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Mini-review

Phosphatidic acid in neuronal development: A node for membrane and cytoskeleton rearrangements

Mohamed-Raafet Ammar, Nawal Kassas, Marie-France Bader, Nicolas Vitale*

Institut des Neurosciences Cellulaires et Intégratives (INCI), UPR-3212 Centre National de la Recherche Scientifique & Université de Strasbourg, 5 rue Blaise Pascal, 67084 Strasbourg, France

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ABSTRACT

Phosphatidic acid (PA) is the simplest phospholipid naturally existing in all-living organisms. It constitutes only a minor fraction of the total cell lipids but has attracted considerable attention being both a lipid second messenger and a modulator of membrane shape. The pleiotropic functions of PA are the direct consequence of its very simple chemical structure consisting of only two acyl chains linked by ester bonds to two adjacent hydroxyl groups of glycerol, whose remaining hydroxyl group is esterified with a phosphomonoester group. Hence the small phosphate head group of PA gives it the shape of a cone providing flexibility and negative curvatures in the context of a lipid bilayer. In addition, the negatively charged phosphomonoester headgroup of PA is unique because it can potentially carry one or two negative charges playing a role in the recruitment of positively charged molecules to biomembranes. In consequence, PA has been proposed to play various key cellular functions. In the brain, a fine balance between cell growth, migration and differentiation, and cell death is required to sculpt the nervous system during development. In this review, we will summarize the various functions that have been proposed for PA in neuronal development.

1. Introduction

During brain development, neurons exhibit various morphological changes including axon and dendrite outgrowth, dendritic branching and spine formation, as well as synapse formation, which are critical events in the establishment of neuronal networks. Because of its complexity and heterogeneity, most molecular aspects of the nervous system development have been studied using cell culture models. These include various types of neurons, but also the rat neural crest derivative PC12 cells that develop neuronal features in response to neuronal growth factor (NGF) treatment.

In culture, hippocampal neurons undergo morphological changes that were first described by Dotti et al. [1]. Shortly after plating, the cells begin to develop lamellipodia around the cell body (Stage 1). The second phase of development is marked by the loss of lamellipodia and the extension of neurites or neurite outgrowth (Stage 2). After 1–2 days *in vitro*, the majority of cells have an asymmetrical morphology characterized by a single and long neurite, which will become the axon, and several extensions shorter than the axon, which will become dendrites (Stage 3 and 4). The last stage (Stage 5) represents the maturation of the axonal and dendritic branching, including dendritic spine formation and synaptogenesis [2,3].

Morphological changes of neurons during development are essentially based on two different cellular mechanisms: i) the reorganization of the cytoskeleton [4] and ii) the enlargement of the cell surface sustained by exocytosis of cytoplasmic vesicles [5].

At the tips of the growing neurites, growth cones are specialized highly mobile cellular compartments consisting of a central region filled with organelles and microtubules and a dynamic peripheral region rich in actin. Growth cone morphology, guidance and motility are driven by cyclical polymerization and depolymerization of actin filaments, whereas the structure of the axon and its elongation rely on microtubules [4,6–8]. Cytoskeletal dynamics also play a key role in dendritic spine formation and maturation. Dendritic spines are small protrusions emerging from parent dendrites that contain postsynaptic structures such as postsynaptic density, actin filaments and microtubules (Reviewed in Ref. [9]). In *vivo*, spines represent the postsynaptic zones that

* Corresponding author.
E-mail address: vitalen@unistra.fr (N. Vitale).

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establish the physical contact with the presynaptic area to form functional synapses that allow the chemical transfer of signal between two neurons.

Morphological maturation of neurons is also accompanied by changes in the membrane surface provided by two membrane trafficking mechanisms: exocytosis and endocytosis. These two mechanisms are highly regulated and provide directional transportation of intracellular membrane components [10]. Indeed, axons or neurites extend sometimes over long distances to reach their synaptic targets. Their elongation requires the increase in membrane surface, which is supported by anterograde vesicular transport from the cell body to the growth cone followed by membrane fusion/exocytosis to ensure the addition of vesicular membrane and surface expansion at the growth cone [5,11,12]. In contrast, endocytosis and the reduction of cell surface is involved in neurite retraction and growth cone collapse [13–15].

Phosphatidic acid (PA) is a pleiotropic lipid that plays an important structural role as a precursor of various glycerophospholipids. PA is also considered as a key player in the transmission, amplification and regulation of a variety of intracellular signaling and cellular functions, such as cell proliferation, vesicle/membrane trafficking and cytoskeletal organization. At the molecular level, PA interacts with various proteins to modulate catalytic activity and/or membrane association [16,17], and these include GTPases, kinases, phosphatases, nucleotide binding proteins and phospholipases [18]. Many PA partners are involved in the regulation of actin dynamics and membrane trafficking and some of them have been involved in neuronal development. In this review, we will summarize the current knowledge linking PA to the cellular/molecular mechanisms that govern neuronal development.

