Neuroscience 290 (2015) 115–125

NICOTINE STIMULATES EXPRESSION OF PROTEINS IMPLICATED IN PERIPHERAL AND CENTRAL SENSITIZATION

J. L. HAWKINS, J. E. DENSON, D. R. MILEY AND P. L. DURHAM *

Center for Biomedical & Life Sciences, Missouri State University, Springfield, MO, USA

Abstract—Pain patients who are nicotine dependent report a significantly increased incidence and severity of pain intensity. The goal of this study was to determine the effects of prolonged nicotine administration on inflammatory proteins implicated in the development of peripheral and central sensitization of the trigeminal system. Behavioral, immunohistochemical, and microarray studies were utilized to investigate the effects of nicotine administered daily for 14 days via an Alzet® osmotic pump in Sprague Dawley rats. Systemic nicotine administration caused a significant increase in nocifensive withdrawals to mechanical stimulation of trigeminal neurons. Nicotine stimulated expression of the pro-inflammatory signal transduction proteins phosphorylated-extracellular signal-regulated kinase (p-ERK), phosphorylated-c-Jun N-terminal kinase (p-JNK), and protein kinase A (PKA) in the spinal trigeminal nucleus. Nicotine also promoted elevations in the expression of glial fibrillary acidic protein (GFAP), a biomarker of activated astrocytes, and the microglia biomarker ionized calcium-binding adapter molecule 1 (Iba1). Similarly, levels of eleven cytokines were significantly elevated with the largest increase in expression of TNF-α. Levels of PKA, p-ERK, and p-JNK in trigeminal ganglion neurons were increased by nicotine. Our findings demonstrate that prolonged systemic administration of nicotine promotes sustained behavioral and cellular changes in the expression of key proteins in the spinal trigeminal nucleus and trigeminal ganglion implicated in the development and maintenance of peripheral and central sensitization. **© 2015 IBRO. Published by Elsevier Ltd. All rights reserved.**

Key words: central sensitization, cytokines, nicotine, spinal trigeminal nucleus, trigeminal ganglion.

*Corresponding author. Address: Center for Biomedical & Life Sciences, Missouri State University, 524 N, Boonville Avenue, Springfield, MO 65806, USA.

E-mail address: pauldurham@missouristate.edu (P. L. Durham).

Abbreviations: cAMP, cyclic adenosine monophosphate; CGRP, calcitonin gene-related peptide; CRE, cAMP response element; GFAP, glial fibrillary acidic protein; Iba1, ionized calcium-binding adapter molecule 1; MAPKs, mitogen-activated protein kinases; nAch, nicotinic acetylcholine; PBS, phosphate-buffered saline; p-ERK, phosphorylated-extracellular signal-regulated kinase; p-JNK, phosphorylated-c-Jun N-terminal kinase; PKA, protein kinase A; ROI, regions of interest; SEM, standard error of the mean.

http://dx.doi.org/10.1016/j.neuroscience.2015.01.034
0306-4522/© 2015 IBRO. Published by Elsevier Ltd. All rights reserved.

INTRODUCTION

Migraine is a painful neurological disorder that affects 18% of the general population, with prevalence highest in women of childbearing age (Buse et al., 2013). Migraineurs are thought to have a hyperexcitable nervous system characterized by a heightened sensitivity toward external stimuli that may be responsible for triggering a migraine attack (Dodick and Silberstein, 2006). Sensitization and activation of trigeminal nerves, which provide a nociceptive pathway from the peripheral tissues to the spinal trigeminal nucleus, is implicated in migraine pathology. A lowering of the activation threshold of trigeminal nociceptive neurons indicative of neuronal sensitization is associated with increased neuron-glia interactions and cellular changes in ion channels, receptors, gap junctions, and signal transduction pathways (Hucho and Levine, 2007). These cellular changes are mediated by increases in neuronal and glial expression of signal transduction proteins and elevated levels of cytokines in both the trigeminal ganglion and spinal trigeminal nucleus. In particular, members of the mitogen-activated protein kinase (MAPK) family and protein kinase A (PKA) are involved in intracellular signaling cascades that promote neuron–glia interactions and maintain a hyperexcitable state of nociceptive neurons (Ji et al., 2009; Seybold, 2009). Cytokines are a large family of proteins that modulate inflammatory responses and also play an important role in the development of peripheral and central sensitization, (Miller et al., 2009) and elevated cytokine levels have been reported in serum and the cerebrospinal fluid in migraine patients (Rozen and Swidan, 2007; Uzar et al., 2011).

Nicotine is a widely abused, alkaloid substance that accounts for about 0.6–2.9% of the dry weight of tobacco (Hoffmann and Hoffmann, 2012) and is reported to cause activation and hypersensitivity of neurons and to elicit pain (Liu and Simon, 1996). In support of this notion, pain patients who are chronic tobacco users and have severe nicotine dependency report significantly greater pain intensities than non-users (Weingarten et al., 2008; Zvolensky et al., 2009; Shi et al., 2010). In a study conducted by the Mayo clinic, pain patients diagnosed with a variety of pathologies and who were nicotine dependent also reported higher pain intensities that interfered with daily activities and mood (Weingarten et al., 2008). Similarly, findings from a later study showed a strong correlation existed between chronic pain and nicotine dependence (Zvolensky et al., 2009). This correlation likely extends to migraine sufferers since smokers are

