Neuroscience Letters 660 (2017) 34–38

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Research article

Artemin transiently increases iNOS expression in primary cultured trigeminal ganglion neurons

Haiqiong Shang[a,b], Yan Wang[a,d], Xiuhua Chao[a,b], Gaoying Sun[b,c], Xiaohui Bai[a,b], Lei Xu[a], Yuechen Han[a], Jianfeng Li[b,c], Haibo Wang[a,b,c], Zhaomin Fan[a,*]

[a] Department of Otolaryngology-Head and Neck Surgery, Shandong Provincial Hospital Affiliated to Shandong University, Jinan, 250021, PR China
[b] Shandong Provincial Key Laboratory of Otology, Jinan, 250022, PR China
[c] Institute of Eye and ENT, Shandong Provincial Hospital Affiliated to Shandong University, Jinan 250022, PR China
[d] Department of Otolaryngology, People's Hospital of Rizhao, 276800, PR China

ARTICLE INFO

Keywords:
Artemin
Trigeminal ganglion
iNOS
GFRα3
TUJ-1

ABSTRACT

Artemin, a member of the glial cell line-derived neurotrophic factor family, is an important cytokine and a critical participant in trigeminal pain disorders such as tongue pain and migraine. However, the mechanisms underlying artemin's activity are largely unknown. In the present study, we used primary cultured trigeminal ganglion neurons (TGNs) to determine the effect of artemin on the expression of the inducible form of nitric oxide synthase (iNOS), which is released in response to painful and inflammatory stimuli. Following artemin treatment, western blot analysis showed that the protein level of iNOS was transiently elevated after artemin treatment for 15 min ($p < 0.05$). Immunofluorescence revealed that both the expressions of iNOS and GFRα3 were significantly up-regulated after artemin treatment for 15 min. In addition, iNOS expression induced by artemin was co-localized with GFRα3 and TUJ-1 in primary cultured TGNs, respectively. Our results indicate a previously unknown role of artemin in regulating iNOS expression in primary cultured TGNs, and regulation of iNOS might be involved in the mechanism through which artemin participates in the trigeminal pain pathway.

1. Introduction

Artemin, a member of the glial cell line-derived neurotrophic factor (GDNF) family ligands, exerts its effects by binding to the GDNF family receptor alpha 3 (GFRα3)/RET receptor complex [3,28]. The GDNF family ligands play critical roles in supporting the development and survival of various kinds of neurons in the peripheral and central nervous systems [2,12]. Recently, artemin has triggered considerable interest because it not only plays a protective role in nerve regeneration, but also contributes to inflammatory pain [13,21]. Peripheral inflammation produces pain by activating the peripheral terminals of primary sensory neurons, which in turn sensitize the central nervous system [15,33]. Previous studies revealed that overexpression of artemin in the tongue can cause oral sensitivity to chemical stimuli and can increase the sensitivity of trigeminal afferents [9,27]. In addition, artemin has been shown to be present in higher concentrations in the dura mater of rodent models of migraine [26]. The trigeminal pain pathway is considered to be a major pathway during migraine and tongue pain, but the exact mechanisms underlying how artemin is involved in the trigeminal pain pathway remain unknown.

Nitric oxide (NO) is one of the main mediators involved in inflammatory pain. As a free radical gasotransmitter, it functions as an important signaling molecule in numerous physiological processes. It is now known that overproduction of NO can have diverse effects such as inflammatory pain, nerve injury, and headache [16,30]. NO is derived from the conversion of L-arginine to L-citrulline by three different isoforms of the nitric oxide synthase (NOS) enzyme, including neuronal type I (nNOS), inducible type II (iNOS), and endothelial type III (eNOS) [1,17]. Among these three enzymes, nNOS and eNOS are constitutively expressed enzymes that are stimulated by increasing Ca^{2+} concentrations to regulate neural and vascular function, respectively. In contrast, iNOS, a Ca^{2+}-independent isoform that is often expressed in response to painful and inflammatory stimuli, is active for extended periods yielding high outputs of NO that modulate various kinds of inflammatory pain pathogeneses [7,29]. Furthermore, a recent study demonstrated that iNOS plays a key role in the trigeminal pain signaling pathway [6]. However, whether iNOS is involved in the mechanisms underlying the action of artemin on the trigeminal pain pathway remains unknown.

