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Behavioral Study of TRPA1 and TRPV1 Channels Relationship in Rats

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ABSTRACT. A number of temperature-sensitive transient receptor potential (TRP) ion channels are studied as nociceptors that respond to extreme temperatures and harmful chemicals. Among them there is a family of six thermo-TRP channels (TRPA1, TRPM8, TRPV1, TRPV2, TRPV3, and TRPV4) that exhibit sensitivity to increases or decreases in temperature, as well as to chemical substances eliciting the respective hot or cold sensations. In this study, we used behavioral method of cold plate test to investigate whether allyl isothiocyanate (AITC), a natural compound of mustard oil, and capsaicin affect the sensitivity to innocuous and noxious cold stimuli in male rats. Obtained results indicate that TRPA1 and TRPV1 channels are clearly involved in pain reactions, and the TRPA1 and TRPV1 agonists AITC and capsaicin enhance the cold pain sensitivity modulating TRPA1 channels co-expressed in nociceptors with TRPV1. Our data support the role of thermosensitive TRPA1 and TRPV1 channels in pain modulation and show that these two thermo receptor channels are in synergistic and/or conditional relationship to innocuous and noxious cold cutaneous stimulation. *© 2014 Bull. Georg. Natl. Acad. Sci.*

Key words: *cold pain, heat pain, hyperalgesia, mechanical allodynia, mustard oil, capsaicin, thermal preference*

Sensitive response of the nervous system to changes in temperature is of predominant importance for homeotherms to maintain a stable body temperature. A number of temperature-sensitive transient receptor potential (TRP) ion channels are studied as nociceptors that respond to extreme temperatures and harmful chemicals. Strong activation of these channels in the nervous system elicits pain [1].

Recent findings have established a family of six thermo TRP channels (TRPA1, TRPM8, TRPV1, TRPV2, TRPV3, and TRPV4) that exhibits sensitivity to increases or decreases in temperature, as well as to chemical substances eliciting the respective hot or cold sensations. Such irritants include capsaicin (from chili pepper), menthol, cinnamaldehyde, gingerol, mustard oil, camphor, eugenol, and others [2-10].

The role of TRP ion channel subtype ankyrin 1 (TRPA1) in thermal and mechanical transduction is controversial. TRPA1 was previously found to be

cold sensitive, being activated at temperatures below 17°C [11-13]. As most of the expression of TRPA1 overlaps with that of TRPV1, it seemed reasonable to link TRPA1 to cold nociception and responsible for chemical hypersensitivity, chronic cough, and airway inflammation in asthma [14]. However, rising data connect TRPA1 with heat sensitivity. Particularly, TRPA1 agonists induced heat and mechanical hyperalgesia and a burning pain sensation, but no cold hyperalgesia [4,8-10,15-21].

Exposure to capsaicin evokes a painful burning sensation through the vanilloid TRPV1 receptor, that is also activated by noxious thermal stimuli above 43°C or by an acidic environment of pH 5.4 [13,22]. In addition to its sensitivity to various pain-inducing stimuli such as capsaicin, heat, and protons, the TRPV1 ion channel has many features that a receptor related to nociception is supposed to have, such as its preferential distribution in the central nervous system within small to medium-sized spinal dorsal root and trigeminal ganglia neurons, which are believed to serve as nociceptive nerve cells [4,9]. Although TRPV1 was shown to be activated by noxious heat, studies in TRPV1 knockout mice reveal intact or partly reduced heat sensitivity [23-25]. This suggests that TRPV1 cannot alone be responsible for heat nociception in the 42–52 °C temperature range, and this also applies to all other heat-activated ion channels as far as knockout mice phenotypes are concerned [16].

In this study, using behavioral cold plate test we examined whether allyl isothiocyanate (AITC), a natural compound of mustard oil and capsaicin affect sensitivity to innocuous and noxious cold stimuli in male rats. We hypothesized here that vanilloid TRPV1 and ankyrin TRPA1 channels could be involved in these processes and that intraplantar injection of high doses of AITC and capsaicin would induce hyper- or hyposensitivity at +5°, and -5°C temperatures.

Materials and Methods

Animals

Behavioral studies using adult male Wistar rats (350-450 g) were singly housed and given rodent chow and water *ad libitum*. 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. Guidelines of the International Association for the Study of Pain regarding animal experimentation were followed throughout [26].

