Heme oxygenase-1 in the spinal cord plays crucial roles in the analgesic effects of pregabalin and gabapentin in a spared nerve-injury mouse model

Kohei Godai*, Takahiro Moriyama

Department of Anesthesiology and Critical Care Medicine, Graduate School of Medical and Dental Sciences, Kagoshima University, 8-35-1 Sakuragaoka, Kagoshima 890-8520, Japan

ARTICLE INFO

Keywords:
Calcium channel α2δ ligands
Heme oxygenase-1
Neuropathic pain
Glia

ABSTRACT

Introduction: Neuropathic pain remains one of the most intractable types of pain; although calcium channel α2δ ligands, such as pregabalin and gabapentin, are classified as first-line drugs, they have only modest efficacy. Heme oxygenase-1 (HO-1) signaling attenuates glial activation during neuropathic pain. Thus, this study aimed to investigate the effects of the blood–brain barrier (BBB)-permeable HO-1 inhibitor, tin protoporphyrin IX (SnPP), or the BBB-impermeable HO-1 inhibitor, zinc (II) protoporphyrin IX (ZnPP), on the analgesic efficacy of pregabalin and gabapentin. Additionally, we examined the effects of co-administration of SnPP with pregabalin or gabapentin on the expression of glial markers or other genes.

Methods: Neuropathic pain was induced by spared nerve injury (SNI) of the sciatic nerve. The mechanical threshold was tested using the von Frey filaments. The expression of spinal glial markers or other genes was examined using reverse transcription-polymerase chain reaction.

Results: Systemic HO-1 inhibition reversed the mechanical antiallodynic effects of pregabalin and gabapentin, although peripheral HO-1 inhibition did not alter the mechanical antiallodynic effects of either pregabalin or gabapentin. Intrathecal injection of SnPP or ZnPP abolished the mechanical antiallodynic effects of pregabalin and gabapentin. Pregabalin and gabapentin increased HO-1, arginase-1, and endogenous opioid precursor preproenkephalin gene expression and decreased the expression of glial markers, interleukin-1β, and inducible nitric oxide synthase.

Conclusions: This study suggests that spinal HO-1 plays a crucial role in the analgesic effects of calcium channel α2δ ligands through the attenuation of glial activation and endogenous opioid release.

1. Introduction

Neuropathic pain is one of the most intractable types of pain [1] and is estimated to affect 3%–17% of the general population [2]. Calcium channel α2δ ligands attenuate central sensitization and excitatory neurotransmitter release in the spinal dorsal horn [3], exerting analgesic effects peripherally (dorsal root ganglia) and centrally (spinal dorsal horn) [4,5]. Thus, calcium channel α2δ ligands are classified as first-line drugs for neuropathic pain [6].

Heme oxygenase (HO)-1 and HO-2 are rate-limiting enzymes in the oxidative degradation of heme into bilirubin, iron, and carbon monoxide (CO), which has anti-inflammatory properties [7]. HO-1 is induced by various stresses, including oxidative stress and inflammation [8,9], and HO-1 signaling plays an important role in the antinociceptive effects of calcium channel α2δ ligands [10]. The induction of HO-1 by carbon monoxide-releasing molecules (CO-RMs) or specific HO-1 inducers, like cobalt protoporphyrin IX (CoPP), alleviates neuropathic pain [10]. Conversely, HO-1 is inhibited by the blood–brain barrier (BBB)-permeable tin protoporphyrin IX (SnPP) and BBB-impermeable zinc (II) protoporphyrin IX (ZnPP) [11].

Glial activation plays an important role in the development of neuropathic pain [12]; microglia are activated during the early course of acute inflammation and promote the development of hyperalgnesia [13]. Macrophages/microglia are of two types: classically activated/pro-

Abbreviations: BBB, blood–brain barrier; CO, carbon monoxide; CO-RMs, CO-releasing molecules; CoPP, cobalt protoporphyrin IX; HO-1, heme oxygenase-1; HIV, human immunodeficiency virus; iNOS, inducible nitric oxide synthase; RT-PCR, reverse transcription-polymerase chain reaction; SNI, spared nerve injury; SnPP, tin protoporphyrin IX; ZnPP, zinc (II) protoporphyrin IX.

* Corresponding author.

