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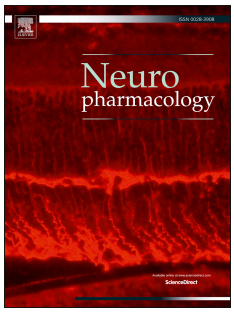

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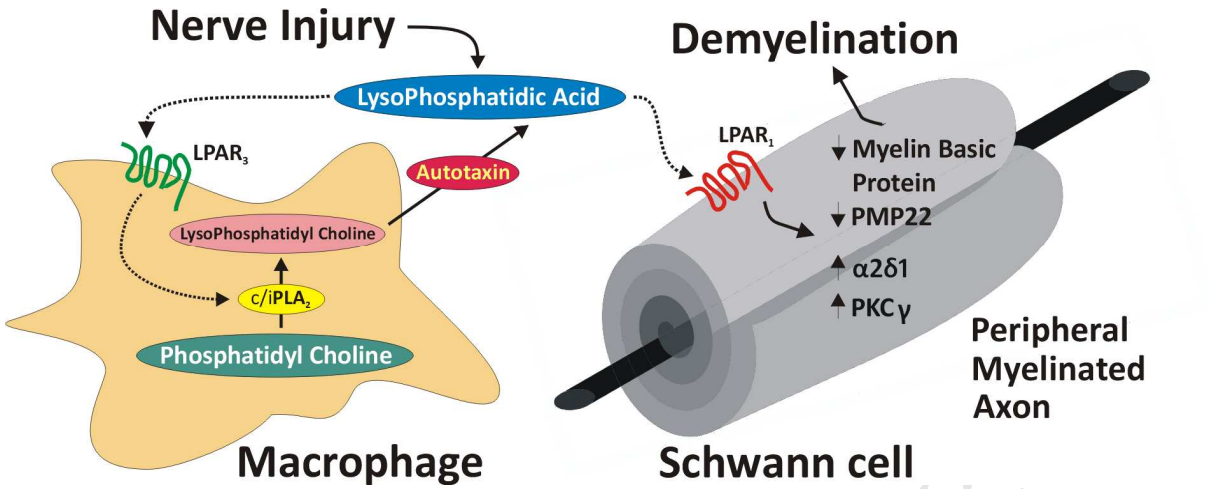

Neuropharmacology Special Issue on lipid-sensing GPCRs

Lysophosphatidic acid receptors (LPARs): potential targets for the treatment of neuropathic pain

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Abstract

Neuropathic pain can arise from lesions to peripheral or central nerve fibres leading to spontaneous action potential generation and a lowering of the nociceptive threshold. Clinically, neuropathic pain can manifest in many chronic disease states such as cancer, diabetes or multiple sclerosis (MS). The bioactive lipid, lysophosphatidic acid (LPA), via activation of its receptors (LPARs), is thought to play a central role in both triggering and maintaining neuropathic pain. In particular, following an acute nerve injury, the excitatory neurotransmitters glutamate and substance P are released from primary afferent neurons leading to upregulated synthesis of lysophosphatidylcholine (LPC), the precursor for LPA production. LPC is converted to LPA by autotaxin (ATX), which can then activate macrophages/microglia and modulate neuronal functioning. A ubiquitous feature of animal models of neuropathic pain is demyelination of damaged nerves. It is thought that LPA contributes to demyelination through several different mechanisms. Firstly, high levels of LPA are produced following macrophage/microglial activation that triggers a self-sustaining feed-forward loop of *de novo* LPA synthesis. Secondly, macrophage/microglial activation contributes to inflammation-mediated demyelination of axons, thus initiating neuropathic pain. Therefore, targeting LPA production and/or the family of LPA-activated G protein-coupled receptors (GPCRs) may prove to be fruitful clinical approaches to treating demyelination and the accompanying neuropathic pain. This review discusses our current understanding of the role of LPA/LPAR signalling in the initiation of neuropathic pain and suggests potential targeted strategies for its treatment.

1. Neuropathic pain

According to the International Association for the Study of Pain (IASP), neuropathic pain (NP) is defined as the pain caused by a lesion to, or a disease of, the somatosensory nervous system. In other words, NP is a complex, chronic pain state resulting from damage to neural tissue such as peripheral afferent neurons, dorsal root ganglia (DRG) or spinal cord neurons. It can also result from direct damage to peripheral and central neurons, which often occur in cancer (Zhao et al., 2010), traumatic brain injury (TBI) (Crack et al., 2014) or multiple sclerosis (MS) (Khan and Smith, 2014). Characteristic symptoms of NP include unpleasant abnormal sensations (dysaesthesia), an enhanced perception of pain in response to noxious stimuli (hyperalgesia), as well as abnormal painful responses to innocuous or tactile stimuli that do not usually cause pain (allodynia) (Frisca et al., 2012; Fujita et al., 2007). While the mechanisms underlying allodynia are still partially unknown, neuropathic hyperalgesia is associated with up-regulation of molecules, such as substance P and the excitatory neurotransmitter glutamate, which enhance the transmission of painful stimuli in DRG and spinal dorsal horn neurons (Fujita et al., 2007; Marchand et al., 2005; Ueda, 2006).

