Lysophosphatidic Acid as the Initiator of Neuropathic Pain

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The injury-induced intense stimulation of spinal cord neurons causes lysophosphatidic acid (LPA) biosynthesis. LPA receptors activate Cav1 in dorsal root ganglion and PKCγ in the dorsal horn, underlying mechanisms for characteristic neuropathic hyperalgesia in myelinated sensory (A-type) fibers. On the other hand, the LPA3 receptor mediates microglia activation at the early stage after nerve injury and LPA-induced LPA biosynthesis. Thus, both the LPA1 and LPA3 receptors play key roles in the initiation step using a feed-forward system for neuropathic pain.

Key words lysophosphatidic acid; demyelination; feed-forward system; neuropathic pain; microglia

1. INTRODUCTION

Chronic pain should be considered disease pain, though acute or nociceptive pain is sometimes viewed as physiological pain because of its bio-alarming roles. Neuropathic pain is a representative chronic pain, and is caused by damage to peripheral or central neurons in the pain pathway.1—4 This type of chronic pain commonly occurs as a secondary symptom in diseases such as diabetes, cancer, and herpes zoster infection, or as a side effect of chemotherapeutic treatments.5—8 Neuropathic pain following partial sciatic nerve injury is often characterized by abnormally hypersensitive sensory perception through A-fibers, called hyperalgesia or allodynia, in which innocuous (tactile) stimuli cause intense pain.9,10 The abnormal sensory perception also includes C-fiber hypoesthesia,10—12 which indicates sensory loss.13,14 A number of studies10,12,15 demonstrate the altered expression of receptors, neuropeptides and ion-channels in sensory fiber neurons, called dorsal root ganglion (DRG) and in the dorsal horn. According to current studies, on the other hand, the sustained activation of non-neuronal cells such as astrocytes and microglia becomes manifest in the mechanisms underlying neuropathic pain.9,11 In addition to these mechanisms, the demyelination is supposedly related to chronic pain diseases, and to the physical cross-talk/sprouting and ectopic discharge in sensory fibers, all which may underlie neuropathic hyperalgesia and allodynia.10,12,16 As neuropathic pain is in general resistant to non-steroidal anti-inflammatory drugs (NSAIDs) or morphine, it has been called intractable pain, though a limited number of medicines are currently available or in progress of development in clinics.17 Accumulating findings reveal the important insights into both the basic biology and biomedical importance of signaling initiated by lipid mediator lysophosphatidic acid (1-acyl 2-hydroxyl glycerol 3-phosphate, LPA), LPA, a simple lipid with glycerol, fatty acid and phosphate in its structure. LPA plays roles in many cellular processes including cellular proliferation, cell migration, prevention of apoptosis, cytokine and chemokine secretion and smooth muscle contraction.18—23 LPA receptors activate multiple signaling pathways and multiple G-proteins,22,23 but LPA1 signaling is unique, since it has a downstream coupling to Go12/13, as well as G13 and G11. Regarding neurobiological actions, LPA causes a growth cone collapse on neurons through its receptor LPA1 and downstream Go12/13-RhoA-Rho kinase (ROCK) system,24,25 and plays crucial roles in neuronal developmental processes, including neurogenesis, neuronal migration, neuritogenesis and myelination.26,27 In terms of pain regulation, we first reported that LPA causes an activation of peripheral nociceptor endings directly and indirectly through a release of histamine from peripheral cells, such as mast cells.28,29 Current studies have revealed that LPA plays a key role in the initiation of neuropathic pain.10,12,30 As the intrathecal LPA-induced abnormal pain shows quite similar characteristics to those in nerve injury-induced neuropathic pain,30 LPA could be considered as a good tool for the studies of in vitro and in vivo mechanisms underlying neuropathic pain.31—37

2. LPA1-MEDIATED INITIATION OF NEUROPATHIC PAIN

Among multiple mechanisms involved in the manifestation of nerve injury-induced neuropathic pain, the enhanced expression of Cav1,2δ1 expression in DRG, PKCγ expression and microglial activation in dorsal horn seem to be representative mechanisms for hyperalgesia, while the demyelination and following physical cross-talk among sensory fibers may underlie the mechanisms for allodynia. We were the first to find that nerve injury-induced neuropathic pain behaviors were substantially abolished in Lpa1−/− mice.30 As there was no significant change in the basal nociceptive threshold, it is evident that endogenous levels of LPA do not affect this threshold and that newly produced LPA following nerve injury causes neuropathic pain mechanisms. In order to study the critical time period for LPA1 receptor-mediated sig-
naling underlying neuropathic pain and its mechanisms, Ki-16425, a short-lived antagonist of LPA₁ receptor was used. The Ki-16425 blockade of nerve injury-induced neuropathic pain and upregulation ofCaₐ₂δ₁ expression was maximal as late as 3 h after the injury but not after this critical period. These results suggest that LPA₁ signaling, which underlies the development of neuropathic pain, works at an early stage of the critical period after nerve injury.