E-mail address: kxg179@icloud.com (K. Godai).

https://doi.org/10.1016/j.neulet.2021.136310
Received 20 September 2021; Received in revised form 17 October 2021; Accepted 22 October 2021
Available online 29 October 2021
0304-3940/© 2021 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).
The effects of HO-1 signaling and the analgesic effects of calcium channel α2δ ligands occur peripherally and centrally [17,18]. However, the location of the interaction between HO-1 signaling and calcium channel α2δ ligands remains to be elucidated. In this study, we investigated 1) the effects of SnPP and ZnPP on the analgesic efficacy of pregabalin and gabapentin and 2) effects of the co-administration of SnPP with pregabalin or gabapentin on the expression of glial markers or other genes.

2. Material and methods

2.1. Animals

Male C57BL6 mice aged 8-10 weeks were obtained from Japan SLIC, Inc. (Hamamatsu, Japan). The Animal Research Committee of Kagoshima University approved all experimental procedures, which were implemented according to the guidelines of the National Institutes of Health and International Association for the Study of Pain (approved number, MD17071) [19]. Four mice weighing 20 to 25 g were housed in each cage (floor area 54 square inch, height 5 in.) for approximately 7 days before the beginning of the study. The mice were provided free access to food and water and were on a 12:12 light/dark schedule at 21 °C and 60% humidity.

2.2. Neuropathic pain model

Neuropathic pain was induced by spared nerve injury (SNI) of the sciatic nerve [20]. The mice were deeply anesthetized by inhalation of 1.5%-2.0% isoflurane (Abbott, Tokyo, Japan) via a nose cone. An incision was made at the mid-thigh level, and a section was made through the biceps femoris. The tibial and common peroneal nerves were ligated and transected using a 6-0 silk suture. A 1- to 2-mm section of the two nerves was removed. The procedure was performed carefully, avoiding any damage to the sural nerve. The muscles and skin were sutured using two 6-0 silk sutures. We have previously reported the development of ipsilateral mechanical allodynia 1 to 8 weeks after inducing SNI [10]. As observed in the previous study, all mice developed neuropathic pain in the present study [20]. We confirmed reduction of the mechanical threshold after surgery. There was no adverse event such as paralysis. All mice used in the study received SN1 surgery. Since neuropathic pain is established on day 7 after SNI surgery, all the experiments (both behavioral and gene expression tests) were performed on the day 7 after SNI surgery.

2.3. Mechanical threshold

The mechanical threshold was determined using calibrated von Frey filaments (0.008-2.0 g; Aesthesio® Precise Tactile Sensory Evaluator; Dannmic Global, San Jose, CA, USA) introduced serially to the hind paw in ascending order of strength. Animals were placed in non-transparent plastic cubicles on a mesh floor for an acclimatization period of at least 30 min on the morning of the test day. A positive response was defined as rapid withdrawal and/or licking of the paw immediately after the application of the stimulus. Filaments were tested five times per paw, and the paw withdrawal threshold was defined as the filament for which three or more withdrawals in five trials were observed [21]. The operators who conducted the tests were blinded to the treatment. Anti-nociception induced by the experimental drugs was expressed as the percentage of the maximal possible effect calculated according to the following equation [10]:

Maximal possible effect (%) = ([drug – baseline]/[cut-off – baseline]) × 100.

The baseline values were obtained before test drugs administration on the day 7 after SNI surgery.

2.4. Reverse transcription-polymerase chain reaction (RT-PCR)

We selected the L3–4 spinal cord level for study as the L3–4 spinal neurons are mainly affected by SNI [22]. Under deep anesthesia using inhalation of 1.5%-2.0% isoflurane, the ipsilateral L3–4 spinal dorsal horn was rapidly removed, frozen on dry ice, and stored at −80 °C. Total RNA from the spinal dorsal horn was extracted using Sepasol reagent (Nacalai Tesque, Kyoto, Japan). First-strand cDNA was synthesized using a High-Capacity RNA-to-cDNA Kit (Applied Biosystems, Carlsbad, CA, USA), according to the manufacturer’s instructions. Quantitative PCR was performed on an ABI Prism StepOnePlus Real-Time PCR System (Applied Biosystems) using TaqMan Fast Advanced Master Mix (Applied Biosystems), according to the manufacturer’s instructions. The primers for the target genes were Aif1 (assay ID, Mm00479862_g1), Arg1 (assay ID, Mm00475986_m1), Gfap (assay ID, Mm01253033_m1), Hmox1 (assay ID, Mm00516005_m1), Il1b (assay ID, Mm00434228_m1), Nos2 (assay ID, Mm00440502_m1), and Pten (assay ID, Mm01212875_m1). Target gene expression was normalized to glyceraldehyde 3-phosphate dehydrogenase expression.

