

## RAPID REPORT

# LYSOPHOSPHATIDYLCHOLINE INDUCES NEUROPATHIC PAIN THROUGH AN ACTION OF AUTOTAXIN TO GENERATE LYSOPHOSPHATIDIC ACID

M. INOUE,<sup>a</sup> W. XIE,<sup>a</sup> Y. MATSUSHITA,<sup>a</sup> J. CHUN,<sup>b</sup> J. AOKI<sup>c</sup> AND H. UEDA<sup>a\*</sup>

<sup>a</sup>Division of Molecular Pharmacology and Neuroscience, Nagasaki University, Graduate School of Biomedical Sciences, 1-14 Bunkyo-machi, Nagasaki, 852-8521, Japan

<sup>b</sup>The Scripps Research Institute, 10550 North Torrey Pines Road, ICND118, La Jolla, CA 92037, USA

<sup>c</sup>Department of Microbiology and Immunology, Tohoku University Graduate School of Medicine, 2-1, Seiryomachi, Aoba, Sendai 980-8575, Japan

**Abstract**—Lysophosphatidic acid receptor (LPA<sub>1</sub>) signaling initiates neuropathic pain and several pathological events in a partial sciatic nerve injury model. Recently, we reported that lysophosphatidic acid (LPA) induces neuropathic pain as well as demyelination and pain-related protein expression changes via LPA<sub>1</sub> receptor signaling. Lysophosphatidylcholine (LPC), also known as lysolecithin, which is hydrolyzed by autotaxin/ATX into LPA, induces similar plastic changes. Here, we attempted to clarify whether ATX and LPA<sub>1</sub> receptor signaling is involved in the LPC-induced neuropathic pain. In wild-type mice, a single intrathecal (i.t.) injection of LPC induced mechanical allodynia and thermal hyperalgesia 2 days after injection; this persisted for 7 days at least. On the other hand, LPC-induced mechanical allodynia and thermal hyperalgesia were completely abolished in mice lacking an LPA<sub>1</sub> receptor gene. Furthermore, the LPC-induced response was also significantly, but partially reduced in heterozygous mutant mice for the ATX gene. These findings suggest that intrathecally-injected LPC is converted to LPA by ATX, and this LPA activates the LPA<sub>1</sub> receptor to initiate neuropathic pain. © 2008 IBRO. Published by Elsevier Ltd. All rights reserved.

**Key words:** LPC, LPA, LPA<sub>1</sub> receptor, autotaxin, neuropathic pain, biosynthesis.

Lysophosphatidylcholine (LPC, lysolecithin) is an important cell signaling molecule among lysophospholipids (Meyer zu Heringdorf and Jakobs, 2007). It is a major plasma lipid component that transports fatty acids and choline to tissues, and is produced under physiological and pathological conditions (Yokota and Hansson, 1995; Murgesan and Fox, 1996). Recent reports suggest that LPC

modulates pain signaling. LPC treatment of the saphenous or sciatic nerve-induced neuropathic pain, such as mechanical allodynia and thermal hyperalgesia, as well as demyelination and up-regulation of pain-related proteins in the dorsal root ganglion (DRG) (Wallace et al., 2003). However, the molecular mechanisms underlying LPC-induced neuropathic pain-like behaviors remain to be determined. More recently, we reported that intrathecal (i.t.) injection of lysophosphatidic acid (LPA) induces mechanical allodynia and thermal hyperalgesia as well as demyelination of dorsal root and upregulation of pain-related proteins in the DRG and spinal cord via the LPA<sub>1</sub> receptor (Inoue et al., 2004). Because autotaxin (ATX), which is responsible for the conversion of LPC to LPA (Shen et al., 1998; Eder et al., 2000; Aoki, 2004; van Meeteren and Moolenaar, 2007), is abundantly expressed in central and peripheral nerve tissues, especially in cerebrospinal fluids (Tanaka et al., 2004; Sato et al., 2005; Tanaka et al., 2006), we hypothesized that ATX and LPA<sub>1</sub> receptor signaling are involved in LPC-induced neuropathic pain-like behaviors.

## EXPERIMENTAL PROCEDURES

### Animals

Male mutant mice for the *lpa<sub>1</sub>* gene (*lpa<sub>1</sub><sup>-/-</sup>*) (Contos et al., 2000), heterozygous mutant mice for *autotaxin* gene (*atx<sup>+/-</sup>*) (Tanaka et al., 2006) and their sibling wild-type mice from the same genetic background, weighing 20–24 g, were used. They were kept in a room maintained at 21±2 °C with free access to standard laboratory diet and tap water. The experiments were designed to minimize the number of animals used and their suffering. Procedures were approved by Nagasaki University Animal Care Committee and were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animal.