Drugs

AITC at doses of 5, 10, and 15%, and capsaicin at concentrations 0.1, 0.2, 0.3 and 0.4% (Sigma-Aldrich, USA) or vehicle control (mineral oil, or Tween 80, Fisher Scientific, USA) were injected intraplantar using 30 - gauge needles.

Behavioral testing by the Cold plate test

To test sensitivity to cold temperatures, rats received bilateral intraplantar injections of capsaicin, AITC and vehicle and 5 min later was placed onto the thermoelectric surface that was set at +5°C, 0°C or –5°C. The latency for nocifensive behavior (lifting and licking one hind paw, or jumping) was measured, at which point the rat was immediately removed. All animals were retested at 5, 30, 60, 90 and 120 min post-injections.

Data analysis

The time spent on the plate in the cold plate test was normalized to baseline averages and subjected to repeated-measures analysis of variance (ANOVA) with *post-hoc* Tukey-Kramer multiple comparison test using software InStat 3.05 (GraphPad Software Inc., USA). A 95 % confidence interval was used for all statistical comparisons, and the error reported is the standard error of the mean (s.e.m.).

Results

Bilateral intraplantar injection of AITC induced a significant, concentration-dependent reduction in cold plate latency compare to vehicle control which we interpret as a cold hyperalgesia. However, there were

