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Phosphatidic acid and neurotransmission

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Abstract

Lipids play a vital role in the health and functioning of neurons and interest in the physiological role of neuronal lipids is certainly increasing. One neuronal function in which neuronal lipids appears to play key roles in neurotransmission. Our understanding of the role of lipids in the synaptic vesicle cycle and neurotransmitter release is becoming increasingly more important. Much of the initial research in this area has highlighted the major roles played by the phosphoinositides (PtdIns), diacylglycerol (DAG), and phosphatidic acid (PtdOH). Of these, PtdOH has not received as much attention as the other lipids although its role and metabolism appears to be extremely important. This lipid has been shown to play a role in modulating both exocytosis and endocytosis although its precise role in either process is not well defined. The currently evidence suggest this lipid likely participates in key processes by altering membrane architecture necessary for membrane fusion, mediating the penetration of membrane proteins, serving as a precursor for other important SV cycling lipids, or activating essential enzymes. In this review, we address the sources of PtdOH, the enzymes involved in its production, the regulation of these enzymes, and its potential roles in neurotransmission in the central nervous system.

Introduction

One of the more exciting developments in understanding regulated neurotransmission has been the increasing interest in the role of lipids (Davletov and Montecucco, 2010, Gauthier-Kemper et al., 2015, Huttner and Schmidt, 2000, Koch and Holt, 2012, Lauwers et al., 2016, Lim and Wenk, 2009, Milovanovic and Jahn, 2015, Posor et al., 2015, Puchkov and Haucke, 2013, Rohrbough and Broadie, 2005, Stutz and Horvath, 2015, Wen et al., 2012). Proper neuronal function depends on neurotransmission to occur in a rapid and reliable manner (Farsad and De Camilli, 2002, Fernandez-Alfonso and Ryan, 2006, Haucke et al., 2011, Kavalali, 2006). It is recognized that this is largely accomplished via a coordinated mechanism at the presynaptic bouton referred to as the synaptic vesicle (SV) cycle where exocytosis of neurotransmitter loaded vesicles is followed by a synchronized endocytosis of

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empty vesicles, which are refilled and subsequently re-released (Falcieri et al., 1992, Royle and Lagnado, 2010, Schweizer and Ryan, 2006, Sudhof, 2004). Many previous studies led to the discovery of an array of proteins responsible for regulating this cycle and it is now recognized that lipids are also important mediators for SV recycling (Ammar et al., 2013, Cremona and De, 2001, Cremona et al., 1999b, Huttner and Schmidt, 2000, Lim and Wenk, 2009, Puchkov and Haucke, 2013, Rohrbough and Broadie, 2005, Tu-Sekine et al., 2015).

As a result of the research to examine the role of lipid involvement in neurotransmission, it is now clear that phosphoinositides (PtdIns), diacylglycerol (DAG), and phosphatidic acid (PtdOH), play key roles (Davletov and Montecucco, 2010, Lauwers, Goodchild, 2016, Lim and Wenk, 2009, Rohrbough and Broadie, 2005, Schwarz et al., 2011, Tu-Sekine, Goldschmidt, 2015, Tu-Sekine and Raben, 2011, Yuan et al., 2015). Given their popularity, it is understandable that PtdIns(4,5)P₂ and PtdIns(3,4,5)P₃ have received a great deal of attention (see (Cremona et al., 1999a, Davletov and Montecucco, 2010, Di Paolo and De Camilli, 2006, Di Paolo et al., 2004, Lauwers, Goodchild, 2016, Lim and Wenk, 2009, Martin, 2015, Micheva et al., 2001, Puchkov and Haucke, 2013). In addition to these lipids, now there is also rather convincing evidence for the roles DAG plays in evoked exocytosis of SV vesicles, largely via its well-documented ability to activate PKCs (Antal and Newton, 2014) or recruit munc-13 (Betz et al., 1998, Rhee et al., 2002). New data suggest that a gradient of this lipid may also be involved in modulating evoked endocytosis (Yuan, Liu, 2015).

