Acta Physiologica Sinica, October 25, 2008, 60 (5): 565-570
http://www.actaps.com.cn

Review

Effects of the neurotrophic factor artemin on sensory afferent development and sensitivity

Shuying WANG, Christopher M. Elitt, Sacha A. Malin, Kathryn M. Albers*

Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15261, USA

Abstract: Artemin is a neuronal survival and differentiation factor in the glial cell line-derived neurotrophic factor family. Its receptor GFRα3 is expressed by a subpopulation of nociceptor type sensory neurons in the dorsal root and trigeminal ganglia (DRG and TG). These neurons co-express the heat, capsaicin and proton-sensitive channel TRPV1 and the cold and chemical-sensitive channel TRPA1. To further investigate the effects of artemin on sensory neurons, we isolated transgenic mice (ARTN-OE mice) that overexpress artemin in keratinocytes of the skin and tongue. Enhanced levels of artemin led to a 20% increase in the total number of DRG neurons and increases in the level of mRNA encoding TRPV1 and TRPA1. Calcium imaging showed that isolated sensory neurons from ARTN-OE mice were hypersensitive to the TRPV1 agonist capsaicin and the TRPA1 agonist mustard oil. Behavioral testing of ARTN-OE mice also showed an increased sensitivity to heat, cold, capsaicin and mustard oil stimuli applied either to the skin or in the drinking water. Sensory neurons from wildtype mice also exhibited potentiated capsaicin responses following artemin addition to the media. In addition, injection of artemin into hindpaw skin produced transient thermal hyperalgesia. These findings indicate that artemin can modulate sensory function and that this regulation may occur through changes in channel gene expression. Because artemin mRNA expression is up-regulated in inflamed tissue and following nerve injury, it may have a significant role in cellular changes that underlie pain associated with pathological conditions. Manipulation of artemin expression may therefore offer a new pain treatment strategy.

Key words: pain; nociceptor; GFRα3; skin; tongue; sensitization

1 Introduction

Neurotrophic growth factors have essential roles in the development, maintenance and function of primary sensory afferents that detect multiple types of physical and chemical stimuli. The role of two neurotrophic factors, the neurotrophin nerve growth factor (NGF) and the glial cell line-derived neurotrophic factor (GDNF), have been well studied in sensory system development and in adult systems. Much less is known however, about the role of the neurotrophic factor artemin (ARTN) and its affect on sensory neurons. ARTN is a GDNF family ligand (GFL) that binds to the GDNF family receptor α3 (GFRα3) GPI-anchored protein. GFRα3-ART forms a receptor complex with the receptor tyrosine kinase Ret through which activation of several signaling pathways is mediated[1]. GFRα3 is highly restricted to neurons of the peripheral nervous system (sensory and sympathetic) and is predominantly expressed in sensory neurons that are TrkA-positive and therefore NGF-responsive[2]. GFRα3 sensory neurons also express the transient receptor potential (TRP) ion channel proteins TRPV1 and TRPA1[2,3]. Noxious heat (> 43 ºC), protons and vanilloid compounds (e.g., capsaicin, resinferatoxin) activate TRPV[4], while TRPA1 is sensitive to noxious cold (10-15 ºC), cinnamonaldehyde and mustard oil[5,6]. The expression of these channels in GFRα3-positive neurons suggests ARTN signaling via GFRα3/Ret binding could modulate neuron sensitivity. ARTN may function primarily in this manner since initial studies in mice that lack either the gene encoding ARTN or GFRα3 genes showed no apparent effect on developmental survival of peripheral sensory neurons[7,8]. Whether the lack of ARTN or GFRα3 causes impairments in sensory function in the adult has not yet been examined.

In recent studies we investigated the effect of ARTN on sensory system properties using transgenic mice that overexpress ARTN in the skin[3]. Studies in which exogenous ARTN was applied to neurons *in vitro* and skin *in vivo* were also done[9]. Our findings indicate that ARTN significantly regulates sensory function by modulating

*Corresponding author. Tel: +1-412-648-9669; Fax: +1-412 -648-9731; E-mail: kaa2@pitt.edu

molecular and physiological properties of sensory neurons that in turn causes changes in behavioral responses to thermal and chemical stimulation. A summary of this work is presented below.