The present study was designed to determine the effect of artemin

* Corresponding author.
E-mail address: fanent@126.com (Z. Fan).

http://dx.doi.org/10.1016/j.neulet.2017.09.016
Received 27 April 2017; Received in revised form 8 September 2017; Accepted 8 September 2017
Available online 09 September 2017
0304-3940/

on modulating iNOS expression in primary cultured trigeminal ganglion neurons (TGNs) and to explore the underlying mechanisms behind this effect. Our results indicate that artemin up-regulates iNOS expression, and this might be the way in which artemin is involved in the trigeminal pain pathway.

2. Materials and methods

2.1. Experimental animals

The experiments were conducted in postnatal 3-day-old Wistar rats from the Animal Center of Shandong University (Jinan, China). The animal care and experimental protocols were approved by the Animal Care Committee of Shandong University, P.R. China.

2.2. Primary cultures of rat TGNs

After anesthesia by chloral hydrate, the postnatal rats were quickly decapitated. The temporal bones and brain halves were immediately removed, and the trigeminal ganglion tissues were separated from the cranial base under a microscope. The tissues were placed in PBS containing 0.125% trypsinase (Gibco, USA) for digestion (20 min, 37 °C). The cells were then mechanically dissociated with a Pasteur pipette in DMEM/F12 medium (Gibco, USA) containing 10% fetal bovine serum (Gibco, USA) and 50 μg/ml ampicillin (Sigma-Aldrich, USA). In each set of western blot experiments, trigeminal ganglion neurons (TGNs) from 15 to 20 rats were plated in poly-L-lysine-coated 6-well plates (Corning, USA). For each immunofluorescence staining experiment, TGNs from 3 to 6 rats were inoculated in 4-well dishes (Greiner Bio-One, Germany) with 10-mm glass coverslips precoated with Cell Tak (BD Biosciences, USA). In order to prevent the division and proliferation of glial cells, once the TGNs adhered to the plastic or glass surfaces (about 3 h), the culture medium was changed to another one without fetal bovine serum. The TGNs were cultured in DMEM/F12 medium (Gibco, USA) supplemented with N2 (1:100 dilution, Invitrogen, USA), B27 (1:50 dilution, Invitrogen, USA), and 50 μg/ml ampicillin (Sigma-Aldrich, USA) for 2 days. The cultures were maintained at 37 °C, 5% CO_2, and 95% humidity.

2.3. Treatment of TGN cultures

In the artemin-treated groups, the TGNs were incubated with 10 ng/ml artemin (R & D Systems, USA) [25] for different times (15 min, 30 min, 1 h, 2 h, and 4 h) followed by the protein extraction procedure. TGNs in the control group were given the same volume of medium without artemin.

2.4. Protein extraction and western blot analysis

Total protein from the cultured TGNs was extracted by radioimmune precipitation in lysis buffer according to the manufacturer's protocols (Beyotime, China). The protein content of the samples was measured by means of the BCA protein assay kit (Beyotime, China). A total of 35 μg of each protein sample was denatured and separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto nitrocellulose membranes. The nitrocellulose membranes were blocked in 5% skimmed dried milk for 1 h at room temperature. Next, the membranes were incubated with rabbit anti-iNOS (1:400 dilution, Abcam Systems, USA, ab3523) or mouse anti-β-actin (1:1500 dilution, ZhongShan Goldenbridge Biotechnology, China, TA-09) primary antibodies in TBST containing 3% fat-free dry milk for 1 h at room temperature and then overnight at 4 °C. After washing three times with TBST, the membranes were incubated with the secondary goat anti-rabbit IgG antibody (1:5000 dilution, Santa Cruz Biotechnology, USA) or goat anti-mouse IgG antibody (1:5000 dilution, Santa Cruz Biotechnology, USA) at room temperature for 1 h. Finally, the immunoblots were detected using an ECL kit (Santa Cruz Biotechnology, USA) and visualized after exposure on X-ray films. The relative optical density ratio was calculated with the Image J software by comparison with β-actin.

