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NAPE Phospholipase D Enzyme and Recent Advances in the Understanding of its Biological Properties

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Abstract: ***N-acylethanolamines (NAEs) are lipids obtained from the membranes that are used as signalling molecules in the nervous system for e.g the endocannabinoid anandamide. An N-acyl phosphatidylethanolamine phospholipase D (NAPE-PLD) that catalyzes formation of NAEs was recently identified as a member of the zinc metallohydrolase family of enzymes. Immunocytochemical analysis has revealed intense NAPE-PLD immunoreactivity in the axons of granule cells. Substantial NAPE-PLD immunoreactivity was also detected in axons of the vomeronasal nerve that project to the accessory olfactory bulb. NAPE-PLD expression was detected in other brain regions also e.g., cortex, hippocampus, thalamus, hypothalamus. It is suggested that NAEs generated by NAPE-PLD in axons may act as anterograde synaptic signaling molecules that regulate the activity of postsynaptic neurons. This article tries to encompass some of the recent advances in this area.***

Keywords: NAPE-PLD, enzyme, endocannabinoid, Anandamide, neuron

1. Introduction

NAPE lipid species that incorporate oleic acid at the amine position generate OEA uponhydrolysis of their distal phosphodiester bond [1-2]. This reaction is catalyzedby a NAPE-selective phospholipase D (NAPE-PLD), which was purified and molecularlycloned in the laboratory of Natsuo Ueda [3]. NAPE-PLD cleaves different members of theNAPE family with similar efficiency and is broadly expressed in mammalian tissues [3]. The second step in the canonical pathway is the release of NAE from NAPE by a PLD-type enzyme known as NAPE-PLD [4]. cDNA cloning revealed that NAPE-PLD belongs to the metallo-b-lactamase family and is molecularly distinguished from the known PLD isoforms, which hydrolyze common glycerophospholipids such as phosphatidylcholine (PtdCho) to produce phosphatidic acid (PA), an intracellular signal molecule [5-6].It has been cloned from mouse, rat, and human and is 393-396 amino acids in length, with an estimated molecular weight of 46 kDa. Both NAPE-PLD mRNA and protein activity have been detected in a wide range of tissues with the highest levels in brain, kidney, and testis [7-8]. In rat, NAPE-PLD activity in the brain is low in neonates and is 15-fold higher in adults, whereas the activity remains constant in the heart during development.NAPE-PLD appears to contain catalytically essential zinc, and the purified recombinant enzyme is specific for NAPE, beingalmost inactive with major glycerophospholipids such as PtdCho and PtdEtn [9]. At this time, NAPE-PLD is the sole enzyme in animal tissues known to directlyrelease NAE from NAPE. This is in marked contrast to plants, which lack NAPE-PLD, but express otherPLD isoforms, such as PLDb and PLDc, thathydrolyze different glycerophospholipids, including NAPE [9]. NAPE-PLD is tightly bound to membranes. The soluble enzyme prepared by treatment with detergent can be stimulated by millimolar concentrations of divalent cations, including Ca^{2+} and Mg^{2+} [10], and 10–100 µM PtdEtn [11].

Ethanol consumption and NAPE-phospholipase D

Excessive ethanol drinking has deleterious effects on the bra as well as human behaviour. However, the effects of alcohol on microglia, the main mediator of the brain's innate immune response are not properly understood. On the other hand, the endocannabinoid system plays a pivotal role in regulating microglial reactivity and function. In a recent study the effects of acute ethanol exposure to murine BV2 microglial cells on N-acyl phosphatidylethanolamine-phospholipase D (NAPE-PLD), a major synthesizing enzyme of anandamide and related N-acylethanolamines. It was found that the ethyl alcohol downregulated microglial NAPE-PLD expression by activating cAMP/PKA and ERK1/2 [12]. These signalling pathways converged on increased phosphorylation of CREB. Moreover, ethanol induced an increase in histone acetyltransferase activity which caused higher levels of acetylation of histone H_3.

Central nervous expression of the NAPE-PLD and relationship with other lipids:

N-acylethanolamines (NAEs) are biosynthesized from phospholipids of the cell membrane assisted by NAPE-PLD hydrolysis. In a recent study, it was shown that a variation was there in the expression of this membrane associated synthesis enzyme, NAPE-PLD, despite its well-preserved sequence from rodents to humans [13]. In the mouse, NAPE-PLD follows the same pattern of expression as CB1R and FAAH, except that it is not found in rods. Moreover, unlike the mouse but like the primate, the rodent tree shrew has a high expression of NAPE-PLD in outer nuclear layer (ONL) and outer plexiform layer (OPL). Studies on primates show for the first time that NAPE-PLD expression in monkeys is exclusively restricted to the photoreceptor layer. Unlike CB1R, NAPE-PLD is ubiquitously expressed in the rat brain with the maximum levels in the thalamus [14]. Besides its role in the endocannabinoid (eCB) biosynthesis, many other physiological roles have been linked to NAPE-PLD such as anti-inflammatory effect [15], anorexic effect