2 Results

2.1 ARTN is a developmental survival factor for GFRα3-positive sensory neurons

Transgenic mice that overexpress ARTN in the skin were isolated by microinjection of a transgene construct in which the human K14 keratin promoter was used to drive expression of the ARTN coding region (Fig. 1*A*). K14 keratin is a keratinized epithelium specific promoter that turns on at approximately embryonic day 11 in mouse whisker pad skin[10] and then remains active into adulthood. In overexpresser mice (ARTN-OEs), enhanced ARTN level in the skin (Fig. 1*B*, 1*C*) caused a 20% increase in total neuron number in sensory ganglia and visibly enlarged trigeminal (TG) and dorsal root ganglia (DRG) (Fig. 1*D*). No major change in innervation to the spinal cord dorsal horn was noted (Fig. 1*E*). In sensory ganglia of both wildtype (WT) and ARTN-OE mice nearly all GFRα3-positive neurons were found to express TRPV1 (95%) and many of these were TRPA1-positive (Fig. 2). The percentage of GFRα3-positive neurons in ganglia of ARTN-OE mice was unchanged compared with WT mice [WT = (19.64±0.57)%; ARTN-OE = (18.07±1.47)%] and TRPV1 [WT = (27.53±1.19)%; ARTN-OE = (28.81±2.75)%)]. Even so, the total number of GFRα3- and TRPV1-positive neurons likely increased since an increase in total neuron number occurred (see above). In addition, both GFRα3 and TRPV1 neurons in ARTN-OE ganglia were hypertrophied and exhibited enhanced peripheral projections to the skin that were GFRα3 and TRPV1-positive (Fig. 3). These findings indicate that the GFRα3/TRPV1 neuron popula-

