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Enzymatic formation of *N*-acylethanolamines from *N*-acylethanolamine plasmalogen through *N*-acylphosphatidylethanolamine-hydrolyzing phospholipase D-dependent and -independent pathways

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ABSTRACT

Bioactive *N*-acylethanolamines include anandamide (an endocannabinoid), *N*-palmitoylethanolamine (an anti-inflammatory), and *N*-oleoylethanolamine (an anorexic). In the brain, these molecules are formed from *N*-acylphosphatidylethanolamines (NAPEs) by a specific phospholipase D, called NAPE-PLD, or through NAPE-PLD-independent multi-step pathways, as illustrated in the current study employing NAPE-PLD-deficient mice. Although *N*-acylethanolamine plasmalogen (1-alkenyl-2-acyl-glycero-3-phospho(*N*-acyl)ethanolamine, pNAPE) is presumably a major class of *N*-acylethanolamine phospholipids in the brain, its enzymatic conversion to *N*-acylethanolamines is poorly understood. In the present study, we focused on the formation of *N*-acylethanolamines from pNAPEs. While recombinant NAPE-PLD catalyzed direct release of *N*-palmitoylethanolamine from *N*-palmitoylethanolamine plasmalogen, the same reaction occurred in the brain homogenate of NAPE-PLD-deficient mice, suggesting that this reaction occurs through both the NAPE-PLD-dependent and -independent pathways. Liquid chromatography-mass spectrometry revealed a remarkable accumulation of 1-alkenyl-2-hydroxy-glycero-3-phospho(*N*-acyl)ethanolamines (lyso pNAPEs) in the brain of NAPE-PLD-deficient mice. We also found that brain homogenate formed *N*-palmitoylethanolamine, *N*-oleoylethanolamine, and anandamide from their corresponding lyso pNAPEs by a Mg^{2+}-dependent "lysophospholipase D". Moreover, the brain levels of alkenyl-type lysophosphatidic acids, the other products from lyso pNAPEs by lysophospholipase D, also increased in NAPE-PLD-deficient mice. Glycerophosphodiesterase GDE1 can hydrolyze glycerophospho-*N*-acylethanolamines to *N*-acylethanolamines in the brain. In addition, we discovered that recombinant GDE1 has a weak activity to generate *N*-palmitoylethanolamine from its corresponding lyso pNAPE, suggesting that this enzyme is at least in part responsible for the lysophospholipase D activity. These results strongly suggest that brain tissue *N*-acylethanolamines, including anandamide, can be formed from *N*-acylated plasmalogen through an NAPE-PLD-independent pathway as well as by their direct release via NAPE-PLD.

1. Introduction

N-acylethanolamines are ethanolamides of long-chain fatty acids and represent a class of endogenous lipid mediator molecules [1,2]. Among different *N*-acylethanolamines, anandamide (*N*-arachidonoylethanolamine) has been well characterized since it exerts cannabimimetic actions as an endogenous agonist of cannabinoid receptors [3]. Other *N*-acylethanolamines that are inactive at cannabinoid receptors also attract attention because of their biological actions. In particular, *N*-palmitoylethanolamine and *N*-oleoylethanolamine have been extensively investigated owing to their anti-inflammatory and analgesic effects [4,5] and anorexic effect [6], respectively.

In the classical "*N*-acylation/phosphodiesterase" pathway, *N*-acylethanolamines are biosynthesized from membrane glycerophospholipids by a two-step enzymatic pathway [7]. The first reaction catalyzed by *N*-acyltransferase is the transfer of the *sn*-1 acyl group of glycerophospholipids to the primary amino group of ethanolamine phospholipid molecules such as diacyl-glycerophosphoethanolamine (phosphatidylethanolamine, PE), alkenylacyl-glycerophosphoethanolamine

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(ethanolamine plasmalogen), and alkylacyl-glycerophosphoethanolamine [1,8]. This *N*-acylation of ethanolamine phospholipids results in the formation of *N*-acylethanolamine phospholipids. When the ethanolamine phospholipid molecule is PE, the product is *N*-acyl-PE (NAPE). *N*-Acylethanolamines are then released directly from NAPEs by a specific phospholipase (PL) D generally referred to as NAPE-hydrolyzing PLD (NAPE-PLD) (Fig. 1A). We previously cloned cDNA of NAPE-PLD and revealed that this enzyme is a member of the metallo-β-lactamase family [9,10].