Based on a review of studies published since 2000, it is estimated that 7% of the world's population is affected by NP (Andrew et al., 2014). However, an epidemiological review of chronic pain found that, within a given population, there are still no accurate estimates available for the prevalence of NP (Smith and Torrance, 2012). Although many investigations into the underlying mechanisms of NP have uncovered perturbations to both peripheral nerves and to spinal cord neurons, most experimental therapeutics tested for their ability to correct distorted pain perception, target peripheral neuron damage. Various types of noxious stimuli, mechanical, chemical or thermal in nature, can activate nociceptors on unmyelinated C-fibres and thinly-myelinated Aδ-fibres leading to increases in ectopic electrical discharges and lowering of the nociceptive threshold. Localised accumulation of voltage-gated ion channels, in particular sodium (Na^+) channels, is responsible for this hyperexcitability (Aurilio et al., 2008; Casals-Diaz et al., 2015). In addition, upregulation of the transient receptor potential cation channel subfamily V member 1 (TRPV1) can also sensitise C-fibres to heat by lowering the nociceptive threshold to under 41^{o}C (Ma et al., 2005). Similar damage-induced hyperexcitability of DRG neurons can also result in NP (Aurilio et al., 2008; Baron, 2006; Baron et al., 2010; Chung and Chung, 2002). In the central nervous system, sensitisation of injured neurons can manifest from molecular perturbations such as phosphorylation of postsynaptic NMDA- and AMPA-type glutamate receptors (Katano et al., 2011; Wang et al., 2014) and activation of

signalling cascades such as extracellular signal-regulated kinase (ERK) pathways (Zhang et al., 2014). Loss of inhibitory GABAergic interneurons and/or reduced GABA release in the spinal cord can also culminate in neuronal hyperexcitability (Moore et al., 2002). Finally, inflammation plays a significant role in sensitising peripheral and CNS neurons following nerve injury (Moalem et al., 2005).

Since NP is a multifactorial disorder affecting both the peripheral nervous system (PNS) and CNS, it is not surprising that the current therapies for NP are largely inadequate and that combination therapies, which target several distinct pathways, may be needed in future. As pain transmission is known to be modulated by excitatory and inhibitory neurotransmitters, as well as calcium (Ca^{2+}) and Na^{+} channels, many of the currently available treatments target these systems. Among currently-approved first-line treatments for NP are the antidepressants Duloxetine and Desipramine and the anticonvulsants Gabapentin and Pregabalin (NICE, 2015). The first two drugs are approved antidepressants and their analgesia-promoting effects may be directly related to their ability to prevent presynaptic reuptake of serotonin and noradrenaline, both of which inhibit descending pain pathways in the CNS (Sawynok et al., 2001). Desipramine is a tricyclic antidepressant categorized as a secondary amine with relatively selective inhibition of noradrenaline reuptake (Maizels and McCarberg, 2005). Duloxetine is a potent and selective serotonin–noradrenaline reuptake inhibitor (SNRI) and lacks affinity for other neurotransmitters (Bymaster et al., 2001). Interestingly, neither Paroxetine (serotonin reuptake inhibitor) nor Thionisoxetine (noradrenaline reuptake inhibitor) displayed efficacy in attenuating pain-related behaviours in mice. However, combining both drugs effectively alleviated pain (Iyengar et al., 2004) suggesting that the likely mechanism of action of antidepressants in reducing chronic pain is mediated through inhibition of both serotonin and noradrenaline reuptake. In contrast, the anticonvulsants Gabapentin and Pregabalin, bind to α2δ1-containing voltage-gated calcium (Ca^{2+}) channels (VGCC) on central terminals of primary afferent nociceptors, leading to decreased release of the excitatory neurotransmitters noradrenaline, glutamate and substance P (Baron et al., 2010; Xu et al., 2012). Second-line treatments for NP usually include selective serotonin reuptake inhibitors (SSRIs) or anticonvulsants in combination with opioids, botulinum toxin type A or lidocaine (NICE, 2015). A major disadvantage of currently-approved therapeutics for NP is that they usually need to be taken chronically. To date, there is no pharmacological therapy available which acts to reverse and cure the underlying molecular mechanisms that cause the pathological state that gives rise to NP.

1.1. Animal models of neuropathic pain

There are more than 40 clinically-relevant animal models of neuropathic pain (Jaggi et al., 2011). Many of these are peripheral nerve injury models such as the neuroma model which mimics *anaesthesia dolorosa*, i.e. a constant burning or aching pain in an area which is otherwise devoid of sensory input (i.e. numb) (Wall et al., 1979); chronic constriction injury (CCI) which models hypersensitivity to non-noxious stimuli and chemical irritants (Bennett and Xie, 1988; Martucci et al., 2008; Sacerdote et al., 2008); the Seltzer model, also known as partial sciatic nerve ligation (PSNL), which induces behavioural alterations including cold allodynia, chemical hypersensitivity and mechanical hyperalgesia and is said to be a good model of causalgiform pain syndromes (i.e. severe burning pain in a limb) (Kim et al., 1997; Malmberg and Basbaum, 1998; Seltzer et al., 1990); and the spared nerve injury (SNI) paradigm in which the sural nerve is 'spared' and the tibial and common peroneal nerves are axotomized, thus producing thermal and mechanical hyperalgesia but, more importantly, facilitates the assessment of nociception in non-injured areas of skin located adjacent to denervated zones (Bourquin et al., 2006; Decosterd and Woolf, 2000; Shields et al., 2003). There are also central pain models, such as the Allen's model of contusive spinal cord injury (SCI) in which a weight is dropped on the exposed spinal cord leading to severe paraplegia and complete segmental necrosis (Allen, 1911; Greenberg et al., 1978); drug-induced neuropathy models, for example, the chemotherapeutic cisplatin which is known to cause peripheral axonal neuropathy that affects both small and large diameter sensory fibres (Cece et al., 1995; Meijer et al., 1999; Shimoyama et al., 2002; Tredici et al., 1999); disease-induced neuropathy models such as diabetes and cancer NP models (Courteix et al., 1993; Lee et al., 1990; Shimoyama et al., 2002); and various other *in vivo* models of NP (Dina et al., 2006; Imamura et al., 1997). The major limitation of current NP animal models is the inherent difficulty in attempting to translate improvements in animal behaviour to clinically-significant alleviation of human pain perception; but despite this, many of these models are accepted as useful tools. However, since NP can manifest in many different ways and has multiple aetiologies, a range of experimental animal models is needed to study the underlying causes of NP.