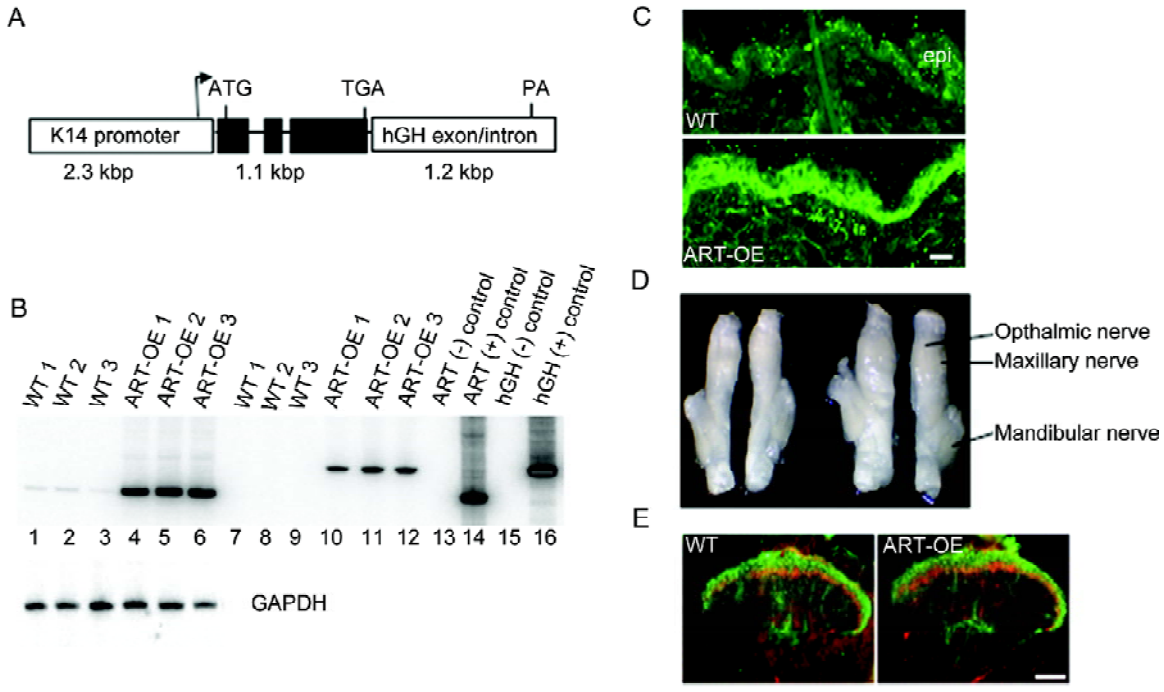

Fig. 1. Overexpression of artemin in the skin is driven by the K14 keratin promoter. *A*: Diagram of transgene construct used for isolation of artemin overexpresser (ARTN-OE) mice. The K14 keratin promoter drives expression of the artemin sequence represented by black boxes. Arrow indicates transcription start site, ATG and TGA are translation start and stop sites, respectively. *B*: RT-PCR analysis of RNA isolated from wildtype (WT, *n*=3) and transgenic (ARTN-OE, *n*=3) back skin showing increased expression of artemin mRNA in ARTN-OE skin. Lanes 1-6 show amplicons obtained using primers to the artemin gene sequence; lanes 7-12 show products using transgene specific PCR primers; lanes 13 and 15 are negative controls for the PCR reaction and lanes 14 and 16 are positive controls for the artemin and transgene sequences, respectively. *C*: Immunolabeling of whisker pad skin from WT (top panel) and ARTN-OE (bottom) mice using an antibody to artemin. epi: epidermis. Scale bar in lower panel, 25 μm. *D*: Trigeminal ganglia from WT and ARTN-OE ganglia. *E*: Spinal cord dorsal horn of WT and ARTN-OE mice labeled with anti-CGRP (green) and isolectin B4 (IB4, red). No significant difference was observed in the distribution or density of CGRP peptidergic or IB4 non-peptidergic afferents. Scale bar in *E*, 100 μm.

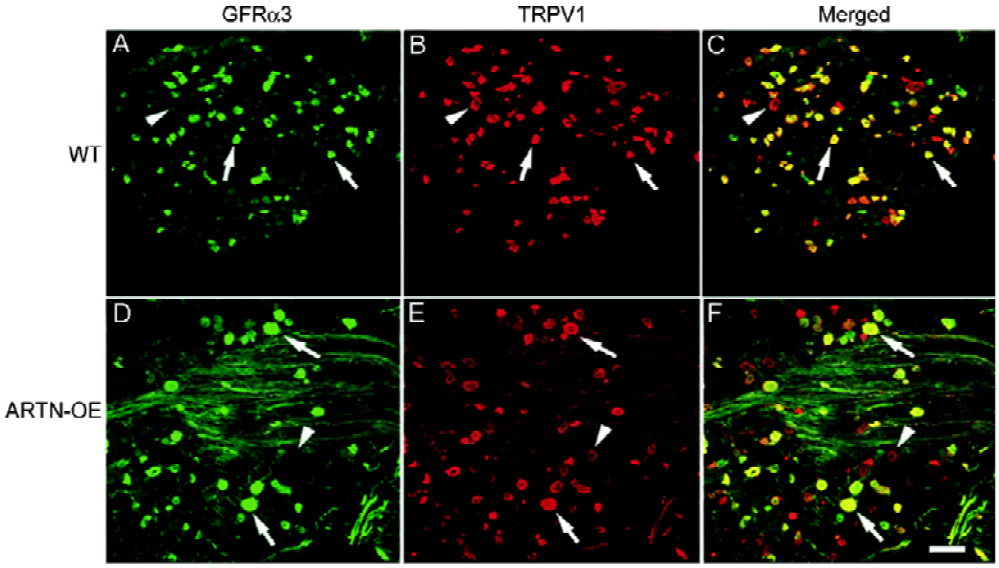

Fig. 2. Sensory neurons responsive to artemin are hypertrophied and express TRPV1. GFRα3 immunolabeling (green) of WT (*A*, *C*) and ARTN-OE (*D*, *F*) DRG show GFRα3-positive neurons are larger in size. Nearly all GFRα3 neurons in WT (*B*) and ARTN-OE (*E*) ganglia express TRPV1 (red; arrows) though several TRPV1-labeled neurons do not express GFRα3 [arrowheads; see merged images of WT (*C*) and ARTN-OE (*F*) ganglia]. Trigeminal neurons showed a similar hypertrophy in TRPV1/GFRα3 neuron size. Scale bar in *C* = 60 μm and applies to all panels.

