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Role of Transient Receptor Potential Channels in Itch and Pain Sensations

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ABSTRACT. Itch and pain are similar in that they signal the organism of potentially dangerous stimuli, and are associated with protective motor responses. Both might share common mechanisms, particularly, light mechanical touch surrounding a region of itch and pain elicits a sensation of itch (alloknesis) or pain (allodynia), respectively. Recent findings support the notion that histamine-evoked itch requires the presence of the transient receptor potential vanilloid 1 (TRPV1) channel in peripheral pruriceptors while the most non-histaminergic itch mediators require the TRPA1 channel. Both of these ion channels have previously been implicated in pain. In the present paper, we investigated whether chemical inducers of itch, including histamine and non-histaminergic mediators, elicit signs of thermal and mechanical hyperalgesia (increased pain to a noxious stimulus). We measured nociceptive thermal paw withdrawal latencies and mechanical thresholds bilaterally in mice at various time points following intraplantar injection of histamine or chloroquine producing hyperalgesia. When pretreated with the TRPV1 antagonist (AMG-517) we found a significant reduction of thermal and mechanical hyperalgesia. In the second session, pretreatment with the TRPA1 antagonist (HC-030031) produced a significant attenuation of thermal and mechanical hyperalgesia evoked by a non-histaminergic pruritogen chloroquine. Thus, we show for the first time that histaminergic and non-histaminergic pruritogens elicit thermal and mechanical hyperalgesia along with and hyperknesis (itch elicited by strong stimulation) through the activation of TRP channels. *© 2018 Bull. Georg. Natl. Acad. Sci.*

Key words: antinociception, hyperalgesia, Hargreaves' test, von Frey's test, pruritus

Pain is associated with a wide range of injury and disease and is sometimes the disease itself. Some conditions may have pain and associated symptoms arising from a discrete cause, such as postoperative pain or pain associated with a malignancy, or may be conditions in which pain constitutes the primary problem, such as neuropathic pains or headaches [1].

Chronic itch is another common and costly problem and the treatment of chronic itch has been largely unmet. Chronic itch or pruritus is a frequent symptom in the general population and in many skin and systemic diseases, such as atopic dermatitis, psoriasis, primary biliary cirrhosis, chronic renal failure, and urticaria as well as several neurological, infectious, neoplastic, haematological, autoimmune,

genetic and drug-induced conditions. Its frequency demonstrates high burden and impaired quality of life [2-5]. There is a need to better understand mechanism of itch and interactions between itch and pain in order to develop novel evidence-based treatments for these chronic conditions [6-14].

There is very little previous evidence that itch mediators elicit hyperalgesia and almost there are no recent studies using modern controlled thermal stimulation methods to confirm if histamine or any other itch mediator elicits heat hyperalgesia, and to our knowledge there are no studies investigating if histamine or non-histaminergic itch mediators elicit thermal or mechanical allodynia/hyperalgesia.

The possibility that itch mediators also influence pain is supported by recent findings that histamine-evoked itch required the presence of the transient receptor potential vanilloid 1 (TRPV1) channel in peripheral itch receptors (pruriceptors) [14], and that most non-histaminergic itch mediators (e.g., chloroquine) require the transient receptor potential ankyrin 1 (TRPA1) channel [15,16]. Both of these ion channels have previously been implicated in pain [17-23]. This, it is conceivable that histamine and other non-histaminergic itch mediators can activate not only pruriceptors but also nociceptors expressing the TRPV1 and TRPA1 channels that signal pain. While the behavioral manifestation is predominantly scratching and alloknesis, in this study we determined that itch behavior is also accompanied by pain, heat and mechanical hyperalgesia in mice.

Materials and Methods

Animals: The experiments were performed on male mice < 50 grams in body weight, bred at the Beritashvili Experimental Biomedicine Center. The animals were kept under standard housing conditions (22±2 °C, 65% humidity, lights from 6:00 a.m. to 8:00 p.m.), and fed by standard dry diet; water freely available. Guidelines of International Association for the study of Pain regarding investigations of experimental pain in conscious animal were followed throughout [34].

Chemical injections: All chemical irritants histamine (0.25, 0.5, and 1M/1μL), chloroquine (0.25, 0.5, and 1M/1μL), and TRPV1 antagonist AMG-517 (10μg/1μL; 20μg/1μL) and TRPA1 antagonist HC030031 (50μg/30μL; 100μg/30μL) were purchased from Sigma-Aldrich Chemicals, Co., (St. Louis, MO, USA). Various doses of these chemicals were injected intradermal through a 30 g needle connected by PE 50 tubing to a Hamilton micro-syringe. The same volumes of vehicle (isotonic saline) were microinjected in the same manner separately as a control. 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.

