Published in final edited form as:

Neurosci Lett. 2010 April 12; 473(3): 233–236. doi:10.1016/j.neulet.2010.02.056.

Behavioral evidence of thermal hyperalgesia and mechanical allodynia induced by intradermal cinnamaldehyde in rats

Merab G. Tsagareli², Nana Tsiklauri², Karen L. Zanutto¹, Mirela Iodi Carstens¹, Amanda H. Klein¹, Carolyn M. Sawyer¹, Gulnazi Gurtskaia², Elene Abzianidze², and E. Carstens¹

¹ Department of Neurobiology, Physiology and Behavior, University of California, Davis

² Dept Neurophysiology, Beritashvili Inst. Physiology, Tbilisi, Rep. of Georgia

Abstract

TRPA1 agonists cinnamaldehyde (CA) and mustard oil (allyl isothiocyanate= AITC) induce heat hyperalgesia and mechanical allodynia in human skin, and sensitize responses of spinal and trigeminal dorsal horn neurons to noxious skin heating in rats. TRPA1 is also implicated in cold nociception. We presently used behavioral methods to investigate if CA affects sensitivity to thermal and mechanical stimuli in rats. Unilateral intraplantar injection of CA (5-20%) induced a significant, concentration-dependent reduction in latency for ipsilateral paw withdrawal from a noxious heat stimulus, peaking (61.7% of pre-injection baseline) by 30 min with partial recovery at 120 min. The highest dose of CA also significantly reduced the contralateral paw withdrawal latency. CA significantly reduced mechanical withdrawal thresholds of the injected paw that peaked sooner (3 min) and was more profound (44.4% of baseline), with no effect contralaterally. Bilateral intraplantar injections of CA resulted in a significant cold hyperalgesia (cold-plate test) and a weak enhancement of innocuous cold avoidance (thermal preference test). The data are consistent with roles for TRPA1 in thermal (hot and cold) hyperalgesia and mechanical allodynia.

Keywords

TRPA1; cinnamaldehyde; nociception; heat hyperalgesia; cold hyperalgesia; mechanical allodynia

INTRODUCTION

Cinnamaldehyde (CA) is a pungent chemical from cinnamon that acts as an agonist of the thermosensitive TRP (transient receptor potential) ion channel TRPA1 that was originally reported to be activated by cold temperatures below 18°C [26]. TRPA1 is activated by several other irritant chemicals including mustard oil (allyl isothiocyanate= AITC) [4,12,18] and is colocalized with TRPV1 in polymodal nociceptors [15,26]. Topical application of CA or AITC elicits a burning sensation and heat hyperalgesia in humans [1,20,22,24] and enhances responses of rat spinal and trigeminal subnucleus caudalis (Vc) dorsal horn neurons to noxious heat [19,25,31]. The role of TRPA1 in cold pain is more controversial, with discrepant reports

© 2010 Elsevier Ireland Ltd. All rights reserved.

Corresponding author: Prof. Earl Carstens Department of Neurobiology, Physiology and Behavior University of California, Davis 1 Shields Avenue Davis, CA 95616 USA Tel.: 1-530-752-7767 Fax: 1-530-752-5582 eecarstens@ucdavis.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

that TRPA1 does [13,26] or does not respond to intense cooling [12]. Knockout mice lacking TRPA1 exhibit normal cold sensitivity [4], or partial [17] or severe [13] deficits in cold pain sensitivity. In humans, topical cutaneous application of CA induces cold hypoalgesia [20] while epilingual CA induces brief cold hyperalgesia [1]. Neither agent affects rat spinal neuronal responses to cooling [19,23].

TRPA1 was originally reported to play a role in mechanotransduction [7]. AITC induced mechanical allodynia in humans [16]. Intraplantar injection of the TRPA1 agonist 4-hydroxynonenal reduced mechanical paw withdrawal thresholds in mice [28] and blockade of TRPA1 by systemically or locally administered antagonists reversed mechanical hyperalgesia in inflammatory and nerve injury models in mice [8,21].

Based on the studies described above, we hypothesized that intraplantar injection of CA would induce (1) hyperalgesia to noxious heat, (2) cold allodynia and/or cold hyperalgesia, and (3) mechanical allodynia. We presently tested these hypotheses using a battery of behavioral tests of thermal and mechanical sensitivity in rats. An abstract of portions of this study has appeared [29].

