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Lysophosphatidylcholine causes neuropathic pain via the increase of neuronal nitric oxide synthase in the dorsal root ganglion and cuneate nucleus

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ABSTRACT

In this study, we investigated the role of nitric oxide (NO) in lysophosphatidylcholine (LPC) induced peripheral neuropathy by the use of nitric oxide synthase (NOS) inhibitors and NO donor. We found that LPC treatment of the median nerve induced neuropathic pain behaviors (allodynia and hyperalgesia) and nerve demyelination. Immunohistochemistry revealed that the amounts of neuronal NOS-like immunoreative (nNOS-LI) neurons in both the dorsal root ganglion (DRG) and cuneate nucleus (CN) increased and peaked at 1 week after LPC treatment. Following electrical stimulation of the LPC-treated nerve, the number of c-Fos-LI neurons in the ipsilateral CN also increased in a dose-dependent manner following LPC injection and peaked at 1 week. Administration of L-NAME (N_{ω}-Nitro-L-arginine methyl ester) or 7-NI (7-nitroindazole) 1 week after 4% LPC injection attenuated tactile allodynia and thermal hyperalgesia. However, the application of the NO donor S-Nitroso-N-acetylpenicillamine (SNAP) only exacerbated thermal hyperalgesia. After electrical stimulation of the LPC-treated median nerve, the number of c-Fos-LI neurons in the CN diminished in the L-NAME and 7-NI groups, but increased in the SNAP group. Taken together, our findings suggest that advanced NO made by the dramatically increased number of nNOS in the DRG and CN might be involved in the neuropathic sensation and boosted neuronal activity in the CN after LPC treatment.

1. Introduction

Lysophosphatidylcholine (LPC) is one of the plasma lipid components that transfers choline and fatty acid to tissues, and is made under both physiological and pathological conditions (Yokota and Hansson, 1995; Murugesan and Fox, 1996). Previous studies revealed that LPC treatment of the sciatic nerve resulted in mechanical allodynia and thermal hyperalgesia (Wallace et al., 2003; Inoue et al., 2008). Also, LPC injection induced nerve demyelination and an increase in pain-related protein levels, including neuropeptide Y (NPY), Nav 1.8, Nav 1.3, chemokines, and their receptors, in the dorsal root ganglion (DRG) (Wallace et al., 2003; Bhangoo et al., 2007). However, the effect of LPC treatment to the median nerve on the development of neuropathic pain behavior remains uncertain.

It is well documented that nitric oxide (NO) is synthesized and regulated by three types of nitric oxide synthases (NOS): neuronal (nNOS), endothelial (eNOS), and inducible (iNOS). Peripheral nerve injury leads to changes in the number of nNOS-like immunoreactive (nNOS-LI) neurons in the DRG, spinal cord, and gracile nucleus (GN) (Zhang et al., 1993; Gonzalez-Hernandez and Rustioni, 1999; Ma et al., 2000; Lukacova et al., 2003; Liu et al., 2005). Although nNOS-LI neurons have been reported to be present in the cuneate nucleus (CN) (Valtschanoff et al., 1995; Wang et al., 2001, 2012), it is seldom known about the changes in the number of nNOS-LI neurons in the DRG and CN after LPC injection to the median nerve.

A recent study further demonstrated that NO regulated the NPY release from the injured median primary afferent terminals (PATs) and upregulated c-Fos expression in the CN after electrical stimulation (Wang et al., 2012). The expression of c-Fos in cuneothalamic projection neurons (CTNs) in rats induced by electrical stimulation of the injured median nerve was associated with behavior signs of mechanical allodynia and thermal hyperalgesia (Day et al., 2001). Several studies have demonstrated that NO might mediate mechanical allodynia and

Abbreviations: cGMP, cyclic guanosine monophosphate; CN, cuneate nucleus; CTNs, cuneothalamic projection neurons; DMSO, dimethyl sulfoxide; DRG, dorsal root ganglion; GN, gracile nucleus; LPC, Lysophosphatidylcholine; L-NAME, N_{ω}-Nitro-L-arginine methyl ester; MNT, median nerve transection; NADPH-d, nicotinamide adenine dinucleotide phosphate-diaphorase; 7-NI, 7-nitroindazole; nNOS, neuronal nitric oxide synthase; NO, nitric oxide; NOS, nitric oxide synthase; NPY, neuropeptide Y; PATs, primary afferent terminals; PB, phosphate buffer; SNAP, S-Nitroso-N-acetylpenicillamine.