Animal models of NP have facilitated the identification of disrupted cellular processes at peripheral, spinal and cerebral cortical levels. In the periphery, sensitisation occurs due to increased excitability and decreased firing thresholds for nociceptor terminals, as well as the release of inflammatory

mediators such as bradykinin and prostaglandin E2 (PGE_2) (Basbaum et al., 2009; Bridges et al., 2001; Xu et al., 2012). At the level of the central spinal cord, excitatory transmission at C-fibre synapses is often potentiated and the firing threshold of dorsal horn neurons is decreased. These phenomena can be caused by the upregulation of AMPA-type glutamate receptors and phosphorylation of several other ion channels leading to postsynaptic hyperexcitability (Ikeda et al., 2006; Latremoliere and Woolf, 2009; Sandkühler, 2007). Other aspects of NP involve somatosensory processing, which occurs in cerebral cortical areas, such as the anterior cingulate cortex (ACC), where protein kinase M zeta (PKMζ) is activated, excitatory neurotransmission at pyramidal neurons is potentiated (LTP), glutamate receptors are upregulated and decreases in long-term depression (LTD) lead to disinhibition and reorganization of neural networks (Li et al., 2010; Vogt, 2005; Wu et al., 2005). NP, therefore, can be viewed as a spectrum of disorders with mechanistically-related, but distinct, aetiologies. A better understanding of the causative mechanisms of NP will be necessary in order to develop new therapeutic strategies for this illness. The following sections focus on the emerging role that lysophosphatidic acid (LPA) plays in triggering and perpetuating molecular and cellular disturbances associated with NP.

2. Lysophosphatidic acid (LPA) signalling

Several studies suggest that increased levels of LPA may contribute to injury-induced demyelination of neurons and can thus cause neuropathic pain. LPA (1-acyl-2-sn-glycerol-3-phosphate) is a well-characterised glycerophospholipid with a molecular weight of approximately 430–480 Dalton (Yung et al., 2014). LPA activates a family of six distinct G protein-coupled receptors (GPCRs), named $LPAR_{1\text{-}6}$ (Callaerts-Vegh et al., 2012) and these GPCRs signal through four distinct G_α protein subtypes (G_i, G_q, G_{12} and G_s) leading to multiple downstream signalling pathways and cellular responses (Frisca et al., 2012; Lin et al., 2010). In addition, LPA can also bind to the intracellular peroxisome proliferator-activated receptor γ (PPARγ) (McIntyre et al., 2003) and the TRPV1 cation channel (Nieto-Posadas et al., 2012), thus playing important roles in gene regulation and nociception, respectively.

LPA is generated by a number of different enzymes such as phospholipases A_1 and A_2, monoacylglycerol kinase, glycerol-3-phosphate acyltransferase and autotaxin (ATX) (Noguchi et al., 2009; Pebay et al., 2007) from various precursors including phosphatidic acid, glycerol-3-phosphate

and several glycerophospholipids. LPA can be produced by platelets (Eichholtz et al., 1993), fibroblasts (Fukami and Takenawa, 1992), mitotic neurons (Fukushima et al., 2000), astrocytes (Savaskan et al., 2007) and also cancer cells (Zhao et al., 2010). High concentrations of LPA have been observed in several pathological conditions such as atherosclerosis (Smyth et al., 2014), traumatic brain injury (Crack et al., 2014), spinal cord injury (Santos-Nogueira et al., 2015), different types of cancer (Eder et al., 2000; Lee and Yun, 2010; Zeng et al., 2009; Zhao et al., 2010), neuropsychiatric disorders (Yung et al., 2014) and neuropathic pain (Ahn et al., 2009; Fujita et al., 2007). Production of LPA during inflammation promotes wound-healing and acts to control the pro-inflammatory environment. Thus, LPA has pleiotropic effects on a wide range of cell and tissue types, including those located within both PNS and CNS and participates in normal physiological functions including cellular development, proliferation and migration (Frisca et al., 2012). Below, we discuss the role of high levels of LPA in the initiation, maintenance and pathophysiological underpinnings of NP and highlight potential therapeutic targets for its treatment.

2.1. Initiation of neuropathic pain by LPA

The role of LPA in the initiation of neuropathic pain is gaining acceptance, but the origin of raised LPA levels is less well established. Normal concentrations of LPA in most cell types are relatively low, but detectable levels (0.1 – 1 μM) have been measured in plasma, serum, saliva, cerebrospinal fluid (CSF) and inflammatory exudates (Frisca et al., 2012). Precursors for LPA include membrane phosphatidylcholines (PC) which can be converted to lysophosphatidylcholine (LPC) by the cytosolic phospholipase A_2 ($cPLA_2$) or calcium-independent PLA_2 ($iPLA_2$). Interestingly, inhibition of both enzymes abolishes nerve injury-induced LPA production (Ma et al., 2010). LPC, in turn, can be converted into LPA by the enzymatic action of ATX, a secreted enzyme present in the extracellular milieu (see **Figure 1**) (Tokumura et al., 2002). Notably, the CSF contains high concentrations (0.4 – 1.3 mg/L) of ATX (Nakamura et al., 2009). Leakage of serum containing LPA into the CNS can occur following injury and damage to the blood-spinal cord barrier and this rise in LPA levels in the CSF can trigger signalling events in neurons which culminate in neuropathic pain (Kusaka et al., 1998). Whilst full $ATX^{-/-}$ knockout mice die early in embryogenesis, heterozygous ATX mutant mice ($ATX^{+/-}$) reportedly show a 50% decrease in ATX activity resulting in lower levels of LPA production and better recovery from NP (Inoue et al., 2008a; Inoue et al., 2008b; Inoue et al., 2008c). Therefore, ATX activity leads to an increase of LPA levels in pathological states and this