Thermal paw withdrawal (Hargreaves) test. Mice first were habituated to stand on a glass surface heated to 30ºC within a Plexiglas's enclosure, over three separate daily sessions. For formal testing, baseline latencies for paw withdrawals evoked by radiant thermal stimulation of each hind paw were measured a minimum three times/ paw, with at least 5 min elapsing between tests on a given paw. 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 mouse was then held gently and one hind paw received an intra-plantar injection of chemicals or vehicle. The mice then was placed back onto the glass plate and withdrawal latencies of both paws were measured at 3, 15, 30, 45, 60 and 120 min post-injection.

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 Anesthesiometer (2390, IITC,

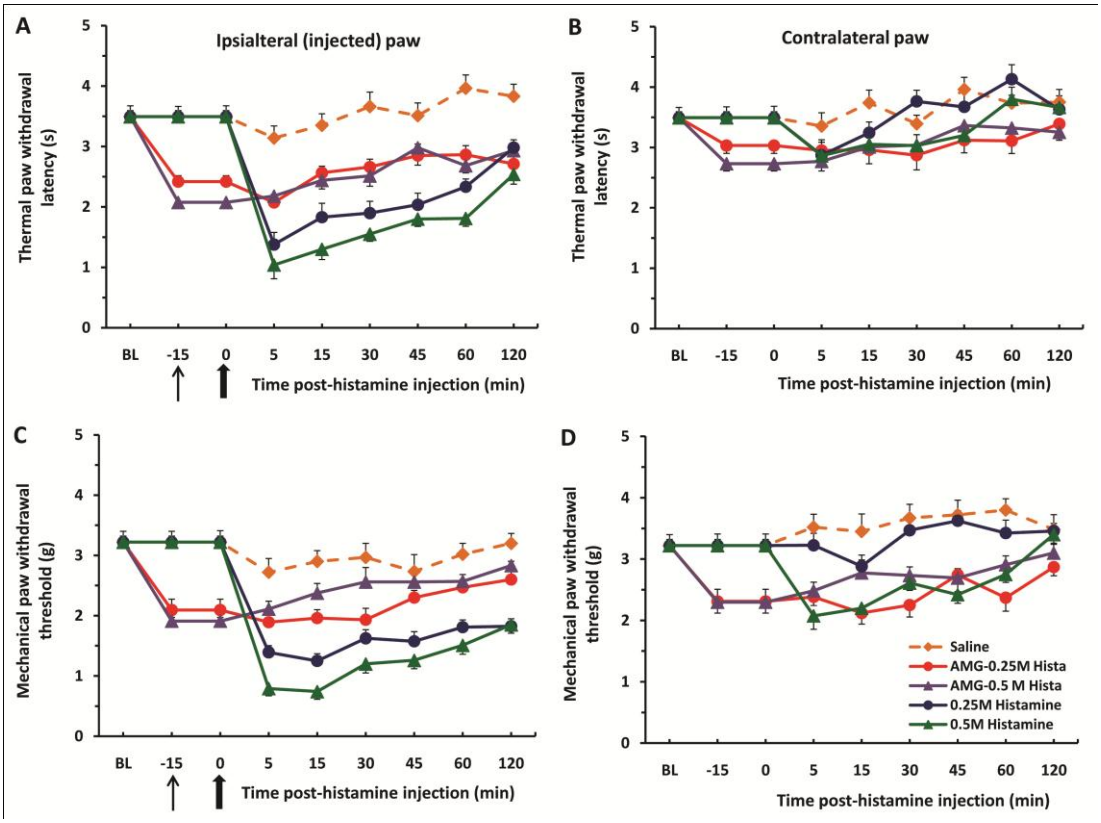

Fig. 1. Intraplantar injection of histamine result in significant decrease of the thermal paw latency (A) and mechanical paw threshold (C), i.e., develops hyperalgesia. However, pretreatment with TRPV1 antagonist AMG-517 reduces these thermal and mechanical hyperalgesia, respectively. There is not observed similar 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 histamine. BL - pre-injection baseline.

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 mouse 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.