3. LPA₁-MEDIATED UPREGULATION OF KEY MOLECULES UNDERLYING HYPERALGESIA

Upregulation of Caₐ₂δ₁ expression and subsequent increased pain transmission may underlie the mechanism for hyperalgesia. Gabapentin and pregabalin, which are widely used in clinics for neuropathic pain, are known to inhibit the pain transmission by inhibiting this subunit Caₐ₂δ activity. The expression of Caₐ₂δ₁ is observed only in the small C-fiber neurons of DRG in naïve animals. After partial injury of the sciatic nerve, most of the A-fiber neurons also express this subunit. Pretreatment with Clostridium botulinum C toxin (BoNT/C3), an inhibitor of RhoA, abolished this additional expression in A-fiber neurons. Quite similar results of additional Caₐ₂δ₁ expression in these neurons and its blockade by BoNT/C3 were observed when LPA was intrathecally injected only once. On the other hand, the nerve injury- or LPA-induced up-regulation of Caₐ₂δ₁ was abolished in neuropathy mice.

N-Methyl-D-aspartate (NMDA) receptor plays key roles in the transmission of pain in naïve and chronic pain status. Recent studies reported that NMDA receptor is transactivated through EphB signaling initiated by interaction with presynaptic Ephrin B1. When the profiling of LPA-induced and BoNT/C3 reversible genes in DRG was performed, ephrin B1 was found in 82 unique genes. Further characterization revealed that antisense oligonucleotide for ephrin B1 largely abolished the LPA-induced mechanical alldynia, thermal hyperalgesia and hypersensitivity to electrical stimuli through Aδ and Aβ-fibers. As Ephrin B1-Fc caused neuropathic pain-like behaviors in an NMDA receptor antagonist MK-801-reversible manner, LPA-mediated Ephrin B1 upregulation may also contribute to the mechanisms underlying neuropathic hyperalgesia.

As seen in the case with nerve injury, intrathecal injection of LPA causes an up-regulation of PKCγ at the substantia gelatinosa of spinal dorsal horn. This change is known as so-called wind-up facilitation or hyperalgesia observed in neuropathic pain. The up-regulation by nerve injury or LPA was also abolished by BoNT/C3 pretreatment and in neuropathy mice.

4. LPA₁-MEDIATED DEMYELINATION UNDERLYING ALLODYNIA

It is known that many demyelinating diseases accompany chronic pain, as seen in the cases with Guillain–Barre syndrome and multiple sclerosis. Demyelination and subsequent physical cross-talk and ectopic discharges due to accumulation of sodium channels have been speculated as the mechanisms underlying neuropathic pain. Nerve injury- and intrathecal LPA₁-induced demyelination of dorsal root fibers through LPA₁ receptor activation are evidenced by the down-regulation of myelin proteins, such as myelin basic protein (MBP), myelin protein zero (P0) and myelin-associated glycoprotein (MAG). The ex vivo studies using dorsal root fibers also demonstrated that the addition of LPA causes demyelination within 24 h in scanning and transmission electron microscopy (SEM and TEM) analyses. As well as typical demyelination of A-fibers, there was direct contact between neighboring C-fibers. In co-culture experiments using myelinated fibers built up with isolated DRG neurons and Schwann cells, the addition of LPA also causes a sequential morphological change, in an order of collapse of growth cone at 1 h, sprouting at the nerve endings at 8 h and axon at 18 h and complete spreading of myelinated Schwann cell at 36 h. As the down-regulation of myelin proteins was abolished by the pretreatment with BoNT/C3 or ROCK inhibitor Y-27632, the major pathway is presumably mediated by the LPA₁-G₁₂/₁₃-RhoA-ROCK system. Indeed, the LPA-induced down-regulation of myelin protein genes in vivo and ex vivo studies using dorsal root fibers was abolished by Y-27632. Most recently, there is a report that calpain plays a negative regulator role in the myelin protein gene expression. As the RhoA-ROCK system is reported to stimulate c-jun expression through JNK activation, it is speculated that the sequential activation of LPA₁-G₁₂/₁₃-RhoA-ROCK-JNK, followed by up-regulation of c-jun, leads to a negative regulation of myelin protein gene expression. In terms of signal transduction, it is also known that LPA₁ causes the stress-fiber formation and actin rearrangement through G₁₂/₁₃-RhoA-ROCK activation. Thus, such LPA₁-mediated morphological changes may contribute to rapid mechanisms underlying demyelination without any changes in myelin protein levels.