2.5. Experimental protocol

The doses of SnPP or ZnPP combined with pregabalin or gabapentin in this study were selected as those that produced a relevant effect in accordance with previous studies [10,18,23]. In the first experiment, we evaluated effects of the systemic (intraperitoneal) administration of SnPP on the mechanical antiallodynic effects produced by pregabalin or gabapentin (n = 8 animals per group). In the second set of experiments, we evaluated effects of the peripheral (intra-planter in the left hind paw) administration of ZnPP on the mechanical antiallodynic effects produced by pregabalin or gabapentin (n = 7–8 animals per group). In the third set of experiments, we evaluated effects of the intrathecal administration of SnPP or ZnPP on the mechanical antiallodynic effects produced by pregabalin or gabapentin (n = 7–8 animals per group).

Finally, in the fourth set of experiments, we evaluated effects of the intrathecal administration of SnPP combined with intraperitoneal administration of pregabalin or gabapentin on the expression of HO-1, a microglial marker (ionized calcium-binding adapter molecule-1, Iba-1), an M1 microglial marker (inducible nitric oxide synthase, iNOS), an M2 microglial marker (arginase-1), the pro-inflammatory cytokine interleukin (IL)-1β, an astrocyte marker (glial fibrillary acidic protein, GFAP), and the endogenous opioid precursor, preproenkephalin. SNI-affected mice treated with intrathecal administration of dimethyl sulfoxide (DMSO) combined with intraperitoneal administration of saline were used as controls (n = 4–6 samples per group).

2.6. Drugs

Pregabalin and gabapentin were purchased from Sigma-Aldrich (St. Louis, MO, USA) and were dissolved in saline solution (0.9% NaCl). In all four sets of experiments, pregabalin and gabapentin were administered intraperitoneally 60 min before the experiment. SnPP and ZnPP purchased from Enzo Life Sciences, Inc. (Farmingdale, NY, USA) were dissolved in DMSO (1% solution in saline). All drugs were freshly prepared before use. In the first experiment, 10 mg/kg of SnPP or 1% DMSO was mixed with pregabalin or gabapentin before intraperitoneal administration. The total amount of solution injected intraperitoneally was 300 μL. In the second experiment, 400 nmol of ZnPP or 1% DMSO was injected 30 min before the behavioral tests. The total amount of solution injected into the hind paws was 20 μL/paw for intra-planter injection. In the third and fourth experiments, percutaneous intrathecal injections between L5 and L6 were performed in unanesthetized mice according to the method described by Hylden and Wilcox, using a...
10-mL Hamilton syringe and disposable 30-gauge 0.5-inch needles [24]. The appropriate needle placement for injection was confirmed by the elicitation of a strong tail-flick reflex. The total amount of intrathecally injected solution was 4 μL.

2.7. Statistical analysis

Data are expressed as mean ± standard error of the mean. To detect a value for sigma 20%–40%, with α = 0.0125–0.05 and power of 80%–99%, we needed a sample size of 5–8 for behavioral testing and 4–6 for gene expression studies, based on our previous publications and preliminary data (R version 4.1.1, R Foundation for Statistical Computing, Vienna, Austria) [18]. Statistical analysis was performed using GraphPad Prism 7.0 (GraphPad Software, La Jolla, CA, USA). All comparisons were two-tailed. The effects of the experimental drugs on gene expression in the ipsilateral spinal dorsal horn were compared using one-way analysis of variance (ANOVA) followed by Tukey’s multiple comparison test. Statistical significance was set at \( P < 0.05 \).

3. Results

3.1. Effects of systemic SnPP administration on the mechanical antiallodynic efficacy of pregabalin and gabapentin

We assessed effects of the intraperitoneal administration of 10 mg/kg of SnPP or vehicle (1% DMSO) on the mechanical antiallodynic effects produced by intraperitoneal administration of pregabalin (30 mg/kg) or gabapentin (30 mg/kg) in mice with SNI. Co-administration of pregabalin with SnPP completely reversed the mechanical antiallodynic effects of pregabalin administered alone (Fig. 1A). Similarly, co-administration of gabapentin with SnPP completely reversed the mechanical antiallodynic effects produced by gabapentin administered alone (Fig. 1B).