Data analysis: All data from behavioral tests were subjected to repeated measures of analysis of variance (ANOVA) and then were compared between chemicals and vehicle treatment groups by paired *t*-test. The data are expressed as Mean±S.E.M. The Kolmogorov-Smirnov test was applied to verify normality and equal variance. Thereafter Kruskal-Wallis ANOVA and subsequent Tukey test was used to assess differences between treatments. Statistical significance is acknowledged if $P < 0.05$. The statistical software utilized was Prism 4.03 (GraphPad Software, Inc, San Diego, CA, USA).

Results and Discussion

Histamine-induced itching is accompanied by hyperalgesia.

Intraplantar injection of histamine resulted in thermal and mechanical hyperalgesia that persisted

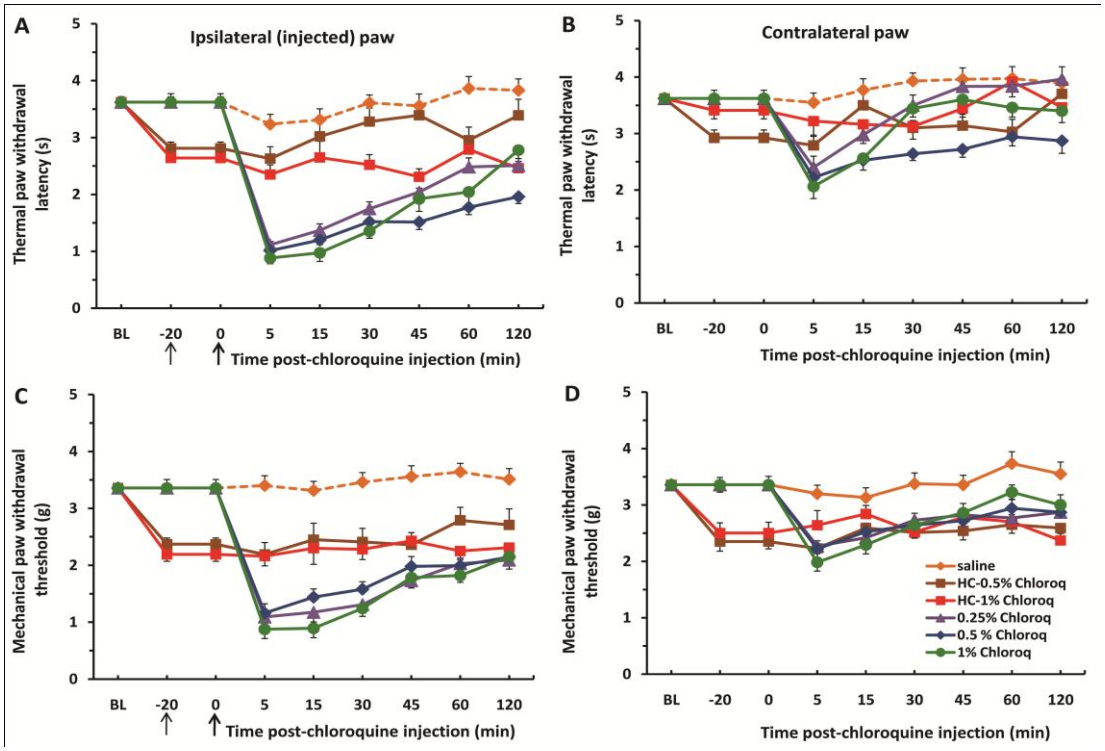

Fig. 2. Intraplantar injection of cloroquine result in significant decrease of the thermal paw latency (A) and mechanical paw threshold (C), i.e., develops hyperalgesia. However, pretreatment with TRPA1 antagonist HC030031 attenuates these thermal and mechanical hyperalgesia, respectively. There is not observed similar effects for the contralateral paw (B, D). The thin black arrow indicates the time of injection of HC030031and the bold arrow indicates the time of injection of chloroquine. BL - pre-injection baseline.

beyond 2 hours for the first 30-40 min compare to the control group (P<0.001) (Fig. 1). These hyperalgesic effects are very similar to those reported previously when we intraplantarly injected the TRPV1 channel agonist capsaicin, and the TRPA1 channel agonists cinnamaldehyde and allyl isothiocyanate (a natural compound of mustard oil) into the rat's hind paw [24-30].

Pretreatment with the TRPV1 channel antagonist AMG-517 significantly reduced the latencies of thermal paw withdrawal reflex and mechanical paw withdrawal threshold, i.e. attenuated histamine-evoked activation of TRPV1. However, we did observed similar effects for the contralateral paw (Fig. 1).