significantly more likely to experience autonomic symptoms during migraine attacks than non-smokers (Rozen, 2011) and heavy smoking is considered a risk factor for migraine (Le et al., 2011; Schramm et al., 2013). Nicotine exhibits high affinity for nicotinic acetylcholine (nAch) receptors, which are expressed on trigeminal neurons as well as astrocytes and microglia (Liu et al., 1998; De Simone et al., 2005; Oikawa et al., 2005). Nicotine activation of nAch receptors leads to an increase of intracellular calcium that facilitates increased neuronal and glial excitability (Delbono et al., 1997; Oikawa et al., 2005; Michel et al., 2011). Although nicotine dependency is associated with elevated pain states, the cellular mechanisms by which nicotine use affects the excitability state of trigeminal neurons are not well understood.

Sensitization of trigeminal nociceptive neurons is implicated in the underlying pathology of migraine (Pietrobon and Moskowitz, 2013). In our study, changes in nocifensive responses to mechanical stimuli over the eyebrow and masseter areas in response to prolonged nicotine administration were investigated. Immunohistochemistry was also utilized to determine changes in protein levels in neurons and glia implicated in the development of peripheral and central sensitization in response to prolonged nicotine administration. In addition, changes in the level of 29 cytokines in trigeminal ganglia and upper spinal cord tissue were investigated using cytokine protein profiling arrays. Results from our study provide evidence that prolonged nicotine administration equivalent to smoking > 20 cigarettes per day increased sensitivity to mechanical stimuli in the facial area, which correlated with increased expression of proteins in the trigeminal ganglion and spinal cord that are known to promote peripheral and central sensitization of nociceptive neurons.

EXPERIMENTAL PROCEDURES

Animals

All animal studies were conducted in accordance with the protocols approved by the Institutional Animal Care and Use Committee at the Missouri State University and were in compliance with all guidelines established in the Animal Welfare Act of 2007 and National Institutes of Health. A mindful effort was made to minimize the number and suffering of the animals used during this study. Animals were housed in standard, clean plastic cages that allowed unrestricted access to food and water. Animals were acclimated to the environment that included a 12-h light/dark cycle upon arrival one week prior to use. All procedures were conducted at the same time of the day, between the hours of 7:00 a.m. and 12:00 p.m.

Administration of nicotine

Adult Sprague–Dawley male rats (200–250 g; Charles River Laboratories, Wilmington, MA, USA) were monitored until growth weights stabilized (400–500 g). Animals were anesthetized by inhalation of isoflurane (3–5%). Incision sites were prepared for pump implantation by trimming the hair located over the surgical area and disinfecting the site with 70% ethanol and Betadine scrub (Patterson Companies Inc, Devens, MA, USA). Breathing rates were monitored visually, and body temperature was maintained throughout the procedure through use of a Gaymar water blanket (Patterson Companies). Using the aseptic technique, an incision was made on the dorsal side inferior to the scapula using a sterile surgical scalpel. An Alzet® Osmotic Pump (Alzet, Cupertino, CA, USA) containing either 1× phosphate-buffered saline (PBS) or a PBS-buffered solution that systemically delivered nicotine hydrogen tartrate at 32-mg/kg/day (10.4-mg/kg/day as a free base; Sigma, St. Louis, MO, USA) when released at a rate of 5.22 μl/h for two weeks. Pumps were subcutaneously implanted and the incision was closed with Weblon nylon sutures (Patterson Companies). Animals were monitored during recovery and again 24 h after the procedure for normal grooming and feeding behaviors. Sutures were removed under 2% isoflurane seven days post-procedure and wounds were checked for infection. Nicotine or PBS was administered for two weeks, at which point animals were euthanized by CO_2 asphyxiation and decapitation. Trigeminal ganglia and C1/C2 spinal cord tissue containing the dorsal medullary horn of the spinal trigeminal nucleus were acquired through cranial dissection. Spinal cord tissues were cut evenly from superior to inferior down the midline with a scalpel. The right side was used for immunohistochemistry and the left side used for cytokine analysis. If at any point during the study an animal showed signs of infection, excessive weight loss, abnormal behavior, or distress, the animal was removed from the study.

Measurement of cotinine levels

Serum levels of cotinine, which is the primary metabolite of nicotine, were measured at the end of the 2 week of administration to validate that the pumps were functioning properly. Blood samples were collected in BD Vacutainer® Plastic Serum Tubes with a serum separator (Becton Dickinson, Franklin Lakes, NJ, USA) upon completion of the study. Tubes were inverted 5 times and allowed to coagulate at room temperature for 2 h. Samples were centrifuged at 300*g* for 3 min and serum transferred to autoclaved tubes. Serum cotinine levels were determined using a selective Cotinine ELISA for rat serum (Calbiotech Inc, Spring Valley, CA, USA) according to manufacturer's protocol. Absorbance was measured at a wavelength of 450 nm after the addition of the stopping solution to each well. Values are reported as the average level of serum cotinine ± standard error of the mean (standard error of the mean (SEM), ng/mL).

Behavioral testing using mechanical stimulation

All behavioral assessments were carried out essentially as described in a previously published study (Garrett

Download English Version:

https://daneshyari.com/en/article/6272626

Download Persian Version:

https://daneshyari.com/article/6272626

Daneshyari.com