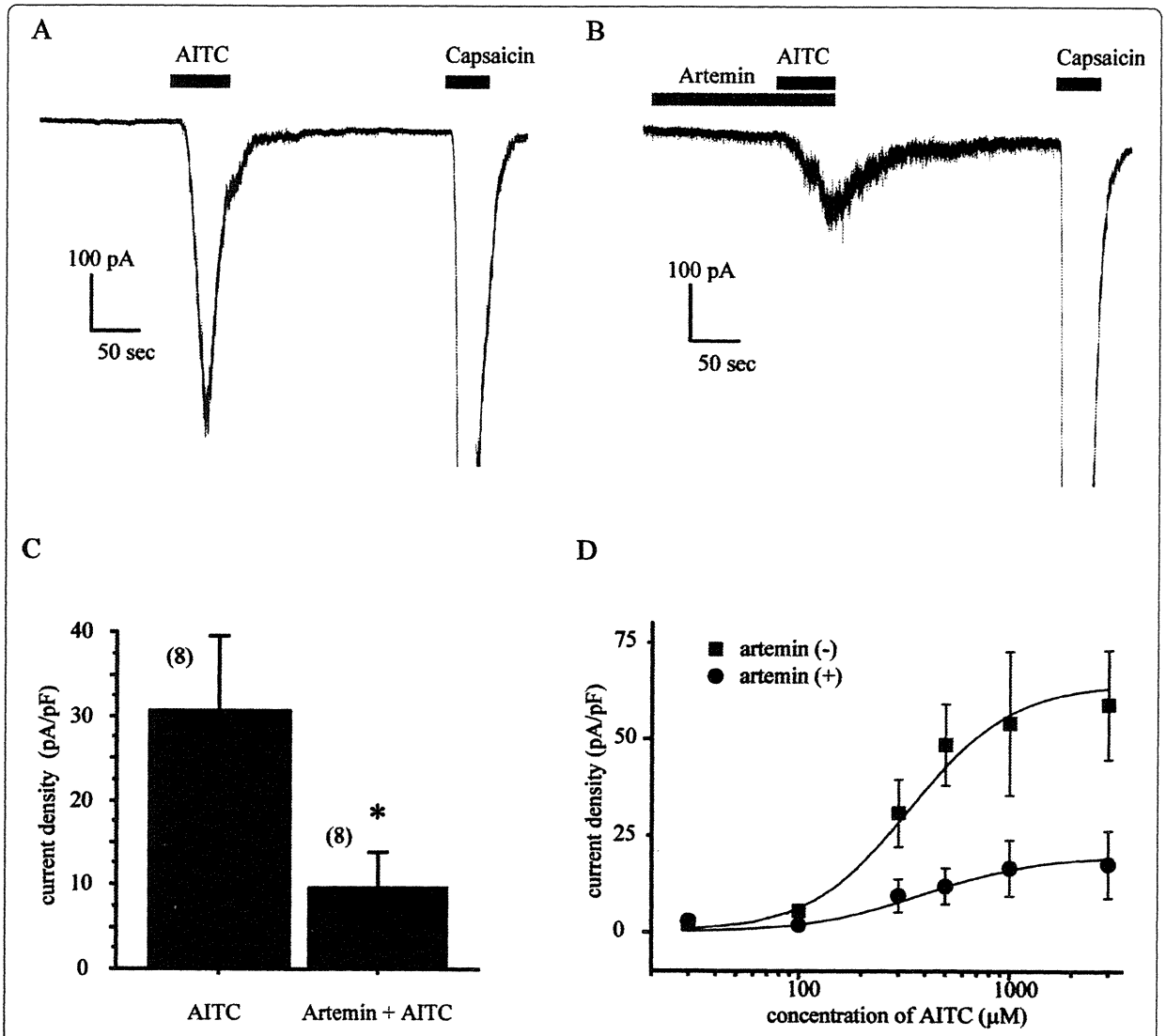

Figure 3 Suppression of AITC-induced current density in DRG neurons by artemin. (A, B): Representative traces show TRPA1 currents produced by AITC application (300 μM, 1 min) in the absence (A) or presence (B) of artemin (100 ng/ml). AITC was applied for 1 min after artemin administration for 2 min. (C): Bar graph shows the effect of artemin on AITC activated current density (pA/pF). Numbers in parenthesis indicate cells tested. * $p < 0.05$ versus control (absence of artemin), unpaired t-test. (D): Concentration-response curves for AITC-induced current in the present or absence of artemin. Figure shows averaged data fitted with the Hill equation (see methods). EC50 = 343 μM and Maximum current density = -64.4 pA/pF in the absence of artemin, EC50 = 396 μM and Maximum current density = -19.8 pA/pF in the presence of artemin. Data for each point were generated from separate neurons by application of AITC for 1 min after pretreatment of artemin or vehicle. Holding potential (Vh) = -60 mV in all experiments.

