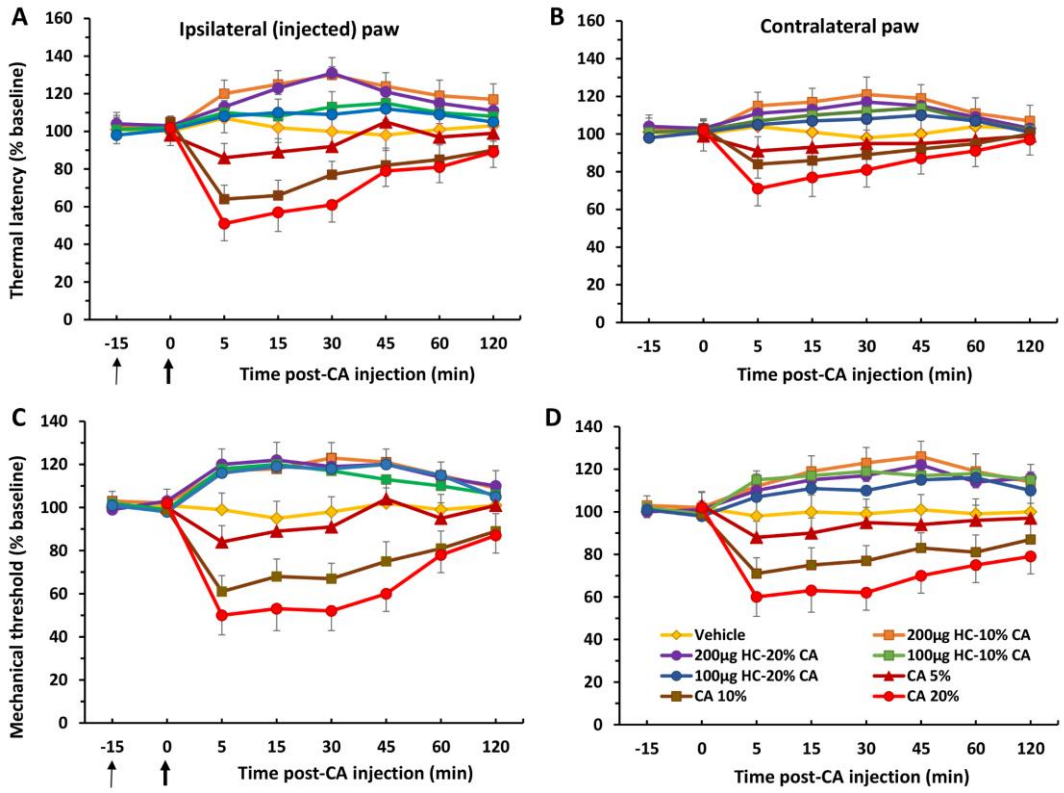

Fig. 2. Intraplantar injection of CA results in significant decrease of the thermal paw latency (A) and mechanical paw threshold (C), i.e., develops thermal hyperalgesia and mechanical allodynia, respectively. However, pretreatment with TRPA1 antagonist HC-030031 attenuates these hyperalgesia and allodynia. There is observed similar week effects for the contralateral paw (B, D). The thin black arrow indicates the time of injection of HC-030031and the bold arrow indicates the time of injection of CA. BL - pre-injection baseline.

The highest dose resulted in a mean reduction to almost 80% of the preinjection baseline value by 5 min. For the contralateral paw, there was an overall significant effect of treatment, with the 15% group being significantly different from the vehicle group (Fig. 3B).

The TRPA1 channel antagonist HC-030031 attenuated thermal hyperalgesia and mechanical allodynia produced by AITC injected in the same hindpaw. For the thermal paw withdrawal test (Hargreaves), there were significant differences between the HC-030031 pre-injected and AITC-only groups ($p<0.001$) (Fig. 3A). For the mechanical paw withdrawal (von Frey) test, similar significant differences were observed ($p<0.001$ for each) (Fig. 3C). The weaker mirror image thermal hyperalgesia and mechanical allodynia induced by AITC were significantly reduced by pretreatment with HC-030031 (Fig. 3B, D).

