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Nerve injury-induced changes in Homer/glutamate receptor signaling contribute to the development and maintenance of neuropathic pain

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SUMMARY

Dynamic but temporally distinct regulation of different Homer isoforms and their interaction with mGluR5 contribute to long-lasting increases in excitatory synaptic efficacy that underlies neuropathic pain.

Abstract

While group 1 metabotropic glutamate receptors (mGluRs) and ionotropic *N*-methyl-D-aspartate (NMDA) receptors regulate nociception, the precise molecular mechanism(s) contributing to glutamate signaling in chronic pain remain unclear. Here we not only confirmed the key involvement of Homer proteins in neuropathic pain, but also distinguished between the functional roles for different Homer family members and isoforms. Chronic constriction injury (CCI) of the sciatic nerve induced long-lasting, time-dependent increases in the postsynaptic density expression of the constitutively expressed (CC) isoforms Homer1b/c and/or Homer2a/b in the spinal dorsal horn and supraspinal structures involved in nociception (prefrontal cortex, thalamus), that co-occurred with increases in their associated mGluRs, NR2 subunits of the NMDA receptor, and the activation of downstream kinases. Virus-mediated overexpression of Homer1c and Homer2b after spinal (intrathecal) virus injection exacerbated CCI-induced mechanical and cold hypersensitivity, however, *Homer1* and *Homer2* gene knockout (KO) mice displayed no changes in their neuropathic phenotype. In contrast, overexpression of the immediate early gene (IEG) Homer1a isoform reduced, while KO of *Homer1a* gene potentiated neuropathic pain hypersensitivity. Thus, nerve injury-induced increases in CC-Homers expression promote pain in pathological states, but IEG-Homer induction protects against both the development and maintenance of neuropathy. Additionally, exacerbated pain hypersensitivity in transgenic mice with reduced Homer binding to mGluR5 supports also an inhibitory role for Homer interactions with mGluR5 in mediating neuropathy. Such data indicate that nerve injury-induced changes in glutamate receptor/Homer signaling contribute in dynamic but distinct ways to neuropathic pain processing, which has relevance for the etiology of chronic pain symptoms and its treatment.

1. Introduction

Peripheral nerve injury leads to the development of neuropathic pain as a consequence of injury-induced central sensitization. This involves increases in excitatory neuronal firing, glutamate release within peripheral and central nervous systems, and subsequent reorganization of the nociceptive network (eg, [9,31,32]). Glutamate transmission, particularly through the Gq/o-coupled Group1 metabotropic glutamate receptors (mGluR1/5), as well as (or in conjunction with) ionotropic *N*-methyl-D-aspartate (NMDA) glutamate receptors, has been highly implicated in nociception (eg, [7,16,45,72]). Thus, a likely molecular candidate contributing to changes in spinal and supraspinal glutamate signaling in chronic pain is the Homer family of proteins, which constitutes a part of the signaling scaffold regulating the trafficking, clustering, and function of both Group1 mGluRs and NMDA receptors (eg, [13,14,53]).

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Homer proteins are encoded by 3 genes (*Homer1*, *2*, *3*) that give rise to constitutively expressed, coiled-coiled (CC; Homer1b/c/d/g/h, Homer2a/b and Homer3; a.k.a. CC-Homers), and immediate early gene (IEG) products (from *Homer1*, Homer1a and ania-3) [13,14]. In contrast to CC-Homers, IEG Homers lack the CC-domain and are incapable of multimerization or multiprotein interactions. Thus, their induction upon synaptic activity destabilizes CC-Homer interactions (including those with glutamate receptors), enabling synaptic rearrangement [6,26]. While the role for Homer proteins in regulating both pre- and postsynaptic aspects of glutamate signaling is well characterized (eg, [53]) and tissue/nerve injury-induced adaptations within glutamate signaling were shown to be critically involved in the development and maintenance of chronic pain [7,16,45,72], there is recently a growing line of evidence implicating Homer proteins in the regulation of nociception. Homer proteins are expressed in brain regions conveying nociceptive information (eg, [33,53,56]), as well as in the spinal dorsal horn neurons that receive sensory input [36,40,57,71]. Both IEG-Homer1a and CC-Homer1b/c are selectively upregulated in the spinal dorsal horn neurons in model of chronic inflammatory pain, and the manipulation of their protein levels alters inflammation-induced mechanical and thermal hyperalgesia [57,71]. While *Homer1a* induction and increased Homer1b/c exhibit distinct temporal profiles within the spinal dorsal horn following chronic constriction injury (CCI) of the sciatic nerve [36,37,40] or chronic compression of dorsal root ganglion [35], the functional relevance of changes in Homers expression has been examined to date only in the early stage (4 hours after CCI) of the development of neuropathic pain [36].