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thermal hyperalgesia after peripheral nerve injury (Meller et al., 1992; Cizkova et al., 2002; Kim et al., 2011). Moreover, recent studies demonstrated that nNOS knockout mice barely developed mechanical allodynia and thermal hyperalgesia after nerve injury (Guan et al., 2007; Hervera et al., 2010). However, the direct evidence of NO involvement in the median nerve neuropathic sensation remains uncertain.

Based on these observations, the present study was aimed to investigate if behavioral signs of neuropathic pain would develop after LPC application on the median nerve. We also examined temporal changes in the morphological structure of the median nerve and in the amounts of nNOS-LI neurons in the C6 DRG and CN after LPC treatment. To further address the role of NO in LPC induced neuropathic pain, we observed LPC-induced neuropathic pain behavior signs and electrical stimulation induced c-Fos expression in the CN following the application of NOS inhibitors, N_{ω}-Nitro-L-arginine methyl ester (L-NAME), 7-nitroindazole (7-NI) and NO donor S-Nitroso-N-acetylpenicillamine (SNAP).

2. Materials and methods

2.1. Animals

All experiments were reviewed and approved by the National Science Council Committee and the Animal Center Committee, College of Medicine, National Taiwan University, Taiwan (IACUC Approval No. 20110436). Male Wistar rats weighing 180–200 g were purchased from BioLASCO (Taiwan), housed under a 12/12 h light/dark cycle, and were able to access food and water ad-libitum.

2.2. Nerve injury surgery

LPC (Sigma, St. Louis, MO) was dissolved in physiological saline to give a final concentration of 1%, 2%, 4% and 8%. Rats were anesthetized by an intraperitoneal (i.p.) administration of 7% chloral hydrate (4.5 ml/kg body weight). The median nerve was exposed at the elbow level proximal to its entering between the two heads of the pronator teres muscle. Two microliters of normal saline or various concentrations of LPC were injected into the median nerve at the elbow level via a glass micropipette connected to a Hamilton microsyringe (Hamilton, Nevada, U.S.A.). Subsequently, the incision site was sutured and animals were allowed to survive for 1, 2 and 4 weeks after injection.

2.3. Behavioral testing and NO related reagent treatment

Von Frey filaments (Somedic Sales AB, Horby, Sweden) were used to test the mechanical withdrawal thresholds (g/mm^2) of the rat forepaws (Day et al., 2001; Tsai et al., 2009). Testing began with the smallest bending force and continued in an increasing order (11 to 122 g/mm^2). Each filament was applied five times to the medial plantar surface of each rat forepaw and the first filament that caused at least three withdrawal responses was defined as the paw withdrawal threshold. The threshold value of each group was presented as the mean ± SEM.

The Plantar apparatus (Ugo Basile, Comerio, Italy) was used to measure the time for forepaw withdrawal in response to a noxious thermal stimulus (Hargreaves et al., 1988). A radiant heat source was aimed at the plantar surface of the forepaw and the withdrawal latency, in seconds, from initial heat source activation to paw withdrawal was measured by the apparatus. Five latency measurements of each forepaw were recorded and averaged to yield a withdrawal latency, which was presented as the mean ± SEM.

