Effect of the Synaptic Scaffolding Protein Homer1a on Chronic Compression of Dorsal Root Ganglion

Zheng-Liang Ma, Wei Zhu, Wei Zhang, and Xiao-ping Gu
Department of Anesthesiology, Drum Tower Hospital, Medical Department of Nanjing University, Nanjing, Jiangsu Province, China

Abstract. Activity-dependent plasticity in the spinal dorsal horn may underlie the development of neuropathic pain following peripheral nerve injury. A product of an immediate early gene (IEG), the synaptic scaffolding protein Homer1a, has received increasing attention because it appears to play a critical role in synaptic plasticity. In this study, we explored the early expression of Homer1 gene in the spinal dorsal horn by using the neuropathic pain model of chronic compression of dorsal root ganglion (CCD). The levels of Homer1a mRNA in the ipsilateral dorsal horn of CCD rats were markedly increased at 4 hr, remained elevated at 8 hr, and then returned to baseline values by 24 hr after CCD treatment. In contrast, there were no significant changes of Homer1a expression in the Sham-operated or Control groups. Significant thermal hyperalgesia appeared at 24 hr post-operation in the CCD rats, but not in the Sham-operated or Control groups. These data show that CCD induces a transient and rapid increase in Homer1a expression in the spinal dorsal horn. These data also suggest that the transient and rapid increase in Homer1a expression may play an important role in the thermal hyperalgesia elicited by neural injury.

Keywords: spinal dorsal root ganglion compression, Homer1a, synaptic plasticity, neuropathic pain

Introduction

Chronic compression of the L4 and L5 dorsal root ganglia (CCD) can result in neuropathic pain syndromes, including hyperalgesia, allodynia, and spontaneous pain. Several factors, such as MCP-1/CCR2 [1], activating transcription factor-3 [2], glial-derived neurotrophic factor (GDNF) protein [3], and nNOS [4] have been implicated in the pathogenesis of this process. Changes in the expression of many genes [5], which underlie injury-elicited plasticity in the spinal dorsal horn, may contribute to neuronal hyperexcitability and neuropathic pain. Homer1, recent identified, appears to play a critical role in the expression of synaptic plasticity in the spinal dorsal horn [6,7].

The Homer protein family is a major constituent of the postsynaptic density (PSD), and much evidence suggests an important role of Homer in synaptic plasticity [8,9]. Recent studies have shown that plastic changes in the processing of nociceptive information in the spinal dorsal horn are involved in the development of neuropathic pain following peripheral nerve injury [10,11]. The data also indicate that Homer proteins play roles in this process [7,12].

Homer proteins are classified as short (Homer1a) and long (Homer1b/c, Homer2, and Homer3) isoforms. Homer1a was the first isoform discovered after induction of excitatory synaptic activity in cerebellar granule neurons [13]. Similar to other immediate early genes (IEGs), Homer1a responds rapidly to neuronal activity [14]. Unlike most IEGs that encode transcription factors, the unique regulation of Homer1a provides important insights into glutamatergic synaptic plasticity [15]. Recently, Homer1a has received increasing attention.
because of its action on post-synaptic structure and function [12,16,17]. A striking functional feature of Homer1a is that it contains the N-terminal Ena/ VASP Homology 1 (EVH1) domain but lacks the coiled-coil domain that mediates multimerization, so it likely functions as a naturally occurring dominant-negative form of Homer [18].

In the present study, we used the well-established chronic compression of dorsal root ganglion (CCD) model of neuropathic pain [19] and focused on changes in the early expression of Homer1a during CCD treatment. We also studied the potential relationship between the expression of Homer1a and the behavioral hyperalgesia elicited by CCD treatment.

Methods

Animal preparation. This study was performed on adult, male Sprague-Dawley rats (n = 60, 200-220 g at the start of each experiment). Rats were divided randomly into three groups as follows: Control group (n = 20), CCD group (n = 20), and Sham group (n = 20). The rats were kept individually in pathogen-free conditions with a 12 hr light/dark cycle and were fed sterile food and water. All experiments were approved by the Animal Care and Use Committee of the university and were in accordance with the guidelines for the use of laboratory animals [20]. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Surgical procedures. In the Control group (n = 20), the surgical procedure was performed aseptically under pentobarbital anesthesia (30 mg/kg, ip). The estimated levels of Homer1a mRNA in these animals served as reference baseline controls. In the CCD group (n = 20), the right L4 and L5 intervertebral foramens were exposed and a stainless steel sil (4 mm in length, 0.7 mm in diameter) was inserted to provide steady compression on the L4 and L5 dorsal root ganglia [19]. In the Sham group (n = 20), the right L4 and L5 intervertebral foramens were exposed without compression. In each group, there were 4 time points, selected as 48 hr before operation, and 4 hr, 8 hr, and 24 hr after operation, for behavioral testing and Homer1a analysis.

