PERSPECTIVE

Modulation of lysophosphatidic acid (LPA) receptor activity: the key to successful neural regeneration?

The central nervous system (CNS) is characterized by a remarkably elaborate cellular architecture comprising large numbers of glial and neuronal cells with enormous functional diversity, organized into highly complex and specific networks. During development, the various neural cell types must first be correctly specified, then assume their appropriate positions through carefully choreographed cellular migration, and finally establish and refine their functional connections, often over long distances. The end result of all these processes is an extraordinarily intricate anatomical structure, able to receive, integrate, and store information and orchestrate appropriate responses.

The molecular mechanisms of the developing CNS are only poorly understood, and due to its outstanding complexity in adulthood, only little regeneration or repair mechanisms occur. The wiring of the normal adult CNS has classically been seen as stable and permanent, but this is not completely true. The neuronal network of the adult CNS does retain a limited capacity for growth and structural change. A large number of regeneration factors have been identified in the recent past, but a general solution for the induction of repair mechanisms after damage is still missing.

So far, the main focus of neural regeneration research has been based on investigating proteins and their signaling cascades. However, the field of lipidomics has been successful in providing information on the crucial involvement of bioactive lipids, such as lysophosphatidic acid (LPA) or sphingosine-1-phosphate, as signaling molecules and regulators in physiological and pathophysiological neuronal processes.

These novel findings raise the question, whether neuronal lipid metabolism could be the future target for therapeutic approaches addressing neural regeneration. One successful example of such a therapy approach is the drug fingolimod (Gilenya®). Marketed in 2010 as an oral treatment for relapsing-remitting multiple sclerosis, it has become the first drug to modulate the sphingolipid signaling pathway. Fingolimod is a substrate of sphingosine kinases, generating fingolimod phosphate which acts as an agonist at sphingosine-1-phosphate receptors. However, this interaction prompts the internalization of the receptors from the membrane, resulting in functional antagonistic action of fingolimod. It was initially discovered for its immunomodulative effects, preventing experimental autoimmune encephalitis in rats by reducing the number of lymphocytes in the blood and CNS. However, pathology improving effects were also observed in lymphocyte-independent multiple sclerosis models, indicating additional CNS specific actions of fingolimod (reviewed in Brinkmann et al. (2010)).

Nevertheless, traumatic injuries of the CNS remain a major challenge and no effective drugs for stimulating regeneration processes are so far in use. The extracellular environment, however, allows neurite elongation only under specific molecular conditions. Molecules involved in neurite outgrowth, such as semaphorins, netrins and ephrins, are able to transduce outgrowth-regulating signals to elongate axons via specific receptors. A phospholipid–rich environment normally inhibits outgrowth of fibers. The bioactive lipid LPA is present in the extracellular space and acts via the LPA receptors involving intracellular activation of small G-proteins that mediate neurite retraction (Yung et al., 2014). Crack et al. showed elevated levels of the pro-inflammatory LPA in cerebrospinal fluid samples from patients with traumatic brain injuries and of mice subjected to control cortical impact injury (Crack et al., 2014).

Interestingly, blocking LPA with a LPA-specific antibody improved the neurological outcome in control cortical impact injury mice, by reducing lesion size and behavioral deficits (Crack et al., 2014). These findings suggest a substantial role of LPA in restraining neural regeneration processes in the adult CNS after injury, making it a highly interesting target lipid.

LPA can bind to at least six known G-protein coupled receptors (LPA_{1-6}). Each receptor can couple with multiple types of G proteins ($G_{12/13}$, $G_{i/o}$, $G_{q/11}$, G_s) to activate a range of downstream signaling pathways inducing pleiotropic effects inside the cell. For example, activation of phospholipase C, Rho, and Akt, and phosphatidylinositol 3-kinase pathways or inhibition of adenylyl cyclase (reviewed in Yung et al. (2014)).

LPA receptor gene products are detectable in most mammalian tissues (reviewed in Yung et al. (2014)). In our recent study, we showed the dynamic temporal and spatial expression of LPA_1, LPA_2, LPA_4 and LPA_6 receptors in the developing mouse brain and in differentiation of neuronal cells (Suckau et al. (2019) and **Table 1**). This dynamic receptor expression proposes a significant role of LPA signaling during fundamental neurodifferentiation processes, like astrogenesis and oligogenesis, axon and dendrite growth or synapse formation and maturation. With this dynamic expression pattern, a highly complex regulation mechanism is generated that further complicates the investigation of neuronal LPA metabolism. The LPA-induced effects may result from differences in concentration and differential expression of various LPA receptor subtypes. Kingsbury et al. showed that LPA exposure to cortical hemisphere cultures induces folding and widening of the cerebral wall, which was absent in cortical hemispheres of LPA_1/LPA_2 double-null-mice, indicating a receptor mediated effect (Kingsbury et al., 2003). Zheng et al. on the other hand demonstrated that, depending on the concentration, LPA can act as both a survival and an apoptotic factor in cultured cortical neurons (Zheng et al., 2004).