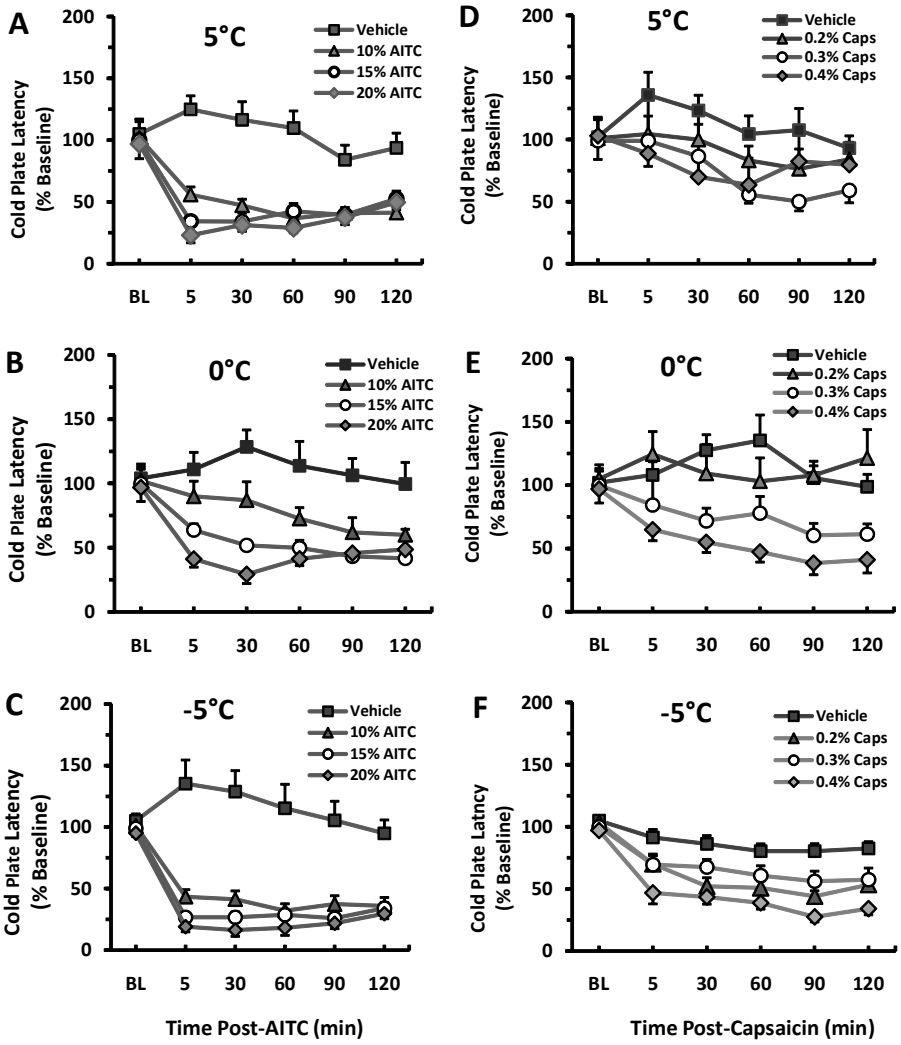

Fig. 1. Intraplantar AITC injections produce a dose-dependent cold hyperalgesia at +5°C (A), 0°C (B) and –5°C (C) temperatures, respectively. Graph plots change in cold plate latency (% of pre-AITC baseline) *vs.* time after topical AITC application at indicated concentrations. Note, the AITC effect is maximal at the first time point (5 min) since AITC diffuses readily through the skin. Intraplantar capsaicin injections also produce a dose dependent cold hyperalgesia at +5°C (D), 0°C (E) and –5°C (F) temperatures, respectively. Graph plots change in cold plate latency (% of pre-capsaicin baseline) *vs.* time after topical capsaicin application at indicated concentrations. Note, the capsaicin effect appears to grow over time, consistent with it diffusing more slowly through the skin, n = 12/group

no antinociceptive effects in latency at the +5°C, 0°C and –5°C temperatures. In the –5°C cold plate test, AITC treatment resulted in the high significant difference between vehicle (mineral oil) and AITC treated groups ($P<0.001$) (Fig. 1). Note a vehicle solution shows some protective effects to cold temperatures, especially to –5°C temperature for a mineral oil control group compare to AITC injections (Fig. 1C).

We received almost similar results with capsaicin injections as with AITC injections except –5°C temperature where the difference between vehicle (tween+saline) treated group and capsaicin treated group is not so significant (Fig. 1F) as with AITC injections (Fig. 1C).

Discussion

The presented data provide a comprehensive view of the effects of intraplantar injections of AITC and capsaicin on the thermal sensitivity. Particularly, capsaicin, AITC and cinnamaldehyde (CA) enhanced lin-

gual heat pain elicited by a 49 °C stimulus. At the same time AITC and CA weakly enhanced lingual cold pain (9.5 °C), whereas capsaicin had no effect [15]. Other investigators found that topical application of AITC produces neurogenic inflammation and, concurrently, heat and mechanical hyperalgesia, presumably via a centrally mediated sensitization process, and that these effects are TRPA1-mediated [3,11,27-30]. Dose-dependent increases in the magnitude and duration of heat hyperalgesia induced by AITC and CA were similar to that induced by intraplantar capsaicin herein. Since TRPA1 is co-expressed in sensory neurons expressing TRPV1 [31], heat hyperalgesia induced by AITC and CA might involve activation of these receptors (sensory intradermal terminals of nociceptor nerve endings) in an intracellular mechanism leading to enhanced heat sensitivity of TRPV1.

Alternatively, AITC and CA may cause intradermal release of inflammatory mediators, which lowers the heat threshold of TRPV1 [23,32]. Capsaicin in higher concentrations may also trigger central sensitization, leading to the observed reduction in the withdrawal latency for the contralateral paw [9,10,33]. Long-lasting enhancement of mechano-sensitivity (i.e., allodynia) following AITC and capsaicin applications is consistent with previous studies that showed a prolonged decrease in the mechanical withdrawal threshold in mice following intraplantar injection of a TRPA1 agonist, bradykinin [34,35], and with allodynia induced in the human skin by topical application of AITC [15]. However, TRPA1 as a ligand-gated ion channel in sensory neurons was initially reported to be activated by cold temperatures (below 18ºC) [15,18,22], although this opinion has been disputed [3,36]. TRPA1 knockout mice exhibited either normal cold sensitivity [3], or mild [15] or severe deficits [18] in human subjects.

Our previous behavioral data support the role of TRPA1s in cold detection, since intraplantar injection of CA in rats resulted in enhanced avoidance from a cold surface (temperature place preference test) and significantly lowered the withdrawal threshold in 0ºC and +5ºC (cold plate test), phenomena indicative of cold hyperalgesia [4,8-10,20,37]. Here in cold plate test we revealed a cold hyperalgesia in ATIC and capsaicin treated groups and did not observe any antinociceptive effects. These results are consistent with the possibility that TRPA1 agonists can enhance cold-evoked gating of TRPA1 channels to increase their cold sensitivity [12,32,38]. Concerning capsaicin-induced effects in previous human experiments, applications of capsaicin on the tongue significantly enhanced heat pain but not cold pain [15]. This finding is consistent with prior psychophysical studies showing that intradermal capsaicin enhanced the heat pain intensity within a small region around the injection site for up to 2 h [39-41]. The TRPV1 channels sensitive to capsaicin respond to temperatures above the pain threshold [22]. The presented results might thus be explained by a capsaicin-induced enhancement of thermal gating of TRPV1 expressed in polymodal nociceptors mediating thermal pain sensation [15,42].