### Drug injection

LPC containing primary palmitic, stearic and oleic acids was purchased from Sigma-Aldrich (St. Louis, MO, USA). The i.t. injection was performed according to the method of Hylden and Wilcox (1980).

### Nociceptive tests

In thermal paw withdrawal tests, nociception was measured as the latency to paw withdrawal evoked by exposure to a thermal stimulus, as described previously (Rashid et al., 2003; Inoue et al., 2004). Unanesthetized animals were placed in Plexiglas cages on

\*Corresponding author. Tel: +81-95-819-2421; fax: +81-95-819-2420. E-mail address: ueda@nagasaki-u.ac.jp (H. Ueda).

Abbreviation: ATX, autotaxin; *atx<sup>+/-</sup>*, heterozygote mutant mice for *autotaxin* gene; DRG, dorsal root ganglion; i.t., intrathecal; LPA, lysophosphatidic acid; *lpa<sub>1</sub><sup>-/-</sup>*, mutant mice for *lpa<sub>1</sub>* gene; LPC, lysophosphatidylcholine; lyso-PLD, lysophospholipase D.

top of a glass sheet, and an adaptation period of 1 h was allowed. A thermal stimulator (IITC Inc., Woodland Hills, CA, USA) was positioned under the glass sheet and the focus of the projection bulb was aimed exactly at the middle of the plantar surface of the animal. A mirror attached to the stimulator permitted visualization of the plantar surface. A cutoff time of 20 s was set in order to prevent tissue damage. The paw pressure test was performed as described previously. Briefly, mice were placed into a Plexiglas chamber on a 6×6 mm wire mesh grid floor, and were allowed to acclimatize for a period of 1 h. A mechanical stimulus was then delivered to the middle of the plantar surface of the right hind-paw using a Transducer Indicator (Model 1601, IITC Inc., Woodland Hills, CA, USA). The pressure needed to induce a flexor response was defined as the pain threshold. All behavioral experiments were carried out by investigators blinded to the drug-treatment.

### Statistical analyses

Statistical analyses were performed using Student's *t*-test. Significance was set to  $P < 0.05$ , # 0.05. All results are expressed as means ± S.E.M.

## RESULTS

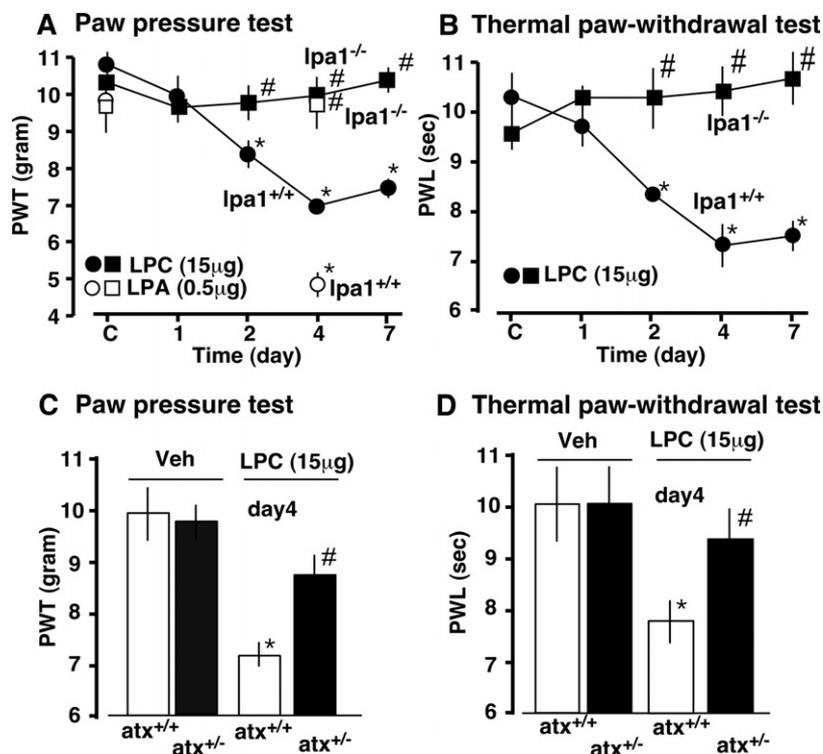
I.t. injection of LPA at 0.5  $\mu\text{g}$  reduced the mechanical nociceptive threshold on day 4 after treatment in wild-type ( $lpa_1^{+/+}$ ) mice (Fig. 1A), consistent with previous data (Inoue et al., 2004). As shown in Fig. 1A, LPA-induced mechanical allodynia was completely abolished in knockout mice ( $lpa_1^{-/-}$ ), which showed the equivalent basal threshold to  $lpa_1^{+/+}$  mice. Similarly, wild-type mice that were given a single i.t. injection of LPC at 15  $\mu\text{g}$  also

showed significant, but slightly weaker mechanical allodynia on days 2–7 (Fig. 1A). However, a higher dose of LPC (50  $\mu\text{g}$  i.t.) caused abnormal behaviors or death (data not shown). On the other hand, LPC-induced mechanical allodynia was completely abolished in  $lpa_1^{-/-}$  mice (Fig. 1A). Similarly, a single i.t. injection of LPC also produced thermal hyperalgesia (Fig. 1B), and this was completely abolished in  $lpa_1^{-/-}$  mice, which showed equivalent basal threshold to  $lpa_1^{+/+}$  mice in thermal nociceptive tests.

