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Hyperalgesia in Non-Histaminergic Itch: the Role of TRPA1 Channel

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ABSTRACT. Itching is a remarkably common symptom of dermatologic and systemic diseases and seriously impacts a patient's quality of life. Itch is broadly categorized as histaminergic (sensitive to antihistamines) or non-histaminergic. The peptide Ser-Leu-Ile-Gly-Arg-Leu (SLIGRL) is an itch-inducing agent widely used to study histamine-independent itch. The Mas-related G-protein-coupled receptors, particularly MrgprC11, rather than protease-activated receptor 2 (PAR2) as previously thought, mediates histamine-independent itch. Chloroquine and the bovine adrenal medulla peptide 8–22 (BAM8–22) produce itch-related scratching through MrgprA3 and MrgprC11, respectively, in mice and in humans. On the other hand, the possibility that itch mediators also influence pain is supported by recent findings that most non-histaminergic itch mediators require TRP ankirin 1 (TRPA1) channel. In the present paper we report that MrgprA3 and MrgprC11 mediated itch by their agonists BAM8-22 and SLIGRL, respectively, is accompanied by heat and mechanical hyperalgesia via the TRPA1 channel. We measured nociceptive thermal paw withdrawal latencies and mechanical thresholds bilaterally in mice at various time points following intraplantar injection of BAM8-22 or SLIGRL producing hyperalgesia. When pretreated with the TRPA1 antagonist (HC-030031) we found a significant reduction of thermal and mechanical hyperalgesia. Thus, we show that non-histaminergic pruritogens (BAM8-22, SLIGRL) elicit thermal and mechanical hyperalgesia through the activation of TRPA1 channel that was attenuated by its antagonist. *© 2019 Bull. Georg. Natl. Acad. Sci.*

Key words: Antinociception, hyperalgesia, pruritus, signal transduction

Pruritus (itch) is defined as the sensation that causes the desire to scratch, and it can be induced by mechanical, thermal and chemical stimuli. Persistent itch accompanying diseases of the skin and other organs can significantly impair the quality of life [1]. Chronic itch is a frequent symptom of dermatologic conditions and a variety of systemic diseases. Common conditions presenting with itch include atopic dermatitis, psoriasis, post-herpetic neuralgia, kidney failure, and liver diseases [2,3]. Acute itch is a commonplace sensation elicited by insect bites and certain plants, and normally serves as a protective warning signal [4].

Histamine is one of the best-evaluated itch mediators producing pruritus accompanied by skin reactions (wheal and flare) in humans. Intradermal injection of histamine elicits scratching in most strains of mice while a high dose elicits both hindlimb scratching and forelimb wiping following cheek injection in rats [5].

We have recently found that intraplantar injection of histamine in mice one of the hindpaws resulted in thermal and mechanical hyperalgesia that persisted beyond 2 hours compared to the control group. However, pretreatment with the transient receptor potential vanilloid 1 (TRPV1) channel antagonist AMG-517 significantly reduced this hyperalgesia revealed in the increase of the latencies of the thermal paw withdrawal reflex and mechanical paw withdrawal threshold, i.e., attenuated histamine-evoked activation of TRPV1 [6].

Protease-activated receptors (PARs) are activated by protease-induced cleavage of a part of the extracellular domain that acts as a tethered ligand and their role in pain and itch receives considerable attention [1,2]. Cowhage spicules from the bean plant (Mucuna pruriens) have long been known to induce itch. Its active component is mucunain, a cysteine protease, which acts at PAR-2 and PAR-4 subtypes to produce histamine-independent itch [2]. A tethered ligand, such as the peptide Ser-Leu-Ile-Gly-Arg-Leu (SLIGRL) (agonist of PAR-2) is also known to elicit scratching in mice [5]. A recent study reported that SLIGRL elicited scratching via Mas-related G-protein-coupled receptors C11 (MrgprC11) rather than PAR-2 [7]. Chloroquine and the bovine adrenal medulla peptide 8–22 (BAM8–22) produce itch-related scratching through MrgprA3 and MrgprC11, respectively, in mice and in humans [2]. In comparison with responses of control mice, contact hypersensitivity mice exhibited a significant increase in the scratching evoked by BAM8-22, a peptide that elicits a histamine-independent itch [8]. In experiments with chloroquine we found that pre-treatment with the TRPA1 channel antagonist HC030031 significantly attenuated thermal and mechanical hyperalgesia for the first 30-45 min [9].

It is important to understand the mechanisms how PAR-2 regulates protease-dependent, but histamine-independent itch. This is also of major medical interest since some of serine proteases have been implicated in various pruritic diseases including atopic dermatitis, psoriasis, dry skin itch, anaphylaxis-associated itch, or renal insufficiency-associated itch [10]. On the other hand, the possibility that itch mediators also influence pain is supported by recent findings that most non-histaminergic itch mediators require TRP ankirin 1 (TRPA1) channel [11,12]. It is unclear whether most pruritic stimuli act through Mrgpr receptors, at least indirectly, but they help clarify the neural mechanisms underlying itch, and to a lesser extent, pain sensation.

