The mouse as a model to understand peroxisomal disorders

Brites, P.

Citation for published version (APA):
Teixeira Brites, P. M. (2009). The mouse as a model to understand peroxisomal disorders

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
CHAPTER 2

FUNCTIONS AND BIOSYNTHESIS OF PLASMALOGENS IN HEALTH AND DISEASE.

P. Brites, H.R. Waterham & R.J.A. Wanders

Published in
Review

Functions and biosynthesis of plasmalogens in health and disease

Pedro Brites, Hans R. Waterham, Ronald J.A. Wanders*

Departments of Clinical Chemistry and Paediatrics, Academic Medical Center, Lab Genetic Metabolic Diseases, F0-224, Meibergdreef 9, Amsterdam 1105 AZ, Netherlands

Received 15 December 2003; accepted 15 December 2003

This article is dedicated to Prof. Henk van den Bosch on the occasion of his retirement

Abstract

Plasmalogens (1-O-alk-1’-enyl-2-acyl glycerophospholipids) constitute a special class of phospholipids characterized by the presence of a vinyl–ether bond at the sn-1 position. Although long considered as biological peculiarities, interest in this group of phospholipids has grown in recent years, thanks to the realization that plasmalogens are involved in different human diseases. In this review, we summarize the current state of knowledge with respect to the enzymatic synthesis of plasmalogens, the characteristic topology of the enzymes involved and the biological roles that have been assigned to plasmalogens.

© 2004 Elsevier B.V. All rights reserved.

Keywords: Plasmalogen; Peroxisome; Metabolic disorder

1. Introduction

Ether-phospholipids constitute a special class of phospholipids characterized by the presence of an ether bond at the sn-1 position of the glycerol backbone rather than an ester bond as in diacylglycerophospholipids. Two types of ether bonds occur in ether-phospholipids: the ether bond and the vinyl–ether bond. The former is found in platelet activating factor (PAF), whereas the vinyl–ether bond is found in a class of ether-phospholipids called plasmalogens. The schematic representation of the different types of glycerophospholipids is shown in Fig. 1. In plasmalogens, the aliphatic moieties at the sn-1 position consist of C16:0 (palmitic acid), C18:0 (stearic acid) or C18:1 (oleic acid) carbon chains, whereas the sn-2 position is occupied by polyunsaturated fatty acids (PUFAs) and the head group is usually either ethanolamine or choline. Different tissues, and possibly even different cell types within one tissue, may have variable amounts of plasmalogens. Brain myelin possesses the highest content of ethanolamine plasmalogens (PE-plasmalogen) whereas heart muscle has a higher content of choline plasmalogens (PC-plasmalogen). Moderate amounts of plasmalogens are found in kidney, skeletal muscle, spleen and blood cells, whereas liver is known for its low plasmalogen content.

For a long time plasmalogens have been considered not more than a biological peculiarity. Over the last 20 years, however, an increased interest has arisen with respect to their occurrence, synthesis and properties. This interest in plasmalogens has been stimulated by their implication in various degenerative diseases and the discovery of genetic disorders in which ether-phospholipids are deficient. Here we review the plasmalogen biosynthetic pathway and human disorders in which the biosynthesis of plasmalogens is impaired and/or reduced levels of plasmalogens have been observed. Although the physiological function(s) of plasmalogens is still not fully resolved, we also review several biological roles that have been assigned to plasmalogens.

2. Biosynthesis of plasmalogens

The first step in the biosynthesis of plasmalogens (Fig. 2) involves the esterification of dihydroxyacetone phosphate (DHAP) with a long-chain acyl-CoA ester and is carried out by dihydroxyacetone phosphate acyltransferase (DHAP-AT;
EC 2.3.1.42) [1]. The characteristic ether-bond at the sn-1 position of plasmalogens is introduced by the replacement of the sn-1 fatty acid with a long-chain fatty alcohol. This reaction is catalysed by alkyl-dihydroxyacetone phosphate synthase (ADHAP-S; EC 2.5.1.26) [2,3] with alkyl-DHAP as product. The fatty alcohol may be derived from dietary intake or by the reduction of long-chain acyl-CoAs through the action of an acyl-CoA reductase. Alternatively, fatty alcohols may derive from chain elongation of dodecanoyl-CoA using the peroxisomal pool of acetyl-CoAs that results in the formation of hexadecanol within peroxisomes [4].

