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Review

Neuronal phospholipid deacylation is essential for axonal and synaptic integrity☆

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ABSTRACT

Recessively-inherited deficiency in the catalytic activity of calcium-independent phospholipase A2-beta (iPLA2β) and neuropathy target esterase (NTE) causes infantile neuroaxonal dystrophy and hereditary spastic paraplegia, respectively. Thus, these two related phospholipases have non-redundant functions that are essential for structural integrity of synapses and axons. Both enzymes are expressed in essentially all neurons and also have independent roles in glia. iPLA2β liberates *sn-2* fatty acid and lysophospholipids from diacyl-phospholipids. Ca^{2+}-calmodulin tonically-inhibits iPLA2β, but this can be alleviated by oleoyl-CoA. Together with fatty acyl-CoA-mediated conversion of lysophospholipid to diacyl-phospholipid this may regulate *sn-2* fatty acyl composition of phospholipids. In the nervous system, iPLA2β is especially important for the turnover of polyunsaturated fatty acid-associated phospholipid at synapses. More information is required on the interplay between iPLA2β and iPLA2-gamma in deacylation of neuronal mitochondrial phospholipids. NTE reduces levels of phosphatidylcholine (PtdCho) by degrading it to glycerophosphocholine and two free fatty acids. The substrate for NTE may be nascent PtdCho complexed with a phospholipid-binding protein. Protein kinase A-mediated phosphorylation enhances PtdCho synthesis and may allow PtdCho accumulation by coordinate inhibition of NTE activity. NTE operates primarily at the endoplasmic reticulum in neuronal soma but is also present in axons. NTE-mediated PtdCho homeostasis facilitates membrane trafficking and this appears most critical for the integrity of axon terminals in the spinal cord and hippocampus. For maintenance of peripheral nerve axons, iPLA2β activity may be able to compensate for NTE-deficiency but not vice-versa. Whether agonists acting at neuronal receptors modulate the activity of either enzyme remains to be determined. This article is part of a Special Issue entitled Phospholipids and Phospholipid Metabolism.

1. Introduction

This review focuses on the intracellular deacylation of phospholipids in neurons by two related phospholipases of the patatin family: calcium-independent phospholipase A2-beta (iPLA2β; PNPLA9; Group VIA PLA2) and neuropathy target esterase (NTE; PNPLA6). While both these enzymes are expressed in a variety of tissues and cell types, attention has been attracted to their neuronal functions because mutations in their genes give rise to human neurological diseases. General features of iPLA2β and NTE together with those of other deacylating phospholipases have been the subject of an extensive recent review [1].

2. Neural and neuronal phospholipid composition

Phosphatidylcholine (PtdCho) is the major glycerophospholipid (GPL) in the brain where it is synthesized by the CDP-choline pathway. The rate-limiting step in this pathway is catalyzed by two isoforms of CTP:phosphocholine cytidylyltransferase: CTα and CTβ2, of which the latter is the dominant form in brain [2]. In brain, as in other tissues, turnover of PtdCho by deacylation may be required to modify PtdCho fatty acid composition for spatial and temporal variations of membrane fluidity; to respond either to varying availability of certain fatty acids via the diet or to membrane lipid peroxidation; to liberate particular fatty acids or lysophospholipids with signaling functions; and to restrict total PtdCho levels within a physiologically-optimal range.

Mice deficient in iPLA2β or NTE have been described and will facilitate examination of the roles of these enzymes in maintaining neuronal phospholipid composition. In such investigations it would be useful to have basic information of mouse neuronal phospholipid composition under normal conditions: however, this is not readily available, due primarily to the complexity of neurons and their physiological environment. For example, an adult mouse brain contains ~70 million neurons and ~40 million non-neuronal or glial cells: astrocytes, oligodendrocytes and microglia [3]. Moreover, ~10% of the dry weight of mouse brain is composed of myelin, the lipid-rich, extended plasma membrane of oligodendrocytes that insulates the axons of some neurons [4].

Neurons are highly polarized cells that receive chemical signals from other neurons via synapses on their cell soma or on dendritic processes; this information is converted into an electrical action potential that is conveyed via an axon, and causes release of another

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chemical signal from this axon terminus that synapses with another neuron or, in some instances, with muscle. Different neurons have differing functions and highly variable morphologies. For example, pyramidal neurons in the hippocampus and Purkinje neurons in the cerebellum have enormous dendritic trees, while inter-neurons in various neural locations have relatively short dendrites and axons. Some primary motor neurons in the cerebral cortex project long axons which terminate in the lumbar spinal cord on secondary motor neurons; long axons from some of the latter neurons terminate on muscles in the feet. Pseudo-unipolar sensory neurons with cell bodies in ganglia just outside the spinal cord have two long axonal branches: one that receives sensory information from the periphery (and is, in this manner, equivalent to the dendrites of other neurons); the other axon conveys information to neurons in the brain. In humans, each axon of a dorsal root ganglion sensory neuron can be up to one meter long and maintenance of these huge structures places a heavy metabolic burden on the cell. Moreover, the two axons of the sensory neuron have different local environments: the central axon is myelinated and supported by oligodendrocytes and astrocytes while, for the peripheral axon, both these functions are fulfilled by Schwann cells [5].

Given this heterogeneity, techniques capable of high spatial resolution are being employed increasingly to define phospholipid composition and turnover in neural tissue. MALDI-imaging mass spectrometry (IMS) has been used to visualize the distribution of different polyunsaturated fatty acid (PUFA)-esterified PtdCho in sagittal sections of the mouse brain: by this means it has been shown that arachidonic acid (AA)-containing PtdCho and docosahexaenoic acid (DHA)-containing PtdCho are enriched in cell layers of the hippocampus and in cerebellar Purkinje cells, respectively [6].