induced a significant lifting and flinching behavior of the injected hindpaw during the 30 or 60 min post-injection period, whereas such behaviors were not observed in vehicle-injected rats (data not shown). The injection of artemin did not cause any inflammatory reactions (e.g. redness, swelling) and acute nocifensive behavior (e.g. paw lifting, flinching or licking). Five min after pre-treatment with the artemin, AITC or formalin was injected into the same area of the hind paw. Pre-treatment with artemin induced a significant decrease of the number of paw lifts and the duration of paw flinches in the initial 30 min post-injection period of AITC, compared to those of rats pre-treated with vehicle (Figure 4). Administration of artemin preceding the formalin injection also reduced the number of flinches observed in both Phase I (0-5 min) and Phase II (10-60 min) (Figure 5).

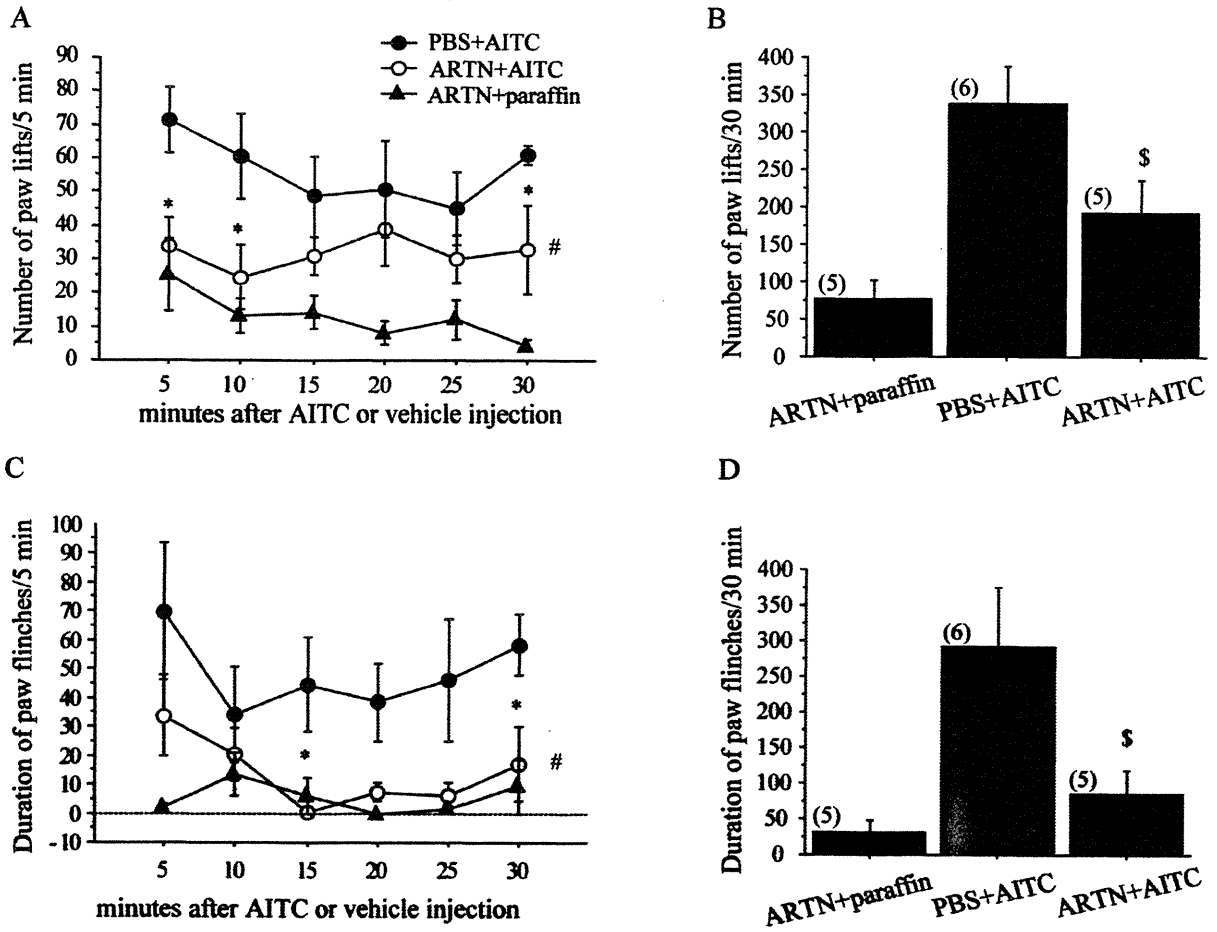

Figure 4 Artemin suppressed nocifensive behaviors induced by AITC intraplantar injection. Artemin (10 μg/ml) or PBS was pre-treated subcutaneously 5 min before AITC (3% in 50 μl liquid paraffin) injection. (A-D): Left panels show the time course of the number of hind paw liftings (A) and the duration of paw flinches (C) after AITC injection with artemin (ARTN) or PBS pre-treatment. The number counted per 5 min interval in the initial 30 min post-injection period. Right panels show cumulative number of paw lifts (B) and cumulative duration of flinches (D) over the first 30 min period after injection of AITC. * $p < 0.05$; versus PBS + AITC at each time point (unpaired t-test), # $p < 0.05$; ## $p < 0.001$; versus PBS + AITC group (two-way repeated ANOVA followed by Fisher's PLSD), $ $p < 0.05$; $$ $p < 0.01$; versus PBS + AITC group (two-way factorial ANOVA). Numbers in parentheses indicate number of rats used in each group.