In this paper we report that MrgprA3 and MrgprC11 mediated itch by their agonists BAM8-22 and SLIGRL respectively, is accompanied by heat and mechanical hyperalgesia via the TRPA1 channel.

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.

Chemical injections: All chemical irritants BAM8-22 (2.5, 5, and 12 mM/1μL), SLIGRL (7.5, 15, and 38 mM/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 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 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 sec later. The mouse was then held gently and one hind paw received an intra-plantar injection of chemicals or vehicle. The mice then were 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, 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

BAM8-22 induced itching is accompanied by hyperalgesia. In the first set of experiments, we tested if pre-treatment with the TRPA1 channel antagonist HC030031 attenuated hyperalgesia produced by BAM8-22. The latter looks like as if chloroquine produces itch-related scratching behavior through receptors MrgprC11 in mice [2]. Intraplantar injection of BAM8-22 resulted in strong thermal and mechanical hyperalgesia compared to saline control group ($p < 0.001$). Pretreatment with HC010031 reduced thermal and mechanical hyperalgesia for ipsilateral, chemicals injected paw, but not contralateral paw (Fig. 1).

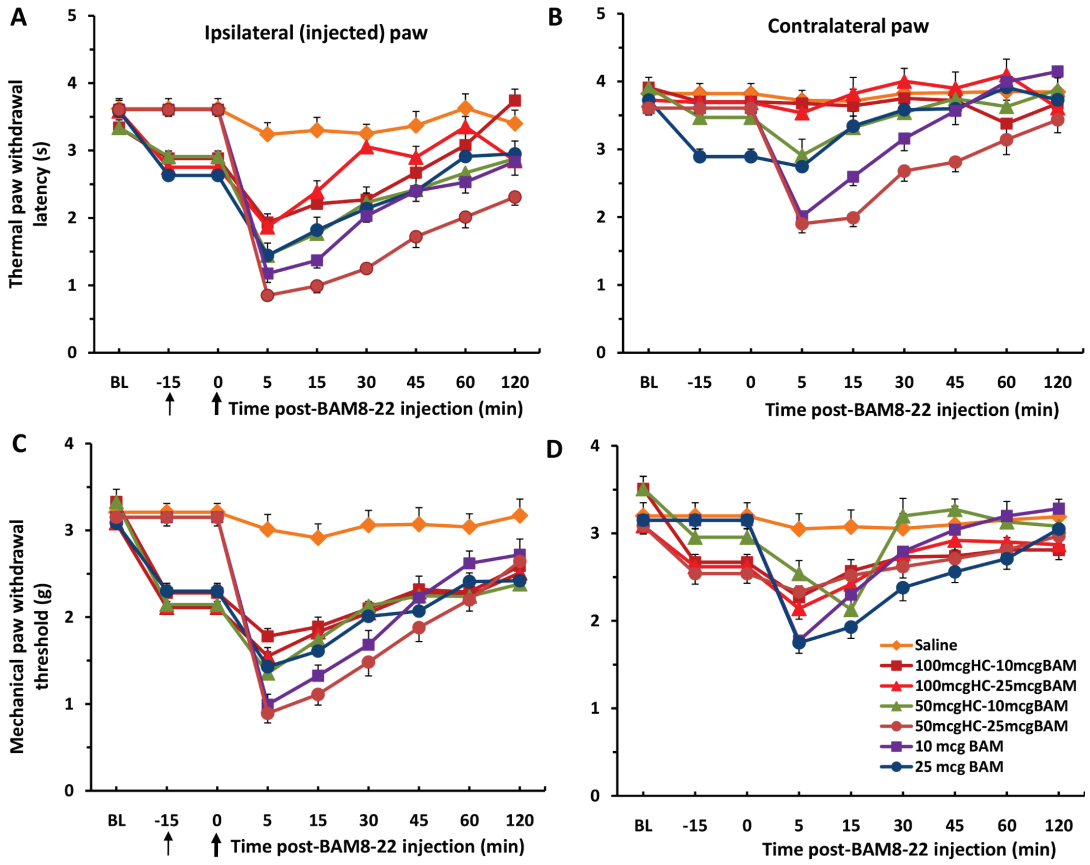

Fig. 1. Intraplantar injection of BAM8-22 result in significant decrease of the thermal paw latency (A) and mechanical paw threshold (C), i.e., develops hyperalgesia. However, pretreatment with TRPA1 antagonist HC030031 reduces these thermal and mechanical hyperalgesia, respectively. There is not observed similar effects for the contralateral paw (B, D). The thin black arrow indicates the time of injection of HC030031and the bold arrow indicates the time of injection of BAM8-22. BL - pre-injection baseline.