METHODS

Behavioral studies using adult male Sprague Dawley rats (393-460 g) were conducted at approximately the same time each day to reduce circadian effects, under a protocol approved by the UC Davis Animal Care and Use Committee.

CA (Sigma-Aldrich, St. Louis MO) at doses of 0.5, 1 or 2 mg/10 μ l (i.e., 5%, 10% and 20% or 378.3 mM, 756.7 mM and 1.5 M; in saline + 5% Tween 80, Fisher Scientific, Waltham, MA) or vehicle was injected intraplantar using a 30 gauge needle. Immediately after intraplantar CA, rats sometimes exhibited weak and variable nocifensive responses including paw withdrawal, licking, flinching or shaking that subsided within 3 min later, consistent with previous reports using TRPA1 [3,8,28] and TRPV1 [9] agonists. For thermal and mechanical paw withdrawal tests, CA or vehicle was injected unilaterally while for cold plate and thermal preference tests, it was injected bilaterally. The rationale for bilateral injections was to ensure that at least one treated hind paw would contact the thermal surface even if the animal guarded the other paw. Thermal [10] and mechanical paw withdrawal tests were conducted using methods described previously [5]. After habituation to the test apparatus, baseline latencies for paw withdrawals evoked by radiant thermal stimulation of each hind paw were measured 3 times/ paw, with at least 5 min elapsing between tests of a given paw. A light beam (Plantar Test 390, IITC, Woodland Hills CA) was focused onto the plantar surface through the glass floor from below, and the latency from onset of the light to brisk withdrawal of the stimulated paw was measured. A cutoff of 20 sec was imposed to prevent tissue damage. The other hind paw was similarly tested 30-60 sec later. After baseline testing, the rat received intraplantar injection of CA or vehicle and withdrawal latencies for both paws were determined at 3, 15, 30, 45, 60 and 120 min post-injection using a paradigm similar to that of Gilchrist et al. [9]. Tests of mechanosensitivity used an electronic von Frey filament (1601C, IITC) that was pressed against the ventral paw from below, through a screen the animal stood on. The device monitored force (g) at the moment of paw withdrawal. Mechanical paw withdrawals were tested using the same experimental design as described for thermal withdrawal tests. The same group of rats was used for thermal and mechanical withdrawal tests, with a minimum of seven days in between successive tests to avoid possible carryover effects. Thermal and mechanical paw withdrawal responses were normalized and subjected to repeated measures analysis of variance (ANOVA) using SPSS 9.0 software (SPSS, Chicago IL).

For thermal preference tests, the rat was placed onto a surface consisting of two adjacent thermoelectric plates (each $13.3 \times 6.37''$; AHP-1200DCP, Teca Thermoelectric, Chicago, IL) that could be independently heated or cooled to a pre-set temperature (-5 to $>50^{\circ}\text{C}$ $\pm 1.0^{\circ}\text{C}$). Both plates were enclosed by an opaque Plexiglas box with a center partition having an opening in the middle to allow the rat to move freely between the two surfaces. One plate at 30°C and the other at 15°C in a counterbalanced design. CA or vehicle was bilaterally injected intraplantar, and the animal was placed onto one of the plates in a matched block design alternating initial position and temperature. The animal was videotaped from above, and the duration of time the animal spent on the 15°C and 30°C plates during the first 20 min was recorded by investigators blind as to treatment and plate temperature, and compared between CA and vehicle treatment groups by paired t-test, with $p < 0.05$ considered significant. The 30°C - 15°C temperature difference was selected based on pilot tests with naïve animals showing that the colder surface was avoided about 80% of the time, thus allowing assessment of further increases or decreases in cold avoidance (i.e., avoiding floor and ceiling effects).

To test sensitivity to noxious cold, the rat received bilateral intraplantar injections of CA, vehicle, or nothing, and 2 min later was placed onto one of the thermoelectric surfaces described above that was set at $+5^{\circ}\text{C}$, 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. A cutoff time of 150 sec was imposed to prevent tissue damage [2]. These temperatures bracket the range from shortest response latency (-5°C) to virtual absence of response ($+5^{\circ}\text{C}$), thereby accounting for potential floor and ceiling effects. All animals were retested 3, 60 and 120 minutes post-injection. The cold plate surface was cleaned, scraped clear of ice if necessary, and dried prior to testing each animal. At least seven days intervened between successive tests of the same rat. Cold plate latencies were normalized to pre-treatment baselines and compared between CA and vehicle treatment groups by repeated measures ANOVA and by paired t-test.