Discussion

The present findings showed significant thermal hyperalgesia and mechanical allodynia induced by CAPS (TRPV1 channel agonist) as well as TRPA1 channel agonists CA and AITC. CAPS-evoked thermal hyperalgesia and mechanical allodynia were completely prevented by pretreatment with a TRPV1 antagonist (AMG-517), indicating that these hyperalgesia and allodynia require a TRPV1 channel. In contrast, 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. These results

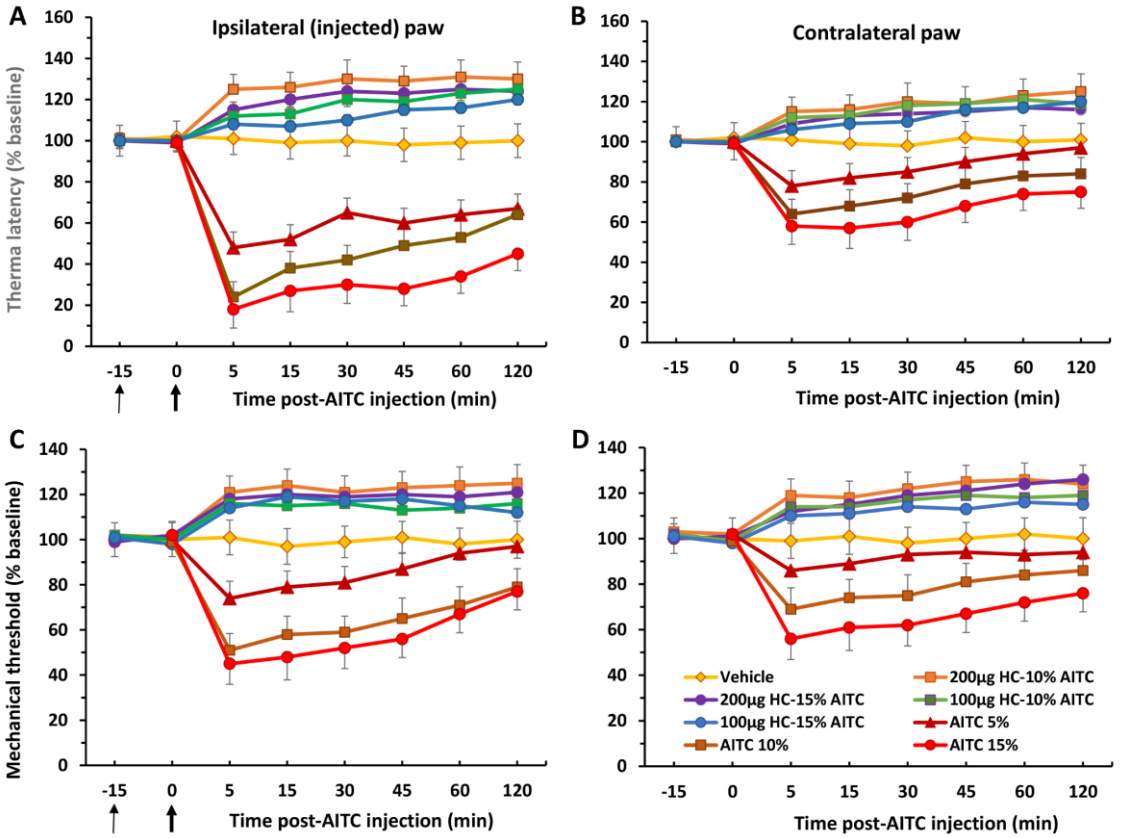

Fig. 3. Intraplantar injection of AITC results in significant decrease of the thermal paw latency (A) and mechanical paw threshold (C), i.e., develops thermal hyperalgesia and mechanical allodynia, respectively. However, pretreatment with TRPA1 antagonist HC-030031 attenuates these hyperalgesia and allodynia. There is observed similar week effects for the contralateral paw (B, D). The thin black arrow indicates the time of injection of HC-030031and the bold arrow indicates the time of injection of AITC. BL - pre-injection baseline.

confirm our previous reports that TRP channels agonists CAPS, CA, and AITC, but not menthol, elicit thermal hyperalgesia and mechanical allodynia expressed in withdrawal/wiping behavior in rodents [6-11].

We have recently shown that commonly used non-steroidal anti-inflammatory drugs (NSAIDs) such as diclofenac, ketoprofen, ketorolac and xefocam also reduced agonist-evoked activation of the TRPA1 and TRPV1 channels [19-21]. As TRPA1 is co-expressed in sensory neurons with TRPV1, heat hyperalgesia induced by CAPS, AITC and CA might involve activation of these receptors through an intracellular mechanism, leading to enhanced heat sensitivity of TRPV1 [15,16].