SLIGRL induced itching is accompanied by hyperalgesia. In the second set of experiments, we verified if pre-treatment with the TRPA1 channel antagonist HC030031 attenuated thermal and mechanical hyperalgesia produced by SLIGRL. The latter produces itch-related scratching through receptors MrgprC11. Intraplantar injection of SLIGRL resulted in similar effects that we observed with BAM8-22. Here also pretreatment with HC010031 in the same manner, but lesser reduced thermal and mechanical hyperalgesia for ipsilateral, chemicals injected paw, but not contralateral paw (Fig. 2).

It is interesting that we have recently shown that histaminergic (histamine) and non-histaminergic pruritogens (chloroquine) 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, respectively [6,9].

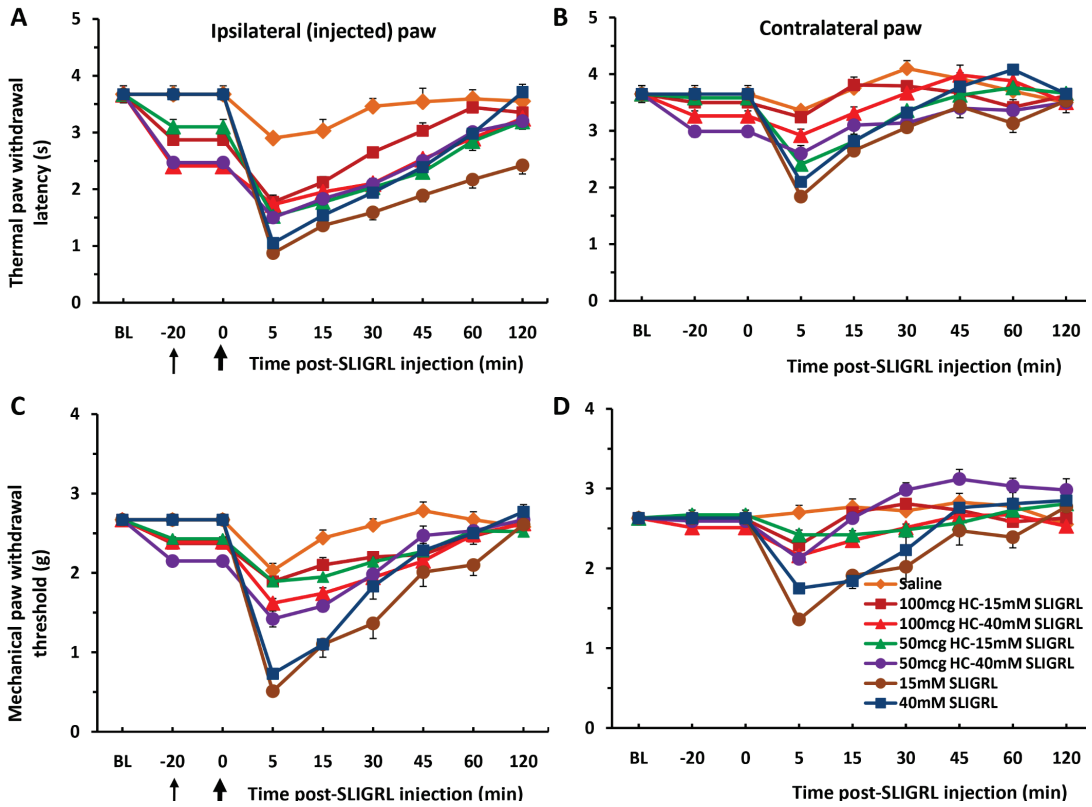

Fig. 2. Intraplantar injection of SLIGRL result in significant decrease of the thermal paw latency (A) and mechanical paw threshold (C), i.e., develops hyperalgesia. However, pretreatment with TRPA1 antagonist HC030031 reduces these thermal and mechanical hyperalgesia, respectively. There is not observed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

In fascinating findings, the dorsal root ganglia (DRG) neurons responding to BAM8-22, chloroquine, and SLIGRL almost completely overlapped, consistent with *in situ* studies showing overlap of Mrgprc11 and Mrgpra3 mRNA, and were also responsive to histamine and capsaicin [13,14]. This engaged the subset of neurons as specialized itch-selective nociceptors (pruriceptors), though the exact significance is still unclear.

As stated above, recent studies have implicated TRP channels as intracellular mediators of multiple receptors involved in nociception and itch [6,9,11,12,15,16]. This exciting adjustment to classical modes of G protein-coupled receptor (GPCR) signaling is expanding to a diverse collection of GPCRs and is in line with experiments showing abundant expression of multiple TRP channels in nociception and itching [11,12].

