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Review

Lysophosphatidic acid: Chemical signature of neuropathic pain ☆

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ABSTRACT

Acute inflammatory pain signal originates from transient hypersensitivity in afferent fibers when depolarized via injured tissues or proinflammatory cells-derived pronociceptive ligand binding. This pain is sensitive to opioids and NSAIDs. In neuropathic pain, however, damage to the nerve along the pain pathway results in spontaneous generation of action potential and lowered nociceptive threshold, as seen in allodynia and hyperalgesia. This abnormal pain transmission had been linked to LPA production in the spinal cord, through activation of NMDA and NK1 activation by glutamate and SP in $iPLA_2$/$cPLA_2$/ATX-dependent pathway. In a bifurcated response involving $G_{q/11}$ and $G_{12/13}$ coupling, Schwann cell LPA_1 mediates degradation and transcriptional suppression of myelin proteins, respectively. The loss of contact inhibition on axonal growth creates cytoskeletal framework for axonal sprouting. LPA causes an amplification of LPA production through activation of LPA_3 signaling in microglia immediately after nerve injury. LPA_1 deficient mice ($LPA_1^{-/-}$) show no neuropathic-pain behavior or demyelination in response to intrathecal LPA injection or nerve injury. Given these bodies of research evidence, LPA therefore presents as the chemical signature for the initiation of neuropathic pain. This article is part of a Special Issue entitled Advances in Lysophospholipid Research.

1. Introduction

The initiation point of acute pain is the action potential generated in nociceptive afferent neurons by injured tissue and pro-inflammatory cells-derived ligands and chemical species [1]. The action potential later elicits excitatory postsynaptic potentials in the dorsal horn of spinal cord, which ultimately relays the signal to the thalamus and cerebral cortex through secondary synapse. Usually, this type of pain sensation is transient and abolished with the removal of the noxious signal by non-steroidal anti-inflammatory drugs (NSAIDs) or the opioid-induced suppression of primary pain signals by driving descending pain-inhibitory system through noradrenergic or serotonergic neurons [2]. In chronic pain paradigm, on the other hand, the damage to peripheral or central neurons in the pain pathway produces a contingent of near-irreversible abnormal sensory perception with resulting long-lasting pain hypersensitivity, which is clinically presented as opioid and NSAIDs-insensitive allodynia, hyperalgesia and hypoesthesia [3–8]. Neuropathic pain a well-documented form of chronic pain accounts for 17.9% of 116 million adults diagnosed with chronic pain in America alone [9,10]. Neuropathic pain is clinically recognized as symptomatic of underlying diseases such as diabetes, cancer, herpes zoster, multiple sclerosis and some other forms of demyelinating diseases [11–13]. Due to complex neurobiological and clinical presentations of neuropathic pain, it has become rather difficult to integrate the contributions of environmental [14], (epi)-genetic [15,16] and etiological factors [10] into one formidable model capable of explaining the plethora of neuro-biochemical events that constitute the initiation point, and how the resulting plasticity in the sensory neurons permanently lowers pain threshold to the point of spontaneity of pain signal transmission.

For over a decade, we have documented a repertoire of anatomical events in mice subjected to different types of neuropathic pain model. We have observed demyelination regardless of model-type. As the cluster of biochemical events involved in demyelination are being deconstructed, the unique role of lysophosphatidic acid (LPA) as the chemical signature in initiation of neuropathic pain through demyelination mechanisms is becoming clearer [7,8,17–19]. Further confirmations have been obtained through neuropathic-like behavior and focal demyelination in animals after intrathecal and intratrigeminal injections of LPA [20,21]. Global demyelination in dorsal root, spinal nerve and sciatic nerve (*ex vivo*) has been documented in LPA receptor-dependent manner [18,19]. New research evidence is now