RESULTS

CA resulted in a significant, dose-dependent reduction in ipsilateral thermal paw withdrawal latency. Fig. 1A shows mean withdrawal latencies of the injected paw vs. time relative to injection of vehicle or CA at each concentration tested. There was a dose-dependent reduction in latency, with the 20% CA concentration significantly different from vehicle and 5% CA treatments. The highest dose resulted in a mean reduction to 61.7% of pre-injection baseline by 30 min with partial recovery at 120 min. For the contralateral paw (Fig. 1B), there was an overall significant effect of treatment with the 20% group being significantly different from saline and 5% CA treatments.

Mechanically-evoked withdrawal thresholds are plotted vs. time for the treated paw in Fig. 1C. At each CA concentration thresholds were significantly different from vehicle, but not from each other, indicating that a maximal reduction in withdrawal threshold (to 44.4% of baseline) was achieved at the lowest (5%) concentration of CA. Mean withdrawal thresholds for the contralateral paw (Fig. 1D) were not significantly affected at any CA concentration.

In the 30° vs. 15°C thermal preference test, rats treated with the highest CA concentration exhibited a small but significant avoidance of the colder plate, spending a significantly ($p < 0.05$, paired t-test) greater percentage of time on the warmer 30°C plate (83.3 % $\pm$ 6.9 SEM) compared to vehicle-treated rats (76.5% $\pm$ 7.1).

When tested on the -5°C cold plate test (3 min post-treatment), there was no significant difference in latency between groups treated with the highest concentration of CA compared to vehicle (26.0 $\pm$ 5.3 [SEM] sec vs. 24.9 $\pm$ 2.24 sec; $p > 0.05$, $n=8$). In the 0°C cold plate

test, CA treatment resulted in a significant concentration- and time-dependent decrease in latency indicative of cold hyperalgesia (Fig. 2A). The mean latency was significantly different between 5% CA vs. 10% or 20% CA treatment groups at the initial (3-min) time point ($p < 0.05$, repeated-measures ANOVA). When tested on the $+5^{\circ}\text{C}$ cold plate, many animals did not display nocifensive behavior up to the 150 sec cutoff consistent with a previous report [11]. CA treatment resulted in a significant reduction in latency (Fig. 2B) with no significant effect of CA concentration over time, indicating that a maximal effect was achieved at the lowest CA concentration.

DISCUSSION

The present data provide a comprehensive view of effects of intraplantar CA on thermal (hot and cold) and mechanical sensitivity. CA induced a dose-dependent heat hyperalgesia lasting >2 hr at the highest dose, mechanical allodynia, and cold hyperalgesia.

CA enhancement of heat sensitivity is consistent with previous studies. Topical application of CA (795 mM) to human forearm skin evoked burning pain and heat hyperalgesia [20]. Epilangual CA (16 mM) produced brief heat hyperalgesia [1]. CA enhanced responses of spinal [19] and Vc neurons to noxious heat [31]. These and the present findings are consistent with a role for TRPA1 in heat pain and heat hyperalgesia.

The dose-dependent increase in magnitude and duration of heat hyperalgesia induced by CA was similar to that induced by intraplantar capsaicin (1–30 μg dose range) in rats using the same method [9]. Since TRPA1 is co-expressed in sensory neurons that express TRPV1 [15, 26], heat hyperalgesia induced by CA might involve its activation of intradermal nociceptor nerve endings to engage an intracellular mechanism leading to enhanced heat sensitivity of TRPV1. Alternatively, CA may cause intradermal release of inflammatory mediators that lower the heat threshold of TRPV1 [6,27]. CA at the highest concentration may have also triggered central sensitization, leading to the observed reduction in withdrawal latency for the contralateral paw (Fig. 1B). Consistent with this, topical application of AITC (TRPA1 agonist) to the lateral hindlimb significantly reduced the tail flick latency in rats in a manner dependent on the integrity of the rostral ventromedial medulla [30].