A further set of experiments showed that TrpA1 was required for chloroquine and BAM8-22 induced rises in intracellular calcium and action potentials in DRG neurons. No scratching was observed in TrpA1 null mice, and both MrgprA3 and MrgprC11 were shown to couple to TrpA1 in heterologous cells. However, these pathways were not identical. MrgprC11 signaling was shown to require phospholipase C (PLC) as an intermediate, while MrgprA3 signaling did not require PLC but instead involved an active Gbeta/gamma complex [12]. This in itself is another interesting modification of the classical GPCR models, in which the alpha subunits are the active messenger proteins [17].

In conclusion, the obtained data showed a significant attenuation of thermal and mechanical hyperalgesia induced by BAM8-22 and SLIGRL for the first 30-45 min. These findings indicate that pretreatment with TRPA1 channel antagonist HC030031 did significantly reduce the magnitude of hyperalgesia and significantly shortened the time-course of hyperalgesia induced by BAM8-22 and SLIGRL. Thus, we revealed that non-histaminergic pruritogens elicit thermal and mechanical hyperalgesia via the activation of TRPA1 channel. This hyperalgesia was attenuated by the TRPA1 channel antagonist HC030031. Therefore, it is very important to get more evidence for the potential role of TRPA1 channel inhibitors as modulators of preclinical and/or clinical itch and pain conditions.

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ადამიანისა და ცხოველთა ფიზიოლოგია

ჰიპერალგეზია არაჰისტამინერგული ქავილის დროს: TRPA1 არხის როლი

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ქავილის შეგრძნება დერმატოლოგიური და სისტემური დაავადებების საკმაოდ ხშირი სიმპტომია, რომელიც სერიოზულად აისახება პაციენტის ცხოვრებაზე. ქავილი განპირობებულია, როგორც ჰისტამინერგული (მგრძნობიარე ანტიჰისტამინური პრეპარატების მიმართ), ისე არაჰისტამინერგული მექანიზმებით. პეპტიდი სერ-ლეუ-ილე-გლი-არგ-ლეუ (**SLIGRL**) არის ქავილის გამომწვევი აგენტი, რომელიც ფართოდ გამოიყენება ჰისტამინ-დამოუკიდებელი ქავილის შესასწავლად. **G** ცილასთან შეწყვილებული რეცეპტორები, კერძოდ **MrgprC11**, განსხვავებით პროტეაზ-გააქტივებული რეცეპტორი-2-სა (**PAR2**), როგორც ადრე ფიქრობდნენ, წარმოადგენს არაჰისტამინერგული ქავილის მედიატორს. ქლოროკინი და მსხვილფეხა რქოსანი პირუტყვის თირკმელზედა ჯირკვლის მედულარული პეპტიდი **8-22** (**BAM8-22**) იწვევს ქავილით გამოწვეულ ფხანის რეაქციას **MrgprA3** და **MrgprC11** რეცეპტორების ჩართულობით, შესაბამისად თაგვებსა და ადამიანში. მეორე მხრივ, შესაძლებლობა, რომ ეს შუამავლები ასევე გავლენას ახდენენ ტკივილზე, მტკიცდება ბოლო აღმოჩენებით, რომ არაჰისტამინური მედიატორების უმრავლესობა მოითხოვს **TRP** ანკირინის **1** (**TRPA1**) არხის ჩართულობას.

წარმოდგენილ შრომაში ჩვენ დავადგინეთ, რომ **MrgprA3** და **MrgprC11** რეცეპტორების შუამავლობითა და **BAM8-22** და **SLIGRL** აგონისტებით განპირობებულ ქავილს თან ახლავს თერმული და მექანიკური ჰიპერალგეზია. ეს უკანასკნელი გამოწვეული უნდა იყოს **TRPA1** არხით. ჩვენ გავაანალიზეთ ლაბორატორიული თაგვების თათის მოცილების ნოციცეპტური რეფლექსის ფარული პერიოდისა და თათის მოცილების მექანიკური ზღურბლის ცვლილებები გამოწვეული **BAM8-22** და **SLIGRL**-ის ტერფქვეშა ინექციებით თაგვებში. **TRPA1** არხის ანტაგონისტის (**HC-030031**) წინასწარი შეყვანა თათში იწვევდა თერმული და მექანიკური ჰიპერალგეზიის სარწმუნო შემცირებას. ამრიგად, ჩვენ ვაჩვენეთ, რომ არაჰისტამინერგული პრურიტოგენები **BAM8-22** და **SLIGRL** იწვევს თერმულ და მექანიკურ ტკივილს (ჰიპერალგეზიას), რომლის განვითარებაშიც ჩართულია **TRPA1** არხი.

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