contributes to the initiation of NP. As such, ATX is an interesting therapeutic target, upstream of LPA, for the treatment of NP (see **Table 1**).

LPA-mediated signalling via $G\alpha_{12/13}$ and activation of the signalling pathways involving RhoA, Rho-kinase, ROCK and Ras appear to be involved in NP (Inoue et al., 2004; Radeff-Huang et al., 2004; Ueda, 2006). Inhibition of Rho signalling pathways by *Clostridium botulinum* C3 exoenzyme (BoTXC3) or the antagonist Y-27632 (see **Table 1**), prior to peripheral nerve injury, blocks hyperalgesia and nociceptive responses in mice (Ahn et al., 2009; Inoue et al., 2004; Inoue et al., 2006). Confirmation that the RhoA pathway is activated through $LPAR_1$ subtype was demonstrated utilising *Lpar1*$^{-/-}$ knock-out mice, which do not exhibit nociceptive responses or sensitivity after LPA injections (Inoue et al., 2006). Furthermore, several studies showed that intrathecal administration of LPA mimics partial sciatic nerve injury in mice (Inoue et al., 2004; Inoue et al., 2006; Ueda, 2006) whereas a related lipid, sphingosine 1-phosphate (S1P), does not produce such allodynia (Ishii et al., 2004), thus highlighting the specificity of LPA. The observation that neuropathic pain can be attenuated by prophylactic treatments which block Rho signalling or LPAR activation (see **Table 1**) when they are administered pre- but not post-injury (Inoue et al., 2004) suggests that LPAR activation may trigger demyelination and initiate NP.

It has recently been shown that both intrathecal injection of LPA into the healthy CNS and increases in endogenous LPA levels, caused by contusive spinal cord injury, trigger demyelination of central neurons (Santos-Nogueira et al., 2015). Similarly, LPA can induce demyelination in the PNS (DRG and trigeminal nerves) (Ahn et al., 2009; Fujita et al., 2007; Inoue et al., 2004; Ogawa et al., 2012; Xie et al., 2010). Both Schwann cells and oligodendrocytes express high levels of $LPAR_1$ both *in vivo* and *in vitro* (Weiner and Chun, 1999) but express relatively low levels of other LPARs suggesting that LPA exerts its main effects on myelin-forming cell types exclusively via $LPAR_1$ activation. Decreases in myelin basic protein (MBP) and peripheral myelin protein 22 (PMP22) expressions after LPA injection in the dorsal root have also been reported and these effects were inhibited by pre-treating mice with BoTXC3 (Fujita et al., 2007; Inoue et al., 2004). Furthermore, blockade of $LPAR_1$ and $LPAR_3$ by diacylglycerol pyrophosphate (DGPP) or inhibition of Rho kinase-activated signalling pathways by Y-27632, prevented demyelination of the trigeminal nerve after intratrigeminal ganglionic injection of LPA (Ahn et al., 2009). Taken together, these studies suggest that demyelination observed on peripheral neurons due to nerve injury occurs through a

direct action of LPA on Schwann cells via $LPAR_1$ (see **Figure 2**). In support of these findings, a recent study has shown that selective blockade of $LPAR_1$, using the antagonist AM095, attenuates CNS demyelination after contusive spinal cord injury in mice and improves locomotor recovery (Santos-Nogueira et al., 2015). In addition to demyelination, $LPAR_1$ mediates the upregulation of the α2δ1 Ca^{2+} channel subunit and the γ-isoform of protein kinase C (PKCγ) in spinal cord (Inoue et al., 2004); both of which are well-established markers of NP (Luo et al., 2002; Weiner and Chun, 1999; Weiner et al., 2001).

Further evidence implicating LPA signalling in NP include a report that thermal hyperalgesia and mechanical allodynia after PSNL are attenuated in *Lpar1*$^{-/-}$ knockout mice or by pre-treatment with antisense oligodeoxynucleotides against $LPAR_1$; but because no significant changes in uninjured *Lpar1*$^{-/-}$ mice were observed, it was suggested that *de novo* LPA produced after the injury was responsible for initiating NP (Inoue et al., 2004). LPA also activates PLC via the $G\alpha_{q/11}$ and $G\alpha_{i/o}$ coupled $LPAR_3$ subtype. Notably, there is minimal LPA production in *Lpar1*$^{-/-}$ and *Lpar3*$^{-/-}$ mice and NP is abolished in this phenotype (Ma et al., 2013; Ma et al., 2009b), suggesting that both receptors mediate the amplification of LPA production, which is necessary to induce NP. Supporting these findings are reports that the chemotherapeutic drug, paclitaxel, triggers NP in mice by stimulating $LPAR_1$- and $LPAR_3$-mediated LPA production (Uchida et al., 2014).