One week before saline or LPC treatment, rats were acclimated and trained for 3 days on all behavioral tests, followed by a baseline measurement of the tests within 1 day before LPC administration. Behavioral tests were repeated once every two days for 2 weeks after saline or various concentrations of LPC treatment (each group, n = 5). Additionally, 4% LPC treated animals were given NOS inhibitors or NO donor. Baseline measurements for the behavioral tests were performed 1 day before LPC application. Five days after 4% LPC treatment, rats displayed mechanical and thermal hypersensitivity, and were further intraperitoneally injected with saline, DMSO, L-NAME (Sigma; 200 mg/kg), 7-NI (Sigma; 50 mg/kg), or SNAP (Sigma; 3 mg/kg) (each group, n = 5). L-NAME was dissolved in physiological saline; 7-NI and SNAP were dissolved in dimethyl sulfoxide (DMSO). The behavioral tests were performed 30 min before and after drug application and continued every 30 min thereafter. All behavioral measurements were obtained by an investigator blind to the treatment groups. At 1 week after 4% LPC treatment, a second dose of saline, DMSO, 200 mg/kg L-NAME (Wang et al., 2012), 50 mg/kg 7-NI (Thippeswamy et al., 2007), or 3 mg/kg SNAP (Naik et al., 2006) was administered 30 min prior to the electrical stimulation of the right LPC-treated median nerve and the rats were sacrificed 2 h after electrical stimulation.

2.4. Electrical stimulation

The right median nerve was isolated upstream of the injection site at the level of brachial plexus under anesthesia. Bipolar silver hook electrodes were placed under the isolated median nerve. The area around the exposed median nerve was covered with paraffin oil to prevent nerves drying out. Using an electrical stimulator (Grass, Quincy, MA, USA) through a constant-current unit (Grass CCU1A), a 10-min pulse train of electrical stimulation with duration of 0.1 ms, frequency of 10Hz, and intensity of 0.1 mA was performed on the median nerve (Day et al., 2001; Tsai et al., 2009). Two hours after electrical stimulation, the animals were sacrificed and processed for c-Fos immunohistochemistry.

2.5. Tissue preparation and morphological investigation

The rats were perfused with 4% (w/v) paraformaldehyde in 0.1 M phosphate buffer (PB) (pH 7.4) under anesthesia. For nerve morphological investigation, 3-mm segment of the median nerve was taken at 6 mm proximal to the site of LPC injection. Isolated median nerve segments were post-fixed with a mixture of 2.5% glutaraldehyde and 4% paraformaldehyde in 0.1 M PB overnight. The nerves were immersed with 1% osmium tetroxide in 0.1 M PB for 90 min, dehydrated in a graded alcohol series, and embedded in Epon 812 (Polyscience, Philadelphia, PA). Semithin sections were cut with an ultra-microtome (Reichert Ultracut E; Leica, Wetlzer, Germany), stained with alkaline toluidine blue, and examined under light microscopy.

The medulla tissues containing middle region, defined as the region 0.3–0.7 mm caudal to the obex (Maslany et al., 1991; Day et al., 2001; Lue et al., 2002; Tsai et al., 2004), of CN were dissected out, stored in 30% (w/v) sucrose in PB, and cut transversely at 30 μm thickness with a cryostat (Leica) for c-Fos immunohistochemistry. The C6 DRGs, predominantly segmental distribution of the median nerve (Tsai et al., 2007), were also removed and post-fixed in the Bouin's fixative and then embedded in paraffin wax. Serial sections were cut at 7 μm thickness and mounted onto 3-aminopropyltriethoxysilane (Sigma)-coated slides. Slides containing sections were deparaffinized with xylene and rehydrated through decreasing concentrations of alcohol ending in distilled water. Immunostaining was performed following antigen retrieval by heating the slides in 0.01 M citrate buffer (pH 6) in a Milestone RHS-1 vacuum microwave (Milestone, Bergamo, Italy).

These medulla and DRG sections were collected and treated with 0.5% (v/v) H_2O_2 in PB for 30 min, washed and blocked with 5% (v/v) normal goat serum (GibcoBRL, Auckland, New Zealand) in PB, and