Thus, in the present study, we used a combination of genetics and immunoblotting approaches to further confirm the important role for Homer proteins in regulating pain sensitivity and, more importantly, to delineate the relative functional roles played by different Homer family members and isoforms in the development and maintenance of neuropathic pain in mice. We first determined the profile of changes in Homer proteins expression after CCI, together with injury-induced alterations in the protein level/activation of Homers-associated mGluRs (mGluR1a/5) and downstream kinases (protein kinase C [PKC]ε, PI3K, and ERK1/2) within structures involved in nociception. Next, we examined the effects of Homers overexpression after spinal (intrathecal) delivery and the global Homers deficiency on both the development and maintenance states of CCI-induced neuropathic pain. Finally, we investigated whether the mGluR5–Homer proteins interaction affects neuropathic pain hypersensitivity.

2. Material and methods

2.1. Subjects

2.1.1. C57BL/6J mice

The expression of the CC-Homer isoform Homer1b/c is upregulated within the spinal cord dorsal horn following sciatic nerve ligation [36,37]. Thus, immunoblotting experiments extended these earlier data to another CC-Homer isoform, Homer2a/b, and related changes in CC-Homer expression to those of their associated Group1 mGluRs (mGluR1a/5) and the NR2a/b subunits of the NMDA receptor, as well as to the activational state of downstream kinases (ERK1/2, PKCε, and PI3K) to determine whether or not CCI of the sciatic nerve induces changes in the spinal and supraspinal expression/activation of Group1 mGluR/Homer signaling pathways. Follow-up behavioral experiments then assayed for the functional relevance of observed changes in Homer protein expression for neuropathic pain hypersensitivity. All of these studies employed adult male C57BL/6J (B6) mice (8 weeks of age; 25–30 g; the Jackson Laboratories, Bar Harbor, ME, USA). B6 mice were allowed to acclimate to the colony room for at least 7 days after arrival and were housed in polyethylene cages (4–5 per cage) in a room controlled for temperature (25°C) and humidity (71%) under a regular 12-hour day/night cycle (lights on at 7:00 AM; lights off at 7:00 PM). Standard laboratory rodent chow and water were available ad libitum. Animals were habituated to testing procedures for at least 3–4 days before experiments. The handling and testing of the animals were conducted during the light phase, between 9:00 AM and 2:00 PM. Every effort was made to minimize the number of animals used in the study. Experimental protocols were approved by the Institutional Animal Care and Use Committee of our respective institutions and were consistent with the guidelines provided by the National Institutes of Health (NIH) Guide for Care and Use of Laboratory Animals and the guidelines of the Committee for Research and Ethical Issues of IASP published in [75].

2.1.2. Homer knockout (KO) mice

Our immunoblotting data revealed time-dependent increases in Homer1b/c and Homer2a/b expression following CCI (Fig. 1), and earlier studies indicated an important role for Homer1a in regulating inflammatory pain [50]. Thus, we employed a knockout (KO) strategy to confirm a necessary role for IEG and CC-Homer isoforms (Homer1a, Homer1, Homer2) in the development and maintenance of neuropathic pain following CCI. For this, the behavior of Homer1a, Homer1, and Homer2 gene KO mice was compared to their respective wild-type (WT) and heterozygous (HET) mutant mice. WT, HET, and KO littermates from all 3 KO lines were bred in-house at the University of California Santa Barbara Biology II vivarium from mating of heterozygous breeder pairs (B6 X 129Xi/SvJ background). Details of the generation of these mice are provided in Hu et al. [21] for Homer1a KO, Yuan et al. [73] for Homer1 KO, and Shin et al. [49] for Homer2 KO. WT, HET, and KO littermate pups from a minimum of 4 different litters for each line were used for each replicate of the behavioral studies to avoid litter confounds. Experimental mice were transferred to the Psychology vivarium at approximately 5 weeks of age and allowed to acclimate to the housing conditions for 2–3 weeks prior to surgery and behavioral testing. All experimental procedures involving WT, HET, and KO mice were conducted on male littermate mice, 7–8 weeks of age, housed and handled under conditions described for the B6 mice above.

2.1.3. mGluR5 transgenic (Tg) mice

Our immunoblotting data indicated the co-regulation of CC-Homer expression and mGluR5 by CCI (Fig. 1). Thus, we examined the functional relevance of mGluR5–Homer interactions in neuropathic pain by assaying the pain hypersensitivity of a transgenic (Tg) mouse with a phenylalanine (F) → arginine (R) point mutation within the Homer binding domain on mGluR5 at amino acid position 1128 (mGluR5^{F1128R}) that impairs the capacity of Homer proteins to physically interact with the receptor [12,61], but does not affect the total protein expression of either mGluR5 or Homers [12]. The generation of Tg littermates and all experiments procedures involving WT, HET, and Tg mice were conducted as described for B6 and KO mice above.

2.2. Induction and assessment of neuropathic pain

2.2.1. Sciatic nerve injury

B6 mice, homozygous and heterozygous KO/Tg mice, as well as their WT counterparts, were subjected to peripheral neuropathy induced by CCI of the sciatic nerve as described by Bennett and Xie [4], with slight modifications for mice [42,45]. The sciatic nerve injury was performed under isoflurane anesthesia delivered via a nose cone (2% isoflurane with oxygen as the carrier gas). The skin

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