2.5. Immunofluorescence staining

After fixing in 4% paraformaldehyde for 30 min, the cultured cells were washed in 0.01 M PBS for 10 min and then blocked in PBS containing 0.3% Triton X-100 (Sigma, USA) and 10% heat-inactivated donkey serum (NQBB, USA) for 1 h at room temperature. Subsequently, the cells were incubated with primary antibodies including polyclonal rabbit anti-NeuN antibody (1:500 dilution, Millipore, USA, MAB377), mouse anti-TUJ-1 antibody (1:500 dilution, Neuromics, USA, MO15013), goat anti-GFRα3 antibody (1:50 dilution, R & D systems, USA, BAF2645) or rabbit anti-iNOS antibody (1:50 dilution, Abcam Systems, USA, ab3523) overnight at 4 °C. The next day, after being washed in PBS, samples were incubated with FITC-conjugated or TRITC-conjugated (1:1000 dilution, Invitrogen, USA) secondary antibody along with DAPI (1:800 dilution, Sigma-Aldrich, USA) in 0.1% Triton X-100 and 1% BSA in PBS at room temperature for 1 h. The samples were rinsed in PBS for 30 min and visualized with an inverted DMI 400CS confocal microscope (Leica, Germany).

2.6. Statistical analysis

The statistical analyses were performed using SPSS 17.0 software (SPSS Inc., USA). Data were presented as the mean ± standard error of the mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA). A p-value < 0.05 was considered statistically significant.

3. Results

3.1. TGNs were identified by anti-NeuN and anti-TUJ-1 antibodies

After being cultured *in vitro* for 2 days, the TGNs were immunostained with the anti-NeuN and anti-TUJ-1 neuronal markers and were observed under a confocal microscope. As shown in Fig. 1, NeuN was expressed in the nuclei of neuronal cells and TUJ-1 was present in the neuronal cell body and neuronal axon. A total of 91.8 ± 2.7% of the trigeminal ganglion cells were NeuN-positive, and 87.9 ± 2.4% of the trigeminal ganglion cells were TUJ-1-positive.

3.2. iNOS protein expression was increased in cultured TGNs after artemin treatment

A western blot assay showed a band of iNOS immunoreactivity at about 135 kDa. The protein level of iNOS was significantly elevated in cultured TGNs after artemin treatment for 15 min compared to the control group ($n = 3$, $p < 0.01$) (Fig. 2). The protein levels of iNOS were then decreased and were similar to controls at 30 min, 1 h, 2 h, and 4 h (Fig. 2).

3.3. Immunofluorescence expression of iNOS was increased in TGNs following artemin treatment

Immunofluorescence staining was performed to detect the expression of iNOS in TGNs after artemin treatment for 15 min. The immunofluorescence signal confirmed that iNOS protein expression was increased in the artemin-treated group compared to the control group and showed that artemin-induced iNOS expression was co-localized with TUJ-1 in the TGNs (Fig. 3).