However, current studies demonstrated a different mechanism independent of the G₁₂/₁₃-RhoA-ROCK system. Intrathecal injection of LPA causes a rapid down-regulation of myelin-associated glycoprotein (MAG). By surveying protease inhibitors, the calcium-activated neutral serine protease, calpain was found to play a major role in the down-regulation of MAG. Pretreatment with calpain inhibitors abolished the MAG down-regulation and significantly attenuated the LPA-induced neuropathic pain-like behaviors. Interestingly, calpain activation in dorsal root was only observed by nerve injury and abolished in neuropathy mice, while it was not observed by the pretreatment with inflammatogenic Complete Freund Adjuvant (CFA). Furthermore, calpain inhibitors reversed the nerve injury-induced neuropathic pain, but not CFA-induced pain. Although details remain elusive, it is suggested that the LPA₁-G₁₂/₁₃-PLC activation system may play a role in the calcium-mediated protein degradation. Thus, several cellular mechanisms following LPA₁ stimulation may contribute to the demyelination.

In relation to allodynia, the loss of innervation of sensory fibers following LPA₁-mediated demyelination may cause a physical cross-talk (or electric synapse/ephapse) among innocuous Aβ fibers and nocuous Aδ-fibers, which in turn leads to an abnormal pain transmission allodynia. The down regulation of MAG may also cause the sprouting, which is induced by a loss of negative signal through the NOGO/p75 receptor complex, as seen in Fig. 1.
The stimulation of LP A, receptor on myelinated Schwann cells causes rapid down-regulation of myelin proteins through calpain-mediated down regulation and gene silencing. The downregulation of compact myelin proteins including myelin binding protein (MBP) and myelin protein zero (P0) leads to a loosening of the myelin sheath. The loss of another myelin protein, myelin associated glycoprotein (MAG), which couples to NOGO/p75 complex (NgR/p75) and RhoA-ROCK system, results in disinhibition of sprouting, possibly through rearrangements of actin and tubulin polymers.

5. LPA\textsubscript{1}-MEDIATED SYNAPTIC REORGANIZATION IN PAIN PATHWAY

Extracellular signal-regulated kinase \(1/2 \) (ERK\(_{1/2}\)), representing one of the major subfamilies of mitogen-activated protein kinases (MAPKs), is phosphorylated following membrane depolarization and Ca\(^{2+}\) influx.\(^{53}\) It is known that ERK\(_{1/2}\) is immediately activated after noxious stimulation in DRG neurons and spinal dorsal horn in a stimulus intensity-dependent manner.\(^{54,55}\) Therefore, ERK phosphorylation (pERK) could be a biochemical marker of activated neurons, allowing us to visualize the pain-signaling pathways and more objective evidence of neurotransmission. A significant number of neurons at the superficial layer of spinal dorsal horn became pERK-positive following the stimulation of nociceptive C- and A\(\delta\)-fibers by use of Neurometer\(^a\), while no neuron became pERK-positive by innocuous A\(\beta\) stimulation.\(^{56}\) However, following sciatic nerve injury, A\(\beta\)-stimulation showed a significant number of pERK-positive neurons at the superficial layer of dorsal horn, where the innervation with noxious C- or A\(\delta\)-fibers is observed. This mechanism seems to explain why the tactile stimuli (through A\(\beta\)) cause intense pain. Such nerve injury-induced synaptic reorganization was also abolished in \(\text{lpaa}^-^-\) mice.\(^{57}\)

6. LPA\textsubscript{3}-MEDIATED LPA BIOSYNTHESIS

The LPA\textsubscript{3}-mediated demyelination following partial injury of sciatic nerve was only observed in dorsal root, but not spinal nerve or sciatic nerve.\(^{35}\) Such dorsal root-specificity was also observed in the down-regulation of myelin-associated glycoprotein (MAG), which plays a key role in the regulation of axonal sprouting.\(^{12}\) As the addition of LPA causes the demyelination or down-regulation of MAG in all these sensory nerve regions, the dorsal root specificity following sciatic nerve injury seems attributable to the localized LPA production. The most probable source of LPA would be from spinal cord, since the dorsal root as well as spinal cord is within the subarachnoid.