3.2. Effects of peripheral ZnPP administration on the mechanical antiallodynic efficacy of pregabalin and gabapentin

We also investigated effects of intra-planter (left hind paw) administration of 400 nmol of ZnPP or vehicle (1% DMSO) on the mechanical anti-allodynic effects produced by intraperitoneal administration of pregabalin (30 mg/kg) or gabapentin (30 mg/kg) in mice with SNI. The intra-plantar injection of ZnPP did not affect the mechanical
anti-allodynic effects produced by pregabalin (Fig. 1C) or gabapentin (Fig. 1D).

3.3. Effects of the intrathecal administration of SnPP or ZnPP on the mechanical anti-allodynic efficacy of pregabalin and gabapentin

We assessed effects of the intrathecal administration of 100 nmol/kg of SnPP or vehicle (1% DMSO) on the mechanical anti-allodynic effects produced by intraperitoneal administration of pregabalin (30 mg/kg) or gabapentin (30 mg/kg) in mice with SNI. Coadministration of pregabalin with SnPP completely reversed the mechanical anti-allodynic effects of pregabalin administered alone (Fig. 2A). Similarly, coadministration of gabapentin with SnPP completely reversed the mechanical anti-allodynic effects produced by gabapentin administered alone (Fig. 2D).

Furthermore, we assessed effects of the intrathecal administration of 100 nmol/kg of ZnPP or vehicle (1% DMSO) on the mechanical anti-allodynic effects produced by intraperitoneal administration of pregabalin (30 mg/kg) or gabapentin (30 mg/kg) in mice with SNI. Coadministration of pregabalin with ZnPP completely reversed the mechanical anti-allodynic effects of pregabalin administered alone (Fig. 2C). Similarly, coadministration of gabapentin with ZnPP completely reversed the mechanical anti-allodynic effects produced by gabapentin administered alone (Fig. 2D).

3.4. Effects of intrathecal administration of 100 nmol/kg of SnPP combined with intraperitoneal administration of 30 mg/kg of pregabalin or gabapentin on the expression of glial markers, IL-1β, and preproenkephalin

Figure 3 shows the mRNA levels of HO-1, Iba-1, GFAP, iNOS, arginase-1, IL-1b, and preproenkephalin in the ipsilateral spinal dorsal horn of mice treated with vehicle, pregabalin alone, or pregabalin combined with intrathecal SnPP. In mice treated with pregabalin alone, increased expression of HO-1, arginase-1, and preproenkephalin and decreased expression of Iba-1, iNOS, IL-1b, and GFAP were noted. The changes in the expression of HO-1, arginase-1, and preproenkephalin were partially
reversed by coadministration of SnPP. The increase in the expression of IL-1β was not altered by coadministration of SnPP.

4. Discussion

In this study, we demonstrated in SNI model mice that the systemic administration of a BBB-permeable HO-1 inhibitor, SnPP, reversed the analgesic effects of pregabalin or gabapentin, while peripheral administration of the BBB-impermeable HO-1 inhibitor ZnPP did not alter these analgesic effects. However, the analgesic effects of pregabalin or gabapentin were reversed by the intrathecal administration of either SnPP or ZnPP. Pregabalin administration increased the expression of HO-1, arginase-1, and preproenkephalin and decreased expression of Iba-1, iNOS, IL-1β, and GFAP. Similarly, gabapentin administration increased expression of HO-1, arginase-1, and preproenkephalin and decreased expression of IL-1β and GFAP. However, the pregabalin-induced alterations in the expression of HO-1, arginase-1, and preproenkephalin were prevented by intrathecal administration of SnPP. Taken together, these results suggest that spinal HO-1 plays a crucial role in the analgesic effects of calcium channel α2δ ligands through the modulation of glial activation and endogenous opioid release.

SnPP and ZnPP are metal-based HO-1 inhibitors [11] that are frequently used in pain studies [10,18,23,25]. Some studies have reported that SnPP does not pass through the BBB [26,27]. However, we and other researchers have shown that the intraperitoneal administration of SnPP significantly inhibits HO-1 in the brain and spinal cord [10,23]. Conversely, the HO-1 inducers CoPP or CORM-2 increase HO-1 expression in the spinal cord, which consequently reduces microglial/astrocytic activation and iNOS levels [28]. We have previously reported that HO-1 induction potentiates analgesic effects of pregabalin/gabapentin in neuropathic pain [10]. Although pregabalin and gabapentin have similar pharmacologic properties, we used both drugs. The two drugs showed similar responses, which strengthens our results’ generalization.