The $LPAR_5$ subtype also plays a key role in triggering NP. Previously known as GPR92 (Lee et al., 2006), $LPAR_5$ is expressed on the cell bodies of C-fibres within the DRG. In contrast, $LPAR_5$ is not found on Aβ fibres which transmit sensations induced by innocuous tactile stimuli (Kinloch and Cox, 2005). *Lpar5*$^{-/-}$ mice subjected to the CCI model of NP display attenuated cold-induced allodynia using the acetone test, despite no changes in mechanical allodynia and spontaneous displays of discomfort using the von Frey test and balance box, respectively (Callaerts-Vegh et al., 2012). *Lpar5*$^{-/-}$ mice also display marked protection against development of NP in the PSNL model (Lin et al., 2012). In this study, there were no differences in sciatic nerve or DRG demyelination, or in expression of the NP markers, PKCγ and Ca^{2+} channel subunit $\alpha_2\delta_1$. In addition, the downstream transcriptional target of $LPAR_5$, i.e. cAMP response element-binding protein (CREB), displayed lower levels of phosphorylation in *Lpar5*$^{-/-}$ mice subjected to the PSNL and CCI models of NP. These results suggest that $LPAR_5$ can trigger NP by a different mechanism to $LPAR_1$, thereby representing and additional drug target for the treatment of NP.

LPA synthesis can also be activated through other mechanisms including the pain-inducing neurotransmitters, glutamate and substance P, which are released from primary afferent fibres in response to nerve injury and activate NMDA and neurokinin-1 (NK1) receptors, respectively (Ueda et al., 2013). Blockade of both NMDA and NK1 receptors prior to nerve injury by their respective antagonists, MK-801 and CP-99994, resulted in the inhibition of LPA production at the dorsal horn (Ma et al., 2013). This suggests that nerve injury results in glutamate and substance P-mediated activation of NMDA and NK1 receptors on neurons and subsequent activation of the enzyme PLA_2 leading to LPA production and initiation of neuropathic pain (see **Figure 2**).

2.2. Effects of LPA on non-neuronal cell types

The induction of neuropathic pain by LPA not only relies on its actions on neurons, Schwann cells and oligodendrocytes, but also through its effects on other cell types such as astrocytes, macrophages/microglia and immune cells (Goldshmit et al., 2012; Ma et al., 2010; Smith et al., 1999; Ueda, 2011). LPA has been shown to increase inflammation and glial cell proliferation in zebrafish and inhibit neural regeneration after traumatic injury (Goldshmit et al., 2012). LPA causes an increase in expression of brain-derived neurotrophic factor (BDNF) in primary cultures of rat microglia which express $LPAR_3$ (Fujita et al., 2008). LPA activates macrophages/microglia, possibly through $LPAR_1$ and $LPAR_3$, and this initiates a self-sustaining feedforward loop of LPA production within macrophages/microglia, which can be inhibited with minocycline (Ma et al., 2013; Uchida et al., 2014). Moreover, intrathecal injection of mice with LPA increases the transcription of genes such as CD11b, leading to activation of microglia and morphological changes from ramified to amoeboid phenotypes. While early treatment with minocycline inhibits microglial activation and attenuates neuropathic pain, administering minocycline at later time-points has no effect (Ma et al., 2010), suggesting that microglia are involved to a greater extent in the initiation of NP more than in its maintenance.

3. Recent developments in the treatment of neuropathic pain

Because NP is a multifactorial pathological state with several distinct triggers and mechanisms involved in its maintenance, a personalised medicines approach to treating patients with NP will likely be needed to achieve optimal analgesia. There are several approaches to consider when testing

potential therapies for NP that target LPA/LPAR signalling. Upstream interventions may include targeting high levels of LPA, produced following nerve injury, by neutralising antibody-based therapies that act as molecular sponges (Crack et al., 2014; Goldshmit et al., 2012). In this regard, the humanized monoclonal antibody, anti-LPA B3, that targets LPA, is currently under investigation (Crack et al., 2014; Goldshmit et al., 2012). An alternative approach may be to target the enzyme ATX (Gierse et al., 2010; Gupte et al., 2011; Hwang et al., 2013), which contributes to LPA production. There are currently several ATX inhibitors in pre-clinical studies, although none are exclusive for NP. PF-8380 is a specific inhibitor that reduces inflammatory hyperalgesia in rats within 3h (Gierse et al., 2010). Other ATX inhibitors, such as 4PBPA (Gupte et al., 2011), Gintonin (Hwang et al., 2013) and ONO-8430506 (Benesch et al., 2014) are indicated for cancer, although they may also have effects on NP (see **Table 1**). The first oral therapy developed for MS, i.e. fingolimod (Kappos et al. 2006), may also act as an inhibitor of ATX (van Meeteren et al., 2008).

Downstream interventions for the treatment of NP may include targeting LPARs with specific antagonists. There are a number of $LPAR_1$ antagonists in early pre-clinical phase studies, such as AM095 (Castelino et al., 2011), Ki16425 (Liao et al., 2013; Ma et al., 2009a), BMS compounds (Nogueira, 2013) and VPC12249 (Okusa et al., 2003). Only the BMS compounds are specifically indicated for neuropathic pain (Nogueira, 2013), where, for example, BMS-986202 is in phase I and BMS-986020 in phase II clinical trials (Bradford, 2012). Blockade of the Rho kinase pathways is also a potential therapeutic strategy for NP since administration of the Rho kinase inhibitors H-1152 or Y-27632, alleviates neuropathic pain in mice and rats (Ramer et al., 2004; Tatsumi et al., 2005).