These inconsistent results demonstrate the complexity and ubiquity of the LPA metabolism during neuronal de- and regeneration processes. It also stresses that more research on the underlying fundamental mechanisms is needed and that an overall understanding is not yet in sight. Moreover, for therapeutic approaches targeting the LPA metabolism, this deeper understanding is vitally important, as due to the ubiquitous actions of LPA, severe side effects can occur, and these must be more assessable.

The complexity of the regulation mechanisms might represent the largest problem in intervention of LPA metabolism. This begins right from LPA synthesis: it can be generated through different metabolic pathways with two major routes of synthesis. One of them is the conversion of lysophospholipids, like lysophosphatidylcholine, lysophosphatidylethanolamine or lysophosphatidylserine via enzymatic action of Autotaxin. In the other one, LPA is derived from membrane phospholipids trough the actions of phospholipases. Consequently, LPA synthesis involves the conversion of precursor phospholipids, like phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine and generates lysophospholipids and phosphatidic acid as intermediate lipid products (reviewed in Yung et al. (2014)). These phospholipids are also involved in other cellular processes and must be considered when interfering with LPA metabolism.

As already pointed out, an important regulation mechanism is the localization and composition of the LPA receptor molecules. As we showed in our recent study, the expression of this high number of specific receptors is a dynamic and complex regulation tool, used for controlling LPA actions over temporal processes. The expression of LPA receptors changes depending on the developmental stage of the mouse brain. We detected only LPA_1, LPA_2, LPA_4, and LPA_6 receptor mRNA transcripts in the developing mouse brain, with different dynamic expression patterns. LPA_3 and LPA_5 meanwhile remained below the detection level (Suckau et al. (2019) and **Table 1**).

To give an example, the LPA_2 receptor showed high expression levels in all examined brain regions until birth, followed by an expression decrease except for in the hippocampus region. The hippocampal formation is involved in learning and memory, and here the LPA_2 receptor remained at a high expression level until adulthood (Suckau et al., 2019). These findings are consistent with others, which show that the LPA_2 receptor is presynaptically localized and plays an important role in the modulatory control of hippocampal excitability (Trimbuch et al., 2009).

We also examined LPA receptor expression in the maturation of different brain cells by analyzing mRNA expression in primary cultured cells. All four detected receptors were expressed in primary cultured neurons and increased expression during the maturation process, with LPA_6 showing the highest expression levels. LPA_1 and LPA_6 mRNA was strongly detectable in cultured astrocytes and only LPA_6 showed high expression in cultured microglia. LPA_1 receptor expression increased during maturation of cultured oligodendrocytes, whereas the other three receptors were expressed weakly or not at all (Suckau et al. (2019) and **Table 1**).