The reduction of the ketone group at the sn-2 position of alkyl-DHAP is catalysed by acyl/alkyl-dihydroxyacetone phosphate reductase (AADHAP-R; EC 1.1.1.101) [5,6] and results in the formation of 1-alkyl-sn-glycero-3-phosphate. Acylation to 1-alkyl-2-acyl-sn-glycero-3-phosphate is catalysed by an alkyl/acyl-glycero-3-phosphate acyltransferase (AAG3P-AT) which is different from the enzyme that uses 1-acyl-glycero-3-phosphate as a substrate [7,8]. The phosphate group is removed by a phosphohydrolase (PH) yielding 1-alkyl-2-acyl-sn-glycerol. Subsequently, cytidine-diphosphate-ethanolamine (CDP-ethanolamine) is incorporated via the action of ethanolamine-phosphotransferase in the presence of magnesium to form 1-alkyl-2-acyl-sn-glycero-3-phosphoethanolamine (alkylacyl-GPE) [9]. The desaturation to 1-alk-1-enyl-2-acyl-GPE (PE-plasmalogen) is carried out by a cytochrome b5-dependent microsomal electron transport system and a Δ1-alkyl desaturase (Δ1-desaturase) whereas 1-alk-1-enyl-2-acyl-GPC (PC-plasmalogen) is primarily formed from 1-alk-1-enyl-2-acyl-GPE via sn-2 and polar-head group modifications [10].

3. Subcellular localization and topology of the enzymes involved in plasmalogen biosynthesis

The different subcellular localizations of the enzymes involved in the biosynthesis of plasmalogens show that the process is initiated in peroxisomes and completed in the endoplasmatic reticulum (ER) (Fig. 3). Indeed, the enzymes DHAP-AT and ADHAP-S, catalysing the first two steps, are localised exclusively in peroxisomes [3,11,12] whereas AADHAP-R has been shown to have a bimodal distribution in peroxisomes and ER [5,13]. The remaining enzymes are localised in the ER, where they are also involved in the biosynthesis of diacylphospholipids [14,15].
The topology of the peroxisomal enzymes involved in plasmalogen biosynthesis differs markedly with respect to their localization within peroxisomes (Fig. 4). DHAP-AT and ADHAP-S are truly intraperoxisomal proteins facing the peroxisomal lumen whereas the long-chain alcohol-forming acylCoA reductase and AADHAP-R are bound to the peroxisomal membrane facing the cytosol. The implication of this topology is that the substrates of the DHAP-AT reaction, i.e. acylCoA and DHAP, must be either transported from the cytosol into peroxisomes or generated inside peroxisomes. Since the fatty acid which enters the plasmalogen biosynthesis pathway in the DHAP-AT step is generated in the ADHAP-S reaction, the fatty acid released simply needs to be reactivated to its CoA-ester form to be a substrate again in the DHAP-AT reaction. Peroxisomes indeed contain an acylCoA synthetase, of which the catalytic side faces the peroxisomal lumen. This synthetase, called very-long-chain acylCoA synthetase (VLACS), can activate a wide range of fatty acids and might well catalyze also the esterification of fatty acids derived from the ADHAP-S reaction [16,17]. The ATP required for this reaction would then come from the cytosol in exchange for AMP, as catalyzed by PMP34, a peroxisomal adenine nucleotide transporter that belongs to the family of mitochondrial solute carrier family of transporters [18]. One other unresolved aspect regarding the peroxisomal steps in the biosynthesis of plasmalogen concerns the origin of DHAP, which can either be transported across the peroxisomal membrane from the cytosol or generated in the peroxisomal matrix by the activity of glycerol-3-phosphate dehydrogenase [19,20].

The peroxisomal localization of DHAP-AT and ADHAP-S is crucial for their enzymatic activity and stability. The two enzymes have been found to form a heterotrimetric complex [21] and although the active functional units of the two enzymes are monomers [22], several direct and indirect observations indicate that the complex may regulate enzymatic activities and facilitate substrate channelling. The peroxisomal localization of DHAP-AT and ADHAP-S is achieved by the presence of peroxisomal targeting signals (PTS) in their amino acid sequences [23]. Interestingly, the
two enzymes have different PTS and follow different PTS-mediated modes of import. In mammals, DHAP-AT has a PTS type 1 (PTS1) signal [24] and follows a PEX5-dependent import into peroxisomes, whereas ADHAP-S has a PTS type 2 signal (PTS2) [25] and follows a PEX7-dependent import.