Just recently, it has been shown that TRPA1 and TRPV1 channels contribute to thermal nociception and both TRPA1 and TRPV1 null mice presented behavioral deficits in heat sensitivity [16]. Furthermore, TRPV1 knockout mice showed both reduced behavioral heat sensitivity and heat-induced calcitonin gen related peptide (CGRP) release. They confirmed that TRPV1 is not the sole noxious heat sensor and other contributors must be expressed in the TRPV1 lineage [16, 43]. These authors hypothesized a possible synergistic or conditional relationship between TRPA1 and TRPV1 receptors involved in the response of cutaneous nociceptors to noxious heat and direct as well as indirect interactions between the two receptor channels can be considered [16].

Conclusions

In conclusion, our findings indicate that thermosensitive ion channels are capable of signaling temperature changes across the range normally en-

countered in the environment. We have a particular interest in the ability of TRP channel agonists to modulate the temperature sensitivity with important ramifications for pain sensation. The TRPV1, being a heat sensor, responds to their agonist capsaicin, which elicits corresponding heating sensations. Capsaicin is known to lower the threshold and enhance heat evoked gating of TRPV1. The TRPA1 is an exception, since when it is stimulated by various agonists (e.g., AITC, CA, etc.), the resultant sensation is one of burning pain rather than of cold. However, the role of TRPA1 in cold reception and cold pain sensitivity remains controversial. Our recent data support the role of TRPA1 in cold detection, as the TRPA1 agonist CA enhanced cold sensitivity in two behavioral assays. The TRPA1 is, no doubt, involved in pain, and TRPA1 agonists enhance the heat pain sensitivity, possibly by indirectly modulating the TRPV1 co-expressed with the TRPA1 in nociceptors.

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ადამიანისა და ცხოველთა ფიზიოლოგია

TRPA1 და TRPV1 არხების ურთიერთქმედებათა ქცევითი შესწავლა ვირთაგვებში

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უკანასკნელი წლების გამოკვლევათა თანახმად გარდამავალ რეცეპტორულ პოტენციალთა (გრპ- transient receptor potential-TRP) არხები მიიჩნევიან ტკივილის სამკურნალო სამიზნეებად. ეს არხები მნიშვნელოვან როლს ასრულებენ თერმული, მექანიკური და ტკივილის შეგრძნებათა აღმოცენებაში. ზოგიერთი TRP არხი მგრძნობელობას ავლენს მაღალი ან დაბალი ტემპერატურის, ისევე როგორც რიგი ქიმიური ნივთიერებების მიმართ, რომლებიც აღძრავენ თერმულ და/ან ტკივილის შეგრძნებას. მათ მიკუთვნება მდოგვის ზეთი, დარიჩინის ალდეჰიდი, პიტნის შემცველი მენთოლი, კაპსაიცინი (შეიცავს ცხარე წიწაკა), მიხაკის ზეთი და სხვა. მდოგვის ზეთის შემცველი აქტიური ნივთიერება ალილ-იზოთიოციანატი (აითც) და კაპსაიცინი წარმოადგენენ TRPA1 და TRPV1 იონური არხების აგონისტებს. ექსპრესირდებიან რა სენსორულ ნეირონებში, ისინი იწვევენ მწველ შეგრძნებასა და სითბურ ჰიპერალგეზიას. წარმოდგენილ შრომაში ჩვენ შევისწავლეთ ამ ნივთიერებების ბილატერალური ტერფქვეშა ინექციების პირობებში დაბალი ტემპერატურის (+5°C, 0°C, -5°C) ზეგავლენა, ე.წ. ცივი ფირფიტის ტესტის გამოყენებით. მიღებულმა შედეგებმა გვიჩვენა, რომ ორივე ლიგანდის შემთხვევაში ადგილი აქვს სიცივეზე მგრძნობელობის მომატებას, ჰიპერალგეზიას, განსაკუთრებით -5°C-ზე. ჩვენი და სხვა ავტორთა მონაცემები ადასტურებს მოსაზრებას, რომ TRPA1 და TRPV1 იონური არხები ჩართულია ტკივილისა და ტემპერატურის შეგრძნებაში და რომ ეს თერმო იონური არხები წარმოადგენს იმედის მომცემ პერიფერიულ სამიზნეებს ახალი ტიპის ანალგეზიური პრეპარატების სინთეზისთვის.

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