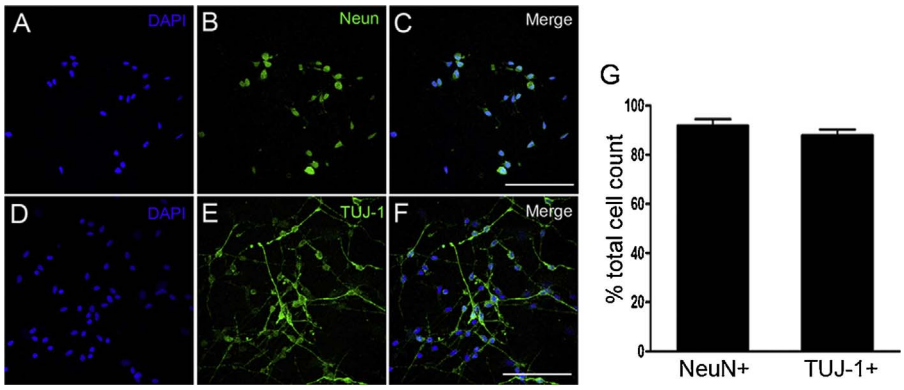

Fig. 1. TGNs were identified by the neuronal markers NeuN and TUJ-1. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
(A, D) DAPI (blue) staining of nuclei. (B) NeuN (green) nuclei marker of neurons. (E) TUJ-1 (green) marker of neurons. (C, F) Merged images. (G) 91.8 ± 2.7% of trigeminal ganglion cells were NeuN-positive, and 87.9 ± 2.4% of trigeminal ganglion cells were TUJ-1-positive. (n = 5, mean ± SEM). Scale bar = 100 μm.

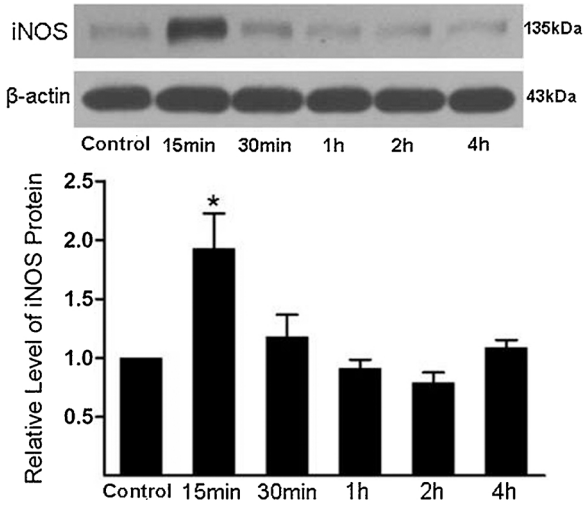

Fig. 2. The protein level of iNOS was up-regulated after artemin treatment.
Western blot analysis showed the protein level of iNOS at different time points after artemin treatment. The expression of iNOS was significantly increased compared to the control group following artemin treatment for 15 min, but was similar to controls at later time points. β-actin served as the loading control in each lane. (n = 3, mean ± SEM, $^{*}p < 0.01$).

3.4. Co-expression of iNOS and GFRα3 in cultured TGNs after artemin treatment

We determined the expressions of iNOS and GFRα3 in cultured TGNs after artemin treatment for 15 min. As shown in Fig. 4, GFRα3 was expressed at a low level in the control group but at a high level in the artemin-treated group. In addition, artemin-induced iNOS expression was co-localized with GFRα3 expression in the TGNs.

4. Discussion

Artemin has recently been shown to have pivotal roles in sensory nociception and trigeminal pain disorders, but the underlying molecular mechanisms behind these effects remain poorly understood. The present study demonstrates for the first time that artemin up-regulates iNOS expression in primary cultured TGNs. This finding implies that iNOS might be a novel downstream target of artemin in the trigeminal pain pathway.

It is known that iNOS, which is responsible for the excessive NO activity associated with inflammation, is an important pain-signaling molecule in the pathogenesis of inflammatory pain [6,11], and studies have shown that iNOS inhibitors attenuate mechanical hypersensitivity after nerve injury [23]. In our study, we found that the level of iNOS protein was significantly increased at the 15 min time point following artemin treatment in TGNs as evidenced by both western blot assay and immunofluorescence, suggesting that artemin rapidly up-regulates iNOS protein expression. Previous studies demonstrated that artemin enhances stimulus-evoked release of calcitonin gene-related peptide (CGRP) in primary cultured neurons [24]. CGRP, an important neuropeptide in the pathology of neurogenic inflammation and pain, can increase the expression of iNOS and stimulate NO release from trigeminal ganglion glial cells [20,32]. Conversely, CGRP promoter activity is also stimulated by NO donors and by the overexpression of iNOS in TGNs [4,8]. Thus, the interplay between CGRP and iNOS in trigeminal pain pathways might contribute to the role of artemin in inflammatory pain.