Non-histaminergic pruritogen chloroquine produces hyperalgesia

In the second set of experiments, we tested if pre-treatment with the TRPA1 channel antagonist HC030031 attenuated hyperalgesia produced by chloroquine. Each of the 6 groups of mice received intraplantar injection of saline in one hindpaw to establish baseline responses. Three days later, 3 groups of mice were injected with chloroquine in the same hindpaw showing strong thermal and mechanical hyperalgesia (P<0.001) (Fig. 2). Two other groups of mice prior to injection of chloroquine pretreated with the TRPA1 channel antagonist HC030031. The obtained data showed a significant attenuation of thermal and mechanical hyperalgesia for the first 30-45 min. These findings indicate that pretreatment with HC030031 did significantly reduce the magnitude of hyperalgesia, as well as significantly shortened the time-course of hyperalgesia induced by chloroquine. We did not observed similar changes for the contralateral paw (Fig. 2).

We have recently shown that commonly used non-steroidal anti-inflammatory drugs (NSAIDs)

such as diclofenac, ketorolac and xefocam also reduced agonist-evoked activation of the TRPA1 and TRPV1 channels [26,31].

As stated above, itch and pain are similar in that they signal the organism of potentially dangerous stimuli, and are associated with protective motor responses [2-5]. Both might share common mechanisms, particularly, light mechanical touch surrounding a region of itch and pain elicits a sensation of itch (alloknesis) or pain (allodynia), respectively. However, the problem remains open whether itch mediators also cause hyperalgesia and allodynia as we have them in pain. This question has not been tested much except for a few older studies that histamine induces heat hyperalgesia [32,33].

In conclusion, here we showed for the first time that histaminergic and non-histaminergic pruritogens elicit thermal and mechanical hyperalgesia along with hyperknesis (itch elicited by strong stimulation) through the activation of TRP channels. This hyperalgesia was attenuated by the TRPV1 channel antagonist AMG-517 and the TRPA1 channel antagonist HC030031.

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ადამიანისა და ცხოველთა ფიზიოლოგია

ტრანზიტორულ რეცეპტორულ პოტენციალთა არხების როლი ქავილისა და ტკივილის შეგრძნებაში

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ქავილი და ტკივილი წარმოადგენს მსგავს შეგრძნებებს იმ თვალსაზრისით, რომ ისინი ატყობინებენ ორგანიზმს დამაზიანებელი სტიმულის არსებობის შესახებ. ორივეს შეიძლება გააჩნდეს მსგავსი მექანიზმები, თუმცა საკმაოდ განსხვავდებიან კიდეც. უკანასკნელმა გამოკვლევებმა ცხადყო, რომ ჰისტამინით გამოწვეული ქავილი საჭიროებს ტრანზიტორული რეცეპტორულ პოტენციალის ვანილოიდური (TRPV1) არხის მონაწილეობას, მაშინ როცა უმრავლესი არაჰისტამინერგული ქავილის გამოსაწვევად საჭიროა ანკირინული (TRPA1) არხის ჩართულობა. უფრო ადრე აღმოჩენილ იქნა, რომ ორივე არხი მონაწილეობს ტკივილის შეგრძნებაში. წარმოდგენილ ნაშრომში ჩვენ შევისწავლეთ, იწვევს თუ არა ქავილის პრურიტოგენები ჰისტამინი და არაჰისტამინერგული მედიატორი ქლოროკინი თერმულ და მექანიკურ ჰიპერალგეზიას. გავაანალიზეთ ლაბორატორიული თაგვების თათის მოცილების ნოციცეპტური რეფლექსის ფარული პერიოდისა და თათის მოცილების მექანიკური ზღურბლის ცვლილებები ჰისტამინითა და ქლოროკინით გამოწვეული ჰიპერალგეზიის შემთხვევაში. თათში TRPV1 არხის ანტაგონისტის (AMG-517) შეყვანა იწვევდა თერმული და

მექანიკური ჰიპერალგეზიის სარწმუნო შემცირებას. ცდების მეორე სერიაში **TRPA1** არხის ანტაგონისტის **(HC030031)** ინექცია თაგვების უკანა თათში ასევე იწვევდა ქლოროკინით განპირობებულ თერმული და მექანიკური ჰიპერალგეზიის სარწმუნო დათრგუნვას. ამრიგად ჩვენ ვაჩვენეთ, რომ ჰისტამინი და ქლოროკინი იწვევს თერმულ და მექანიკურ ტკივილს (ჰიპერალგეზიას), რომლის განვითარებაშიც ჩართულია TRP არხები.

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