Discussion

In recent years, the role of artemin, a GDNF family member, in mediating neuropathic and inflammatory pain has been received much attention. Artemin is known to be one of the survival factors for sensory and sympathetic neurons *in vitro* and *in vivo*. The long-term intrathecal or systemic administration of artemin prevented many of the nerve injury-induced changes in the histochemistry of nociceptor neurons, and produced dose- and time-related reversal of nerve injury-induced pain behaviors [26-28]. On the other hand, overexpression of artemin up-regulated expression of TRPV1 and TRPA1 channels and subsequently led to an increase of neuronal activity and hyperalgesia [2,20]. Acute application of artemin induced a significant potentiation of TRPV1 function and produced acute thermal hyperalgesia [21]. In the present study, in contrast to the potentiation of TRPV1 function by artemin, we observed that a short-term application of artemin significantly suppressed TRPA1 channels activity and the TRPA1-mediated pain behaviors.

Artemin binds to GFR α3/Ret to induce extracellular signals [1]. We found TRPA1 was highly coexpressed with GFR α3 and RET (Figure 1). This finding is consistent with a previous report that indicates nearly all GFR α3-positive neurons express TRPV1 immunoreactivity, and most of these neurons are also TRPA1 positive [2]. The high co-localization of TRPA1 and GFR α3 provides a possible histological prerequisite of the functional interaction between these two molecules.

