Neuroscience 248 (2013) 145–153

THE EXPRESSION OF SUCCINATE SEMIALDEHYDE DEHYDROGENASE IN THE CAUDAL PART OF THE SPINAL TRIGEMINAL NUCLEUS IS DOWN-REGULATED AFTER ELECTRICAL STIMULATION OF THE DURA MATER SURROUNDING THE SUPERIOR SAGITTAL SINUS IN CONSCIOUS RATS

X. L. WANG, J. Y. LIANG, R. Z. LIU, Z. DONG AND S. Y. YU*

Department of Neurology, Chinese PLA General Hospital, Beijing 100853, China

Abstract—Using two-dimensional gel electrophoresis (2-DE), we examined proteomic changes in the caudal part of the spinal trigeminal nucleus induced by electrical stimulation of the dura mater surrounding the superior sagittal sinus (ES-SSS) in conscious rats. After image analysis of 2-DE gels, nine protein spots of interest were excised from the gels and identified by liquid chromatography–tandem mass spectrometry. Among the nine, succinate semialdehyde dehydrogenase (SSADH) was found to be down-regulated after ES-SSS. This result was validated with Real-time polymerase chain reaction and Western blot analyses. Because SSADH degrades GABA, decreased expression of it increases the local concentration of GABA in the caudal part of the spinal trigeminal nucleus after ES-SSS; this has not been reported before and may participate in the modulation of trigeminovascular headache.

Key words: comparative proteomics, succinate semialdehyde dehydrogenase, trigeminovascular headache, electrical stimulation, rat.

INTRODUCTION

Migraine is a common disabling neurological disorder, characterized by severe headache, which gravely affects quality of life and productivity. Although the mechanisms underlying migraine are still unclear, activation of the trigeminovascular nociceptive pathway is widely accepted (Goadsby et al., 2009). In the clinical setting, drugs for symptom control in an ictal period can be categorized into two classes: migraine-specific drugs such as ergot-derivates and 5-$HT_{1B/1D}$ receptor agonist triptan and migraine-nonspecific drugs such as simple analgesics and nonsteroidal anti-inflammatory drags (NSAIDS). For prevention in the interictal period, there are many more categories from which to choose. They include: beta-blockers such as propranolol, tricyclic antidepressant amitriptyline, anticonvulsants such as valproate and topiramate, calcium channel blocker flunarizine and serotonin antagonists such as methysergide (Geraud et al., 2004; Treatment guidelines for acute migraine attacks, 2007; Sprenger and Goadsby, 2009; Akerman et al., 2011). These various drugs with differing effects further reflect the complexity of the mechanisms underlying migraine.

To better understand migraine, an animal model was successfully established by electrical stimulation of the dura mater surrounding the superior sagittal sinus (ES-SSS) in the conscious rat (Dong et al., 2011). After ES-SSS, neurons are activated in many nuclei, such as those in the caudal part of the spinal trigeminal nucleus (Sp5C), the raphe magnus nucleus (RMg), the periaqueductal gray (PAG) and others (Wang et al., 2011). Among these activated nuclei, the Sp5C is special for it not only is the primary afferent center of head and facial pain, but also is the final target of the descending pain inhibitory system (Storer and Goadsby, 1997). The activity of neurons in the Sp5C should be categorized and, perhaps even opposing, but must be integrated in the development and maintenance of migraine. In order to better understand the molecular changes in the Sp5C during migraine, proteomics was introduced to the present study. Proteomics comprises a set of tools for the large-scale study of gene expression at the protein level (Ding et al., 2006). By identifying altered proteins involved in disease, the underlying molecular mechanisms can be explored.

In this study, we examined proteomic changes in the Sp5C induced by ES-SSS in conscious rats. Using two-dimensional gel electrophoresis (2-DE) and liquid chromatography–tandem mass spectrometry (LC–MS/MS), proteins that were

*Corresponding author. Tel: +86-10-55499318.
E-mail addresses: Wangxl80@126.com (X. L. Wang), yingyao582@sina.com (J. Y. Liang), liurz301@sina.com (R. Z. Liu), dong_zhaozhao@126.com (Z. Dong), yusy1963@126.com (S. Y. Yu).

Abbreviations: AEDs, antiepileptic drugs; CBB, coomassie brilliant blue; CHAPS, 3-[3- (bile amide propyl) two a 3-aminopropanesulfonic acid hydrate; 2-DE, two-dimensional gel electrophoresis; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; ES-SSS, electrical stimulation of the dura mater surrounding the superior sagittal sinus; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GPT, gabapentin; IEF, isoelectric focusing system; IPG, immobilized ph gradient; LC–MS/MS, liquid chromatography–tandem mass spectrometry; NCBInr, National Center for Biotechnology Information; NSAIDS, nonsteroidal anti-inflammatory drags; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; Sp5C, caudal part of the spinal trigeminal nucleus; SSADH, succinate semialdehyde dehydrogenase; TCA, trichloroacetic acid; TPM, topiramate; VPA, valproate.