Artemin exerts its effects in intracellular signaling pathways by binding to the GFRα3 receptor, which is highly selective for artemin and is expressed in a subpopulation of nociceptive sensory and sympathetic neurons [10,31]. In this study, immunofluorescence staining showed that the expression of GFRα3 protein was also increased after artemin treatment for 15 min. In addition, artemin-induced iNOS

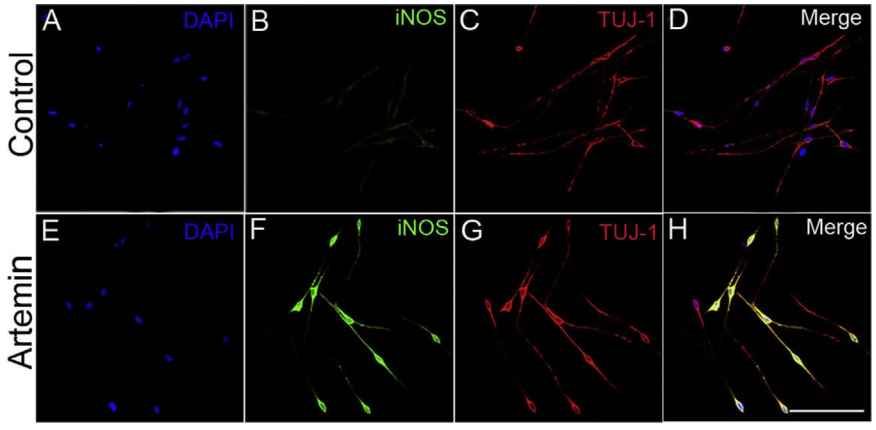

Fig. 3. The immunofluorescence expression of iNOS was co-localized with TUJ-1 after artemin treatment. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
(A–D) Representative images of iNOS (green), TUJ-1 (red), and DAPI (blue) in the control group and the merged image showing co-localization of DAPI, iNOS, and TUJ-1. (E–H) Representative images of iNOS (green), TUJ-1 (red), and DAPI (blue) in the artemin-treated group. The expression of iNOS was increased after artemin treatment, and artemin-induced iNOS expression was co-localized with TUJ-1. Scale bar = 100 μm.

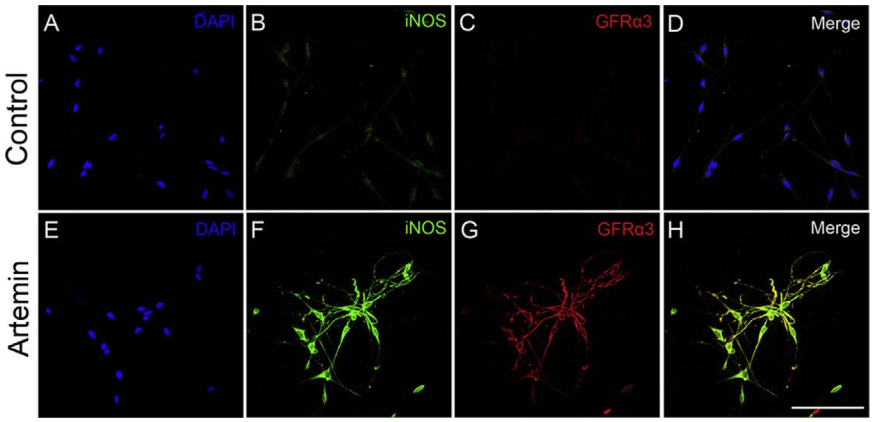

Fig. 4. The immunofluorescence expression of iNOS and GFRα3 was up-regulated after artemin treatment. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
(A-D) Representative images of iNOS (green), GFRα3 (red), and DAPI (blue) in the control group and the merged image showing co-localization of DAPI, iNOS, and GFRα3. (E-H) Representative images of iNOS (green), GFRα3 (red), and DAPI (blue) in the artemin-treated group. The expression of iNOS and GFRα3 was increased after artemin treatment, and artemin-induced iNOS expression was co-localized with GFRα3. Scale bar = 100 μm.