Behavioral testing. Thermal hyperalgesia was assessed by the paw withdrawal thermal latency (PWTL) to radiant heat according to the protocol of Hargreaves et al [21]. Rats were placed in clear plastic cages on an elevated glass plate and allowed to acclimatize for 30 min before testing. A radiant thermal stimulator (BME410A, Institute of Biological Medicine, Academy of Medical Science, China) was focused onto the plantar surface of the hindpaw through the glass plate. The nociceptive endpoints in the radiant heat test were the characteristic lifting or licking of the hindpaw, and the time to the endpoint was considered the PWTL [22]. To avoid tissue damage, a cut-off time of 30 sec was used [23]. There were 5 trials per rat and 5-min intervals between trials. The mean PWTL was obtained from the last 3 stimuli.

Real time PCR. All animals used for Homer1a analysis were anesthetized with pentobarbital sodium at 48 hr before operation and at 4 hr, 8 hr, and 24 hr after operation. The lumbar spinal cords of all animals were exposed by laminectomy at vertebral level L1–2 (spinal cord L4–5), excised, divided into dorsal and ventral halves, and the dorsal halves further subdivided into ipsilateral and contralateral quadrants. The quadrants were kept at -80°C until analysis.

Homer1a expression in individual dorsal horn quadrants was determined by real time PCR based on TaqMan fluorescence methodology to quantify the full range of Homer1a mRNA copy numbers. Briefly, total RNA was isolated from homogenized tissues using 1 ml of Trizol Reagent (Invitrogen, Carlsbad, CA). The total RNA (1 µg) was used for cDNA synthesis. PCR primers and probes for Homer1a and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) genes were designed with Primer Express 2.0 software (Applied Biosystems, Foster City, CA) (Table 1) and synthesized by Genecore (Shanghai, China). For amplification of Homer1a and GAPDH genes, real-time PCR was performed in triplicate for each sample in a 20 µl reaction mixture, which consisted of template DNA (2 µl), primers (900 nmol), probe (250 nmol), Mg²⁺ (5 mmol), and Hotaq PCR Reaction Mix. PCR was performed in a Stratagene Mx3005P instrument using the following thermal cycles: one cycle of 10 min at 95°C, and 55 cycles of 5 sec at 95°C and 30 sec at 60°C. Amplification efficiency of each individual sample was calculated by version

### Table 1. Sequences of primers and probes used in this study.

<table>
<thead>
<tr>
<th>Gene and oligonucleotide</th>
<th>Sequence</th>
<th>PCR product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAPDH</td>
<td>5'-CAAGTTCAACGGCCACAGTCAA-3'</td>
<td>149</td>
</tr>
<tr>
<td>Upper primer</td>
<td>5'-TGTTGAGAAGCCAGTAGACT-3'</td>
<td></td>
</tr>
<tr>
<td>Lower primer</td>
<td>5'-CAGGATCACCCGATTAACCT-3'</td>
<td></td>
</tr>
<tr>
<td>Probe</td>
<td>5'-(FAM)FTCTTCCAGGAGGAGATCCCTAACP(TAMRA)-3'</td>
<td></td>
</tr>
<tr>
<td>Homer1a</td>
<td>5'-CCAGAAAGATCTGAATGGAAGACAGTG-3'</td>
<td>123</td>
</tr>
<tr>
<td>Upper primer</td>
<td>5'-TGTTGAATTGAATGTGTACCTATGTG-3'</td>
<td></td>
</tr>
<tr>
<td>Lower primer</td>
<td>5'-CGTCTGAATGGAATGTGTACCTATGTG-3'</td>
<td></td>
</tr>
<tr>
<td>Probe</td>
<td>5'-(FAM)FTGAGAAGACCCGATTAACCTAACP(TAMRA)-3'</td>
<td></td>
</tr>
</tbody>
</table>

---

**Annals of Clinical & Laboratory Science, vol. 39, no. 1, 2009**

72
7.0 of LinRegPCR program (a gift from c.r.ramakers@amc.uva.nl). According to the method tested by Pfaffl [24], the relative expression ratio (RR) of a targeted gene was calculated based on Eff and the Ct compared to the reference gene (GAPDH).