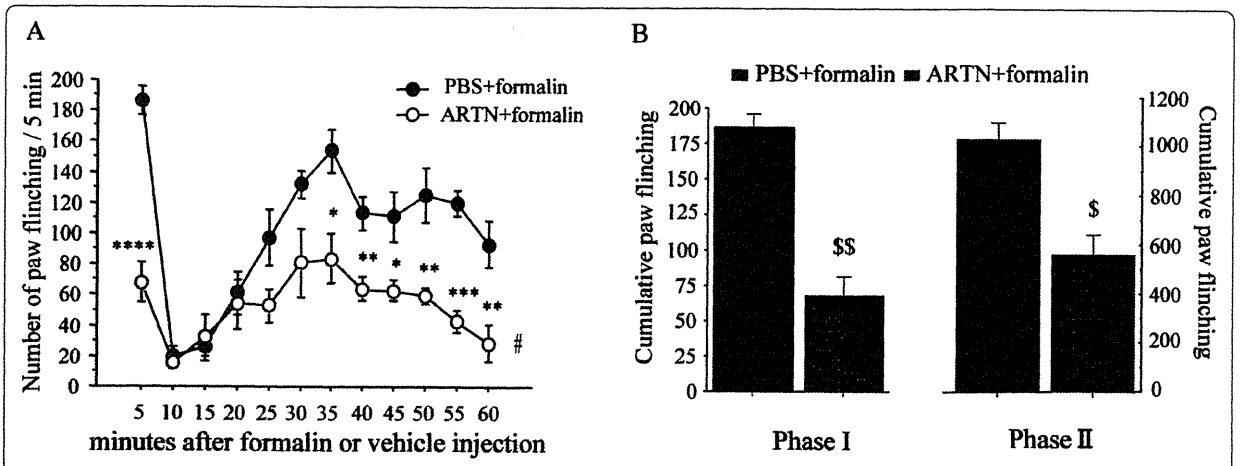

Figure 5 Artemin suppressed nocifensive behaviors induced by formalin. Artemin (10 μg/ml) or PBS was pre-treated subcutaneously 5 min before formalin (3% in 50 μl saline) injection. Time course (A) and cumulative data (B) of the number of hind paw flinches after formalin injection with artemin or PBS pre-treatment were measured. The number counted per 5 min interval in the initial 1 h post-injection period in A. B shows cumulative number of paw flinches during the Phase I (0-5 min) and the second Phase II (10-60 min) after injection of formalin. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.0005$; **** $p < 0.0001$ versus PBS + formalin (unpaired t-test), # $p < 0.001$; versus PBS + formalin (two-way repeated ANOVA followed by Fisher's PLSD), $ $p < 0.005$; $$ $p < 0.0001$ versus PBS + formalin (two-way factorial ANOVA). Numbers in parentheses indicate number of rats used in each group.

We found in the present study that a short term application of artemin significant reduced the AITC-induced TRPA1 current. This inhibitory regulation was examined in two ways. First, artemin was delivered between two applications of AITC and then the normalized current (the next AITC-current versus the first one) was collected (Figure 2). This approach allowed us to confirm that the tested neuron was indeed a TRPA1-expressing one, but tachyphylaxis of AITC-induced current may have interrupted the analysis and made it difficult to explain the reduced normalized current solely as an inhibition of TRPA1 channels, rather a cooperative effect of an artemin-induced inhibition and the tachyphylaxis may have taken place. Therefore, we also performed an experiment with sensory neurons pretreated by artemin and then applied AITC to evaluate the current density. Both of these results clearly indicated inhibitions of TRPA1 channel function by acute application of artemin.

Acute application of artemin has been reported to induce potentiation of another TRP channel, TRPV1, and produce acute thermal hyperalgesia [21], TRPV1 and TRPA1 are structurally endowed with the same TRP domain but have distinctly different intracellular loops. AITC activates TRPA1 by covalently modifying cysteine residues located in the N terminus of the channel, different from a classical lock-and-key binding mechanism for capsaicin-TRPV1. Thus it is not a wonder that the regulatory mechanism of TRPV1 and TRPA1 may differ. For example, PKC has been reported to potentiate TRPV1 [22,29,30] but did not have any effect on TRPA1[23,24]. On the other hand, Elitt et al have reported that overexpression of artemin up-regulated expression of TRPV1 and TRPA1 channels and subsequently led to greater neuronal activity and hyperalgesia [2,20]. These reports suggested a post-transcriptional regulation of artemin (which might contribute as a neurotrophic factor) on TRPV1 and TRPA1 channels, are not discordant with our findings.

TRPA1 is known to be expressed on sensory neurons and acted as an important component of pain. If artemin can suppress the TRPA1activation, pain sensation that is caused through the TRPA1 channel may also be suppressed by the artemin activation. Topical application of AITC has been reported to excite sensory nerve fibers, thereby producing acute pain [5,17,31]. Consistent with our electrophysiological data, artemin produced a significantly persistent suppression of the duration of paw flinch and the number of paw lifts induced by intraplantar injection of AITC (Figure 3). A recent study indicates that TRPA1 is the principal site of formalin's pain-producing action in rodents [19]. The formalin test is a widely used model of continuous pain resulting from formalin-induced tissue injury. Subcutaneous injection of formalin into the rat hindpaw produces a biphasic pain response that consists of an early, acute phase (Phase I) and a late, tonic phase (Phase II) that is manifested behaviorally as lifting, flinching or licking of the affected paw, and these behaviors are robust and readily quantifiable [32,33]. Phase I is thought to result from direct activation of primary afferent sensory neurons, whereas Phase II has been

proposed reflect the combined effects of afferent input and central sensitization in the dorsal horn. In our present study, we found artemin treatment significantly suppressed the formalin-induced pain behaviors at both two phases (Figure 3). Direct inhibition of TRPA1 activity by local injection of artemin in the plantar nerve terminal may result the suppression of behaviors in Phases I, then the reduced input of primary afferent may contribute the reduction of pain behaviors in Phase II. These findings suggest that a short-term application of artemin inhibits the TRPA1 channel's activity and the sequential pain behaviors.