[16], and pro-apoptotic effect [17]. Moreover, the NAE products in axons suggest a role in the regulation of postsynaptic neuron activity as anterograde synaptic signaling molecules [18]. This pattern of expression also implicates alternative direct role of NAEs in primate phototransduction.Long-term depression (LTD) of synaptic transmission dependent on retrograde endocannabinoid signaling (eCB-LTD) has been found in several areas of the brain. eCB-LTD occurs at excitatory synapses in the dorsal striatum, nucleus accumbens, cerebral cortex, dorsal cochlear nucleus,cerebellum, and hippocampus, and at inhibitory synapses in the hippocampus, amygdala and ventral tegmental area [19-20].Endocannabinoid-LTD is induced mostly by repetitive afferent stimulation with or without postsynaptic depolarization, and also by postsynaptic firing and TRPV1 mediated Long term depression (Refer Fig.1). It appears that NAPE-PLD enzymes may contribute effectively in deep central anaesthesia and their role in onset and maintenanace of anaesthesia needs to be probed.

Anandamide and 2-AG and role of NAPE-PLD and lipid metabolizing enzymes:

The endocannabinoid, Anandamide is produced in a two-step process involving N-arachidonoylation of the membrane phospholipid, phosphatidylethanolamine, to form N-arachidonoyl phosphatidylethanolamine (NAPE) by a calcium-dependent N-acyltransferase, followed by hydrolysis by a NAPE-selective phospholipase D (NAPE-PLD) to form N-arachidonoylethanolamine (anandamide) [21]. Anandamide levels are regulated by its breakdown through the action of fatty acid amide hydrolase (FAAH) [22]. 2-arachidonoyl glycerol (2-AG) is synthesized in a two-step process, in which diacylglycerol (DAG) is first produced by the phospholipase C from inositol phospholipids, followed by the hydrolysis of DAG to 2-AG by plasma membrane-associated sn1-DAG lipase (DAGL) [23]. Once formed, 2-AG levels are regulated by monoacylglycerol lipase (MAGL), which is about ~85% of the hydrolysis and by α/β hydrolase domain containing 6 (ABHD6) and ABHD12, which also hydrolyzes 2-AG to arachidonic acid and glycerol[24] In addition to hydrolysis, 2-AG is acted on by cyclooxygenase-2 [25] and lipoxygenase [26], to form prostaglandin glyceryl esters and other related bioactive compounds.

Enzyme assays of the NAPE-PLD isozymes:

The phospholipase-D activities of purified NAPE-1 and NAPE-2 can beestimated in 200 µL Tris buffer (100 mM, pH 7.6) containing 0.5 mM dodecylmaltoside and 1 mM $CaCl_2$ and using*N*-arachidonoyl PE or *N*-palmitoyl PE as a substrate. Predetermined amounts of the substrate are dried and re-suspended in the buffer mixture followed by addition of 1 µg of purified protein. The reactions are carried out for 4 hours at 20°C, and terminated by extraction twice with 400 µL hexane. Lipid extracts are dried down, derivatized with BSTFA and analyzed by GC-MS as described previously [27]. AEA-*d*4 and PEA-*d*4 are used as a standard. NAPE-phospholipase D can also be determined by a radiochromatographic assay [28].

Crystal structure of NAPE-PLD

The crystal structure of human NAPE-PLD has revealed that this enzyme has adapted to perform basically three key processes needed to initiate FAE signaling in cells. First, an extended lipid-binding surface spanning the NAPE-PLD dimer – unprecedented in the MβL superfamily – allows the enzyme to associate with membranes. Secondly, a hydrophobic nook facing the lipid bilayers provides a recognition filter for NAPEs as glycerophospholipid class, while simultaneously accommodating different *N*-acyl substituents. The enzyme has a positively charged zinc center that overlooks the membrane, which provides an attraction point for anionic NAPEs and helps coordinate their hydrolysis [29]. Cryo-electron microscopy studies show that NAPE-PLD is found mainly in proximity of cellular membranes. This is consistent with the presence in the structure of an extended hydrophobic surface that spans both protein dimer subunits and includes residues of the N-terminus, loop L1, helix α3 and loop L3. At the membrane interface, the NAPE-PLD dimer forms a hydrophobic nook that has two molecules of PE, one bound to each monomer . This phospholipid constitutes about ~75-80 mol % of the cell membranes of E. coli [30] that was used to produce the recombinant protein for crystallization. PE is not a true substrate for NAPE-PLD, but its structural similarity with NAPE suggests that it occupies a portion of the site reserved to this molecule.