The continuous study of peroxisomal disorders has revealed that in different disorders the localization of DHAP-AT and ADHAP-S affects not only the rate of plasmalogen biosynthesis but also the activity of DHAP-AT (Fig. 5). In the Zellweger syndrome the mislocalisation of DHAP-AT and ADHAP-S to the cytosol affects the stability of the two proteins, leading to low residual activities and consequently deficient plasmalogen synthesis. In rhizomelic chondrodysplasia punctata (RCDP) type-1 patients, the specific impairment in PTS2-mediated import due to deficient Peroxin 7 results in the cytosolic localization of ADHAP-S. ADHAP-S is unstable in the cytosol, which explains the low residual activity of this enzyme in RCDP type-1 fibroblasts, which is comparable to that of Zellweger-derived fibroblasts. Although DHAP-AT is correctly localised in peroxisomes of RCDP type-1 patients, its activity is reduced to 20–30% of control as a consequence of the absence of ADHAP-S in peroxisomes. Interestingly, de novo plasmalogen biosynthesis is usually much more deficient in RCDP type-1-derived fibroblasts than in Zellweger-derived fibroblasts, although the residual activity of DHAP-AT is higher in RCDP type-1. We speculate that this phenomenon is caused by the spatial separation between DHAP-AT and ADHAP-S in RCDP type-1 cells, whereas in Zellweger cells DHAP-AT and ADHAP-S are localized in the same compartment, i.e. the cytosol.

RCDP type-2 is caused by mutations in the DHAP-AT gene [26] whereas RCDP type-3 is caused by mutations in ADHAP-S gene [27]. Interestingly, the molecular and biochemical characterisation of RCDP type-3 patients revealed that a patient with a nonsense mutation had reduced DHAP-AT activity, whereas a patient with a missense mutation and no ADHAP-S protein detected on Western blotting had normal DHAP-AT activity [28]. These observations are in line with the notion described above that the activity of DHAP-AT is dependent on the physical presence of ADHAP-S in peroxisomes even if in a mutated form. Moreover, they provide an explanation as to why in RCDP type-1 the cytosolic localization of ADHAP-S leads to the reduced activity of DHAP-AT.

4. Topology and localization of plasmalogens in membranes

In the sarcolemmal membrane, PE-plasmalogens are predominantly found in the inner leaflet [29,30]. In red blood cells, PE-plasmalogens have been found to be mainly localized in the inner leaflet of the plasma membrane whereas only a small percentage of PC-plasmalogens is detected in the inner leaflet. These results suggest that, like the corresponding diacylphospholipids, plasmalogens follow an asymmetric distribution [31].

Recently, plasmalogens have been found enriched in lipid rafts of a human epidermal carcinoma cell line [32]. Lipid rafts are lipid microenvironments found in the plasma membrane that play an important role in signal transduction as they can include or exclude proteins and facilitate protein-protein interactions [33]. Plasmalogens have the ability to form non-lamellar structures and can allow the transition of membranes from a lamellar gel to
liquid-crystalline form at lower temperatures. These physical properties may contribute to or modulate the formation and/or maintenance of lipid rafts. The enrichment of plasmalogens in lipid rafts and the role of lipid rafts in signal transduction may provide an explanation for the impaired cellular functions and signalling events identified in human disorders with reduced levels of plasmalogens (see below).

5. Turnover/degradation of plasmalogens

The turnover rates of plasmalogens, as determined in brain, show that they are short lived with PE-plasmalogens having half-lives of approximately 3 h and PC-plasmalogens approximately 30 min [34]. The presence of the characteristic vinyl–ether bond at the sn-1 position of the glycerol moiety in plasmalogens makes them sensitive to radical attack (see below). The hydrolysis of plasmalogens by the selective action of phospholipases is of special interest as it may contribute to plasmalogen turnover but also to the release of bioactive molecules. The selective action of at least three different phospholipases A2 (PLA2) towards plasmalogens over diacylphospholipids is also supported by the different physicochemical and kinetic properties when compared to PLA2 with activities towards diacylphospholipids. These PLA2s have been known as plasmalogenases or plasmalogen-selective phospholipase A2 (psPLA2). psPLA2 have been purified from bovine brain [35,36], canine [37] and rabbit [38] myocardium and rabbit kidney [39,40]. The selectivity of psPLA2 may be attributed to the conformational organisation of the plasmalogen molecule [37]. This is further supported by the observation that a psPLA2 isolated from rat pancreas preferentially hydrolysates plasmalogens with arachidonic acid versus oleic acid in the sn-2 position [41]. The hydrolysis by psPLA2 releases the fatty acid at the sn-2 position, which yields lysoplasmalogens. The released free fatty acids may function as second messengers (see later section). Recently, it has been shown that the hydrolysis of brain plasmalogens by psPLA2 is regulated by ceramide [42]. Lysoplasmalogens may then be degraded further or reacylated as accumulation of lysoplasmalogens in membranes may affect their physical properties. High levels of lysoplasmalogens produced during hypoxia inhibit the plasma membrane Na+, K+-ATPase [43] and can cause severe perturbations in the action potentials of cultured myocytes [44] and may even lead to disruption of cell membranes [45]. Lysoplasmalogens can be degraded by the action of lysoplasmalogenase, an enzyme that catalyses the hydrolysis of the alkyl-ether bond of lysoplasmalogens [46–49]. Despite the need for a tight control over the levels of lysoplasmalogens, they have also been shown to mediate signal transduction events as choline lysoplasmalogens can activate cAMP-dependent protein kinase in the absence of cAMP [50].