Finally, peptide-based therapeutics such as GsMTx4, a component of the *Grammostola spatulata* tarantula venom, which blocks several mechanically-activated (MA) cation channels involved in the transmission of pain-related signals, has shown positive results in alleviating mechanical hyperalgesia and NP (Park et al., 2008). Interestingly, LPA has been shown to sensitise MA channels in a dose-dependent manner. Epithelial and smooth muscle cells exposed to LPA display a marked increase in Ca^{2+} influx through MA channels in response to mechanical stress, but no sensitising effects on Ca^{2+} release from intracellular stores were observed in the presence of LPA (Ohata et al., 1995; Ohata et al., 1996; Ohata et al., 1997a). This enhancement of MA channel responses by LPA can be attenuated by phospholipase C (PLC) inhibitors (Ohata H et al., 1997b), suggesting that it may be an indirect effect through activation of LPAR-mediated GPCR signalling. Therefore,

developing treatments for NP that block mechanically-activated ion channels on neurons and/or glial cell types may also inhibit the downstream sensitising effects of LPA and alleviate symptoms such as hyperalgesia and allodynia.

4. Conclusion

LPA is a highly bioactive molecule with a number of cellular sources and exerts its actions through a range of GPCRs and ion channels present on various cell types. Here, we have highlighted the role that LPA plays in the initiation of NP which is a diverse spectrum of disorders affecting millions of people worldwide for which there appears little *bona fide* treatment to date. There are multiple molecular mechanisms underlying the pathophysiology of NP thus increasing the complexity of developing effective targeted therapeutics. Nevertheless, it appears that LPA production and signalling plays a major role in the initiation of NP, exerting downstream effects on many cell types including neurons, Schwann cells, oligodendrocytes, macrophages, microglia, astrocytes and other immune cells. Therefore, targeting the production of LPA by inhibition of ATX, the enzyme primarily responsible for its synthesis, or blocking $LPAR_1$ and/or $LPAR_5$ activation and downstream signalling cascades could prove to be fruitful strategies to alleviate NP. Taken together, the LPA/LPAR signalling pathway provides multiple targets for the development of new therapeutics for NP and related pathologies as evidenced by recent pre-clinical, phase I and phase II studies.

Figure Legends

Figure 1: Synthesis of lysophosphatidic acid (LPA). Precursors of LPA synthesis include phosphatidylcholine (PC) which is converted to lysophosphatidylcholine (LPC) by the enzymatic actions of cytosolic phospholipase A_2 ($cPLA_2$) and calcium-independent phospholipase A_2 ($iPLA_2$). LPC, in turn, is converted to LPA by autotaxin (ATX); a secreted enzyme present at relatively high concentrations in extracellular fluids.

Figure 2: Potential mechanisms of neuropathic pain initiation by LPA. Traumatic nerve injury can cause the release of glutamate and substance P from peripheral neurons leading to NMDA and NK1 receptor activation, respectively. This can lead to influx of extracellular Ca^{2+} through NMDA receptors and activation of phospholipase A_2 ($cPLA_2$). PLA_2 can also be activated in a non-calcium dependent manner ($iPLA_2$) through NK1-mediated G-protein signalling. $c/iPLA_2$ converts phosphatidylcholine (PC) to lysophosphatidylcholine (LPC) and this, in turn, is enzymatically processed into LPA via the actions of extracellular autotaxin (ATX). LPA can activate macrophages/microglial cell types where it signals through $LPAR_3$ to initiate self-sustaining feedforward production of LPA via the enzymatic activity of $c/iPLA_2$ and ATX. Macrophage-derived LPA can also activate $LPAR_1$ on myelinating Schwann cells leading to demyelination through various mechanisms, including down-regulation of myelin basic protein (MBP) and peripheral myelin protein 22 (PMP22) as well as upregulation of protein kinase C-γ (PKCγ) and the voltage-gated Ca^{2+} channel subunit α2δ1.