Alternatively, NAPE-PLD-independent pathways have been reported in which one or both *O*-acyl chains of NAPEs are eliminated, followed by hydrolysis of the phosphodiester bond of the resultant lyso NAPEs (1-acyl-2-hydroxy-glycero-3-phospho (*N*-acyl)ethanolamines or 1-hydroxy-2-acyl-glycero-3-phospho (*N*-acyl)ethanolamines) or glycerophospho-*N*-acylethanolamines, respectively, to *N*-acylethanolamines (Fig. 1A) [11]. We found that group IB, IIA, and V secretory PLA_2s ($sPLA_2$s) could catalyze the elimination of one *O*-acyl chain from NAPEs to yield lyso NAPEs [12]. We also characterized a lysophospholipase D (lyso PLD) hydrolyzing lyso NAPEs to generate *N*-acylethanolamines in rat brain. However, the enzyme remains to be cloned and characterized. Leung et al. analyzed NAPE-PLD-deficient (NAPE-PLD$^{-/-}$) mice and showed that NAPE-PLD-independent pathways contribute to the *in vivo* biosynthesis of *N*-acylethanolamines, especially polyunsaturated ones such as anandamide [13]. The same group proposed a pathway composed of double-*O*-deacylation of NAPEs via lyso NAPEs and further hydrolysis of resultant glycerophospho-*N*-acylethanolamines to *N*-acylethanolamines [14]. They also found that this pathway could be catalyzed sequentially by α/β-hydrolase 4 (Abh4) and the glycerophosphodiesterase GDE1 as shown in Fig. 1A [14,15]. Moreover, Liu et al. revealed a two-step route, in which PLC-mediated hydrolysis of *N*-arachidonoyl-PE and

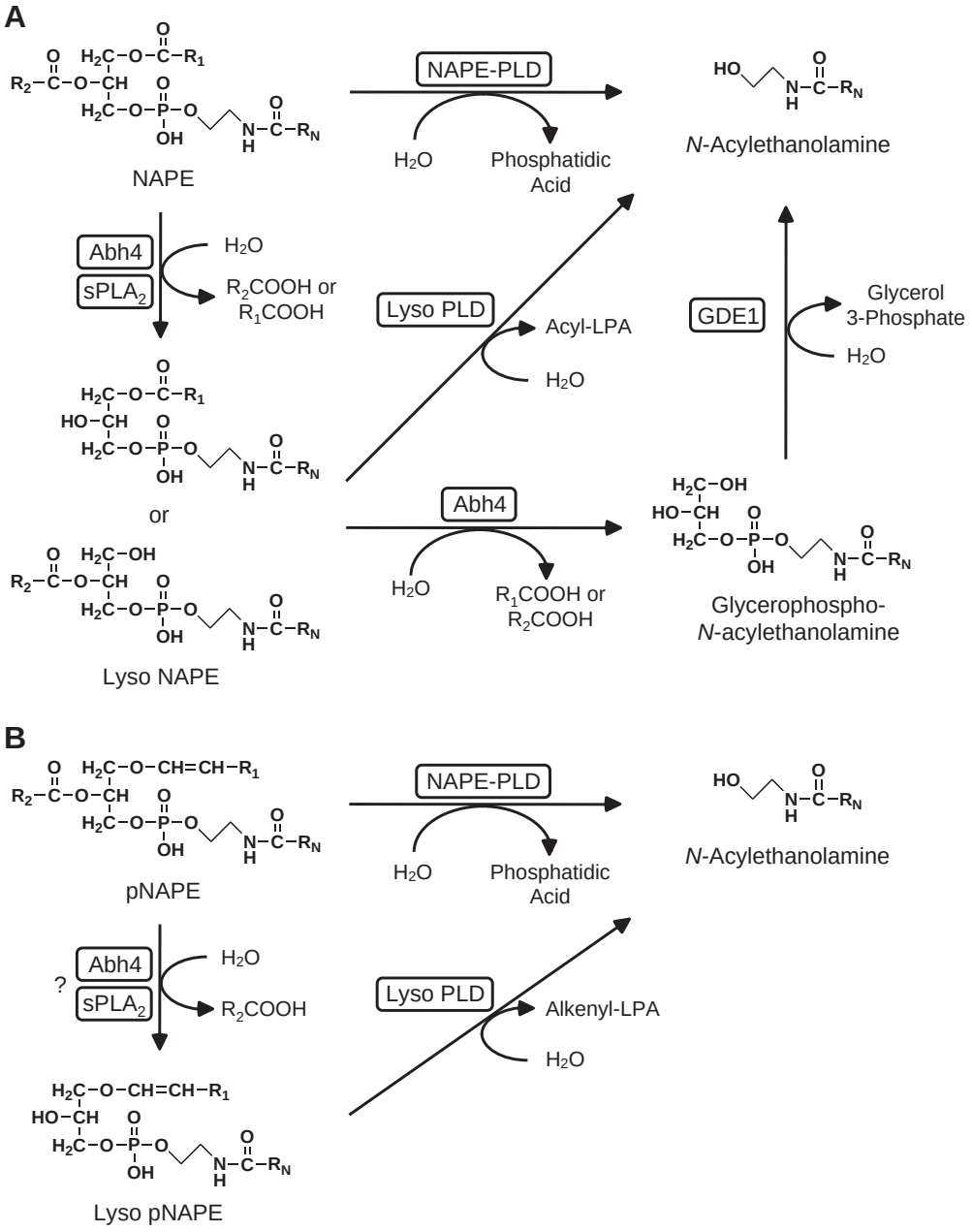

Fig. 1. NAPE-PLD-dependent and -independent pathways for the formation of *N*-acylethanolamines from NAPEs (A) and pNAPEs (B).