Available online at www.sciencedirect.com

SCIENCE DIRECT

Molecular Brain Research 137 (2005) 152 – 158

MOLECULAR BRAIN RESEARCH

www.elsevier.com/locate/molbrainres

Research report

Involvement of phosphorylated extracellular signal-regulated kinase in the mouse substance P pain model

Seong-Soo Choi, Young-Jun Seo, Min-Soo Kwon, Eon-Jeong Shim, Jin-Young Lee, Young-Ok Ham, Soo-Hyun Park, Hong-Won Suh*

Department of Pharmacology, Institute of Natural Medicine, College of Medicine, Hallym University, 1 Okchun-Dong, Chuncheon, Gangwon-Do, 200-702, South Korea

Accepted 3 March 2005
Available online 19 April 2005

Abstract

In the present study, we investigated the role of pERK in nociceptive processing at the spinal and supraspinal levels in the substance P (SP)-induced mouse pain model. In the immunoblot assay, intrathecal (it) injection with SP increased pERK level at the spinal cord and an immunohistochemical study showed that increase of pERK immunoreactivity mainly occurred in the lamina I and II areas of the spinal dorsal horn. At the supraspinal level, pERK was increased in hippocampus and hypothalamus by i.t. SP injection, and an increase of pERK immunoreactivity mainly occurred in the dentate gyrus and CA3 region of hippocampus and paraventricular nucleus on hypothalamus. The nociceptive behavior induced by Sub P administered either i.t. or intracerebroventricularly (i.c.v.) was attenuated by PD98059 (a MEK 1/2 inhibitor) in a dose-dependent manner. Our results suggest that pERK located at both spinal cord and supraspinal levels plays as an important regulator during the nociceptive process activated by SP administered it.

Theme: Sensory systems
Topic: Pain modulation: pharmacology

Keywords: Phospho-ERK; Substance P test; Nociceptive behavior; Spinal cord; Hippocampus; Hypothalamus; Intrathecal; Intracerebroventricular

1. Introduction

Extracellular signal-regulated protein kinase (ERK) is one the mitogen-activated protein kinase family member. Several lines of evidence have demonstrated that phosphorylation of ERK (pERK) is involved in the regulation of nociception. For example, ERK phosphorylation is increased in the spinal cord level during the noxious, but not the innocuous, stimuli [20]. It is believed that pERK is involved in the activation of NMDA or metabotropic glutamate receptors followed by transcriptional activation such as NK-1 or prodynorphin, leading to central or peripheral sensitization [13,20,21,23]. Additionally, it has been suggested that ERK activation is involved in spinal nociceptive processing and secondary hyperalgesia in carrageenan-induced inflammation animal model [13,19,21]. Furthermore, a partial sciatic nerve ligation induces an increase of pERK in astrocytes in the lumbar spinal dorsal horn and the gracile nucleus [27].

In supraspinal regions, pERK-positive cells are observed in the hypothalamus, thalamus, amygdala, hippocampus, and perirhinal cortex in acetic acid-induced visceral inflammatory pain model [15] and in the hypothalamus and spinal cord in the kainic acid-induced hyperalgesia model [38]. Moreover, pERK is also increased in pons/medulla area and dorsal root ganglion during the development of antinociceptive tolerance induced by chronic morphine [28,35]. Of particular interest is the hypothalamus and hippocampus of supraspinal subcortical regions. There are massive spinal projections to the hypothalamus directly

* Corresponding author. Fax: +82 33 248 2612.
E-mail address: hwsuh@hallym.ac.kr (H.-W. Suh).

0169-328X/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.molbrainres.2005.03.007

or indirectly that are involved in autonomic, neuroendocrine, and emotional responses to somatosensory stimulation, including painful stimuli in the rat [4–6,14]. Neuronal pathways from the spinal cord to limbic or cortico-limbic structures indicate the involvement of affective aspects of pain and integration of nociceptive input with contextual memory [36]. Moreover, it is evident that adaptive mechanism in limbic areas may be involved in the decreased response to painful stimuli after stress exposure [2,3,22]. The electrical stimulation into dorsal or ventral hippocampus induces painful expression in guinea pig [26] and monkey [12]. Moreover, analgesia is produced by lidocaine microinjection or blockade of NMDA receptor of dentate gyrus of hippocampus [30,31]. However, the involvement of pERK in the hypothalamus and hippocampus in the regulation of nociception has not been well known yet.