Abbreviations: ATX, autotaxin; CaM, calmodulin; CCR-2, chemokine (C-C motif) receptor-2; CIF, calcium influx factor; DRG, dorsal root ganglia; ER, endoplasmic reticulum; LPA, lysophosphatidic acid; LPC, lysophosphatidyl choline; MAG, myelin-associated glycoprotein; MALDI-TOF-MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; MBP, myelin basic protein; MPZ, myelin protein zero; mTOR, mammalian target of rapamycin; NALDI-TOF-MS, nanostructure-assisted laser desorption/ionization time-of-flight mass spectrometry; NgR, Nogo-66 receptor; NRG, neuregulin; NSAIDs, non-steroidal anti-inflammatory drugs; NuRD, nucleosome remodeling and histone deacetylation; PIP_2, phosphatidylinositol-4,5-triphosphate; PIP_3, phosphatidylinositol-3,4,5-triphosphate; SC, Schwann cell; SP, substance P

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emerging through transcriptional profiling data indicating the possible role of LPA signaling in cell reprogramming. This data provides useful insight into the transcriptional regulation of genes involved CNS plasticity and central sensitization and the modulatory roles of LPA [8,22].

2. Lysophosphatidic acid: chemical signature in neuropathic pain initiation

2.1. LPA: intermediate turned bioactive ligand

Lysophosphatidic acid (1-acyl-glycerol-3-phosphate, LPA) previously recognized as a precursor of phospholipid biosynthesis in both eukaryotic and prokaryotic cells is now emerging as an intercellular signaling molecule [23–26]. Its landmark roles in cell survival, neurite retraction, cancer cell migration and invasion, fertilization, embryonic implantation, spermatogesis, vasculogenesis, angiogenesis, proliferation and differentiation of neural progenitor cells have earned LPA a reputation as a bioactive ligand [26–30]. These biological functions have been linked to specific receptor class such as endothelial differentiation gene-family of receptors including LPA_1 (endothelial differentiation gene − 2/EDG_2), LPA_2 receptor (EDG_4), LPA_3 receptor (EDG_7), purinergic class of receptors LPA_4 receptor (P_2Y_9), LPA_5 receptor (GPR_{92}) and LPA_6 receptor (P_2Y_5) and most recently, TRPV-1 [29,31]. These receptors have wide tissue distribution, and some of the classes have been well characterized in the peripheral nociceptive fibers [29,32,33].

Recently there is a paper that LPA_5 may be also involved in the injury-induced neuropathic pain as well as LPA_1 and LPA_3 in respective knockout mice [34]. In $LPA_5^{-/-}$ mice, the neuropathic pain partially attenuated, but demyelination was not affected [34]. Although the study proposed the involvement of pCREB signaling, details remain to be determined.

2.2. LPA: product of intense afferent fiber stimulation

In response to intense noxious stimulation causing neuropathic pain, glutamate and substance P (SP), the major excitatory neurotransmitters are released at the central terminals of the primary afferent nociceptive neurons and are supposed to simultaneously activate the same neuron. Largely, glutamate and SP activate ionotropic NMDA subtype receptor and metabotropic NK1 receptor at the dorsal horn respectively. These abnormal pain transmissions cause a production of LPA, which was evidenced by measurements of LPA in *ex vivo* studies using spinal cord slices and *in vivo* studies (unpublished data) combined with intrathecal injection of NMDA or NK1 receptor antagonists. *Ex vivo* reconstruction of the intense stimulation events using capsaicin (TRPV-1 agonist)-treated spinal cord slices also resulted in LPA production through activation of glutamate/NMDA and SP/NK1 receptors. In this pathway, $G_{q/11}$-coupling and activation of downstream PLC-β following intense activation of NK1 results in IP_3-mediated depletion of endoplasmic reticulum (ER) store of Ca^{2+} which in turn enhances transmembrane Ca^{2+}-influx via store-operated Ca^{2+} entry pathways through calcium influx factor (CIF) mechanisms [7]. CIF also activates $iPLA_2$ by dissociating calcium-calmodulin (CaM) from CaM/$iPLA_2$ complex [35,36]. Also, activated $ERK_{1/2}$ activates $cPLA_2$ in synergy with Ca^{2+} [37]. $cPLA_2$ and/or $iPLA_2$ are directly involved in the production of lysophosphatidyl choline (LPC) from phosphatidyl choline [38–40], and LPC is extracellularly release, possibly through ATP-binding cassette-mediated hydrophobic lipid efflux activities [41]. In relation to the calcium-involvement in $cPLA_2$-mediated LPC (LPA) producton, there is an interesting paper, which demonstrates that LPA binds to the intracellular domain of TRPV-1 ion channel and activates [31]. Caterina et al. reported that TRPV-1 knockout mice showed a significant blockade the thermal hyperalgesia, but not mechanical allodynia in mice with mustard oil- or complete Freund Adjuvant-induced inflammatory pain model [42]. However, the TRPV-1 knockout mice showed no effect on both abnormal pain behaviors in mice with partial ligation of the ipsilateral sciatic nerve (neuropathic pain model) in this report. Therefore it is evident that TRPV-1 mechanisms are unlikely related to the LPA-induced neuropathic pain development, even though LPA activates TRPV-1 in vivo. Although TRPV-1-mediated Ca^{2+} influx may activate $cPLA_2$ through an elevation of intracellular calcium levels, this mechanism seems to be insufficient for the LPC and LPA biosynthesis, since the simultaneous activation of Ca^{2+}-independent PLA_2 ($iPLA_2$) as well as $cPLA_2$ have been proved to be important for the (SP + NMDA)-induced LPA-biosynthesis [38].