2. Discussion and Conclusion

Although endocannabinoids i.e (N-palmitoylethanolamine, anandamide, N-oleoylethanolamine, and N-stearoylethanolamine) signalling is involved in various forms of short-term and longterm synaptic modulation, it remains largely unknown how each form contributes to particular aspects of neural functions [31]. Future studies will elucidate the molecular, cellular and neural circuit mechanisms as to how endocannabinoid-mediated synaptic modulation contributes to various brain functions and the pathophysiology of neuropsychiatric disorders. There are several studies suggesting the role of NAPE-PLD enzyme products in the regulation of gastrointestinal homeostasis as even bile acids bind with high affinity to selective pockets in this cavity, enhancing dimer assembly and enabling catalysis. NAPE-PLD facilitates crosstalk between bile acid signals and lipid amide signals[32]. Whether this is just due to the local expression of endocannabinoids in the gastrointestinal or through central formation and henceforth transport to the peripheral system needs to be properly explored (Refer Fig.2).A novel desketoraloxifene-based scaffold that inhibited not only the two mammalian PLDs but also structurally divergent PldA and NAPE-PLDhas recently been described by experimental studies [33]. This finding represents an important first step toward the development of small molecules possessing universal inhibition of divergent PLD enzymes to advance the field. This could be an important step inblocking the synthesis of endocannabinoids for any possible experimental and therapeutic intervention.Thus it appears that products of the NAPE-PLD system may be involved in the response of human body to external stress, xenobiotics, in hormonal control and in anti-nociception and anaesthesia [34-35].

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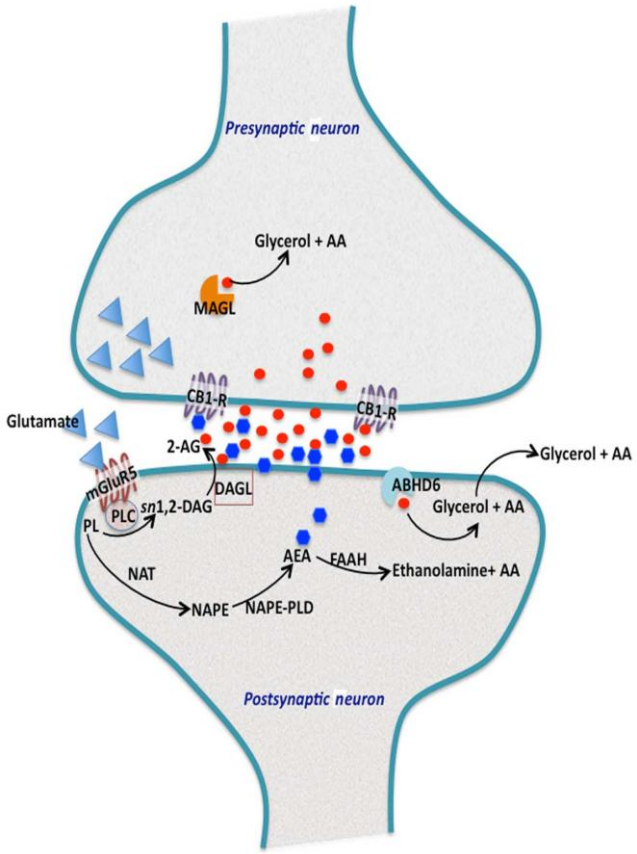

Figure 1: The endocannabinoid system and NAPE-PLD(Courtesy: Wen-Juan Huang, Wei-Wei Chen, Xia Zhang, Mol.Med.Reports, 2016, 14 (4) 2899-2903)

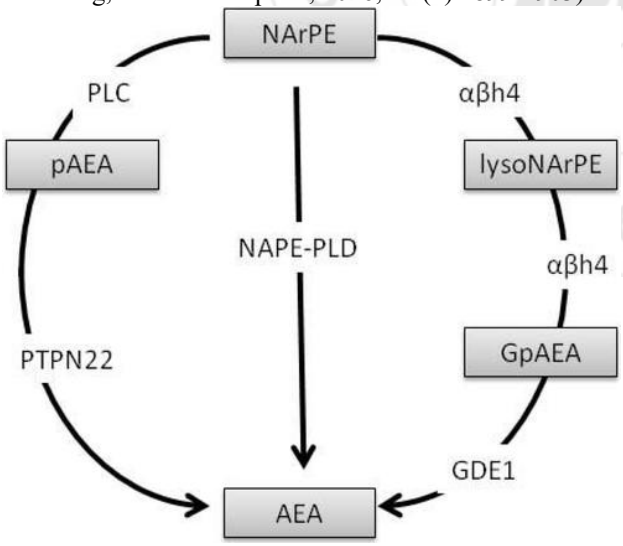

Figure 2: Biosynthetic pathway of N-Acylphosphatidylethanolamine (Courtesy: DR Sagar et al, . Molecular Pain. 2009,5:59)

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