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Review

New players in the fatty acyl ethanolamide metabolism

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ABSTRACT

Fatty acyl ethanolamides represent a class of endogenous bioactive lipid molecules and are generally referred to as *N*-acylethanolamines (NAEs). NAEs include palmitoylethanolamide (anti-inflammatory and analgesic substance), oleoylethanolamide (anorexic substance), and anandamide (endocannabinoid). The endogenous levels of NAEs are mainly regulated by enzymes responsible for their biosynthesis and degradation. In mammalian tissues, the major biosynthetic pathway starts from glycerophospholipids and is composed of two enzyme reactions. The first step is *N*-acylation of ethanolamine phospholipids catalyzed by Ca^{2+}-dependent *N*-acyltransferase and the second step is the release of NAEs from *N*-acylated ethanolamine phospholipids by *N*-acylphosphatidylethanolamine (NAPE)-hydrolyzing phospholipase D (NAPE-PLD). As for the degradation of NAEs, fatty acid amide hydrolase plays the central role. However, recent studies strongly suggest the involvement of other enzymes in the NAE metabolism. These enzymes include members of the HRAS-like suppressor family (also called phospholipase A/acyltransferase family), which were originally discovered as tumor suppressors but can function as Ca^{2+}-independent NAPE-forming *N*-acyltransferases; multiple enzymes involved in the NAPE-PLD-independent multi-step pathways to generate NAE from NAPE, which came to light by the analysis of NAPE-PLD-deficient mice; and a lysosomal NAE-hydrolyzing acid amidase as a second NAE hydrolase. These newly recognized enzymes may become the targets for the development of new therapeutic drugs. Here, we focus on recent enzymological findings in this area.

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1. Introduction

Fatty acyl ethanolamides are a class of endogenous lipid molecules with different long-chain fatty acids, and are generally referred to as *N*-acylethanolamines (NAEs) [1,2]. NAEs exist ubiquitously in animal tissues and have received much attention due to a variety of biological activities [3,4]. NAEs include palmitoylethanolamide (*N*-palmitoylethanolamine, PEA), stearoylethanolamide (*N*-stearoylethanolamine, SEA), oleoylethanolamide (*N*-oleoylethanolamine, OEA), linoleoylethanolamide (*N*-linoleoylethanolamine, LEA), and arachidonoylethanolamide (*N*-arachidonoylethanolamine, anandamide) (Fig. 1) [5]. Although anandamide has been extensively studied because of its behavior as an endocannabinoid (endogenous ligand of cannabinoid receptors CB1 and CB2) [6], anandamide is a minor component in most animal tissues comparing with other NAEs such as PEA, SEA, OEA, and LEA [6,7]. These NAEs except anandamide do not bind to cannabinoid receptors, but exert a variety of biological actions through several other receptors. PEA shows anti-inflammatory, analgesic, and neuroprotective actions through

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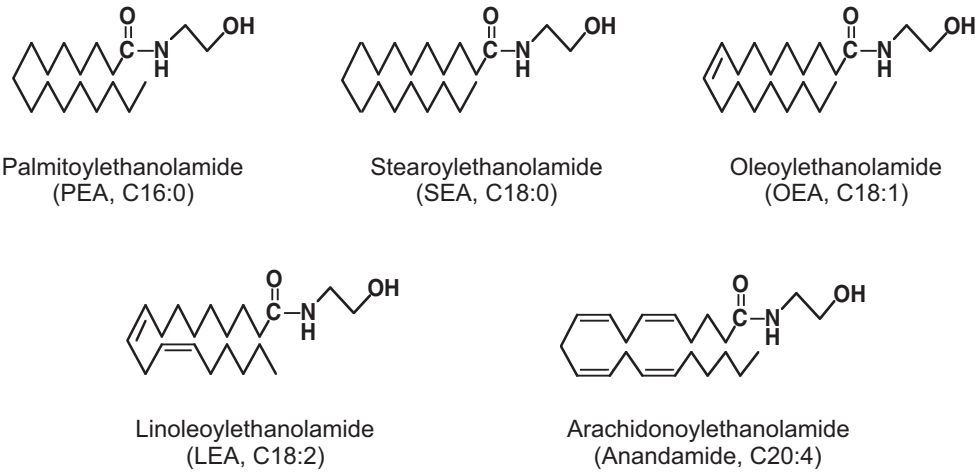

Fig. 1. Chemical structures of representative NAEs.

peroxisome proliferator-activated receptor-α (PPAR-α) [5,8,9] and G protein-coupled receptor GPR55 [10]. OEA exerts an anorexic action, which is mediated by its binding to PPAR-α [11]. On the other hand, GPR119 and transient receptor potential vanilloid type 1 (TRPV1) may mediate the effects of OEA on glucose handling and pain behavior, respectively [11]. Furthermore, SEA was shown to be pro-apoptotic [12] and anorexic [13]. Whereas anandamide as a partial agonist of CB1 shows cannabimimetic activities such as analgesia, neuroprotection, hypotension, and appetite stimulation [14], this molecule also shows a variety of central and peripheral activities by acting as an endovanilloid (endogenous ligand of TRPV1) [15]. For example, postsynaptic TRPV1 affects AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptor endocytosis to mediate anandamide-induced long-term depression in the hippocampus and nucleus accumbens [16,17].