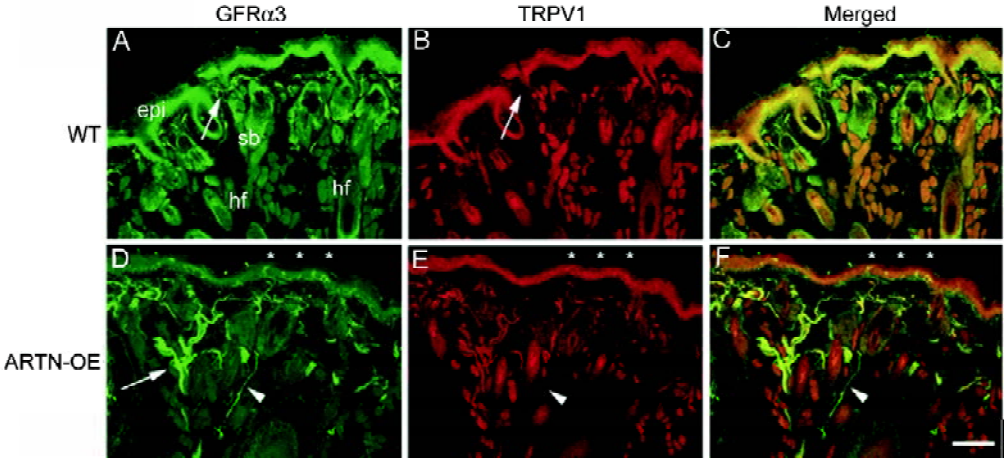

Fig. 3. Skin-derived artemin increases cutaneous GFRα3-positive afferents and intensity of TRPV1 labeling. Low power view compares innervation of whisker pad skin of WT (*A-C*) and ARTN-OE (*D-F*) animals. An increase in GFRα3-positive afferents (green, arrows) occurs in dermis of ARTN-OE skin. Immunolabeling with anti-TRPV1 antibody (red, *B*, *E*) shows higher expression in ARTN-OE afferents (*E*). Few TRPV1 fibers were seen in WT skin (*B*). TRPV1 labeling of GFRα3 afferents of ARTN-OE skin was particularly evident in merged images (*F*) where overlap appears yellow. Overlap was rarely seen in WT skin (*C*). Asterisks (panels *D*, *E* and *F*) indicate appearance of GFRα3 and TRPV1 afferents in epidermis of ARTN-OE skin. Arrowheads in *D-F* indicate a GFRα3-positive fiber that is not TRPV1-positive and may represent sympathetic innervation. Scale bar in *F* = 100 μm and applies to all panels. Epi, epidermis; sb, sebaceous gland; hf, hair follicle.

tion is responsive to the level of target-derived ARTN. This *in vivo* survival action of ARTN is also consistent with previous observations showing increased survival of sensory, sympathetic and enteric neurons cultured in the presence of ARTN[11]. Thus, ARTN can function as a survival factor for sensory neurons during embryonic development.

2.2 Modulation of gene expression in sensory ganglia by overexpression of ARTN in the skin

Reverse transcriptase-PCR assays showed that ARTN overexpression had a significant effect on neuronal gene expression (Table 1). The relative level of GFRα3 mRNA was increased by 34% in lumbar DRG and 81% in the TG of ARTN-OE transgenics. This increase in GFRα3 mRNA

Table 1. Changes in the expression of receptor and TRP channel genes in lumbar DRG (L3, L4 and L5) of ARTN-OE mice compared with WT mice

Gene assayed	GFRα3	Ret	TrkA	TRPV1	TRPV2	TRPV3	TRPV4	TRPA1
Percent change	+34% *	+6%	+37% *	+61% **	+6%	-28%	+8%	+210% **

RT-PCR assays using RNA from DRG of individual animals were done using 4-6 animals per group. $^{**}P \leq 0.0005$, $^{*}P < 0.005$.