Notwithstanding the potential of newer techniques such as IMS, much of the information on the phospholipid composition and turnover of neural tissue has been obtained from assays on whole brain homogenates or sub-cellular fractions enriched in membrane fragments derived from myelin or synaptic plasma membranes: representative data are shown in Table 1. Another approach has been to culture neurons from neonatal or embryonic rodent tissue under conditions that greatly reduce the number of surviving glial cells before phospholipid analysis. PtdCho is the major GPL in cultured rat cerebellar granule neurons [7] as well as murine whole brain [8] (Table 1). However, while GPL-esterified PUFA are present at substantial levels in the whole brain [8], they are barely detectable in the neuronal cultures [7] (Table 1): this is because neurons lack the required desaturases and rely instead on supply of PUFA from glial cells, particularly astrocytes [9]. It is apparent also that PUFA are esterified with phosphatidylethanolamine (PtdEtn) more than with PtdCho [8] and that, in myelin, PtdEtn is the dominant GPL [4] (Table 1).

The observation that synaptic plasma membrane preparations are highly enriched in PUFA-esterified GPL [10] (Table 1) hints at the heterogeneity in GPL composition that exists within different domains of neurons themselves: this may arise by several possible mechanisms, two of which have been studied using cultures of sensory neurons from rodent superior cervical ganglia (SCG). These neurons can be cultured under conditions that allow independent compartmentalized analysis of the ability of soma and axons to incorporate [^{3}H]choline into PtdCho. It has been shown that PtdCho is synthesized in both these compartments [11]. Moreover, while CTα is expressed only in the soma, CTβ2 is also expressed in the distal axon. In CTβ2-null mice [^{3}H]PtdCho synthesis is no longer detected in the distal axon compartment and neurons cultured from these mice show reduced amounts of axonal branching suggesting that locally-synthesized PtdCho may be important for axonal bifurcation [12].

In a different approach, IMS has been used to characterize the PUFA composition of PtdCho in SCG explant cultures that contain glial cells as well as neurons. While only very low levels of PUFA-esterified PtdCho were present in the ganglia (*i.e.*, in neuronal soma), levels several-fold higher were found in the axons and increased in a proximal to distal fashion. Evidence was presented that this gradient of PUFA-esterified PtdCho was maintained by energy- and actin-dependent transport [13]. Thus, local PtdCho synthesis and specific transport mechanisms may contribute to GPL heterogeneity within different regions of neurons.

The foregoing demonstrate that useful preliminary data on brain phospholipid composition and some aspects of its turnover have been obtained from experiments involving measurement of various GPL species in homogenates or subcellular fractions from whole brain or cultured neurons, in the presence or absence of radiolabelled precursors such as choline or PUFA. Such methods have been used to examine the biochemical consequences of inactivating iPLA2β or NTE and these will be described later in this review. However, to discern phospholipid turnover in the major compartments (soma, axon, dendrites) of neurons under physiologically-realistic conditions – with local provision of PUFA from glia – presents substantial technical challenges for the future.

Table 1
Rodent brain tissue preparations: glycerophospholipid (GPL) composition.

Preparation	PtCho/PtdEtn/(PtdSer + PtdIns)	GPL, PUFA (mol%)	Ref.
Whole brain	0.45/0.37/0.18	PtdCho, 7; PtdEtn, 28	[8]
Myelin	0.35/0.49/0.16	All GPL, 59	[4]
Synaptic PM	0.51/0.32/0.17	PtdCho, 48; PtdEtn, 93	[10]
Cultured neurons	0.56/0.34/0.10	All GPL, <5	[7]

PtdCho, phosphatidylcholine; PtdEtn, phosphatidylethanolamine; PtdSer, phosphatidylserine; PtdIns, phosphatidylinositol; PUFA, poly-unsaturated fatty acid; PM, plasma membranes.

3. Neural phospholipid deacylation: iPLA2β and NTE

To consider the contributions of iPLA2β and NTE to GPL deacylation in the brain, the remainder of this review describes their biochemistry and their distribution in, and consequences of their deletion from, the nervous system (Table 2).

3.1. Biochemistry of iPLA2β and NTE

3.1.1. The domain architectures of iPLA2β and NTE

A region of ~160 amino acids within the catalytic (patatin-like) domains of the two enzymes has 28% sequence identity, including key serine and aspartate residues, and a putative nucleotide binding motif, GXGXXG (Fig. 1; for alignment see [14]). The N-terminal domain of iPLA2β is dominated by 8 ankyrin repeats that may allow tetramerization of the enzyme [15]. Two catalytically-active isoforms of iPLA2β result from alternative splicing such that the larger form (type 2), but not the smaller (type 1) has a 54-amino acid insertion in its eighth ankyrin repeat. Type 2- but not type 1-iPLA2β is activated by ATP [16] and associates with particulate rather than soluble subcellular fractions [17]. A region of ~140 residues near the C-terminus of iPLA2β has multiple contact points for binding Ca^{2+}-calmodulin [18] (Fig. 1).

At its N-terminus NTE has a single transmembrane segment that serves to tether the protein to the cytoplasmic face of the endoplasmic reticulum (ER) (Fig. 1; [19]). Two regions within the N-terminal putative regulatory domain of NTE (183–318 and 494–745) have modest (~10% and ~16%) identity to the cAMP-binding regulatory subunit of protein kinase A (PKA) (Fig. 1): to date, no evidence has been reported that these regions functionally bind cAMP (see below). A short region (130–158) closer to the N-terminus of human NTE has homology to the motif on the PKA regulatory subunit that mediates binding to the catalytic subunit (Fig. 1). Recombinant polypeptides containing the equivalent region in Swiss cheese protein (SWS), the *Drosophila* NTE orthologue, bind to the catalytic subunit of PKA-C3 but not