The mechanism of artemin-induced inhibition of TRPA1 activity is not clear. Two potential mechanisms are suggested to be involved in this regulation. One would be that artemin increases EC_{50} by suppressing the binding affinity of AITC to TRPA1. The other mechanism would be that artemin lowers the channel density of TRPA1 on cell membranes through promoting receptor internalization or by inhibiting membrane insertion of TRPA1 channels. In the present study, the concentration-response curves of AITC resulting from the effect of artemin (Figure 3C) revealed that artemin lowered the maximal response of AITC without conspicuously altering EC_{50}, indicating that artemin may suppress AITC response in DRG neurons principally by lowering the channel density. Distinct constitutive and regulated vesicular trafficking mechanisms have critical roles not only in controlling the surface expression of TRP channels but also their activation in response to stimuli [34]. TRPA1 may cycle between the plasma membrane and intracellular compartments, and the balance between membrane insertion and retrieval determines its surface abundance and activity [35]. A recent report indicated that TRPA1 channel desensitization in sensory neurons was regulated by internalization of itself [36]. Artemin-induced GFR α3/RET activation results in stimulation of multiple signal transduction pathways, including the MAP kinase/Erk and PI3 kinase/Akt pathways. A potential mechanism of inhibition of TRPA1 by artemin in the present study may be the internalization of the channels, which is regulated by the downstream of GFR α3/RET intracellular signals. On the other hand, it has been reported that TRPA1 is functionally inactivated in sensory neuron by extracellular Ca^2 influx [37]. Although activation of GFR α3/RET signals results in intracellular Ca^2 mobilization, however, it is not a likely mechanism for the AITC-evoked current decrease observed in our experiment. The reason simply comes from that cytosolic-free Ca^2 is tightly chelated with the 5 mM EGTA included in the pipette solution. Anyway, detailed mechanisms of artemin regulation on TRPA1 need to be further studied.

Conclusions

Artemin is widely expressed in nervous system [38], supports the survival and regulates the differentiation of peripheral neurons, including sympathetic, parasympathetic, sensory and enteric neurons [3,39-41]. We now show the interaction of artemin and TRPA1 in the rat nervous system and provide a novel role of artemin in inhibitory regulation of sensory neurons in the current study. Artemin may have certain functions in both the neurotrophic effects and inhibitory actions on sensory neurons.

Acknowledgements

This work was supported by Grants-in-Aid for Scientific Research KAKENHI 20602017 (YD), 21026028 (KN), Hyogo Science and Technology Association Research Grant 22S014 (YD) and the Research Basis Formation Supporting Project for Private University S0901048 (KN). The authors thank Dr. D. A. Thomas and Professor P. D. Andrew for correcting the English usage on this manuscript.

Author details

[1]Department of Pharmacy, School of Pharmacy, Hyogo University of Health Sciences, Kobe, Hyogo 650-8530, Japan. [2]Department of Anatomy and Neuroscience, Hyogo College of Medicine, Nishinomiya, Hyogo 663-8501, Japan. [3]Department of physiology, Inner Mongolia Medical College, China.

Authors' contributions

NY carried out the electrophysiological and behavioral studies, performed the statistical analysis, and participated in drafting the manuscript. KK carried out the histochemistry studies. LY, GN, SW and SY participated in the electrophysiological studies. SW also participated in drafting the manuscript. KN supervised the project and edited the manuscript. YD conceived of the project, designed and coordinated the studies, and drafted the manuscript. All authors contributed to data interpretation, have read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Received: 11 January 2011 Accepted: 27 May 2011
Published: 27 May 2011