While not receiving a lot of the initial attention, there are now compelling data implicating PtdOH in modulating exocytosis and endocytosis (Antonescu et al., 2010, Burger et al., 2000, Chasserot-Golaz et al., 2010, Lim and Wenk, 2009, Paillart et al., 2003, Puchkov and Haucke, 2013, Rohrbough and Broadie, 2005, Schwarz, Natarajan, 2011, Takei et al., 1998, Xie et al., 2015) although its precise role is not well defined. This lipid could play roles by altering membrane architecture necessary for membrane fusion (see (Lauwers, Goodchild, 2016, Rohrbough and Broadie, 2005)), mediating membrane penetration of proteins such as dynamin (Burger, Demel, 2000, Takei, Haucke, 1998); (see (Doherty and McMahon, 2009, Ferguson and De Camilli, 2012, Kokotos and Cousin, 2015)), serving as a precursor for other important SV cycling lipids such as PtdIns(4,5)P₂, or activating enzymes such as a PI kinase (Jenkins et al., 1994, Moritz et al., 1992) needed for the synthesis of this lipid (Thieman et al., 2009, Volpicelli-Daley et al., 2010). In this review, we address the sources of PtdOH, the enzymes involved in its production, the regulation of these enzymes, and its potential roles in neurotransmission in the central nervous system.

Sources and Enzymes

There are four major routes of cellular PtdOH production (Figure 1): a) *de novo* synthesis, b) phospholipase D (PLD)-mediated hydrolysis of a phospholipid, most likely phosphatidylcholine (PtdCho), c) a diacylglycerol kinase (DGK) catalyzed phosphorylation of diacylglycerol (DAG), or d) an acylation of lysoPtdOH catalyzed by lysoPtdOH acyl transferase (LPAAT). All four will be discussed and their role in generating PtdOH to modulate the SV cycle will be addressed.

In neurons, as in most tissues, PtdOH is an intermediate in the synthesis of phospholipids. PtdOH is generated via the acylation of glycerol-3-phosphate. This metabolic intermediate can be generated via reduction of dihydroxyacetone-3-phosphate or via the phosphorylation of glycerol catalyzed by glycerol kinase (except in adipocytes which lack this enzyme). While this is a well-studied process, there is no evidence that the *de novo* synthesis of PtdOH plays a role in SV cycling.

Six isoforms of PLDs have been identified based on sequence homology (Frohman, 2015). Of these, PLD1 and PLD2 are the major isoforms found in neuronal tissues (Kim et al., 2007). Their precise roles in SV cycling and neurotransmitter release remains uncertain. PLD2 appears to play a role in the endocytosis of some receptors (Bhattacharya et al., 2004, Du et al., 2004, Koch et al., 2006, Padron et al., 2006, Rankovic et al., 2009) but more data are needed to suggest it plays a role in SV cycling. In contrast, there is evidence that PLD1 is involved in neurotransmitter release (see (Humeau et al., 2001, Klein, 2005, Rohrbough and Broadie, 2005, Sun et al., 2013)) and in dendritic branching? PLD1 is a mammalian synaptosomal protein (Chalifa et al., 1990) that interacts with synaptosome-specific proteins (Chung et al., 1997, Lee et al., 1997). More directly, Humeau et al. showed PLD1, but not PLD2, induced neurotransmitter release from *Aplysia californica* neurons (Humeau, Vitale, 2001). Additionally, this isoform has been implicated in the sensitivity to pain in response to prenatal stress (Sun, Gooding, 2013). PLD2, on the other hand, has been implicated in regulating the glutamate transporter (Mateos et al., 2012) the internalization of mGluR (Bhattacharya, Babwah, 2004) and the synaptotoxic action of the A β protein in Alzheimer's disease (AD). Interestingly, ablation of PLD2 rescues memory deficits and provides synaptic protection in an AD transgenic mouse model (SwAPP) (Oliveira et al., 2010). A nice review of the role of PLD2 in neurological dysfunctions has been recently presented (Ghim et al., 2016).