CA significantly lowered nocifensive response latencies in the 0 and $+5^{\circ}\text{C}$ cold plate tests, which we interpret as cold hyperalgesia. The lack of effect of CA in the -5°C cold plate test may reflect a floor effect, since the response latency at this temperature was maximal and thus could not be reduced further by CA. CA also weakly but significantly increased cold avoidance in the thermal preference test. The latter may reflect cold allodynia, on the assumption that the 15°C surface is normally innocuous but became painful following CA treatment. Cold avoidance progressively increases as the temperature decreases from 25 – 0°C (authors' unpublished observations), and it is possible that following CA treatment the 15°C surface was perceived to be colder and hence aversive but not necessarily painful.

In humans, CA on forearm skin induced cold *hypo*algesia, [20] whereas epilangual CA or AITC briefly enhanced cold pain [1]. Lingual application of CA significantly enhanced cold-evoked responses of superficial Vc neurons in rats [31] but did not affect responses of lumbar spinal wide dynamic range (WDR)-type neurons to skin cooling [19]. These discrepancies regarding the effects of CA on cold pain perception and neuronal responses may partly involve the route of delivery and accessibility of CA to intradermal nociceptors. In the present study, intradermal injection of CA allowed for a direct access to nociceptive nerve endings to result in significant cold hyperalgesia and enhancement of cold avoidance.

The prolonged enhancement of mechanosensitivity (i.e. allodynia) following CA (Fig. 1C) is consistent with previous studies showing a prolonged decrease in mechanical withdrawal

threshold in mice following intraplantar injection of a TRPA1 agonist [28], and with allodynia induced in human skin by topical application of AITC [16]. A role for TRPA1 in mechanical allodynia is further supported by reports that TRPA1 antagonists attenuated inflammation- or nerve injury-induced decreases in mechanical paw withdrawal thresholds in mice [8,21] and decrease mechanically evoked responses in C fibers in mice [14]. However, these behavioral data are inconsistent with our electrophysiological data showing that neither CA nor AITC had any significant effect on mechanical sensitivity of spinal WDR neurons [19]. Similarly, only 1 of 9 subjects experienced mechanical allodynia following application of 10% CA to forearm skin [20]. The mismatch between our behavioral observation of a CA-induced increase in mechanosensitivity and lack of CA effect on neuronal mechanosensitivity [19] may involve the route of administration as noted earlier.

Acknowledgments

Funded by grants from the US Civilian Research and Development Foundation (GEB1-2883-TB07) and the National Institutes of Health (DE013685). The authors thank Susan Cheung, Cindy Kwok, and Margaret Ivanov for their technical assistance.

REFERENCES

1. Albin KC, Carstens MI, Carstens E. Modulation of oral heat and cold pain by irritant chemicals. *Chem. Senses* 2008;33:3–15. [PubMed: 17728277]
2. Allchorne A, Broom D, Woolf C. Detection of cold pain, cold allodynia and cold hyperalgesia in freely behaving rats. *Molecular Pain* 2005;1:36. [PubMed: 16354295]
3. Andrade EL, Luiz AP, Ferreira J, Calixto JB. Pronociceptive response elicited by TRPA1 receptor activation in mice. *Neuroscience* 2008;152:511–520. [PubMed: 18272293]
4. Bautista DM, Jordt SE, Nikai T, Tsuruda PR, Read AJ, Poblete J, Yamoah EN, Basbaum AI, Julius D. TRPA1 mediates the inflammatory actions of environmental irritants and proalgesic agents. *Cell* 2006;124:1269–1282. [PubMed: 16564016]
5. Carstens E, Anderson KA, Simons CT, Carstens MI, Jinks SL. Analgesia induced by chronic nicotine infusion in rats - Differences by gender and pain test. *Psychopharmacology* 2001;157:40–45. [PubMed: 11512041]
6. Chuang, H.-h.; Prescott, ED.; Kong, H.; Shields, S.; Jordt, S-E.; Basbaum, AI.; Chao, MV.; Julius, D. Bradykinin and nerve growth factor release the capsaicin receptor from PtdIns(4,5)P2-mediated inhibition. *Nature* 2001;411:957–962. [PubMed: 11418861]
7. Corey DP, Garcia-Anoveros J, Holt JR, Kwan KY, Lin SY, Vollrath MA, Amalfitano A, Cheung ELM, Derfler BH, Duggan A, Geleoc GSG, Gray PA, Hoffman MP, Rehm HL, Tamasauskas D, Zhang DS. TRPA1 is a candidate for the mechanosensitive transduction channel of vertebrate hair cells. *Nature* 2004;432:723–730. [PubMed: 15483558]
8. Eid S, Crown E, Moore E, Liang H, Choong K-C, Dima S, Henze D, Kane S, Urban M. HC-030031, a TRPA1 selective antagonist, attenuates inflammatory- and neuropathy-induced mechanical hypersensitivity. *Molecular Pain* 2008;4:48. [PubMed: 18954467]
9. Gilchrist HD, Allard BL, Simone DA. Enhanced withdrawal responses to heat and mechanical stimuli following intraplantar injection of capsaicin in rats. *Pain* 1996;67:179–188. [PubMed: 8895246]
10. Hargreaves K, Dubner R, Brown F, Flores C, Joris J. A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. *Pain* 1988;32:77–88. [PubMed: 3340425]
11. Jasmin L, Kohan L, Franssen M, Janni G, Goff JR. The cold plate as a test of nociceptive behaviors: description and application to the study of chronic neuropathic and inflammatory pain models. *Pain* 1998;75:367–382. [PubMed: 9583773]
12. Jordt SE, Bautista DM, Chuang HH, McKemy DD, Zygmunt PM, Hogestatt ED, Meng ID, Julius D. Mustard oils and cannabinoids excite sensory nerve fibres through the TRP channel ANKTM1. *Nature* 2004;427:260–265. [PubMed: 14712238]