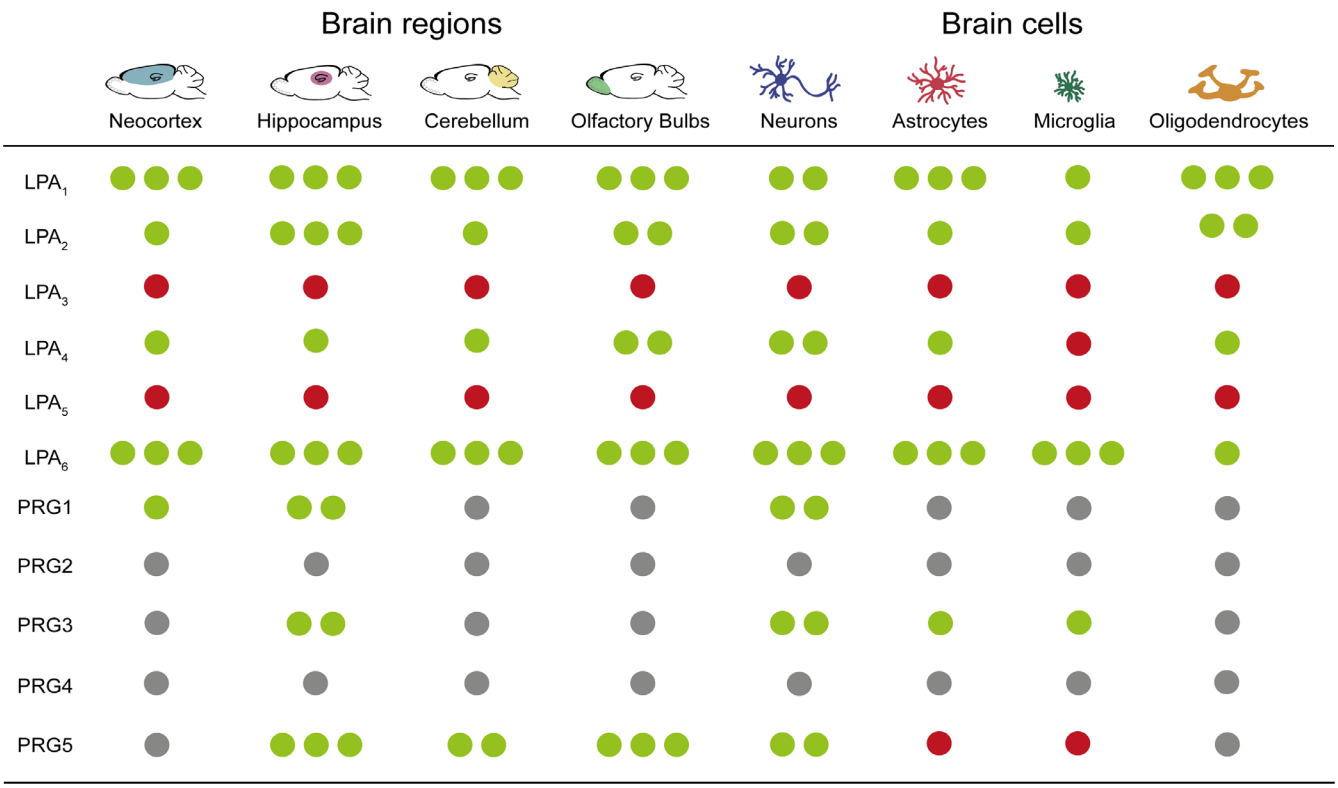
The balancing and interfering of LPA signaling could be mediated by receptor inactivation, or by metabolizing and caging of its ligand. The latter is controlled by lipid phosphate phosphatases (LPPs), an enzyme family which is not neuron-specifically expressed. These ecto-phosphatases can control the extracellular availability and thus the signaling of LPA and other phospholipids and can in turn also be regulated by their expression pattern. A structural homologue to LPPs and a highly brain-specific class of proteins, the plasticity-related genes (PRGs), were shown to be involved in both regeneration processes and attenuation of LPA-induced effects (reviewed in Bräuer and Nitsch (2008)).

Five PRGs have been identified so far, but their distinct roles are understood partially or not at all. Nevertheless, individual expression patterns during brain development in mice have given rise to the assumption that PRGs have different regulatory mechanisms and neuronal functions in the CNS. They interfere with lipid phosphate signaling through various mechanisms (Bräuer and Nitsch (2008), Velmans et al. (2013) and **Table 1**).

PRG1 can enhance axon outgrowth during development and after appearance of lesions, and reduces LPA-induced axon collapse (Bräuer and Nitsch, 2008). It also modulates the LPA-mediated control of neuronal transmission specifically at glutamatergic synapsis *via* the presynaptic LPA_2 receptor (Trimbuch et al., 2009).

However, while phosphatase activity has been shown in LPPs, PRGs lack

Table 1 Reported mRNA expression of lysophosphatidic acid (LPA) receptors and plasticity-related genes (PRGs) in adult mouse brain areas and murine brain cell types



Data were sourced from Bräuer and Nitsch (2008), Velmans et al. (2013), Coiro et al. (2014), Broggini et al. (2016), Suckau et al. (2019). ● Expressed; ● under detection level; ● no data available.

critical amino acids within the conserved domains. This indicates that PRGs are not able to dephosphorylate LPA by the same mechanism that has been proposed for the LPPs. Another member of the family, PRG5, promotes spine formation in primary cultured hippocampal neurons, proposing a specific role in neurodifferentiation processes that are also essential for effective neural regeneration (Coiro et al., 2014).

Brain trauma, cancer and chronic inflammatory diseases leave irreparable damage to the CNS with only limited therapeutical options. Modulating LPA receptor activity can be a tool for addressing the problem of neural regeneration and previous results point to a high number of opportunities. On the other hand, LPA metabolism is characterized by high complexity and a multitude of regulation mechanisms that are still far from being understood. Thus, a key understanding of LPA induced processes and regulation mechanisms is of vital importance before lipid-mediated therapies can be expanded and used as a reliable and effective tool in neural regeneration.

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