Microglial and astrocytic activation is a key component of neuropathic pain [12,13]; there is clinical evidence of glial activation in cases...
of neuropathic pain [29]. SNI increases expression of Iba-1 and GFAP in the spinal cord compared to sham operated mice [30]. HO-1 promotes macrophage/microglia polarization towards the anti-inflammatory M2 type [16], and endogenous opioids are secreted by leukocytes, including M2 macrophages [31]. Ahmad et al. showed that intrathecal administration of calcium channel $\alpha_2\delta$ ligands increased the expression of anti-inflammatory cytokine IL-10 and $\beta$-endorphin in microglia, but not in neurons or astrocytes [32]. However, no study has shown that microglia secrete enkephalin, an endogenous opioid [33]. Enkephalin is expressed by astrocytes and GABA-ergic interneurons [34]. Spinal astrocytes are activated in the spinal dorsal horn of patients with human immunodeficiency virus (HIV) infection who also have chronic pain [35], but not in that of HIV-infected patients without chronic pain. Herein, pregabalin attenuated astrogliosis, and SnPP reversed these effects; therefore, astrocytes may be the source of increased enkephalin. Furthermore, pregabalin and gabapentin increase glutamate-induced intracellular calcium concentrations in astrocytes to stimulate descending inhibition [36,37]. The endoplasmic reticulum and mitochondria are important cytosolic calcium controllers; endoplasmic-reticulum stress and mitochondrial oxidative stress are involved in neuropathic pain induced by nerve injury [38,39].

This study has limitations. First, because we did not modulate glial activity, we could identify the cells (microglia, astrocytes, or other cells) that secrete inflammatory cytokines or endogenous opioids. Second, although we observed gene expression induced by calcium channel $\alpha_2\delta$ ligand, it is unclear if the changes in gene expression are a result of or occur simultaneous to glial modulation. Calcium channel $\alpha_2\delta$ ligand-induced modulation in each glial cell needs to be explored in future studies.

In conclusion, spinal HO-1 plays a crucial role in the analgesic effects of calcium channel $\alpha_2\delta$ ligands through the modulation of glial activation and endogenous opioid release. The current results provide important information for understanding precise mechanisms of calcium channel $\alpha_2\delta$ ligands.

CRediT authorship contribution statement

Kohei Godai: Conceptualization, Data curation, Formal analysis,
Funding acquisition, Investigation, Methodology, Resources, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. Takahiro Moriyama: Project administration, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We thank Dr. Yuichi Kamnura, Ms. Nasa Kuroe, and Ms. Naomi Tokuda, Professor Hiroyuki Okuno, and Dr. Yui Kiyama for helping with experiments, as well as all staff members of the Institute of Laboratory Animal Sciences, Kagoshima University (Frontier Science Writing editing. Validation, Visualization, Writing original draft, Writing – review & editing.

The work reported in this paper. Funding

This work was supported by JSPS KAKENHI (grant numbers JP 17K16740 and 20K17815 to K. Godai).