Another class of enzymes implicated in generating PtdOH in neurons is the DGKs. Of the ten known DGKs, all are found in the brain with nine of them in neurons. Only DGK- α is not found in neurons but in glia type cells (Ishisaka and Hara, 2014, Tu-Sekine and Raben, 2011). These enzymes are particularly interesting as their substrate, DAG, is a product of phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P₂) hydrolysis which is also an important neuronal lipid. This suggests a scenario whereby DAG generated by a phosphoinositide specific phospholipase C (PtdIns-PLC)-mediated hydrolysis of PtdIns(4,5)P₂ serves as the substrate for a DGK-mediated production of PtdOH. In a recent study, we showed one isoform, DGK- θ , is important for modulating evoked SV endocytosis. Ablation of DGK- θ suppressed evoked endocytosis, which is accentuated with increasing strength and duration of action potentials. This defect is rescued by catalytically active DGK- θ but not an inactive enzyme (Goldschmidt et al., 2016). These data demonstrate that the conversion of DAG to PtdOH catalyzed by DGK- θ plays a role in SV cycling. Another DGK isoform implicated in neurotransmission is DGK- κ . Yang et al. provided data suggesting this isoform plays a role in mGluR-dependent long-term depression (Yang et al., 2011). The authors of this report indicated that DGK- κ ablation affects presynaptic release probability. This effect, however, was only observed when a number of data sets were pooled. Furthermore, induced (DHPG, dihydroxyphenylglycine) levels of PtdOH were not significantly altered in the DGK- κ KO neurons. While intriguing, more work is required to define the role of DGK- κ in neurons.

DGK- ϵ has also been implicated in modulating long-term potentiation and seizure susceptibility (Cole-Edwards and Bazan, 2005, Goldschmidt, Tu-Sekine, 2016, Musto and Bazan, 2006, Rodriguez de Turco et al., 2001), and may play a role in Huntington disease (Zhang et al., 2012) but the molecular mechanisms remain obscure. Finally, a recent report suggest DGK- κ associates with the fragile X mental retardation protein (FMRP) in neurons and suggest this enzyme may indirectly impact on the pathology of fragile X syndrome (Tabet et al., 2016).

Another route for PtdOH production in neurons is via the acylation of a lysoPtdOH catalyzed by a lysoPtdOH acyltransferase (LPAAT). This enzyme appears to play an important role in ribbon synapses (Schwarz, Natarajan, 2011). These synapses are found in certain sensory neurons requiring large amount of vesicle recycling in a fast and efficient manner; photoreceptor cells, retinal bipolar cells, and inner ear hair cells. One of the distinguishing features of these neurons is their high rates of synaptic vesicle exocytosis and vesicle release (Wan and Heidelberger, 2011). The name of these synapses derives from a rather unique presynaptic structure that is perpendicular to the plasma membrane, called the synaptic ribbon, on which are found a large number of synaptic vesicles. These vesicles are primed for exocytosis and represent a readily releasable pool of synaptic vesicles. One of the critical proteins in this ribbon is termed RIBEYE. This protein has both a structural and enzymatic role. Its enzymatic activity was shown to be an LPAAT generating PtdOH important for exocytosis at these synapses (Schwarz, Natarajan, 2011). We note that endophilin I, a protein involved in endocytosis that interacts with synaptojanin and dynamin, was once believed to possess LPAAT activity (Schmidt et al., 1999). This conclusion, however, is at best controversial as this activity was later shown to be an artifact (Farsad et al., 2001, Gallop et al., 2005).

Regulation of Phosphatidic Acid Producing Enzymes

PtdOH may play a variety of roles in the nervous system (below). Given this, it is becoming more important to understand the factors controlling the localization and regulation of the enzymes involved in its production. Regulated de novo synthesis or LPAAT activity (above) have not been addressed and the regulation of these PtdOH pathways does not appear to be involved in neurons. There are data, on the other hand, supporting roles for the regulation of PLDs and DGKs in neurons. A recent review outlines some of regulatory roles of these enzymes are outlined in a recent review (Tu-Sekine, Goldschmidt, 2015).

The observations that PLD and DGK (Davletov and Montecucco, 2010, Humeau, Vitale, 2001, Liu et al., 2005, Rohrbough and Broadie, 2005, Waring et al., 1999)(Almena and Merida, 2011, Kanoh et al., 2002, Merida et al., 2008, van Blitterswijk and Houssa, 2000)) play roles in neurotransmitter release strongly implicate this class of enzymes in modulating the levels of PtdOH in neurons (Cremona, Di, 1999b, Davletov and Montecucco, 2010, Goldschmidt, Tu-Sekine, 2016, Huttner and Schmidt, 2000, Lauwers, Goodchild, 2016, Lim and Wenk, 2009, Puchkov and Haucke, 2013, Rohrbough and Broadie, 2005, Schwarz, Natarajan, 2011, Tu-Sekine, Goldschmidt, 2015, Tu-Sekine and Raben, 2011, Yuan, Liu, 2015). Much has been written about the regulation of PLDs and has been the subject of numerous other reviews (Bruntz et al., 2014, Exton, 2002a, b, Klein, 2005, Lauwers,