Next, we examined the involvement of ATX in LPC-induced neuropathic pain. We used  $atx^{+/-}$  heterozygous mutant mice, since it has been reported that homozygous mutant mice die during the early stage of embryogenesis (Tanaka et al., 2006). Similar to the case with  $lpa_1^{-/-}$  mice, there were no differences in mechanical and thermal nociceptive thresholds between wild-type ( $atx^{+/+}$ ) and heterozygous mutant ( $atx^{+/-}$ ) mice with vehicle-treatment. LPC-induced mechanical allodynia and thermal hyperalgesia on day 4 were significantly, but not completely attenuated in  $atx^{+/-}$  mice (Fig. 1C, 1D).

## DISCUSSION

In the present study we found that LPC (i.t.)-induced neuropathic pain-like behaviors are mediated by  $LPA_1$  receptor signaling, since they were completely abolished in  $lpa_1^{-/-}$  mice. Furthermore, we also found that  $atx^{+/-}$  heterozygous mutant mice, which express 50% of the ATX levels and lysophospholipase D (lyso-PLD) activity of wild-



**Fig. 1.** LPC-induced mechanical allodynia and thermal hyperalgesia mediated by the  $LPA_1$  receptor and ATX. (A, B) Complete blockade of LPC (15  $\mu\text{g}$ ) or LPA (0.5  $\mu\text{g}$ )-induced mechanical allodynia responses (A) and thermal hyperalgesia (B) in  $lpa_1^{-/-}$  mice. (C, D) Partial blockade of LPC (15  $\mu\text{g}$ )-induced mechanical allodynia (C) and thermal hyperalgesia (D) in  $atx^{+/-}$  mice. All data represent the mean ± S.E.M. from five to six separate experiments. \*  $< 0.05$  Compared with vehicle treatment, #  $< 0.05$  compared with wild-type mice.

type mice (Tanaka et al., 2006), show a partial attenuation of LPC-induced actions, as seen by an assessment of mechanical allodynia and thermal hyperalgesia. LPC delivered to these animals is converted to LPA by the lysophospholipase activity of ATX in the cerebrospinal fluid (Sato et al., 2005), but at a reduced rate and level. The synthesized LPA can activate the LPA<sub>1</sub> receptor to initiate the neuropathic pain phenotype. However, as shown in Fig. 1A, LPA at 0.5 μg induced more intense allodynia and hyperalgesia than LPC at 15 μg, suggesting that only a small percentage of the injected LPC was converted to LPA by ATX.

Here a question is raised where LPC comes from in pathological conditions. The LPC level in plasma ranges from 150 to 500 μM depending on the mammalian species. By contrast, the LPC level in cerebrospinal fluid is much lower. The LPC concentration in mouse cerebrospinal fluid is 5 μM (Sakagami et al., unpublished observations). Hemorrhage is often associated with nerve injury. Thus, it is reasonable to assume that LPC is derived from plasma upon nerve injury. It is also well accepted that upon injury or in inflammation sites, LPC-generating enzymes such as phospholipase A<sub>2</sub> are induced and activated (Farooqui et al., 2006). Such phospholipase A<sub>2</sub> may also be responsible for the production of LPC.

LPC is widely used as a demyelinating agent (Jeffery and Blakemore, 1995; Ousman and David, 2001; Kotter et al., 2005); it also induces mechanical allodynia and thermal hyperalgesia (Wallace et al., 2003). On the other hand, abnormal sensory phenomena, including hyperalgesia and allodynia, are associated with human peripheral demyelinating neuropathies such as Charcot-Marie-Tooth disease and Guillain-Barré syndrome (Carter et al., 1998; Boerkoel et al., 2001). The present report provides an important finding that at least some of LPC's effects might be related to its conversion to LPA, which could cause neuropathic pain-like phenotypes. However, it remains to be determined whether LPC also causes demyelination or altered expression of key molecules underlying neuropathic pain mechanisms mediated by the LPA<sub>1</sub> receptor.

*Acknowledgments*—This work was supported by MEXT KAKENHI (17109015 to H.U.; 18689010 to M.I.) and NIH grant NS048478 to J.C.