13. Karashima Y, Talavera K, Everaerts W, Janssens A, Kwan KY, Vennekens R, Nilius B, Voets T. TRPA1 acts as a cold sensor in vitro and in vivo. *Proceedings of the National Academy of Sciences* 2009;106:1273–1278.
14. Kerstein P, del Camino D, Moran M, Stucky C. Pharmacological blockade of TRPA1 inhibits mechanical firing in nociceptors. *Molecular Pain* 2009;5:19. [PubMed: 19383149]
15. Kobayashi K, Fukuoka T, Obata K, Yamanaka H, Dai Y, Tokunaga A, Noguchi K. Distinct expression of TRPM8, TRPA1, and TRPV1 mRNAs in rat primary afferent neurons with adelta/c-fibers and colocalization with trk receptors. *The Journal of Comparative Neurology* 2005;493:596–606. [PubMed: 16304633]
16. Koltzenburg M, Lundberg LER, Torebjörk HE. Dynamic and static components of mechanical hyperalgesia in human hairy skin. *Pain* 1992;51:207–219. [PubMed: 1484717]
17. Kwan KY, Allchorne AJ, Vollrath MA, Christensen AP, Zhang DS, Woolf CJ, Corey DP. TRPA1 contributes to cold, mechanical, and chemical Nociception but is not essential for hair-cell transduction. *Neuron* 2006;50:277–289. [PubMed: 16630838]
18. McNamara CR, Mandel-Brehm J, Bautista DM, Seimans J, Deranian KL, Zhao M, Hayward NJ, Chong JHA, Julius D, Moran MM, Fanger CM. TRPA1 mediates formalin-induced pain. *Proc. Natl. Acad. Sci. USA* 2007;104:13525–13530. [PubMed: 17686976]
19. Merrill AW, Cuellar JM, Judd JH, Carstens MI, Carstens E. Effects of TRPA1 agonists mustard oil and cinnamaldehyde on lumbar spinal wide-dynamic range neuronal responses to innocuous and noxious cutaneous stimuli in rats. *Journal of Neurophysiology* 2008;99:415–425. [PubMed: 17942619]
20. Namer B, Seifert F, Handwerker HO, Maihöfner C. TRPA1 and TRPM8 activation in humans: effects of cinnamaldehyde and menthol. *NeuroReport* 2005;16:955–959. [PubMed: 15931068]
21. Petrus M, Peier AM, Bandell M, Hwang SW, Huynh T, Olney N, Jegla T, Patapoutian A. A role of TRPA1 in mechanical hyperalgesia is revealed by pharmacological inhibition. *Molecular Pain* 2007;3:8. [PubMed: 17391515]
22. Prescott J, Swain-Campbell N. Responses to Repeated Oral Irritation by Capsaicin, Cinnamaldehyde and Ethanol in PROP Tasters and Non-tasters. *Chem. Senses* 2000;25:239–246. [PubMed: 10866983]
23. Sawyer CM, Carstens MI, Carstens E. Mustard oil enhances spinal neuronal responses to noxious heat but not cooling. *Neuroscience Letters* 2009;461:271–274. [PubMed: 19545607]
24. Simons CT, Carstens MI, Carstens E. Oral Irritation by Mustard Oil: Self-desensitization and Cross-desensitization with Capsaicin. *Chem. Senses* 2003;28:459–465. [PubMed: 12907583]
25. Simons CT, Sudo S, Sudo M, Carstens E. Mustard oil has differential effects on the response of trigeminal caudalis neurons to heat and acidity. *Pain* 2004;110:64–71. [PubMed: 15275753]
26. Story GM, Peier AM, Reeve AJ, Eid SR, Mosbacher J, Hricik TR, Earley TJ, Hergarden AC, Andersson DA, Hwang SW, McIntyre P, Jegla T, Bevan S, Patapoutian A. ANKTM1, a TRP-like Channel Expressed in Nociceptive Neurons, Is Activated by Cold Temperatures. *Cell* 2003;112:819–829. [PubMed: 12654248]
27. Sugiura T, Tominaga M, Katsuya H, Mizumura K. Bradykinin lowers the threshold temperature for heat activation of vanilloid receptor 1. *Journal of Neurophysiology* 2002;88:544–548. [PubMed: 12091579]
28. Trevisani M, Siemens J, Materazzi S, Bautista DM, Nassini R, Campi B, Imamachi N, Andre E, Patacchini R, Cottrell GS, Gatti R, Basbaum AI, Bunnett NW, Julius D, Geppetti P. 4-Hydroxynonenal, an endogenous aldehyde, causes pain and neurogenic inflammation through activation of the irritant receptor TRPA1. *Proc. Natl. Acad. Sci. U. S. A* 2007;104:13519–13524. [PubMed: 17684094]
29. Tsagareli, M.; Iodi Carstens, M.; Tsiklauri, N.; Gurtskai, GP.; Zanolto, KL.; Abzianidze, EV.; Carstens, E. Behavioral evidence of heat hyperalgesia and mechanical allodynia induced by TRPA1 agonists in rats.. *Neuroscience Meeting Planner*, Vol. Online, Program No. 266.9; Washington, D.C.. 2008;
30. Urban MO, Zahn PK, Gebhart GF. Descending facilitatory influences from the rostral medial medulla mediate secondary, but not primary hyperalgesia in the rat. *Neuroscience* 1999;90:349–352. [PubMed: 10215139]