References

1. Baloh RH, Tansey MG, Lampe PA, Fahrner TJ, Enomoto H, Simburger KS, Leitner ML, Araki T, Johnson EM Jr, Milbrandt J: **Artemin, a novel member of the GDNF ligand family, supports peripheral and central neurons and signals through the GFRalpha3-RET receptor complex.** *Neuron* 1998, 21:1291-1302.
2. Elitt CM, McIlwrath SL, Lawson JJ, Malin SA, Molliver DC, Cornuet PK, Koerber HR, Davis BM, Albers KM: **Artemin overexpression in skin enhances expression of TRPV1 and TRPA1 in cutaneous sensory neurons and leads to behavioral sensitivity to heat and cold.** *J Neurosci* 2006, 26:8578-8587.
3. Orozco OE, Walus L, Sah DW, Pepinsky RB, Sanicola M: **GFRalpha3 is expressed predominantly in nociceptive sensory neurons.** *Eur J Neurosci* 2001, 13:2177-2182.
4. Jaquemar D, Schenker T, Trueb B: **An ankyrin-like protein with transmembrane domains is specifically lost after oncogenic transformation of human fibroblasts.** *J Biol Chem* 1999, 274:7325-7333.
5. 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.
6. 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. *J Comp Neurol* 2005, **493**:596-606.
7. Nagata K, Duggan A, Kumar G, Garcia-Anoveros J: **Nociceptor and hair cell transducer properties of TRPA1, a channel for pain and hearing.** *J Neurosci* 2005, **25**:4052-4061.
8. Bandell M, Story GM, Hwang SW, Viswanath V, Eid SR, Petrus MJ, Earley TJ, Patapoutian A: **Noxious cold ion channel TRPA1 is activated by pungent compounds and bradykinin.** *Neuron* 2004, **41**:849-857.
9. Fujita F, Moriyama T, Higashi T, Shima A, Tominaga M: **Methyl p-hydroxybenzoate causes pain sensation through activation of TRPA1 channels.** *Br J Pharmacol* 2007, **151**:153-160.
10. Hinman A, Chuang HH, Bautista DM, Julius D: **TRP channel activation by reversible covalent modification.** *Proc Natl Acad Sci USA* 2006, **103**:19564-19568.
11. Macpherson LJ, Dubin AE, Evans MJ, Marr F, Schultz PG, Cravatt BF, Patapoutian A: **Noxious compounds activate TRPA1 ion channels through covalent modification of cysteines.** *Nature* 2007, **445**:541-545.
12. Macpherson LJ, Geierstanger BH, Viswanath V, Bandell M, Eid SR, Hwang S, Patapoutian A: **The pungency of garlic: activation of TRPA1 and TRPV1 in response to allicin.** *Curr Biol* 2005, **15**:929-934.
13. 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.
14. 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 USA* 2007, **104**:13519-13524.
15. Fujita F, Uchida K, Moriyama T, Shima A, Shibasaki K, Inada H, Sokabe T, Tominaga M: **Intracellular alkalization causes pain sensation through activation of TRPA1 in mice.** *J Clin Invest* 2008, **118**:4049-4057.
16. Wang YY, Chang RB, Liman ER: **TRPA1 is a component of the nociceptive response to CO2.** *J Neurosci* 2010, **30**:12958-12963.
17. 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.
18. 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.
19. McNamara CR, Mandel-Brehm J, Bautista DM, Siemens J, Deranian KL, Zhao M, Hayward NJ, Chong JA, Julius D, Moran MM, Fanger CM: **TRPA1 mediates formalin-induced pain.** *Proc Natl Acad Sci USA* 2007, **104**:13525-13530.
20. Elitt CM, Malin SA, Koerber HR, Davis BM, Albers KM: **Overexpression of artemin in the tongue increases expression of TRPV1 and TRPA1 in trigeminal afferents and causes oral sensitivity to capsaicin and mustard oil.** *Brain Res* 2008, **1230**:80-90.
21. Malin SA, Molliver DC, Koerber HR, Cornuet P, Frye R, Albers KM, Davis BM: **Glial cell line-derived neurotrophic factor family members sensitize nociceptors in vitro and produce thermal hyperalgesia in vivo.** *J Neurosci* 2006, **26**:8588-8599.
22. Dai Y, Moriyama T, Higashi T, Togashi K, Kobayashi K, Yamanaka H, Tominaga M, Noguchi K: **Proteinase-activated receptor 2-mediated potentiation of transient receptor potential vanilloid subfamily 1 activity reveals a mechanism for proteinase-induced inflammatory pain.** *J Neurosci* 2004, **24**:4293-4299.