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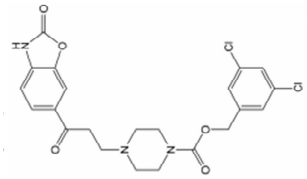
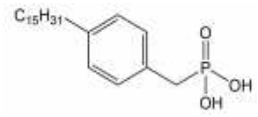
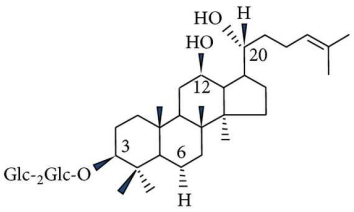
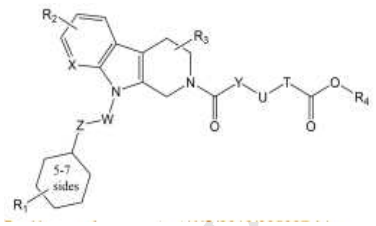
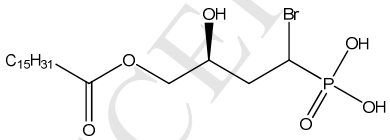
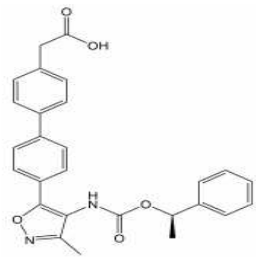
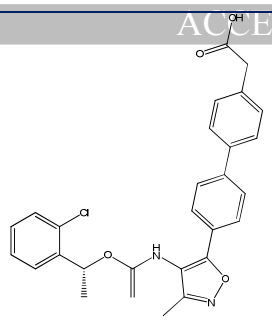
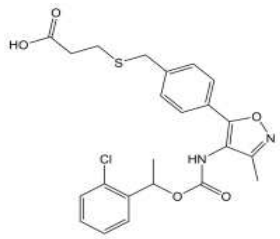
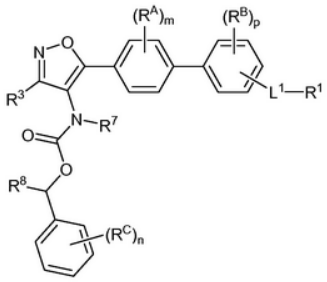
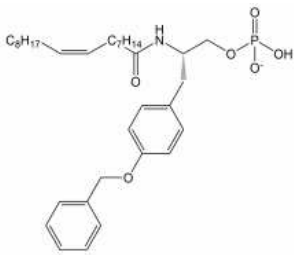
Name	Structure	Target	Phase (Sanofi, 2014)	Reference
Anti-LPA B3	Antibody	LPA signalling	Pre-clinical	(Crack *et al.*, 2014) (Goldshmit *et al.*, 2012)
PF-8380		ATX inhibitor	Pre-clinical	(Gierse *et al.*, 2010)
4PBPA		ATX inhibitor	Pre-clinical	(Gupte *et al.*, 2011)
Gintonin	Glycolipoprotein 	ATX inhibitor	Pre-clinical	(Hwang *et al.*, 2013)
ONO-8430506		ATX inhibitor	Pre-clinical	(Benesch *et al.*, 2014)
BrB-LPA		Dual LPAR antagonist/ATX inhibitor	Pre-clinical	(Xu et al., 2009)
AM095		$LPAR_1$ antagonist	Pre-clinical	(Castelino *et al.*, 2011)
AM966		$LPAR_1$ antagonist	Pre-clinical	(Swaney et al., 2010)

				
KI-16425		$LPAR_1$ antagonist	Pre-clinical	(Liao *et al.*, 2013) (Ma *et al.*, 2009a)
BMS compound		$LPAR_1$ antagonist	Pre-clinical	(Nogueira, 2013)
VPC-12249		$LPAR_1$ antagonist	Pre-clinical	(Okusa *et al.*, 2003)
BMS-986202/AM152	See patent WO/2012/162592 A1 for more details	$LPAR_1$ antagonists	Phase I-Idiopathic Pulmonary Fibrosis	(Bradford, 2012) (BMS, 2011)
BMS-986020	See patent WO/2012/162592 A1 for more details	$LPAR_1$ antagonist	Phase II- Idiopathic Pulmonary Fibrosis- (NCT01766817) Phase II- Systemic sclerosis (NCT02588625)	(Bradford, 2012)
SAR 100842	See patent WO/2012/162592 A1 for more information	$LPAR_1$ $LPAR_3$ antagonist	Phase II- Systemic sclerosis (NCT01651143)	(Sanofi, 2014)
H-1152		Rho kinase inhibitor	Pre-clinical	(Tatsumi *et al.*, 2005)

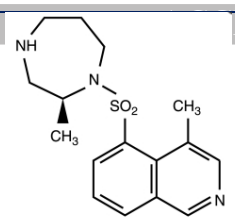
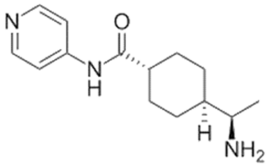
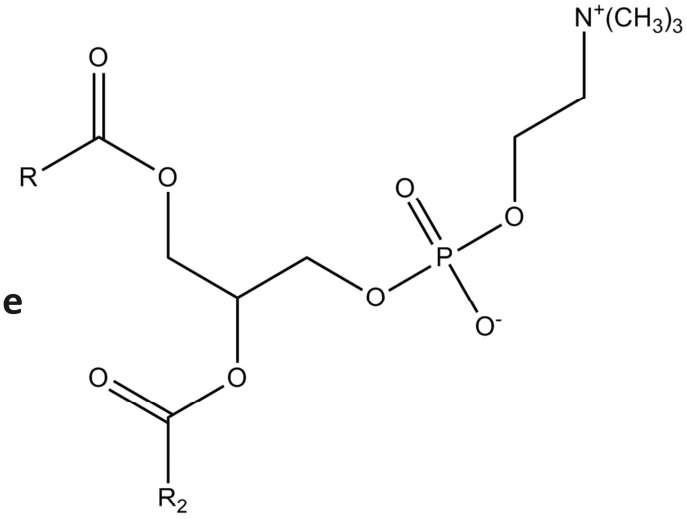
				
Y-27632		Rho kinase inhibitor	Pre-clinical	(Ramer *et al.*, 2004)

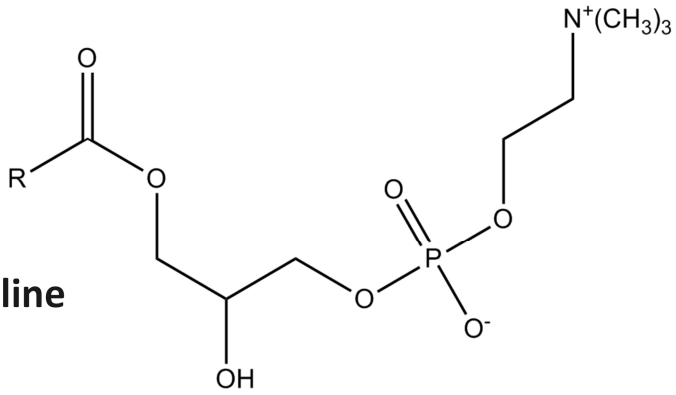
Table 1: LPA signalling and LPA receptor inhibitors with potential efficacy in neuropathic pain treatment.

