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Homeostasis of phospholipids — The level of phosphatidylethanolamine tightly adapts to changes in ethanolamine plasmalogens

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ABSTRACT

Ethanolamine plasmalogens constitute a group of ether glycerophospholipids that, due to their unique biophysical and biochemical properties, are essential components of mammalian cellular membranes. Their importance is emphasized by the consequences of defects in plasmalogen biosynthesis, which in humans cause the fatal disease rhizomelic chondrodysplasia punctata (RCDP). In the present lipidomic study, we used fibroblasts derived from RCDP patients, as well as brain tissue from plasmalogen-deficient mice, to examine the compensatory mechanisms of lipid homeostasis in response to plasmalogen deficiency. Our results show that phosphatidylethanolamine (PE), a diacyl glycerophospholipid, which like ethanolamine plasmalogens carries the head group ethanolamine, is the main player in the adaptation to plasmalogen insufficiency. PE levels were tightly adjusted to the amount of ethanolamine plasmalogens so that their combined levels were kept constant. Similarly, the total amount of polyunsaturated fatty acids (PUFAs) in ethanolamine phospholipids was maintained upon plasmalogen deficiency. However, we found an increased incorporation of arachidonic acid at the expense of docosahexaenoic acid in the PE fraction of plasmalogen-deficient tissues. These data show that under conditions of reduced plasmalogen levels, the amount of total ethanolamine phospholipids is precisely maintained by a rise in PE. At the same time, a shift in the ratio between ω-6 and ω-3 PUFAs occurs, which might have unfavorable, long-term biological consequences. Therefore, our findings are not only of interest for RCDP but may have more widespread implications also for other disease conditions, as for example Alzheimer's disease, that have been associated with a decline in plasmalogens.

1. Introduction

The lipid composition of biological membranes is of great importance for cellular functions. Its dynamic adaptation to specific requirements is a crucial feature of lipid membranes in mammalian tissues. The most abundant membrane lipids are phospholipids, which are typically amphipathic and, thereby, make up the characteristic lipid bilayer structure of biological membranes. Among the phospholipids forming the bilayer, the major classes are the glycerophospholipids phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS) and the ether phospholipids (including plasmalogens), as well as sphingomyelin (SM), a sphingolipid. Each of these is further subdivided into a variety of distinct species based on their fatty acid chains. Eukaryotic cells are able to fine-tune the mixture of these lipids according to their biological task [1]. Furthermore, membranes adapt to various environmental conditions, like for example circadian rhythms or changes in temperature, by altering their lipid composition [2,3]. The dynamic aspect of lipid membranes is additionally underscored by the existence of membrane rafts (formerly termed lipid rafts), small protein–lipid domains enriched in sphingolipids and sterols, two other prevalent membrane lipid classes in addition to glycerophospholipids. Membrane rafts assemble transiently and compartmentalize important biological functions like signal transduction [4].

Abbreviations: AA, arachidonic acid; AGPS, alkylglycerone phosphate synthase; BA, batyl alcohol; CDP, cytidine diphosphate; DHA, docosahexaenoic acid; GNPAT, glyceronephosphate acyltransferase; HDG, hexadecylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PlsEtn, ethanolamine plasmalogen; PS, phosphatidylserine; PUFA, polyunsaturated fatty acid; RCDP, rhizomelic chondrodysplasia punctata; SM, sphingomyelin

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Ether phospholipids constitute a special class of glycerophospholipids that have an O-alkyl group at their *sn*-1 position, which distinguishes them from the diacyl phospholipids. The most abundant subclass of ether phospholipids are the plasmalogens, which account for almost 20% of the phospholipid mass in humans [5]. These compounds carry a double bond adjacent to the ether bond, together forming a vinyl ether bond, which is characteristic of plasmalogens (Fig. 1A). Other ether phospholipids include plasmanyl phospholipids (containing a saturated ether moiety at the *sn*-1 position), platelet-activating factor, seminolipid, and, partly, the glycosylphosphatidylinositol (GPI) anchor of membrane proteins. The biosynthesis of ether phospholipids requires peroxisomes. In the lumen of these organelles, the concerted action of the two enzymes glyceronephosphate acyltransferase (GNPAT; alternative name: dihydroxyacetone phosphate acyltransferase (DHAPAT, DAPAT)) and alkylglycerone phosphate synthase (AGPS) generates the characteristic ether bond. The remaining biosynthetic steps, including the introduction of the vinyl double bond in case of plasmalogens, are subsequently accomplished at the endoplasmic reticulum (ER). The generation of the alkyl group at *sn*-1 depends on fatty alcohols that are either derived from dietary intake or synthesized by the fatty acyl-CoA reductases FAR1 or FAR2 [6]. Recently, FAR1, which preferably accepts saturated or monounsaturated C16 or C18 acyl-CoA esters as substrates, was suggested as the main reductase involved in plasmalogen biosynthesis [7]. Accordingly, C16:0, C18:0 and C18:1 are the major fatty alcohol species at the *sn*-1 position of plasmalogens. The fatty acid composition at *sn*-2 strongly depends on the cell type. In general, plasmalogens are enriched in polyunsaturated fatty acids (PUFAs), a fact that is especially pronounced in neurons. In brain white matter, however, monounsaturated species prevail to ensure myelin stability [8]. In most tissues, ethanolamine is the dominating head group. Choline plasmalogens play an important role in cardiac tissue, but represent a minor species in most other organs. Other head groups, like serine or inositol, are extremely rare. As major constituents of cellular membranes, plasmalogens shape membrane structure and dynamics. They also have been shown to be enriched in membrane rafts [9]. Furthermore, in some cell types, the frequent occurrence of PUFAs in the side chains of plasmalogens engages them as a storage depot for these essential fatty acids [10,11]. Additional functions like antioxidative action [12,13], stimulation of invariant natural killer T cells [14], membrane fusion [15,16] or constriction [17] and in generating lipid second messengers [18] have been proposed based on in vitro findings and experiments in ether phospholipid-deficient mouse models [19–22]. However, the exact biological roles of these lipids and the underlying molecular mechanisms are still enigmatic.