## REFERENCES

- Aoki J (2004) Mechanisms of lysophosphatidic acid production. *Semin Cell Dev Biol* 15:477–489.
- Boerkoel CF, Takashima H, Stankiewicz P, Garcia CA, Leber SM, Rhee-Morris L, Lupski JR (2001) Periaxin mutations cause recessive Dejerine-Sottas neuropathy. *Am J Hum Genet* 68:325–333.
- Carter GT, Jensen MP, Galer BS, Kraft GH, Crabtree LD, Beardsley RM, Abresch RT, Bird TD (1998) Neuropathic pain in Charcot-Marie-Tooth disease. *Arch Phys Med Rehabil* 79:1560–1564.
- Contos JJ, Fukushima N, Weiner JA, Kaushal D, Chun J (2000) Requirement for the lpA1 lysophosphatidic acid receptor gene in normal suckling behavior. *Proc Natl Acad Sci U S A* 97:13384–13389.
- Eder AM, Sasagawa T, Mao M, Aoki J, Mills GB (2000) Constitutive and lysophosphatidic acid (LPA)-induced LPA production: role of phospholipase D and phospholipase A2. *Clin Cancer Res* 6:2482–2491.
- Farooqui AA, Ong WY, Horrocks LA (2006) Inhibitors of brain phospholipase A2 activity: their neuropharmacological effects and therapeutic importance for the treatment of neurologic disorders. *Pharmacol Rev* 58:591–620.
- Hylden JL, Wilcox GL (1980) Intrathecal morphine in mice: a new technique. *Eur J Pharmacol* 67:313–316.
- Inoue M, Rashid MH, Fujita R, Contos JJ, Chun J, Ueda H (2004) Initiation of neuropathic pain requires lysophosphatidic acid receptor signaling. *Nat Med* 10:712–718.
- Jeffery ND, Blakemore WF (1995) Remyelination of mouse spinal cord axons demyelinated by local injection of lysolecithin. *J Neurocytol* 24:775–781.
- Kotter MR, Zhao C, van Rooijen N, Franklin RJ (2005) Macrophage-depletion induced impairment of experimental CNS remyelination is associated with a reduced oligodendrocyte progenitor cell response and altered growth factor expression. *Neurobiol Dis* 18:166–175.
- Meyer zu Heringdorf D, Jakobs KH (2007) Lysophospholipid receptors: signalling, pharmacology and regulation by lysophospholipid metabolism. *Biochim Biophys Acta* 1768:923–940.
- Murugesan G, Fox PL (1996) Role of lysophosphatidylcholine in the inhibition of endothelial cell motility by oxidized low density lipoprotein. *J Clin Invest* 97:2736–2744.
- Ousman SS, David S (2001) MIP-1α, MCP-1, GM-CSF, and TNF-α control the immune cell response that mediates rapid phagocytosis of myelin from the adult mouse spinal cord. *J Neurosci* 21:4649–4656.
- Rashid MH, Inoue M, Kondo S, Kawashima T, Bakoshi S, Ueda H (2003) Novel expression of vanilloid receptor 1 on capsaicin-insensitive fibers accounts for the analgesic effect of capsaicin cream in neuropathic pain. *J Pharmacol Exp Ther* 304:940–948.
- Sato K, Malchinkhuu E, Muraki T, Ishikawa K, Hayashi K, Tosaka M, Mochiduki A, Inoue K, Tomura H, Mogi C, Nochi H, Tamoto K, Okajima F (2005) Identification of autotaxin as a neurite retraction-inducing factor of PC12 cells in cerebrospinal fluid and its possible sources. *J Neurochem* 92:904–914.
- Shen Z, Belinson J, Morton RE, Xu Y, Xu Y (1998) Phorbol 12-myristate 13-acetate stimulates lysophosphatidic acid secretion from ovarian and cervical cancer cells but not from breast or leukemia cells. *Gynecol Oncol* 71:364–368.
- Tanaka M, Kishi Y, Takanezawa Y, Takehi Y, Aoki J, Arai H (2004) Prostatic acid phosphatase degrades lysophosphatidic acid in seminal plasma. *FEBS Lett* 571:197–204.
- Tanaka M, Okudaira S, Kishi Y, Ohkawa R, Iseki S, Ota M, Noji S, Yatomi Y, Aoki J, Arai H (2006) Autotaxin stabilizes blood vessels and is required for embryonic vasculature by producing lysophosphatidic acid. *J Biol Chem* 281:25822–25830.
- van Meeteren LA, Moolenaar WH (2007) Regulation and biological activities of the autotaxin-LPA axis. *Prog Lipid Res* 46:145–160.
- Wallace VC, Cottrell DF, Brophy PJ, Fleetwood-Walker SM (2003) Focal lysolecithin-induced demyelination of peripheral afferents results in neuropathic pain behavior that is attenuated by cannabinoids. *J Neurosci* 23:3221–3233.
- Yokota T, Hansson GK (1995) Immunological mechanisms in atherosclerosis. *J Intern Med* 238:479–489.