expression was co-localized with GFRα3 in TGNs. These findings suggest that treatment of artemin can lead to a sharp increase in the expression of its receptor GFRα3 and that this activity might be involved in artemin-induced iNOS expression. Previous studies have demonstrated that GFRα3 is present in sensory neurons and is co-expressed with nociceptive neuronal markers, including TRPV1 and TRPA1. TRPV1 and TRPA1 are nociceptive receptors that contribute to sensitization to nociceptive signaling from noxious heat, cold, and inflammation [19,22]. Thus, it is likely that the up-regulation of iNOS induced by artemin in TGNs contributes to the sensitization of the nociceptive neurons and thus participates in inflammatory pain. The double-labeling experiments in the present study also revealed high co-localization of the neuronal marker TUJ-1 with iNOS after artemin treatment. This is consistent with a previous study that demonstrated that pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 stimulate iNOS gene transcription in various cell types, including endothelial cells, neurons, and microglia [18]. Similar to other inducible genes, iNOS is considered to be modulated primarily at the transcriptional level through the activities of diverse intracellular signaling pathways [5,14]. Hence, the intracellular signaling pathways involved in artemin-induced iNOS expression require further elucidation.

5. Conclusion

The present study reveals for the first time that artemin can induce iNOS expression in cultured TGNs, and this iNOS expression is accompanied by increased expression of GFRα3. Together, these findings suggest that artemin might be involved in the trigeminal pain pathway by up-regulating iNOS expression.