**Statistical analysis.** Differences in values for paw withdrawal thermal latency (PWTL) and expression of Homer1a over time were tested by one-way ANOVA followed by Newman-Keuls post-tests. Student’s t-test was used to identify the significant differences in PTWL and Homer1a mRNA between the controls and the operated groups. All data were reported as means ± SE; p <0.05 was considered the criterion for statistical significance.

**Results**

The level of Homer1a expression of the CCD group increased significantly at 4 and 8 hr post-operation, compared to the Control group (p <0.01) and Sham group (p <0.01), respectively (Fig.1). The Homer1a expression of the CCD group was greatly increased at 4 and 8 hr post-operation compared to 48 hr pre-operation. At 24 hr post-operation, the Homer1a expression in the CCD group decreased to the baseline level at 48 hr pre-operation. Homer1a expression in the Control or Sham-operated groups did not show significant changes at the four specified time points (p >0.05). The expression of Homer1a in the contralateral side of the CCD, Control, and Sham-operated groups did not change significantly at any of the time points (p >0.05).

At 4 and 8 hr post-operation, PWTL showed no significant differences in the CCD, Sham, and Control groups (p >0.05) (Fig. 2). PWTL was significantly decreased at 24 hr post-operation in the CCD group compared to 48 hr pre-operation (p <0.01), and there was also a significant decrease of PWTL compared to the Sham and Control groups (p <0.01). PWTL in the Control and Sham-operated groups showed no significant changes at 24 hr after operation. The PWTL on the contralateral side of the CCD, Control, and Sham groups did not change significantly at any of the time points (p >0.05).

**Discussion**

Glutamatergic signaling and intercellular calcium mobilization in the spinal cord are crucial for the development of nociceptive plasticity, which is associated with chronic pathological pain [4,25]. Homer1a, the short, activity-dependent splice variant of Homer1b/c, lacks the ability of linking mGluR1/5 to synaptic proteins and functions as an endogenous negative modulator of the mGluR1/5 inositol-1,4,5-trisphosphate (IP3) receptor signaling complex [14,26-30]. Recent investigations showed that Homer1a may be selectively expressed as an immediate early gene after synaptic activity [14,15,17]. The main goal of this study was to detect...
the gene expression of Homer1a in an earlier period of CCD, which would in part reflect the change of Homer1a protein levels. In our current study, we demonstrated that CCD treatment induces Homer1a rapid expression in the spinal dorsal horn. The time-dependence of changes in expression of Homer1a was similar to that reported for cerebellar granule cells in which the induction of Homer1a mRNA appeared slower (peak at 4 hr) and was sustained longer than typical immediate early genes such as c-fos. Miyabe et al [6] also showed that changes in the expression of Homer1a may underlie injury-elicited plasticity in the spinal dorsal horn.

Our work showed some association between the expression of thermal hyperalgesia and changes in the level of Homer1a message in the spinal dorsal horn. The Homer1a expression increased at 4 and 8 hr, but returned to baseline values at 24 hr after CCD. On the contrary, thermal withdrawal latencies were unchanged at 4 and 8 hr, but decreased significantly at 24 hr after CCD. A previous study showed that activity-dependent up-regulation of Homer1a is needed to promote its accumulation at post-synaptic sites to facilitate the redistribution of Homertlb/c and reorganize postsynaptic structures to promote enhancement of synaptic function [31], ie, synaptic plasticity, and to facilitate synaptic remodeling and contribute to the long-lasting increases in synaptic efficacy. Furthermore, the endogenous Homer1a may bind directly to G-protein-coupled receptors (GPCR), such as metabotropic glutamate receptors (mGluR) [32]. Homer1a may also protect inflammatory pain in a manner that attenuates calcium mobilization as well as MAP kinase activation induced by glutamate receptors and reduce synaptic contacts on spinal cord neurons that process pain inputs [12].

In conclusion, CCD elicits early and transient up-regulation of Homer1a gene expression, which may play a role, at least in part, in the production of thermal hyperalgesia. As an immediate early gene, Homer1a may facilitate synaptic remodeling, mutate GPCR, and attenuate calcium mobilization that underlie the subsequent induction and development of pain.

Acknowledgement

This study was supported by the Department of Education Foundation of Jiangsu Province (Grant 02KJB320012).

References

22. Chen J, Luo C, Li H, Chen H. Primary hyperalgesia to mechanical and heat stimuli following subcutaneous bee venom injection into the plantar surface of hindpaw in the conscious rat: a comparative study with the formalin test. Pain 1999;83:67-76.