2.3. NALDI-TOF/MS validates LPC accumulation in neuropathic pain-model

Nanostructure-assisted laser desorption/ionization time-of-flight mass spectrometry (NALDI-TOF-MS) has been developed as an alternative to matrix-free/surface-assisted matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) [43]. The NALDI surface of silicon nanowires provides an effective tool for the analysis of limiting concentration of small to medium-sized, polar organic molecules possessing net positive charge after ionization [44]. Using this technique, time course for *in vivo* production of LPC (16:0, 18:0, and 18:1) as the limiting intermediate in LPA production was monitored in the unilateral dorsal half including dorsal horn (laminae I–V) of the lumbar (L4–L6) spinal cord and L4–L6 dorsal roots in response to partial ligation of sciatic nerve (Fig. 1). LPC production exhibited laterality as only the ipsilateral side of the tissues isolated at various time intervals up to 180 min after nerve injury showed significant spike in LPC at 75 min, but not the contralateral side. It is however important to state that spinal cord slices respond to nerve injury by releasing LPC three times higher than the values recorded for dorsal root [45]. This data therefore reinforce noxious stimulation-dependent afferent fiber transmission coupling to LPA production while it ablates the direct contribution of nerve injury to LPA production. Confirmatory evidence of stimulation-dependent LPA production was obtained when morphine is intracerebroventricularly administered 30 min before partial sciatic nerve injury. This pre-emptive opioid administration abolished LPC burst at 75 min post injury in both dorsal root and spinal cord. This result suggests that the inhibition of nociceptive transmission at the spinal level through opioid-related mechanisms may be exploited in future therapeutic development of neuropathic pain chemotherapy [9,46,47].

3. Demyelination: committing LPA/LPA_1 signaling to neuropathic pain

3.1. LPA-mediated demyelination: the committed step in neuropathic pain

Demyelination at dorsal root of the lumbar (L4–L6) spinal cord after partial sciatic nerve injury has been reproduced in dorsal root after LPA treatment [17,19]. LPA commits nerve injury to neuropathic phenotype via LPA_1-mediated demyelination with subsequent loss of the structural and functional integrity of neuron [7,8]. Due to complex chemistry of extracellular environment that bath the neuron/Schwann cell (SC) complex *in vivo*, we resorted to co-culture experiment of DRG neurons and SCs to validate LPA signaling pathways involved in demyelination [8]. In these co-culture studies we find two differential observations. The first observation is the delayed sprouting, contrasting to the rapid growth cone collapse. The rapid growth cone collapse appears to be the direct action of LPA through LPA_1 and RhoA–ROCK mechanisms [48] while the delayed sprouting to be the indirect action, loss of neuronal outgrowth inhibition following demyelination. The plausible LPA degradation during culture may contribute to this functional switch from collapse to sprouting.