In humans, deficiency of ether phospholipids evokes rhizomelic chondrodysplasia punctata (RCDP), a rare, autosomal recessive disorder caused by mutations in the genes encoding the peroxisomal enzymes GNPAT (RCDP type 2) and AGPS (RCDP type 3) or peroxin 7 (PEX7; RCDP type 1), the receptor needed for peroxisomal import of AGPS [23–27]. Affected individuals suffer from a variety of severe symptoms, including growth and mental retardation, shortening of the proximal long bones, epiphyseal stippling, cataracts and joint contractures. In its severest form, RCDP is fatal during the first months of life, predominantly due to respiratory failure [28]. However, also less severe variants ("intermediate phenotype") of the disease have been described, where residual plasmalogen biosynthesis slightly alleviates the symptoms [29–34]. Ether phospholipid deficiency is also a key feature of peroxisome biogenesis disorders, such as the Zellweger syndrome, in which peroxisomes cannot be properly assembled [35]. Moreover, reduced plasmalogen levels have also been observed in several more common disorders like Alzheimer's disease [36–38], Parkinson's disease [39], Down syndrome [40], or schizophrenia [41] and have been suggested to constitute a part of the pathological mechanism during disease development.

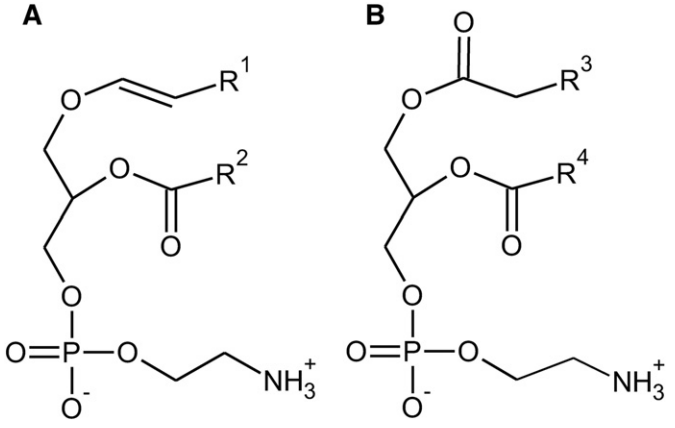

Fig. 1. Structures of PlsEtn (A) and PE (B). R^1 indicates the alkyl residues originating from the primary alcohols C16:0, C18:1 or C18:0. R^2, R^3 and R^4 can be various fatty acyl residues.

Ethanolamine plasmalogens (PlsEtn, also called plasmenylethanolamines) and the diacyl glycerophospholipid PE (Fig. 1B) have an ethanolamine head group in common. To date, two main biosynthesis pathways for PE are known. The Kennedy pathway generates PE de novo and is crucial also for the final steps of PlsEtn production [42]. Here, PE synthesis starts from ethanolamine, which becomes phosphorylated and then activated by cytidine triphosphate (CTP). Cytidine diphosphate (CDP)-ethanolamine subsequently reacts with diacylglycerol completing the biosynthesis of PE [43]. Alternatively, PE can be produced via decarboxylation of PS at the inner mitochondrial membrane [44,45]. The importance of both of these pathways is stressed by the fact that targeted inactivation of either *Pcyt2*, the gene coding for the rate-limiting enzyme of the Kennedy pathway, or *Pisd*, the gene coding for PS decarboxylase, leads to embryonic death in mice [46,47]. Being the second most abundant phospholipid in eukaryotic cells, PE is essential for both structure and function of membranes. It is usually located at the cytoplasmic face of membranes, where, due to its cone shape, it supports the formation of non-lamellar structures. In addition, PE has been found to stabilize membrane proteins and to assist in their folding [48].

In the present study, we investigated how the level and side chain composition of PE respond to PlsEtn deficiency and to excess of PlsEtn induced by exogenous supplementation with precursors. To this end, we used fibroblasts derived from RCDP patients and gray matter brain tissue of *Gnpat* knockout mice as in vitro and in vivo models of ether lipid deficiency. The main objective was to determine how alterations in the PlsEtn concentration affect: (i) the total level of ethanolamine phospholipids (PE + PlsEtn); (ii) the total PUFA level; and (iii) the side chain composition at the *sn*-2 position of ethanolamine phospholipids.

2. Materials and methods

2.1. Patient phenotype classification

Clinical and biochemical phenotypes of patients, whose cells were studied here, have been described previously [32,49,50]. Phenotype severity classes were based on clinical features, red blood cell (RBC) plasmalogen levels and plasmalogen synthesis in patient fibroblasts (Fig. 2A and Table 1). Patients were classified as either severe or intermediate RCDP. Compared with severe RCDP, the intermediate form is characterized by improved growth and development and may not involve rhizomelia. In the intermediate form, residual plasmalogen synthesis and RBC plasmalogen levels are at least 30% of the control mean and more than two standard deviations above the severe RCDP mean.

2.2. Determination of plasmalogen biosynthesis rate and RBC plasmalogens

Plasmalogen biosynthesis rates and RBC plasmalogen levels were determined by established methods in the Peroxisomal Diseases Laboratory at the Kennedy Krieger Institute [51–53]. Briefly, for the measurement of plasmalogen biosynthesis, cultured cells were incubated with