Anandamide and other NAEs are enzymatically biosynthesized from the membrane glycerophospholipids *via* their corresponding *N*-acylphosphatidylethanolamines (NAPEs) (Fig. 2) [18–20]. Since the endogenous levels of NAEs are principally regulated by enzymes responsible for their formation and degradation [18,21], the full characterization of the NAE-metabolizing enzymes is obligatory for better understanding of the physiological and pathological roles of bioactive NAEs. Classically, NAEs were believed to be biosynthesized through "*N*-acylation-phosphodiesterase" pathway. This pathway is composed of the generation of NAPE by Ca^{2+}-dependent *N*-acyltransferase (Ca-NAT) and phospholipase D (PLD)-type hydrolysis of NAPE by NAPE-hydrolyzing PLD (NAPE-PLD). cDNA cloning of NAPE-PLD from human, mouse, and rat by our group facilitated molecular biological approaches to elucidate NAE biosynthetic pathways [22]. The generated NAEs are then degraded by fatty acid amide hydrolase (FAAH) to their corresponding free fatty acids and ethanolamine (Fig. 3) [23–25]. Alternatively, polyunsaturated NAEs such as anandamide can be subjected to the oxygenation of their polyunsaturated fatty acyl moieties. Namely, anandamide can be converted to prostaglandin-like ethanolamides (prostamides) by cyclooxygenase-2 (COX-2) [26,27], hydroperoxy-eicosatetraenoylethanolamides by lipoxygenases [28,29], and hydroxy-eicosatetraenoylethanolamides by cytochrome P-450 [30].

In addition to these enzymes, more recent studies revealed the involvement of new players in NAE metabolism [18–21]. These players include members of the phospholipase A/acyltransferase (PLA/AT) family, which have Ca^{2+}-independent NAPE-forming *N*-acyltransferase activity, multiple enzymes involved in the NAPE-PLD-independent multi-step pathways to generate NAE from NAPE, and a lysosomal hydrolase called NAE-hydrolyzing acid amidase (NAAA). In this mini-review, after an introduction of the classical *N*-acylation-phosphodiesterase pathway, we will outline these new enzymes one by one. Although their physiological significance is not fully understood, these new enzymes/pathways may serve as novel targets for the development of therapeutic drugs.

2. The classical pathway for the biosynthesis and degradation of NAEs

In animal tissues, anandamide and other bioactive NAEs such as PEA and OEA are biosynthesized from glycerophospholipids by a combination of Ca-NAT and NAPE-PLD. This classic NAE-generating pathway, composed of two consecutive enzymatic reactions, is referred to as "transacylation-phosphodiesterase pathway" or "*N*-acylation-phosphodiesterase pathway" [1].

The first reaction is the transfer of a fatty acyl chain from *sn*-1 position of the donor glycerophospholipid molecule such as phosphatidylcholine (PtdCho) to the primary amino group of phosphatidylethanolamine (PtdEt) to form NAPE (Fig. 2). Similarly, plasmenylethanolamine (PlsEt), a plasmalogen-type analog of PtdEt, is considered to be a precursor of *N*-acyl-PlsEt (Fig. 4) [2]. This Ca-NAT activity is found in mammalian tissues including brain, heart, and testis [2,18]. The following three properties are generally accepted [2,31,32]. First, Ca-NAT uses glycerophospholipids (PtdCho, 1-acyl-lyso PtdCho, PtdEt, and cardiolipin) as donor substrates and extracts an acyl group selectively from the *sn*-1 position. Thus, 2-acyl-lysophospholipid, considered to be the product in phospholipase (PL)A_1 reaction, is produced together with NAPE. The polyunsaturated fatty acyl chains like arachidonoyl chain are principally linked to the *sn*-2 instead of *sn*-1 position of donor glycerophospholipids. Since the *N*-acyl chain of NAPE or *N*-acyl-PlsEt, generated by Ca-NAT, is retained in NAEs as *N*-acyl chain, this selective extraction of *sn*-1 fatty acyl chain is a main reason why the tissue levels of anandamide (*N*-arachidonoylethanolamine) are usually much lower than those of saturated or monounsaturated NAEs. Secondly, the enzyme is associated with membranes, and can be solubilized with non-ionic detergent like Nonidet P-40. Thirdly, the enzyme activity is enhanced by submillimolar concentrations of Ca^{2+}. It is well known that NAPE accumulates during cellular stresses and tissue damage such as cerebrovascular ischemia and myocardial ischemia. A recent imaging technique with mass spectrometry also revealed that ischemia–reperfusion causes a large increase in the levels of many species of NAPEs in the whole injured area of neonatal rat brain [33]. This phenomenon may be explained by the remarkable increase in intracellular Ca^{2+} concentration, which results in the activation of Ca-NAT. Furthermore, physiologically