Recent studies demonstrated that the intense stimulation of spinal cord neurons causes synthesis of lysophosphatidyl choline (LPC), which is in turn converted to LPA by lysophospholipase D or autotaxin (ATX).\(^{21,58}\) In these experiments, the combination of SP and NMDA, both of which cause an activation of representative target receptors for different types of primary afferent neurons, produces LPC.\(^{58}\) As no significant synthesis of LPC occurs by single application of either compound, it is presumed to require an intense signal caused by nerve injury, but not by regular pain transmission. Recent studies revealed that phosphatidyl choline is converted to LPC by cPLA\(_2\) or iPLA\(_2\), both of which are regulated by Ca\(^{2+}\)-related mechanisms following NK\(_1\) and NMDA receptor activation. Thus produced LPC in the spinal cord is converted to LPA at the spinal cord and dorsal root by an action of ATX leading to demyelination and \(\text{lpaa}^-^-\) upregulation.\(^{30}\)

Current studies demonstrated that the intrathecal injection of LPC caused neuropathic pain-like behaviors, and these behaviors were abolished in \(\text{lpaa}^-^-\) mice or diminished by 50% in ATX\(^^{-/-}\) mice.\(^{34}\) The study to examine the biochemical evidence for this LPC-induced LPA production revealed that the amounts of LPA were much higher than that expected from the simple conversion through ATX.\(^{53}\) Detailed \textit{ex vivo} culture studies using spinal cord slices revealed that LPA-induced amplified production of LPA was abolished in the preparation derived from \(\text{lpaa}^-^-\) mice. This observation was supported by the behavioral studies, in which nerve injury-induced neuropathic pain was abolished in \(\text{lpaa}^-^-\) as well as \(\text{lpaa}^-^-\) mice. Thus, it is suggested that LPA\(_1\) receptor plays direct roles in molecular machineries underlying neuropathic pain, while LPA\(_3\) receptor and ATX play roles in the synthesis of LPA.

7. MICROGLIA-MEDIATED AMPLIFICATION OF LPA BIOSYNTHESIS

It is now considered that microglia and astrocytes as well as neurons have functional roles in the creation and maintenance of chronic neuropathic pain. Spinal cord glial activation seems to be a common underlying mechanism that leads to chronic pain.\(^{59,60}\) However, it remains to be learned what signal initiates the glial activation, which is assumed to play a key role in the maintenance of neuropathic pain. An attempt to see the effects of LPA in activating microglia revealed that LPA caused an increase in the expression of brain-derived neurotrophic factor (BDNF) in a primary culture of rat microglia, which express LPA\(_3\), but not LPA\(_1\) or LPA\(_2\) receptors.\(^{61}\) These actions were mediated by a release of ATP through activation of LPA\(_3\), G\(_{\alpha/11}\) and phospholipase C. The released ATP or ectopically converted ADP may in turn cause membrane ruffling (a sign of chemotaxis) via P2Y\(_{12}\) receptors and G\(_{\alpha/11}\) activation, and BDNF expression via activation of P2X\(_4\) receptors. Current studies using the microglia inhibitor minocycline revealed that LPA-induced microglia activation functions in the early stage development, but not in the late stage maintenance of neuropathic pain.\(^{62}\) In this study, the early treatment with minocycline abolished...
the LPA-induced and nerve injury-induced neuropathic pain, LPA synthesis and its underlying activation of synthetic enzymes, cytosolic phospholipase A$_2$ (cPLA$_2$) and calcium-independent PLA$_2$ (iPLA$_2$). As the post-treatment with minocycline failed to attenuate the established neuropathic pain, microglial activation following LPA$_2$ signaling seems to take part in the initiation mechanisms for neuropathic pain.

8. CONCLUSION

In the proposed working hypothesis, the LPA-mediated feed-forward system underlying molecular mechanisms for neuropathic pain, the LPA production following intense and mixed pain signals to spinal dorsal horn neurons is the initial mechanism (Fig. 2). Thus produced LPA has two mechanisms: one is related to the actions as an amplifying signal for further LPA production through LPA$_2$ and microglial activation. The other mechanism is related to the LPA$_2$-mediated actions as a reverse signal to cause dorsal root demyelination, and upregulation of Ca$_a$, $\delta$, and Ephrin B$_1$ in DRG. Demyelination and subsequent sprouting may lead to a pathological pain synapse by A$\beta$-fibers, underlying allodynia. Upregulation of key molecules in DRG enhances the pain transmission and may cause subsequent upregulation of PKC$\gamma$ in the dorsal horn. Enhanced and pathological pain transmission may also contribute to a biosynthesis of LPA through direct and indirect mechanisms. When we consider the drug development to cure neuropathic pain, LPA receptor antagonists or inhibitors of LPA synthesis would be candidates. For this purpose, we need to examine whether this feed-forward system through LPA biosynthesis also occurs in the late phase of neuropathic pain. Assuming that the feed-forward system through LPA synthesis by intense pain signal (or injury) is true in the central nervous system, the hypothesis may be further extended to central pain induced by various kinds of stress, spinal (brain) injury or stroke.

REFERENCES