2. Synthesis and degradation pathways for PA

Within cells, PA occurs as a structural intermediate or as a signaling molecule. In mammals, structural PA is synthesized through two acylation reactions: the glycerol 3-phosphate (G-3-P) pathway and the dihydroxyacetone phosphate (DHAP) pathway (Fig. 1). These reactions catalyzed by the glycerol-3-P acyltransferase (G-3-P AT) and the dihydroxyacetone phosphate acyltransferase (DHAP AT) produce a monoacylated form of PA, the lysoPA (LPA) transformed in a second acylation step into PA [19]. PA generated from these *de novo* pathways is a key intermediate in the synthesis of all glycerophospholipids and triacylglycerols. This structural PA is rapidly converted by phosphatidic acid phosphohydrolase (PAP) to

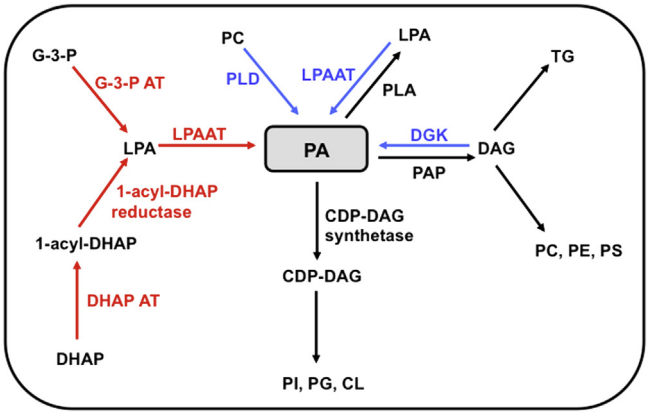

Fig. 1. Biosynthetic pathways of structural and signaling PA. PA is a central phospholipid for biosynthetic and signaling reactions. Red arrows denote biosynthetic reactions that lead to structural PA synthesis whereas formation of signaling pools of PA is shown with blue arrows.

diacylglycerol (DAG), which is an important source for the biosynthesis of triacylglycerols and phospholipids including phosphatidylcholine (PC), phosohatidylethanolamine (PE) and phosphatidylserine (PS) [20]. In addition, PA can be deacylated by phospholipases A (PLA) to LPA or degraded by CDP-DAG synthetase into CDP-diacylglycerol, which is responsible for the synthesis of phosphatidylinositol (PI), phosphatidylglycerol (PG) and cardiolipin (CL) [19] (Fig. 1).

Signaling forms of PA can be formed by three alternative biosynthetic pathways (Fig. 1). The first pathway involves phospholipase D (PLD), which catalyzes the hydrolysis of the distal phosphodiester bond in PC to form PA and choline. PLD preferentially catalyzes a transphosphatidylation reaction to produce phosphatidylalcohols at the expense of PA production in the presence of primary alcohols, such as ethanol and 1-butanol. Measuring the production of labeled phosphatidylalcohols has been until recently an important method used to study cellular functions of PLD [21,22]. Cloning and characterization of genes encoding PLD proteins have identified at first two isoforms (PLD1 and PLD2) in animal cells. Two alternative splicing variants of PLD1 (PLD1a and PLD1b) and three for PLD2 (PLD2a, PLD2b and PLD2c) were subsequently identified. Both PLD isoforms contain two HKD motifs, which are critical for enzymatic catalysis, a phox (PX) domain, a pleckstrin homology (PH) domain and a PIP2 binding domain [23]. In addition, PLD1 contains a "loop domain" that is not found in PLD2 and seems to be involved in the auto-inhibition of PLD1 [24,25]. PLD1 which is characterized by low basal activity, is activated principally by three classes of proteins: ARF GTPases, Rho GTPases and several members of the family of protein kinases including PKCs and RSK2. PLD2 which exhibits a higher basal activity, is also modulated by ARF and Rac proteins [26–28]. More recently, structurally different isoforms of PLD were identified. These include PLD3, PLD4 and a mitochondrial PLD (mito-PLD). Mito-PLD in contrast to classical mammalian PLDs uses cardiolpin as substrate to generate PA. An important structural difference between mito-PLD and PLD1/2 is that mito-PLD has only a single HKD half-catalytic site, requiring it to dimerize to create an active enzymatic complex. Furthermore, mito-PLD displays no PX, PH or PIP2 membrane binding domains and needs its N-terminal sequence for mitochondrial surface anchoring. Regarding PLD3 and PLD4 proteins, although their catalytic activity has not been established to date PLD3 appears to be involved in cell differentiation [29] and PLD4 in phagocytosis of microglial cells [30].

The phosphorylation of DAG using ATP as a phosphate source by DAG kinase (DGK) produces PA. Ten isoforms for mammalians DGK (α, β, γ, δ, η, k, ε, ζ, ι, and θ) have been cloned and characterized. These isoforms are classified in five subtypes and all of them have a conserved catalytic domain and at least two cysteine rich domains. The three type I DGKs (α, β and γ) contain calcium-binding EF hands motifs in their N-termini, while type II (δ, η and k) have pleckstrin homology (PH) domains at their N termini. The type III DGK (ε) has the simplest structure and does not contain regulatory units. The types IV DGK (ζ and ι) have ankyrin repeats at their C termini and a region homologous to the MARCKS protein's phosphorylation site. Finally, DGK θ defines type V, has three cysteine-rich domains and a PH domain [31].

The acylation of LPA by lysoPA-acyltransferases (LPAAT) also leads to PA formation [32]. Six human LPAAT isoforms have been cloned and characterized [33]. The isoforms LPAAT 1 & 2 have 34% sequence homology and the highest LPAAT activity [34]. These isoforms also contain from two to four transmembrane domains and two highly conserved motifs (H (X) 4D and EGT), which are essential for catalytic activity of the family of acyltransferases [35,36]. Interestingly, more proteins displaying LPAAT activities have been recently identified. For instance the protein RIBEYE in