is likely due to elevation in mRNA expression on a per cell level since the percent of GFRα3-positive neurons (~19%) was unchanged. Transcripts encoding the receptor tyrosine kinases Ret and TrkA, which are coexpressed in ~80% of GFRα3-positive neurons, were differentially regulated in ARTN-OE ganglia. Relative to WT, the Ret mRNA level was unchanged whereas TrkA transcript levels were increased by 37% in lumbar DRG and 56% in TG. Compared to WT mice, TRPV1 expression was 61% and 190% higher in the lumbar DRG and TG of the ART-OE mice, respectively. No change in mRNA encoding the TRPV2, TRPV3 or TRPV4 channels was measured. In contrast, TRPA1 mRNA increased by 210% in DRG and 403% in TG of ARTN-OE mice compared to WT.

The relative expression of genes encoding members of the acid sensing ion channel (ASIC) family were also found to be changed. ASICs are proton-gated sodium channels that are proposed to underlie acid-evoked pain and at least some aspects of mechanical sensation[12]. A decrease was measured for all ASIC subtypes in lumbar DRG from female ARTN-OE mice (ASIC1, –44%; ASIC2, –73%; ASIC2b, –44%; ASIC3, –61%). Interestingly, male ARTN-OE mice showed a decrease only in ASIC2a where a decline of 48% was measured in lumbar DRG.

2.3 ARTN protein sensitizes sensory neurons to chemical and thermal stimuli

Calcium imaging studies of dissociated and cultured sensory neurons from WT and ARTN-OE mice indicate that ARTN sensitizes the response of sensory neurons to capsaicin and mustard oil. This sensitization is consistent with the upregulation of TRPV1 and TRPA1 mRNA in sensory ganglia by ARTN overexpression. Both the percentage of capsaicin-responsive neurons and the magnitude of capsaicin-evoked calcium transients were significantly increased in DRG neurons of ARTN-OE mice compared to that in WT DRG neurons. Ratiometric imaging using fura-2 dye showed capsaicin elicited calcium responses in significantly more ARTN-OE DRG neurons [(66±6)%; n=5 mice, 50 cells] than WT DRG neurons [(46±2)%; n=5 mice, 26 cells, $P < 0.020$)]. The magnitude of the capsaicin-evoked calcium transient was also increased in ARTN-OE neurons [(1.24±0.1) ΔF] relative to that in WT DRG neurons [(0.84±0.08) ΔF) ($P < 0.002$). In TG the magnitude of capsaicin-induced calcium transients was also increased in the ARTN-OE mice although the number of responsive neurons to capsaicin was unchanged[13]. ARTN-OE mice also had more mustard oil-responsive TG neurons that exhibited a larger magnitude of calcium transients evoked by mustard oil relative to WT TG neurons. This sensitization by ARTN appeard to be restricted to TRPV1 and TRPA1-dependent responses since overexpression of ARTN did not affect calcium responses induced by ATP, a ligand that activates P2X3 and P2Y receptors in sensory neurons. A more detailed and specific study of lingual afferents that innervate the tongue showed a similar result, i.e., lingual afferents in the ARTN-OE mice were hypersensitive to capsaicin and mustard oil[13].

In addition to the calcium imaging data, thermal hypersensitivity of C-fiber afferents in ARTN-OE mice, manifested by a decrease in heat threshold and an increase in sensitivity, was also detected in ARTN-OE mice using a skin-nerve-DRG-spinal cord *ex vivo* preparation[14,15]. In contrast to thermal responses, the average mechanical threshold of the C-fiber population was unchanged in ARTN-OE mice. These findings suggest that ARTN modulates chemical and thermal responses mediated by TRPV1 and TRPA1.

2.4 ARTN-OE mice exhibit behavioral sensitivity to hot and cold thermal stimuli

The increased expression of TRPV1 and TRPA1 in sensory ganglia and the sensitized chemical and thermal responses of sensory neurons from ARTN-OE mice was predicted to result in altered behavioral responses of these mice. Testing was therefore done using behavioral measures to determine if ARTN-OE mice exhibited altered responses to noxious thermal and chemical stimuli. ARTN-OE mice displayed shorter paw withdrawal latencies [(6.33±0.27) s] to radiant heat applied to the plantar surface of the hind paw (Hargreave's test) compared to that of WT mice [(8.05±0.46) s, P=0.004], indicating that ARTN-OE mice were hypersensitive to noxious heat.