Just recently we have found that histamine, chloroquine, the bovine adrenal medulla peptide (BAM8-22) and the tethered peptide Ser-Leu-Ile-Gly-Arg-Leu (SLIGRL) elicited thermal hyperalgesia and mechanical allodynia in adult male mice. Intraplantar injection of histamine resulted in significant thermal hyperalgesia and mechanical allodynia ipsilaterally that persisted for 1 hour. Pretreatment with the TRPV1 antagonist AMG-517, but not the TRPA1 antagonist HC-030031, significantly attenuated the magnitude and time course of thermal hyperalgesia and mechanical allodynia elicited by histamine, indicating that these effects are mediated by TRPV1 [22]. In contrast, pretreatment with the

TRPA1 antagonist significantly reduced thermal hyperalgesia and mechanical allodynia elicited by chloroquine, BAM8-22, and SLGRL indicating that effects elicited by these non-histaminergic itch mediators require TRPA1 [22-25].

Pain and itch are similar in that they signal the organism of potentially dangerous stimuli, and are associated with protective motor responses. Both might share common mechanisms, and TRPA1 and TRPV1 play roles in chronic as well as acute itch and pain [26-29]. We have recently observed that hyperalgesia and allodynia elicited by acute injection of CAPS were significantly reduced or prevented by pretreatment with the TRPV1 antagonist (AMG-517) but not the TRPA1 antagonist (HC-030013), consistent with a role for TRPV1 in the acute histamine-mediated itch [22]. The prevention by the TRPA1 antagonist of hyperalgesia and allodynia elicited by non-histaminergic pruritogens (chloroquine, BAM8-22, SLIGRL) is consistent with a role for TRPA1 in the acute non-histaminergic itch [22]. These results imply that TRPA1-dependent peripheral sensitization of mechano-sensitive C-fiber afferents contributes to mechanical allodynia under conditions of inflammatory pain [22], and also to thermal (heat and/or cold) pain.

In conclusion, here we showed that chemical irritants (CAPS, CA, and AITC) elicited thermal hyperalgesia and mechanical allodynia via the activation of TRP channels. This hyperalgesia and allodynia were attenuated by the TRPV1 channel antagonist AMG-517 and the TRPA1 channel antagonist HC-030031. 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.

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ადამიანისა და ცხოველთა ფიზიოლოგია

TRPV1 და TRPA1 არხები ჩართულია ტკივილის შეგრძნებაში

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ტკივილის შეგრძნება ატყობინებს ორგანიზმს პოტენციურად სახიფათო გამღიზიანებლების შესახებ და უკავშირდება თავდაცვით მოტორულ რეაქციებს. ბოლოდროინდელი მონაცემები მხარს უჭერს მოსაზრებას, რომ კაპსაიცინით გამოწვეული ტკივილი მოითხოვს გარდამავალი რეცეპტორულ პოტენციური ვანილოიდის 1 (TRPV1) არხის მონაწილეობას პერიფერიულ ნოციცეპტორებში, ხოლო აგონისტების უმეტესობა, როგორიცაა დარიჩინის ალდეჰიდი ან მდოგვის ზეთი, საჭიროებს TRPA1 არხის ჩართულობას. წინამდებარე ნაშრომში შესწავლილია ვირთაგვების ნოციცეპტური თერმული თათის მოცილების ფარული პერიოდი და მექანიკური დაწოლის ზღურბლი კაპსაიცინის, დარიჩინის ალდეჰიდის და ალილ იზოთიოციანიტის (მდოგვის ზეთის ძირითადი კომპონენტი) ტერფქვეშა ინექციის შემდეგ, რაც იწვევს თერმულ ჰიპერალგეზიასა და მექანიკურ ალოდინიას. TRPV1 არხის ანტაგონისტის (AMG-517) წინასწარი ინექცია იწვევდა ტკივილის თავდაცვითი რეფლექსური რეაქციების მნიშვნელოვან შემცირებას. ცდების შემდგომ სერიებში, TRPA1 არხის ანტაგონისტის (HC-030031) წინასწარი შეყვანა ასევე განაპირობებდა დარიჩინის ალდეჰიდისა და ალილ იზოთიოციანიტის შედეგად გამოწვეული თერმული ჰიპერალგეზიის და მექანიკური ალოდინიის სარწმუნო შემცირებას. ამრიგად, ჩვენ ვაჩვენეთ, რომ ორგანიზმისთვის დამაზიანებელი მტკივნეული სტიმულებით გამოწვეული თერმული ჰიპერალგეზია და მექანიკური ალოდინია განპირობებულია TRPV1 და TRPA1 კატიონური არხების გააქტივებით.

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