Goodchild, 2016, Lee, Kang, 1997, Lee et al., 2000, Sarri et al., 1998, Selvy et al., 2011, Vitale et al., 2005, Waring, Drappatz, 1999, Wenk and De Camilli, 2004). With respect to neurons, the regulated metabolism of PtdIns(4,5)P₂ is involved in neurotransmission and this metabolism is also involved in the regulation of a PLD (PLD1) (Powner and Wakelam, 2002). It is also interesting that a number of synaptic vesicle proteins are also implicated in regulating PLDs and this was nicely summarized in a review by Bruntz et al (Bruntz, Lindsley, 2014). At the present time, however, there are no compelling data regarding the precise proteins or mechanisms by which these proteins regulate a PLD in a physiological manner. In addition to the synaptic proteins, other proteins have been shown to regulate PLDs. PLD1, for example, is activated by small GTP-binding proteins such as ARF and Rho proteins (Exton, 1999, 2000, 2002a, b, Frohman, 2015, Powner and Wakelam, 2002). A number of proteins have also been shown to interact with PLD2 (Gomez-Cambronero, 2011) and phosphorylation may also play a role in regulating PLD1 and PLD2 (Henkels et al., 2010).

In 2011, we provided a perspective regarding the localization, function and regulation of neuronal DGKs (Tu-Sekine and Raben, 2011). Very little progress has been made regarding the neuronal roles and regulation of catalytic activity of these enzymes with the exception of DGK- θ . Shortly after this review was published, we provided evidence for a polybasic protein in regulating DGK- θ (Tu-Sekine and Raben, 2012), but the precise protein(s) in neurons has not been identified. Interestingly, DGK- θ 's activity is also increased in the presence of its product PtdOH (Tu-Sekine et al., 2007, Tu-Sekine and Raben, 2012), suggesting a feed-forward regulation in neurons.

As noted above, Rho proteins, such as RhoA, are known to activate PLD1 (Exton, 1999, 2000, 2002a, b, Frohman, 2015, Powner and Wakelam, 2002). It is particularly interesting that RhoA also modulates DGK- θ by inhibiting its activity (Houssa et al., 1999). This tempts the suggestion that RhoA may reciprocally regulate PLD1 and DGK- θ in neurons although this hypothesis has not been fully tested. In support of this notion, however, RhoA is also implicated in regulating neurotransmission (Hiley et al., 2006, McMullan et al., 2006, Wang et al., 2005), and associates with PLD1 in a light-dependent manner in photoreceptor rod outer segments (Salvador and Giusto, 2006).

Neuronal Roles of Phosphatidic Acid

Perhaps the most challenging question with regard to PtdOH in neurotransmission pertains to defining its precise role. The role of this lipid in neurotransmission is often ascribed to its reported ability to serve as a “fusogenic” lipid in that it alters membrane architecture to assist in membrane fusion. PtdOH, however, may also affect the localization and activities of proteins. While a full review of these properties is beyond the scope of this review, studies by Kooijman and colleagues have provided some of the most exciting studies and reviews on this topic (Kooijman and Burger, 2009, Kooijman et al., 2005a, Kooijman et al., 2003, Kooijman et al., 2005b, Kooijman et al., 2007, Loew et al., 2013) and the reader is referred to these articles for a more in depth discussion.

The notion that PtdOH represents a fusogenic lipid has probably attracted the most attention with respect to neurotransmitter release. This property of PtdOH is largely ascribed to its well-documented tendency to generate non-bilayer, hexagonal II phase, membrane domains particularly in the presence of calcium ions (Jouhet, 2013). The ability of PtdOH to be fusogenic is in part due to the fact that it is one of the cone-shaped lipids known to promote negative membrane curvature and fusion. This is important for neurotransmission where vesicles have a large positive curvature and the formation of non-bilayer phases may help to lower the energy needed for fusion (Kozlovsky et al., 2002).