6. Biological roles attributed to plasmalogens

6.1. Plasmalogens as antioxidants

The presence of a vinyl–ether bond makes plasmalogens more susceptible to oxidative attack compared to their 1-acyl analogues [51]. This has prompted the hypothesis that plasmalogens may act as scavengers, protecting other phospholipids, lipids and lipoprotein particles from oxidative reactions [52]. Plasmalogens seem to have an antioxidant effect towards a wide variety of reactive species including, amongst others, reactive oxygen species [53] and iron-induced peroxidation [54]. Plasmalogens may have a crucial role as endogenous antioxidants during states of increased oxidative burden, e.g. during and after spinal cord ischemia [55] and hypoxia [56]. The decrease in plasmalogen content under such oxidative conditions may indicate that plasmalogens are functioning as scavengers [57], but a careful analysis and interpretation of these results may be warranted as it has been shown that some of these oxidative scenarios may activate psPLA2, thus leading to a decrease in plasmalogen content [44].

In addition to being prone to free radical attack on their vinyl–ether bond, plasmalogens have also been shown to have a role in inhibiting iron-induced peroxidation of PUFAs [54] and copper-induced oxidation of low-density lipoproteins [58,59]. The inhibitory effects seem to be related to ion “chelation” in addition to the ability of plasmalogens to interfere with the propagation of lipid peroxidation. Free radical attack and induced lipid peroxidation is a complex process known to occur in various pathological conditions including chronic inflammation, atherosclerosis, aging and cancer [60] in which plasmalogens may be of special importance.

6.2. Plasmalogens as mediators of membrane dynamics

Variations in the length and saturation of the alkyl- and acyl-chains at sn-1 (R1) and sn-2 (R2), respectively, and in the head group of glycerophospholipids lead to altered physicochemical characteristics of the membranes in which they are inserted. The vinyl–ether bond in plasmalogens may also affect the hydrophobic–hydrophilic interface region of phospholipid aggregates [61].

Phospholipid mixtures containing plasmalogens have been shown to undergo the transition from lamellar gel to liquid-crystalline at lower temperatures compared to phospholipid mixtures containing the corresponding diacylphospholipids [62–64]. This propensity to form hexagonal structures has been linked to a role of plasmalogens in facilitating membrane fusion processes [62,64,65]. Plasmalogens also have the ability to reduce the surface tension and the viscosity of phospholipid mixtures [66,67]. The observation that plasmalogens are major constituents of synaptic vesicle membranes [63,68] and are found in alveolar surfactants [69,70] may indicate that plasmalogens are in-
volved in the expedite process of synaptic transmission and membrane dynamics during breathing cycles. Plasmalogens and the products of their catabolism may also potentiate alterations in membrane dynamics during signal transduction in plasmalogen-enriched membranes [71].

Plasmalogens have also been implicated in the process of HDL-mediated cholesterol efflux. Human cell lines with impairment in plasmalogens biosynthesis, due to deficient DHAP-AT activity, have been found to have reduced HDL-mediated cholesterol transport [72]. Moreover, cholesterol transport from the plasma membrane to the ER is impaired in Chinese Hamster Ovary (CHO) mutant cell lines with defective DHAP-AT and ADHAP-S [73].