23. Dai Y, Wang S, Tominaga M, Yamamoto S, Fukuoka T, Higashi T, Kobayashi K, Obata K, Yamanaka H, Noguchi K: **Sensitization of TRPA1 by PAR2 contributes to the sensation of inflammatory pain.** *J Clin Invest* 2007, **117**:1979-1987.
24. Wang S, Dai Y, Fukuoka T, Yamanaka H, Kobayashi K, Obata K, Cui X, Tominaga M, Noguchi K: **Phospholipase C and protein kinase A mediate bradykinin sensitization of TRPA1: a molecular mechanism of inflammatory pain.** *Brain* 2008, **131**:1241-1251.
25. Dai Y, Wang H, Ogawa A, Yamanaka H, Obata K, Tokunaga A, Noguchi K: **Ca2+/calmodulin-dependent protein kinase II in the spinal cord contributes to neuropathic pain in a rat model of mononeuropathy.** *Eur J Neurosci* 2005, **21**:2467-2474.
26. Gardell LR, Wang R, Ehrenfels C, Ossipov MH, Rossomando AJ, Miller S, Buckley C, Cai AK, Tse A, Foley SF, Gong B, Walus L, Carmillo P, Worley D, Huang C, Engber T, Pepinsky B, Cate RL, Vanderah TW, Lai J, Sah DW, Porreca F: **Multiple actions of systemic artemin in experimental neuropathy.** *Nat Med* 2003, **9**:1383-1389.
27. Bennett DL, Boucher TJ, Michael GJ, Popat RJ, Malcangio M, Averill SA, Poulsen KT, Priestley JV, Shelton DL, McMahon SB: **Artemin has potent neurotrophic actions on injured C-fibres.** *J Peripher Nerv Syst* 2006, **11**:330-345.
28. Wang R, King T, Ossipov MH, Rossomando AJ, Vanderah TW, Harvey P, Cariani P, Frank E, Sah DW, Porreca F: **Persistent restoration of sensory function by immediate or delayed systemic artemin after dorsal root injury.** *Nat Neurosci* 2008, **11**:488-496.
29. Bhave G, Hu HJ, Glauner KS, Zhu W, Wang H, Brasier DJ, Oxford GS, Gereau RWt: **Protein kinase C phosphorylation sensitizes but does not activate the capsaicin receptor transient receptor potential vanilloid 1 (TRPV1).** *Proc Natl Acad Sci USA* 2003, **100**:12480-12485.
30. Tominaga M, Numazaki M, Iida T, Moriyama T, Togashi K, Higashi T, Murayama N, Tominaga T: **Regulation mechanisms of vanilloid receptors.** *Novartis Found Symp* 2004, **261**:4-12, discussion 12-18, 47-54.
31. Bandell M, Story G, Hwang S, Viswanath V, Eid S, Petrus M, Earley T, Patapoutian A: **Noxious cold ion channel TRPA1 is activated by pungent compounds and bradykinin.** *Neuron* 2004, **41**:849-857.
32. Tjolsen A, Berge OG, Hunskaar S, Rosland JH, Hole K: **The formalin test: an evaluation of the method.** *Pain* 1992, **51**:5-17.
33. Wheeler-Aceto H, Cowan A: **Standardization of the rat paw formalin test for the evaluation of analgesics.** *Psychopharmacology (Berl)* 1991, **104**:35-44.
34. Ambudkar IS: **Trafficking of TRP channels: determinants of channel function.** *Handb Exp Pharmacol* 2007, 541-557.
35. Schmidt M, Dubin AE, Petrus MJ, Earley TJ, Patapoutian A: **Nociceptive signals induce trafficking of TRPA1 to the plasma membrane.** *Neuron* 2009, **64**:498-509.
36. Akopian AN, Ruparel NB, Jeske NA, Hargreaves KM: **Transient receptor potential TRPA1 channel desensitization in sensory neurons is agonist dependent and regulated by TRPV1-directed internalization.** *J Physiol* 2007, **583**:175-193.
37. Wang YY, Chang RB, Waters HN, McKemy DD, Liman ER: **The nociceptor ion channel TRPA1 is potentiated and inactivated by permeating calcium ions.** *J Biol Chem* 2008, **283**:32691-32703.
38. Yang C, Hutto D, Sah DW: **Distribution of GDNF family receptor alpha3 and RET in rat and human non-neural tissues.** *J Mol Histol* 2006, **37**:69-77.
39. Bolon B, Jing S, Asuncion F, Scully S, Pisegna M, Van GY, Hu Z, Yu YB, Min H, Wild K, Rosenfeld RD, Tarpley J, Carnahan J, Duryea D, Hill D, Kaufman S, Yan XQ, Juan T, Christensen K, McCabe J, Simonet WS: **The candidate neuroprotective agent artemin induces autonomic neural dysplasia without preventing peripheral nerve dysfunction.** *Toxicol Pathol* 2004, **32**:275-294.
40. Golden JP, DeMaro JA, Osborne PA, Milbrandt J, Johnson EM Jr: **Expression of neurturin, GDNF, and GDNF family-receptor mRNA in the developing and mature mouse.** *Exp Neurol* 1999, **158**:504-528.
41. Young HM, Anderson RB, Anderson CR: **Guidance cues involved in the development of the peripheral autonomic nervous system.** *Auton Neurosci* 2004, **112**:1-14.