References

[1] W.K. Alderton, C.E. Cooper, R.G. Knowles, Nitric oxide synthases: structure, function and inhibition, Biochem. J. 357 (2001) 593–615.
[2] R. Andres, A. Forgie, S. Wyatt, Q. Chen, F.J. de Sauvage, A.M. Davies, Multiple effects of artemin on sympathetic neurone generation, survival and growth, Development 128 (2001) 3685–3695.
[3] R.H. Baloh, M.G. Tansey, P.A. Lampe, T.J. Fahrner, H. Enomoto, K.S. Simburger, M.L. Leitner, T. Araki, E.M. Johnson Jr., J. Milbrandt, Artemin, a novel member of the GDNF ligand family, supports peripheral and central neurons and signals through the GFRalpha3-RET receptor complex, Neuron 21 (1998) 1291–1302.
[4] J. Bellamy, E.J. Bowen, A.F. Russo, P.L. Durham, Nitric oxide regulation of calcitonin gene-related peptide gene expression in rat trigeminal ganglia neurons, Eur. J. Neurosci. 23 (2006) 2057–2066.
[5] N. Biswas, S.K. Mahato, A.A. Chowdhury, J. Chaudhuri, A. Manna, J. Vinayagam, S. Chatterjee, P. Jaisankar, U. Chaudhuri, S. Bandyopadhyay, ICB3E induces iNOS expression by ROS-dependent JNK and ERK activation for apoptosis of leukemic cells, Apoptosis 17 (2012) 612–626.
[6] B.V. Daiutolo, A. Tyburski, S.W. Clark, M.B. Elliott, Trigeminal pain molecules, allodynia, and photosensitivity are pharmacologically and genetically modulated in a model of traumatic brain injury, J. Neurotrauma 33 (2016) 748–760.
[7] J. De Alba, N.M. Clayton, S.D. Collins, P. Colthup, I. Chessell, R.G. Knowles, GW274150, a novel and highly selective inhibitor of the inducible isoform of nitric oxide synthase (iNOS), shows analgesic effects in rat models of inflammatory and neuropathic pain, Pain 120 (2006) 170–181.
[8] A. Dieterle, M.J. Fischer, A.S. Link, W.L. Neuhuber, K. Messlinger, Increase in CGRP- and nNOS-immunoreactive neurons in the rat trigeminal ganglion after infusion of an NO donor, Cephalalgia 31 (2011) 31–42.
[9] C.M. Elitt, S.A. Malin, H.R. Koerber, B.M. Davis, K.M. Albers, 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. 1230 (2008) 80–90.
[10] C.M. Elitt, S.L. McIlwrath, J.J. Lawson, S.A. Malin, D.C. Molliver, P.K. Cornuet, H.R. Koerber, B.M. Davis, K.M. Albers, 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. 26 (2006) 8578–8587.
[11] C.M. Hancock, C. Riegger-Krugh, Modulation of pain in osteoarthritis: the role of nitric oxide, Clin. J. Pain 24 (2008) 353–365.
[12] Y. Honma, T. Araki, S. Gianino, A. Bruce, R. Heuckeroth, E. Johnson, J. Milbrandt, Artemin is a vascular-derived neurotropic factor for developing sympathetic neurons, Neuron 35 (2002) 267–282.
[13] Y. Ikeda-Miyagawa, K. Kobayashi, H. Yamanaka, M. Okubo, S. Wang, Y. Dai, H. Yagi, M. Hirose, K. Noguchi, Peripherally increased artemin is a key regulator of TRPA1/V1 expression in primary afferent neurons, Mol. Pain 11 (2015) 4.
[14] Y.M. Janssen-Heininger, I. Macara, B.T. Mossman, Cooperativity between oxidants and tumor necrosis factor in the activation of nuclear factor (NF)-kappaB: requirement of Ras/mitogen-activated protein kinases in the activation of NF-kappaB by oxidants, Am. J. Respir. Cell Mol. Biol. 20 (1999) 942–952.
[15] F. Karim, C.C. Wang, R.W.T. Gereau, Metabotropic glutamate receptor subtypes 1 and 5 are activators of extracellular signal-regulated kinase signaling required for inflammatory pain in mice, J. Neurosci. 21 (2001) 3771–3779.
[16] A.B. Knott, E. Bossy-Wetzel, Nitric oxide in health and disease of the nervous system, Antioxidants Redox Signal. 11 (2009) 541–554.
[17] R.G. Knowles, S. Moncada, Nitric oxide synthases in mammals, Biochem. J. 298 (Pt 2) (1994) 249–258.
[18] K.D. Kroncke, K. Fehsel, V. Kolb-Bachofen, Inducible nitric oxide synthase in human diseases, Clin. Exp. Immunol. 113 (1998) 147–156.
[19] T.K. Lapointe, L. Basso, M.C. Iftinca, R. Flynn, K. Chapman, G. Dietrich, N. Vergnolle, C. Altier, TRPV1 sensitization mediates postinflammatory visceral pain following acute colitis, Am. J. Physiol. Gastrointest. Liver Physiol. 309 (2015) G87–99.
[20] J. Li, C.V. Vause, P.L. Durham, Calcitonin gene-related peptide stimulation of nitric oxide synthesis and release from trigeminal ganglion glial cells, Brain Res. 1196 (2008) 22–32.
[21] E.K. Lippoldt, R.R. Elmes, D.D. McCoy, W.M. Knowlton, D.D. McKemy, Artemin, a glial cell line-derived neurotrophic factor family member, induces TRPM8-dependent cold pain, J. Neurosci. 33 (2013) 12543–12552.
[22] N. Malek, A. Pajak, N. Kolosowska, M. Kucharczyk, K. Starowicz, The importance of TRPV1-sensitisation factors for the development of neuropathic pain, Mol. Cell. Neurosci. 65 (2015) 1–10.
[23] J.E. Payne, C. Bonnefous, K.T. Symons, P.M. Nguyen, M. Sablad, N. Rozenkrants, Y. Zhang, L. Wang, N. Yazdani, A.K. Shiau, S.A. Noble, P. Rix, T.S. Rao, C.A. Hassig, N.D. Smith, Discovery of dual inducible/neuronal nitric oxide synthase (iNOS/nNOS) inhibitor development candidate 4-((2-cyclobutyl-1H-imidazo[4,5-b]pyrazin-1-yl)methyl)-7,8-difluoroquinolin-2(1H) −one (KD7332) part 2: identification of a novel, potent, and selective series of benzimidazole-quinolinone iNOS/nNOS dimerization inhibitors that are orally active in pain models, J. Med. Chem. 53 (2010) 7739–7755.
[24] B.S. Schmutzler, S. Roy, C.M. Hingtgen, Glial cell line-derived neurotrophic factor family ligands enhance capsaicin-stimulated release of calcitonin gene-related peptide from sensory neurons, Neuroscience 161 (2009) 148–156.
[25] B.S. Schmutzler, S. Roy, S.K. Pittman, R.M. Meadows, C.M. Hingtgen, Ret-dependent and Ret-independent mechanisms of Gfl-induced sensitization, Mol. Pain 7 (2011) 22.
[26] H.Q. Shang, Y. Wang, Y.Y. Mao, L.G. Kong, G.Y. Sun, L. Xu, D.G. Zhang, Y.C. Han, J.F. Li, H.B. Wang, Z.M. Fan, Expression of artemin and GFRalpha3 in an animal model of migraine: possible role in the pathogenesis of this disorder, The Journal of Headache and Pain 17 (2016) 81.