References

[1] N. Attal, M. Lanteri-Minet, B. Laurent, J. Fermanian, D. Bouhassira, The specific disease burden of neuropathic pain: results of a French nationwide survey, Pain 152 (2011) 2386–2343, https://doi.org/10.1016/j.pain.2011.09.014.
[2] O. van Hecker, S.K. Austin, R.A. Khan, B.H. Smith, N. Torrance, Neuropathic pain in the general population: a systematic review of epidemiological studies, Pain 155 (2014) 654–662, https://doi.org/10.1016/j.pain.2013.11.013.
[3] C.Y. Li, X.L. Zhang, E.A. Matthews, G. Feng, Z.D. Luo, Calcium channel alpha2delta1 subunit mediates spinal hyperexcitability in pain modulation, Pain 125 (2006) 20–34, https://doi.org/10.1016/j.pain.2006.04.022.
[4] J.E. Biggs, P.A. Boakye, N. Ganesan, P.L. Stemkowski, A. Lantero, K. Ballanyi, P. Flavell, A.M.K. Choi, Carbon monoxide has anti-inflammatory effects involving the mitogen-activated protein kinase pathway, Nat. Med. 6 (4) (2000) 422–428, https://doi.org/10.1038/74680.
[5] K. Godai, M. Hasagewa-Moriyama, T. Kurihito, M. Nakama, Peroxisome proliferator-activated receptor-gamma agonist rosiglitazone attenuates postinflammatory pain by regulating macrophage polarization, Biochem. Biophys. Res. Commun. 426 (1) (2012) 76–82, https://doi.org/10.1016/j.bbrc.2012.08.039.
[6] M. Hasagewa-Moriyama, T. Kurimoto, M. Nakama, K. Godai, M. Kojima, T. Kusuki, Y. Kamnura, Peroxisome proliferator-activated receptor-gamma agonist rosiglitazone attenuates inflammatory pain through the induction of heme oxygenase-1 in macrophages, Pain 154 (2013) 1402–1412, https://doi.org/10.1016/j.pain.2013.04.039.
[7] A. Hervera, S. Leanez, R. Negrete, R. Motterlini, O. Pol, A. Siegel, Carbon monoxide reduces neuropathic pain and spinal microglial activation by inhibiting nitric oxide synthesis in mice, PLoS One 7 (8) (2012) e43693, https://doi.org/10.1371/journal.pone.0043693.
[8] K. Godai, M. Hasagewa-Moriyama, T. Kurimoto, T. Saito, T. Yamada, T. Sato, M. Kojima, Y. Kamnura, Peripheral administration of morphine attenuates postinflammatory pain by regulating macrophage polarization through COX-2-dependent pathway, Mol. Pain 10 (2014) 36, https://doi.org/10.1186/1744-8069-10-36.
[9] M. Zimmermann, Ethical guidelines for experimental investigations in pain conscious animals, Pain 16 (2) (1983) 109–110.
[10] A.B. Bourquin, M. Sovegen, M. Pertin, N. Gilliard, S. Arcey, A.C. Davison, D. R. Spahn, I. Decosterd, Assessment and analysis of mechanical allodynia-like behavior induced by spared nerve injury (SNI) in the mouse, Pain 122 (2014) e1–e14, https://doi.org/10.1016/j.pain.2010.05.036.
[11] M. Kremer, L. Vodehnal, L. Wurts, D. Grubic, D. Daniel, R.A. Hawkes, E. Salvat, M. Barrot, The antiallodynic action of pregabalin in neuropathic pain is independent from the opioid system, Mol. Pain 12 (2016), https://doi.org/10.1186/s12984-016-03347-7.
[12] C.J. Lazarewicz, M. Pertin, H.R. Suter, I. Decosterd, Voltage-gated sodium channel expression in mouse DRG after SNL leads to re-evaluation of projections of injured fibers, Mol. Pain 10 (2014) 19, https://doi.org/10.1186/1744-8069-10-19.
[13] T. Yamamoto, N. Nosaki-Tanouchi, Zinc protoporphyrin IX, an inhibitor of the enzyme that produces carbon monoxide, blocks spinal nociceptive transmission evoked by formalin injection in the rat, Brain Res. 704 (2) (1995) 256–262, https://doi.org/10.1016/0006-8993(95)90112-3.
[14] J.L.K. Hylden, G.L. Wilcox, Intrathecal morphine in mice: a new technique, Eur. J. Pharmacol. 67 (2-3) (1980) 313–316, https://doi.org/10.1016/0014-2999(80)90515-4.
[15] S. Castany, M. Caruelle, S. Leanez, O. Pol, The anticonvulsant effects of a δ-opioid receptor agonist in mice with painful diabetic neuropathy: involvement of heme oxygenase 1, Neurosci. Lett. 614 (2016) 49–54, https://doi.org/10.1016/j.neulet.2015.12.059.
[16] C.E. Cornelius, P.A. Rodgers, Prevention of neonatal hyperbilirubinemia in rhesus monkeys by tin-protoporphyrin, Pediatr. Res. 1149, https://doi.org/10.1038/s41598-018-2843, https://doi.org/10.1038/jci112398.
[17] A. Hervera, S. Leanez, R. Motterlini, O. Pol, Treatment with carbon monoxide-releasing molecules and an HO-1 inducer enhances the effects and expression of µ-opioid receptors during neuropathic pain, Neuroscience 118 (2013) 1180–1197, https://doi.org/10.1016/j.neuroscience.2013.02.041.
[18] A. Hervera, S. Leanez, R. Motterlini, O. Pol, Treatment with carbon monoxide-releasing molecules and an HO-1 inducer enhances the effects and expression of µ-opioid receptors during neuropathic pain, Neuroscience 118 (2013) 1180–1197, https://doi.org/10.1016/j.neuroscience.2013.02.041.
[19] J.F. Ewing, S.N. Haber, M.D. Maines, Normal and heat-induced patterns of expression of heme oxygenase-1 (HSP32) in rat brain: hyperthermia causes rapid induction of mRNA and protein, J. Neurochem. 58 (1992) 1140–1149.
[20] K. Godai, Y. Kamnura, Heme oxygenase-1 inducer and carbon monoxide-releasing molecule enhance the effects of gabapentinoids by modulating glial activation during neuropathic pain in mice, Pain Res. 3 (5) (2018) e677, https://doi.org/10.1097/01.jnr.000060000000677.
[21] O. Bing, L. Grundman, L. Ny, C. Möller, M. Heilig, Modulation of carbon monoxide production and enhanced spatial learning by tin protoporphyrin, Neuroreport 6 (1995) 1369–1372, https://doi.org/10.1093Neuroreport/6.16.1369.
[22] Z.Y. Zhang, P. Gerner, C.J. Woolf, R.J. Ri, ERK is sequentially activated in neurons, microglia, and astrocytes by spinal nerve ligation and contributes to mechanical allodynia in this neuropathic pain model, Pain 114 (2005) 149–159, https://doi.org/10.1016/j.pain.2004.12.022.
[23] M. Tsuda, Y. Shigemoto-Mogami, S. Koizumi, A. Mizokoshi, S. Kohsaka, M. W. Salter, K. Ioune, P2X4 receptors induced in spinal microglia gate tactile alldynia after nerve injury, Nature 424 (6550) (2003) 778–783, https://doi.org/10.1038/nature01976.
[24] J. Huang, Y. Wang, W. Wang, Y. Wei, Li, Y. Lü, S. Wu, Preproenkephalin mRNA is expressed in a subpopulation of GABAergic neurons in the spinal dorsal horn of the GA67G-GFP knock-in mouse, Anat. Rec. (Hoboken). 291 (101 (2008) 1334–1341, https://doi.org/10.1002/ar.20775.
[25] Y. Shi, B.B. Gelman, J.G. Lisnicinich, S.J. Tang, Chronic pain-associated astrocytic reaction in the spinal cord of neonatal mice with maternal infection-induced viral infection in mice, J. Neurosci. 32 (2012) 10832–10840, https://doi.org/10.1523/JNEUROSCI.5628-11.2012.