PtdOH also plays an important role in neurotransmission by binding specific proteins. Further, some of proteins essential for the exocytosis/endocytosis of synaptic vesicles localize to regions of membrane curvature (e.g. see (Jung et al., 2010, Martens, 2010, Milosevic et al., 2011, Shen et al., 2014)). PtdOH mediates the membrane penetration of dynamin (Burger, Demel, 2000, Takei, Haucke, 1998); a protein involved in SV endocytosis (see (Doherty and McMahon, 2009, Ferguson and De Camilli, 2012, Kokotos and Cousin, 2015)) and binds to syntaxin1A, a protein known to be involved in vesicle exocytosis. This protein binds and actually recruits PtdOH to vesicle release sites (Aalmo et al., 1984). A functional interaction between PtdOH and syntaxin1A is believed to be necessary for regulating the energetics of membrane fusion (Lam et al., 2008). Indeed, mutations that disrupt the recruitment of PtdOH suppress vesicle secretion in PC12 cells and overexpression of PLD1 overcomes the secretory defect (Lam et al., 2008). As indicated above, PtdOH activates a PI kinase (Jenkins, Fiset, 1994, Moritz, De Graan, 1992) needed for the synthesis of another lipid important for neurotransmission- PtdIns(4,5)P₂ (Thieman, Mishra, 2009, Volpicelli-Daley, Lucast, 2010).

Summary

One of the captivating aspects of scientific research is the well-known phenomenon that as we discover answers we illuminate more questions. Our recognition regarding the role of PtdIns(4,5)P₂ in neurons has led to question the roles of its metabolites DAG and PtdOH. Given the popularity of PtdIns(4,5)P₂ turnover and the generation of DAG we've learned how important both of these lipids are to neurotransmission. We are now also increasing our appreciation for the potential role of another metabolite PtdOH and becoming more interested in the other pathways responsible for generating and regulating the levels of this lipid. PtdOH plays a number of roles in establishing important membrane architectural changes, binding specific proteins, and modulating essential enzyme activities. Both DGKs and PLDs appear to play important roles in neuronal functions such as modulating the SV cycle (Figure 2), while LPAAT may play a role in very specialized neurons such as sensory neurons with ribbon synapses. Clearly, this field is ripe for further discoveries.

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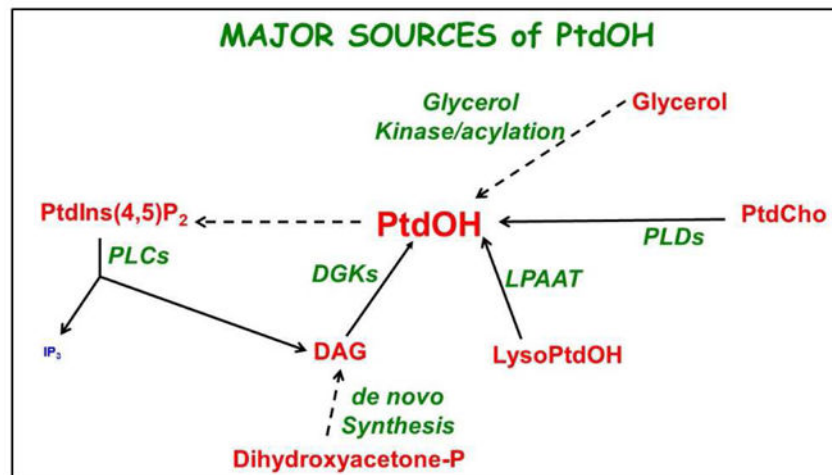


Figure 1. Sources of Phosphatidic Acid

PtdIns(4,5)P₂ = phosphatidylinositol-4,5-bisphosphate; PtdOH = phosphatidic acid; lysPtdOH = lysophosphatidic acid; PtdCho = phosphatidylcholine; DGK = diacylglycerol kinase; PLD = phospholipase D; PLC = phospholipase C; LPAAT = lysophosphatidic acid acyltransferase.

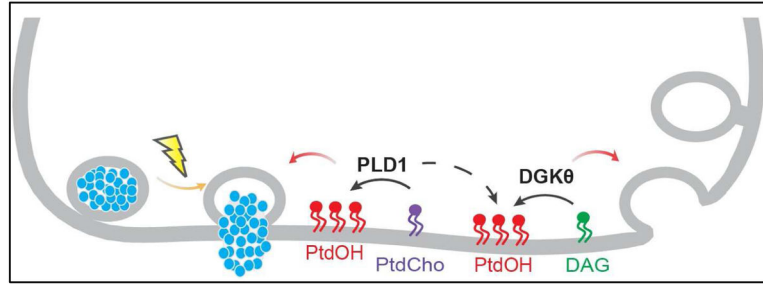


Figure 2.
Proposed Roles of PLD1 and DGK- θ Generated PtdOH in the SV Exocytosis and Endocytosis.