[27] M. Shinoda, M. Takeda, K. Honda, M. Maruno, A. Katagiri, S. Satoh-Kuriwada, N. Shoji, M. Tsuchiya, K. Iwata, Involvement of peripheral artemin signaling in tongue pain: possible mechanism in burning mouth syndrome, Pain 156 (2015) 2528–2537.
[28] R.M. Soler, X. Dolcet, M. Encinas, J. Egea, J.R. Bayascas, J.X. Comella, Receptors of the glial cell line-derived neurotrophic factor family of neurotrophic factors signal cell survival through the phosphatidylinositol 3-kinase pathway in spinal cord motoneurons, J. Neurosci. 19 (1999) 9160–9169.
[29] M. Tanabe, Y. Nagatani, K. Saitoh, K. Takasu, H. Ono, Pharmacological assessments of nitric oxide synthase isoforms and downstream diversity of NO signaling in the maintenance of thermal and mechanical hypersensitivity after peripheral nerve injury in mice, Neuropharmacology 56 (2009) 702–708.
[30] C. Tassorelli, R. Greco, A. Morocutti, A. Costa, G. Nappi, Nitric oxide-induced neuronal activation in the central nervous system as an animal model of migraine: mechanisms and mediators, Funct. Neurol. 16 (2001) 69–76.
[31] P. Thornton, J.P. Hatcher, I. Robinson, B. Sargent, B. Franzen, G. Martino, L. Kitching, C.P. Glover, D. Anderson, H. Forsmo-Bruce, C.P. Low, F. Cusdin, B. Dosanjh, W. Williams, A.C. Steffen, S. Thompson, M. Eklund, C. Lloyd, I. Chessell, J. Hughes, Artemin-GFRalpha3 interactions partially contribute to acute inflammatory hypersensitivity, Neurosci. Lett. 545 (2013) 23–28.
[32] C.V. Vause, P.L. Durham, CGRP stimulation of iNOS and NO release from trigeminal ganglion glial cells involves mitogen-activated protein kinase pathways, J. Neurochem. 110 (2009) 811–821.
[33] C.J. Woolf, M.W. Salter, Neuronal plasticity: increasing the gain in pain, Science 288 (2000) 1765–1769.