Similar to TRPV1, transcript levels of TRPA1 were also elevated in ARTN-OE ganglia and TRPA1 expression overlapped with GFRα3 expression. Because TRPA1 may

contribute to sensing of cold, we tested whether ARTN-OE mice were more responsive to cold stimuli. Response to cold was tested by tail immersion in a bath set at either 0 ºC or at –15 ºC using two sets of 10 WT and 10 ARTN-OE mice (40 mice total). ARTN-OE mice exhibited lower thresholds at the 0 ºC bath temperature [WT=(10.64 ±1.67) s; ARTN-OE=(5.15±1.42) s, n=10, P=0.010] and at the –15 ºC bath temperature [WT=(15.09±2.78) s; ARTN-OE=(5.22±1.03) s, n=10, P=0.002]. In other assays, a place preference test was used, where mice were given the choice between a surface at room temperature and another surface at 5 ºC. ARTN-OE mice spent much less time on the cold side compared to WT mice, further showing that ARTN-OE mice are hypersensitive to noxious cold. These behavioral assays show that ARTN overexpression in the skin can therefore modulate cutaneous sensitivity to both heat and cold.

2.5 ARTN-OE mice display hypersensitivity to chemical stimulation

Mustard oil is a ligand for TRPA1 and it was therefore of interest to test whether ARTN-OE mice were more sensitive to its application. Previous studies have shown that the K14-keratin gene promoter drives transgene expression in the keratinized tongue epithelium of the mouse[16]. We therefore tested whether oral sensitivity to mustard oil was altered in ARTN-OE mice using a two-choice drinking test in which one bottle contains water with mustard oil and another bottle contains normal water. As predicted from the increase in TRPA1 gene expression, ARTN-OE mice drank much less of the mustard oil containing water, indicating oral hypersensitivity of ARTN-OE mice to mustard oil. Similar to mustard oil, ARTN-OE mice also showed hypersensitivity to capsaicin, an agonist for TRPV1. ARTN-OE mice drank much less capsaicin containing water than WT mice. In addition, injection of capsaicin into the hind paw induced more nocifensive responses and a longer duration of nocifensive behavior in ARTN-OE mice compared to WT mice. These findings suggest that ARTN-OE mice are hypersensitive to capsaicin application cutaneously and orally, consistent with increased expression of TRPV1 in the sensory afferents.

2.6 ARTN may have a role in mediating inflammatory pain

Imaging studies to measure changes in intracellular calcium in cultured sensory neurons have shown that exogenous application of ARTN has effects similar to NGF and causes sensitization of primary afferents to capsaicin (see review by Malin and Davis, in this issue, 571-578). The ARTN-evoked sensitization suggests that ARTN acts as a modulator of pain pathways and as such, may have a role in pathological conditions accompanied by inflammatory and neuropathic pain transmission. Investigations of ARTN expression during inflammation and nerve injury support this possibility. In an animal model of inflammation in which complete Freunds adjuvant (CFA) was injected into the glabrous skin of the hind paw, thermal hyperalgesia was elicited. This hyperalgesia could last for up to 7-10 days. Sensitivity to heat was accompanied by an increase in ARTN mRNA expression and development of thermal hyperalgesia. Interestingly, the change in ARTN expression during inflammation appeared to be much greater than NGF expression. These findings suggest that ARTN and GFRα3-postivite neurons have a key role in modulating inflammatory pain[9].

In contrast to inflammatory pain, a possible role for ARTN in neuropathic pain is less clear. In animal models of nerve injury ARTN or its receptor GFRα3 expression was upregulated in the peripheral nervous tissue following injury[1,17]. Expression of ARTN was also studied in human chronic pancreatitis and found to be significantly overexpressed. The level of increased ARTN correlated with pain severity, inflammation, neural density and hypertrophy[18]. Overexpression of ARTN may therefore contribute to neuropathic pain following nerve injury. However, systemic administration of exogenous ARTN produces dose- and time-related reversal of nerve injury-induced pain behavior in rats[19]. This reversal of injury-related sensitivity was not seen however in another spinal nerve ligation study where ARTN was applied following nerve injury[20]. Whether this discrepancy reflects differences in model systems or amount of ARTN applied is unclear.