Phosphatidylcholine (PC)



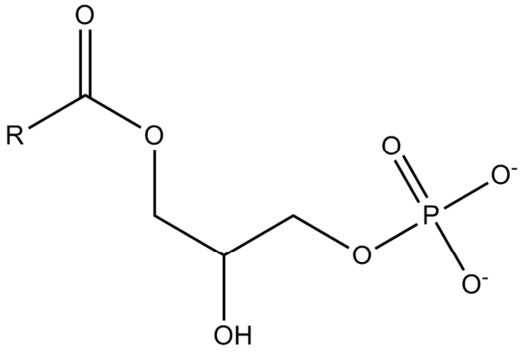
cPLA$_2$
iPLA$_2$

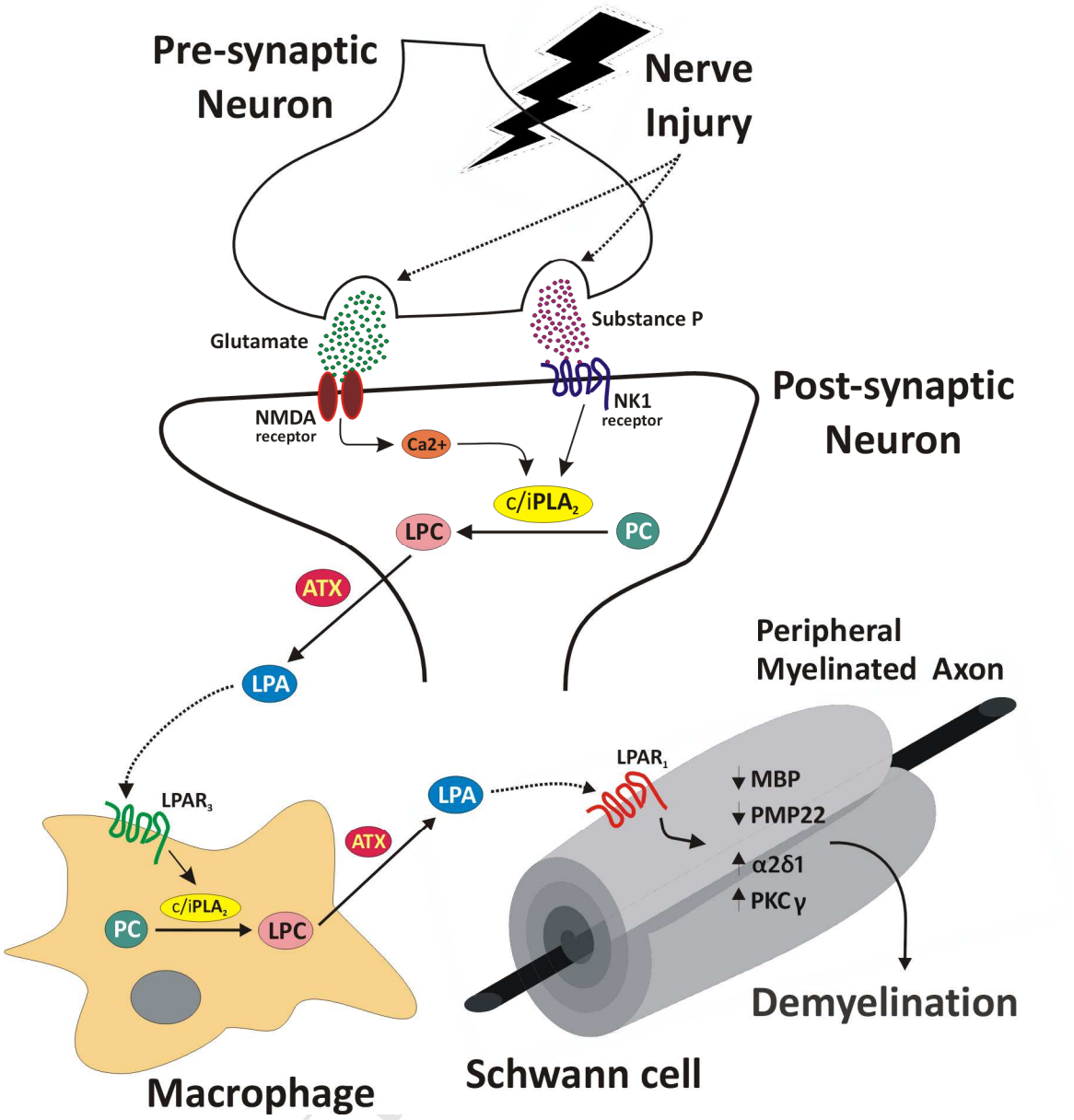
Lysophosphatidylcholine (LPC)



Autotaxin (ATX)

Lysophosphatidic acid (LPA)




Pre-synaptic Neuron
Nerve Injury
Glutamate
Substance P
NMDA receptor
NK1 receptor
Post-synaptic Neuron
Ca2+
c/iPLA2
LPC
PC
ATX
LPA
Peripheral Myelinated Axon
LPAR3
LPA
LPAR1
MBP
PMP22
α2δ1
PKCγ
ATX
PC
c/iPLA2
LPC
Demyelination
Macrophage
Schwann cell

Highlights

- Neuropathic pain (NP) is a multifactorial pathological state with no cure.
- Increased LPA synthesis after nerve injury can trigger demyelination, causing NP.
- LPA/LPAR signalling contributes to maintenance of NP.
- New therapeutics targeting LPA production and signalling may be effective in alleviating NP.