Neuroscience Letters 767 (2022) 136310
[36] T. Suto, A.L. Severino, J.C. Eisenach, K. Hayashida, Gabapentin increases extracellular glutamatergic level in the locus coeruleus via astroglial glutamate transporter-dependent mechanisms, Neuropharmacology. 81 (2014) 95–100, https://doi.org/10.1016/j.neuropharm.2014.01.040.

[37] M. Yoshizumi, J.C. Eisenach, K.-I. Hayashida, Riluzole and gabapentinoids activate glutamate transporters to facilitate glutamate-induced glutamate release from cultured astrocytes, Eur. J. Pharmacol. 677 (1-3) (2012) 87–92, https://doi.org/10.1016/j.ejphar.2011.12.015.

[38] E. Zhang, M.H. Yi, N. Shin, H. Baek, S. Kim, E. Kim, K. Kwon, S. Lee, H.W. Kim, Y. Chul Bae, Y. Kim, O.Y. Kwon, W.H. Lee, D.W. Kim, Endoplasmic reticulum stress impairment in the spinal dorsal horn of a neuropathic pain model, Sci. Rep. 5 (2015) 11555, https://doi.org/10.1038/srep11555.

[39] K. Godai, K. Takahashi, Y. Kashiwagi, C.-H. Liu, H. Yi, S. Liu, C. Dong, D. A. Lubansky, S. Hao, Ryanodine receptor to mitochondrial reactive oxygen species pathway plays an important role in chronic human immunodeficiency virus gp120MN-induced neuropathic pain in rats, Anesth. Analg. 129 (1) (2019) 276–286, https://doi.org/10.1213/ANE.0000000000003916.