3 Summary and conclusions

Transgene-driven overexpression of ARTN in skin keratinocytes enhanced developmental survival and functional properties of sensory neurons that are GFRα3, TRPV1, and TRPA1-positive. Increased ARTN expression in epithelium of skin and tongue of transgenic mice enhanced the level of TRPV1 and TRPA1 mRNA in sensory ganglia and resulted in hyperinnervation. Behavioral sensitivity to noxious heat, cold and pungent compounds, i.e., capsaicin and mustard oil, also developed in ARTN-OE transgenic mice. In WT mice, an up-regulation of ARTN mRNA was measured in inflamed tissue. These observations suggest ARTN is a modulator of neuron sensitivity

and as such, a potential target for pain therapies. Additional studies are required to understand mechanistically how ARTN expression affects neuron response properties and its putative role in pain associated with pathological conditions.

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 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.

3 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.

4 Caterina MJ, Julius D. The vanilloid receptor: a molecular gateway to the pain pathway. Annu Rev Neurosci 2001; 24: 487-517.

5 Jordt SE, Bautista DM, Chuang HH, McKemy DD, Zygmunt PM, Högestött ED, Meng ID, Julius D. Mustard oils and cannabinoids excite sensory nerve fibres through the TRP channel ANKTM1. Nature 2004; 427: 260-265.

6 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.

7 Honma Y, Araki T, Gianino S, Bruce A, Heuckeroth R, Johnson E, Milbrandt J. Artemin is a vascular-derived neurotropic factor for developing sympathetic neurons. Neuron 2002; 35: 267-282.

8 Nishino J, Mochida K, Ohfuji Y, Shimazaki T, Meno C, Ohishi S, Matsuda Y, Fujii H, Saijoh Y, Hamada H. GFR alpha3, a component of the artemin receptor, is required for migration and survival of the superior cervical ganglion. Neuron 1999; 23: 725-736.

9 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.

10 Figueiredo HF, Davis BM, Albers KM. Skin-derived nerve growth factor blocks programmed cell death in the trigeminal ganglia but does not enhance neuron proliferation. Mech Dev 2001; 109: 205-214.

11 Baudet C, Mikaels A, Westphal H, Johansen J, Johansen TE, Ernfors P. Positive and negative interactions of GDNF, NTN and ART in developing sensory neuron subpopulations, and their collaboration with neurotrophins. Development 2000; 127: 4335-4344.

12 Krishtal O. The ASICs: signaling molecules? Modulators? Trends Neurosci 2003; 26: 477-483.

13 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.

14 Albers KM, Woodbury CJ, Ritter AM, Davis BM, Koerber HR. Glial cell-line-derived neurotrophic factor expression in skin alters the mechanical sensitivity of cutaneous nociceptors. J Neurosci 2006; 26: 2981-2990.

15 Ritter AM, Woodbury CJ, Albers K, Davis BM, Koerber HR. Maturation of cutaneous sensory neurons from normal and NGF-overexpressing mice. J Neurophysiol 2000; 83: 1722-1732.

16 Krimm RF, Miller KK, Kitzman PH, Davis BM, Albers KM. Epithelial overexpression of BDNF or NT4 disrupts targeting of taste neurons that innervate the anterior tongue. Dev Biol 2001; 232: 508-521.

17 Bennett DL, Boucher TJ, Armanini MP, Poulsen KT, Michael GJ, Priestley JV, Phillips HS, McMahon SB, Shelton DL. The glial cell line-derived neurotrophic factor family receptor components are differentially regulated within sensory neurons after nerve injury. J Neurosci 2000; 20: 427-437.

18 Ceyhan GO, Bergmann F, Kadihasanoglu M, Erkan M, Park W, Hinz U, Giese T, Müller MW, Büchler MW, Giese NA, Friess H. The neurotrophic factor artemin influences the extent of neural damage and growth in chronic pancreatitis. Gut 2007; 56: 534-544.

19 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.

20 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.