31. Zanutto KL, Iodi Carstens M, Carstens E. Cross-desensitization of responses of rat trigeminal subnucleus caudalis neurons to cinnamaldehyde and menthol. *Neurosci Lett* 2008;430:29–33. [PubMed: 18060696]

Fig. 1.

CA: heat hyperalgesia and mechanical allodynia. A: Normalized mean thermal paw withdrawal latency vs. time following ipsilateral intraplantar injection of vehicle (control) or CA at each indicated concentration. There was a significant effect of CA concentration (repeated measures ANOVA, $F_{3,28}=3.1$, $p<0.05$) with the 20% CA group significantly different from vehicle and 5% CA groups (LSD; $p<0.05$ for both). BL: pre-injection baseline. Error bars: SEM (n=8). B: As in A for paw contralateral to CA injection. There was a significant effect of CA concentration ($F_{3,28}=3.13$, $p<0.05$) with the 20% group significantly different from vehicle and 5% groups (LSD; $p<0.05$ for both). C: As in A for von Frey mechanically-evoked withdrawal of the injected paw. There was a significant effect of CA concentration ($F_{3,28}=4.4$, $p<0.05$) with 5, 10 and 20% CA all different from vehicle but not from each other. D: As in C for contralateral paw. There was no significant effect of CA concentration ($F_{3,28}=0.14$, $p>0.5$).

Fig. 2.

Intraplantar CA decreases cold-plate latency. Graphs plot normalized mean latencies (error bars: SEM; n=8) to lick hindpaw or jump after being placed on a 0°C (A) or +5°C (B) cold plate surface over a 2 hr period of time. A: Mean latency was significantly different between 5% CA vs. 10% or 20% CA treatment groups at the first (3-min) time point (repeated-measures ANOVA, $p < 0.05$). B: There was a significant reduction in latency for each CA treatment group with no significant effect of CA concentration over time.