0306-4522/13 $36.00
http://dx.doi.org/10.1016/j.neuroscience.2013.05.028

differentially expressed in the Sp5C of stimulated and un-stimulated rats were identified, and proteins of interest were validated by RT-PCR and Western blot analysis.

EXPERIMENTAL PROCEDURES

Materials

immobilized ph gradient (IPG) buffer (pH 3–10 NL), 18 cm Immobiline Drystrips (pH 3–10 NL), Drystrip cover fluid, urea, bromophenol blue, 3-[3- (bile amide propyl) two a 3-aminopropanesulfonic acid hydrate (CHAPS), agarose, coomassie brilliant blue (CBB) R-250 and G-250, acrylamide, Bis, Tris, SDS, and tetramethylethylenediamine (TEMED) were obtained from Sigma (St. Louis, MO, USA). 2-DE sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) standards were from BioRad (Hercules, CA, USA). Dithiothreitol (DTT) and sequencing grade modified trypsin (porcine pancreas source) was supplied by Promega (Madison, WI, USA). Ammonium persulfate was obtained from Gibco BRL (Grand Island, NY, USA). TRIzol Reagent and SuperScript III came from Invitrogen (Carlsbad, CA, USA). The primary antibodies for succinate semialdehyde dehydrogenase (SSADH) (rabbit polyclonal IgG; 1:200 dilution) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (1:1 000 dilution) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Other analytical grade chemicals used in this study were supplied by domestic sources. All buffers were prepared with Milli-Q deionized water. The IPGphor isoelectric focusing system (IEF) system, the Ettan Dalt II vertical electrophoresis system, Hoefer processor plus (automated gel stainer) and the ImageMaster® 2-D platinum 5.0 software were purchased from Amersham Pharmacia Biotech (Uppsala, Sweden). A Unicam UV-330 spectrometer (Cambridge, UK) was used to determine protein concentration.

Animals and grouping

Thirty Sprague–Dawley male rats, each weighing 200–250 g, were randomly selected. The rats were divided into experimental and control groups, with 15 rats in each group. The rats in each of these two groups were randomly divided into three subgroups. Subgroup 1 was used for 2-DE and LC–MS/MS, subgroup 2 was used for quantitative RT-PCR and subgroup 3 was used for Western blot analysis. The experimental procedures were approved by the Committee of Animal Use for Research and Education of the Laboratory Animals Center of Chinese PLA General Hospital (Beijing, PR China) and were consistent with the ethical guidelines recommended for pain research in conscious animals by the International Association for the Study of Pain (Zimmermann, 1983).

Our studies generally consisted of two parts, an animal part (Surgery and electrical stimulation and sample preparation) and a molecular biology part (proteomics and RT-PCR and Western blotting). The researchers conducting the animal part differ from those conducting the molecular biology part, and those who did the 2-DE image analysis were blinded to the group.

Surgery and electrical stimulation

The rats in the experimental group underwent surgery and electrical stimulation to establish the migraine model. The rats in the control group underwent surgery, but did not receive electrical stimulation. The rats in subgroups 2 and 3 in the experimental group did not receive surgery or electrical stimulation until proteins of interest were identified in the 2-DE and LC–MS/MS experiment, and then they were used for RT-PCR (subgroups 2) and Western blot analysis (subgroups 3).

Surgery

The procedures for surgery and electrical stimulation have been described previously in detail (Dong et al., 2011; Wang et al., 2011). The rats were anesthetized with 10% chloral hydrate (4 ml/kg, i.p.) and then placed into a stereotactic frame. The scalp covering the dorsal surface of the skull was incised and the connective tissue and muscle were removed, leaving the parietal bone exposed. Two cranial windows (4 mm before and 6 mm after bregma on the midline suture, 1 mm in diameter) for electrical stimulation were carefully drilled into the parietal bone and the skull was opened to expose the dura mater adjacent to the superior sagittal sinus. Care was taken to drill with a constant application of ice-cold saline. A pair of stimulation electrodes (0.8 mm in diameter, from Donghengyu Ltd., China) were oriented in the drilled holes and placed onto the dural surface. Except for their bottom ends, all electrodes were insulated. Care must be taken to avoid lacerating the dural mater. Then, the electrodes were attached to the skull, using 502 glue and dental cement and the sectioned skin was sutured, leaving only the wire connection outside. All animals received prophylactic treatment with antibiotic injections (Penicillin, 0.04 million IU/100 g) for two days following surgery. All of the rats then were housed individually at a constant ambient room temperature and a 12-h light–dark cycle and given unrestricted access to food and water following the surgical procedure. Testing took place on the fourth day in the test room, which was maintained at a temperature of about 25 °C.

Electrical stimulation

Rats were placed into a cage that was 40 cm in diameter and 17.5 cm in height, which is a convenient size for observation. The electrode on each rat's head was connected to a stimulator (SEN-7103, Japan) and separator (SS-102J, Japan) by a matching wire connection. The electrical stimulus parameters were fixed and based on our preliminary experiment (monophasic square-wave pulses, 0.25 ms in pulse duration and 20 Hz in stimuli frequency). Before the electrical stimulus step of the experimental procedures, the rats in the experimental group received a transient electrical stimulation (5 min) to test the electrode