doi:10.1186/1744-8069-7-41
Cite this article as: Yoshida *et al.*: **Inhibition of TRPA1 channel activity in sensory neurons by the glial cell line-derived neurotrophic factor family member, artemin.** *Molecular Pain* 2011 **7**:41.

脊髄障害性疼痛症候群の実態の把握と病態の解明に関する研究班

氏　名	所　属　等	職　名
牛田　享宏	愛知医科大学・医学部・学際的痛みセンター	教　授
戸山　芳昭	慶應義塾大学・医学部・整形外科	教　授
菊地　臣一	福島県立医科大学・本部・脊椎外科	学　長
谷　俊一	高知大学・医学部・整形外科教室	教　授
田口　敏彦	山口大学・医学部・整形外科教室	教　授
山下　敏彦	札幌医科大学・医学部・整形外科教室	教　授
柴田　政彦	大阪大学大学院・医学系研究科・疼痛医学寄付講座	教　授
竹下　克志	東京大学・医学部・整形外科	講　師
中村　雅也	慶応義塾大学・医学部・整形外科	講　師
内田　研造	福井大学・医学部・整形外科	准教授
井上　和秀	九州大学大学院・薬学研究院・薬理学・神経薬理学	教　授
野口　光一	兵庫医科大学・医学部・解剖学・神経解剖学	教　授
上野　雄文	九州大学大学院 医学研究院 精神病態医学分野	特任准教授
高安　正和	愛知医科大学・医学部・脳神経外科	教　授
中村　裕之	金沢大学・医薬保健研究域医学系・環境生態医学・公衆衛生学	教　授
舟木　剛	大阪大学大学院・工学研究科・電気電子情報工学	教　授
矢吹　省司	福島県立医科大学病院・リハビリテーションセンター	部　長
竹林　康雄	札幌医科大学・医学部・整形外科教室	講　師
富永　俊克	山口労災病院・リハビリテーション科	部　長
山縣　正庸	千葉労災病院・整形外科	部　長
中江　文	大阪大学大学院・医学系研究科・麻酔集中治療講座	特任准教授
神谷　光弘	愛知医科大学･医学部･整形外科	特任准教授
鈴木　秀典	山口大学･医学部･整形外科	助教
津田　誠	九州大学大学院・薬学研究院・薬理学	准教授
川上ちひろ	横浜市立大学大学院医学研究科	特任講師
西原　真理	愛知医科大学・医学部・学際的痛みセンター	講　師
井村　貴之	北里大学・整形外科	助　教
山田　圭	久留米大学・整形外科	助　教
大迫　洋治	愛知医科大学・医学部・解剖学講座	講師
謝　典頼	神戸百年記念病院	副院長
奥田　康介	松原メイフラワー病院・整形外科	副部長
仲摩　憲次郎	久留米大学・整形外科	助　教
波多野　栄重	金沢医科大学・整形外科	医　師
池本　竜則	須崎くろしお病院	医師
谷口　慎一郎	関西医科大学滝井病院	講　師
末冨　勝敏	愛知医科大学・医学部・学際的痛みセンター	主任研究員
下　和弘	愛知医科大学・医学部・学際的痛みセンター	研究員
奥田鉄人	金沢医科大学・整形外科整形外科	医師
櫻井　博紀	愛知医科大学・医学部・学際的痛みセンター	講師
菊地　尚久	横浜市立大学学術院医学群附属病院リハビリテーション科	准教授
田中　雅人	岡山大学・医学部・整形外科	准教授
鉄永　倫子	岡山大学・医学部・整形外科	医師
越智　和子	琴平町社会福祉協議会	事務局長
新原　隆一	琴平町社会福祉協議会	