Although the intrathecal (i.t.) SP model [8,9,18] has been widely used to study the nociceptive/antinociceptive mechanisms, there is little known about the involvement of pERK, especially at supraspinal level. Thus, the present study was designed to assess the expression of pERK by i.t. SP at spinal and supraspinal levels. Furthermore, effect of PD98059 (a MEK 1/2 inhibitor) on pain behavior induced by SP administered i.t. was examined.

2. Materials and methods

These experiments were approved by the University of Hallym Animal Care and Use Committee. All procedures were conducted in accordance with the 'Guide for Care and Use of Laboratory Animals' published by the National Institutes of Health and the ethical guidelines of the International Association for the Study of Pain.

2.1. Experimental animals

Male ICR mice (MJ Ltd., Seoul, Korea) weighing 23–25 g were used for all the experiments. Animals were housed 5 per cage in a room maintained at 22 ± 0.5°C with an alternating 12-h light–dark cycle for at least 5 days before the experiments were started and food and water were available ad libitum. The animals were allowed to adapt to the laboratory for at least 2 h before testing and were used only once. To reduce variation, all experiments were performed during the light phase of the cycle (10:00–17:00).

2.2. Intrathecal (i.t.) and intracerebroventricular (i.c.v.) injection of drugs

The i.t. administration was performed in conscious mice following the previously established method [17,18] using a 30-gauge needle connected to a 25-μl Hamilton syringe with polyethylene tubing. The i.t. injection volume was 5 μl and the injection site was verified by injecting a similar volume of 1% methylene blue solution and determining the distribution of the injected dye in the spinal cord. The dye injected i.t. was distributed both rostrally and caudally but with short distance (about 0.5 cm) and no dye was found in the brain.

The i.c.v. injection was performed following the procedure established previously [25]. Briefly, each conscious mouse was injected at bregma with a 50-μl Hamilton microsyringe fitted with a 26-gauge needle that was inserted to a depth of 2.4 mm. Bregma could be found about 1–3 mm rostral to a line drawn between the anterior base of the ears after feeling the suture line by lightly rubbing the point of needle. I.c.v. injection volume was 5 μl and the injection sites were verified by injecting a similar volume of 1% methylene blue solution and determining the distribution of the injected dye in the ventricular space. The dye injected i.c.v. was found to be distributed through the ventricular spaces and reached the ventral surface of the brain and upper cervical portion of the spinal cord.

2.3. Substance P treatment and pain behavior analysis

Five animals of each group were dissected for Western blot analysis. Mice were injected i.t. with SP (0.7 μg). Mice were killed by cervical dislocation at 10, 30, and 120 min after SP injection. Lumbar spinal cord, hippocampus, and hypothalamus were dissected and pooled for Western blot.

For behavioral study, eight to ten animals of each group were pretreated i.t. or i.c.v. once with 10% DMSO(a vehicle) or PD98059(a MEK 1/2 inhibitor) dissolved in 10% DMSO 20 min prior to the SP injection. This pretreatment time was determined from preliminary studies in which the effects reached a maximum after injection. Immediately after the i.t. injection with SP, the mice were placed in a glass cylinder chamber (20 cm high, 20 cm diameter) and the duration of nociceptive behavioral responses, which was manifested by licking, biting, and scratching directed toward the lumbar and caudal region of the spinal cord, was measured for 30 min [18].

2.4. Western immunoblot analysis

Total cellular protein (50 μg) was separated by electrophoresis in 12% sodium dodecyl sulfate (SDS)–polyacrylamide gels [24]. A prestained rainbow protein mixture (Amersham Co., Arlington, USA) was used as a molecular weight standard. Proteins were transferred from acrylamide gel onto polyvinylidene difluoride filters (Amersham Pharmacia Biotech, Little Chalfont, Buckinghamshire, England) according to published procedures [39]. Electrotransferred polyvinylidene difluoride filters were first incubated in blocking buffer [3% skim milk, 1% BSA, 10 mM Trizma base (pH 8.0), and 150 mM NaCl] and then allowed to interact with antibody against phosphor-ERK (Cell Signaling Technology, Beverly, MA, USA; 1:1000 dilution) and ERK (New England Biolabs, USA; 1:1000