6.3. Plasmalogens as storages of PUFAs and lipid mediators

The fatty acids located at the sn-2 position of the glycerol backbone of plasmalogens may be released upon the action of phospholipase A2. The high level of PUFAs in plasmalogens when compared to their diacyl counterparts has prompted the suggestion that they can act as storage depots of docosahexaenoic acid (DHA) and arachidonic acid (AA) [74–76]. Despite the fact that PUFAs, including DHA, cannot be biosynthesised, they comprise 25–30% of the fatty acids in human brain and have been implicated in normal brain development and cognitive function. Outside the central nervous system, DHA is also preferentially incorporated in photoreceptors where it has been shown to be important for signalling events leading to visual response [77,78].

Plasmalogens have been suggested to be involved in the initial phase(s) of signal transduction as receptor-mediated degradation of plasmalogens via psPLA2 may release free DHA and AA, which when metabolised to eicosanoids, may serve as second messenger [79,80]. Recently, it has also been found that G-protein-coupled signal transduction is influenced by the phospholipid composition of membranes and that DHA-rich membranes have faster and more efficient G-protein-coupled signal transduction [81].

7. Plasmalogens and disease

7.1. Zellweger syndrome

The importance of plasmalogens in human health is highlighted by the identification of human disorders in which plasmalogen biosynthesis is deficient. The first inborn error of metabolism in which plasmalogens were found to be deficient was the Zellweger syndrome [82], an often lethal autosomal recessive disorder in which peroxisome biogenesis is impaired, leading to a generalised loss of peroxisomal functions. In cells and tissues derived from Zellweger patients, morphologically identifiable peroxisomes are lacking, and often only the membrane remnants of peroxisomal membranes (denominated peroxisomal ghosts) may be present. Genetic and phenotypic heterogeneity is a feature of PBDs with at least 12 different genes involved and three major clinical presentations, which include the Zellweger syndrome as the most severe disorder and neonatal adrenoleukodystrophy and infantile Refsum disease as milder variants [83]. Zellweger patients accumulate very-long-chain fatty acids, bile acid intermediates, phytanic acid and pristanic acid and have reduced levels of plasmalogens which makes it difficult to correlate the pathology with a given metabolic abnormality. Clinically, Zellweger patients are born with a characteristic craniofacial dysmorphism (a high forehead, large anterior fontanel, hypoplastic supraorbital ridges and low implantation of ears) and show profound hypotonia, seizures, impaired hearing, severe growth retardation and neurological abnormalities. Brain pathology reveals dysmyelination and a severe impairment in neuronal migration with cortical dysplasia and neuronal heterotopias. The comparison between Zellweger patients and patients with single peroxisomal biochemical abnormalities, namely the accumulation of phytanic acid (as in Refsum disease), the accumulation of VLCFA (as in X-linked Adrenoleukodystrophy) or the accumulation of branched chain fatty acids (as in racemase deficiency), or the defect in plasmalogen biosynthesis (as in RCDP type-2 and type-3), suggests that the reduced content of plasmalogens in brain tissue of Zellweger patients may be critical for the neurological abnormalities [84]. The reduced levels of plasmalogens may modulate the effect(s) of fatty acid accumulation at the molecular and cellular level.

Fibroblasts from Zellweger patients have been shown to have impaired muscarinic cholinergic signal transduction activity as measured by the low-Km GTPase activity after carbachol induction and a reduction in secretion of amyloid precursor protein (APP) [85]. These results and the observation that membrane fluidity is significantly higher in fibroblasts derived from Zellweger patients when compared to control fibroblasts [61] substantiate the proposed role of plasmalogens in signal transduction and membrane dynamics. In a recent study, Styger et al. [86] also showed defective signal transduction in Zellweger fibroblasts despite normal membrane fluidity. Regarding this latter point, it should be mentioned that membrane fluidity was higher in two of the three Zellweger cell lines studied. The designation of Zellweger syndrome relates to a clinical presentation and a generalised loss of peroxisomal functions, although genetically this syndrome may be caused by mutations in at least nine different genes [87–89]. It will be important to relate these findings of abnormal signal transduction and membrane fluidity with the assignment of the different cell lines to a given complementation group.

7.2. Rhizomelic chondrodysplasia punctata (RCDP)

RCDP is an autosomal recessive disorder of peroxisome metabolism characterized by the presence of morphologi-
cally distinguishable peroxisomes and multiple, but not generalised, loss of peroxisomal functions. RCDP patients show bone abnormalities (namely shortening of proximal long bones, calcific stippling of epiphysis, and vertebral clefts in vertebrae), contractures, cataracts, and severe growth and mental retardation. The disorder is phenotypically heterogeneous with different degrees of severity in addition to atypical clinical presentations [90–94]. Furthermore, RCDP is genetically heterogeneous with three distinct genetic forms. RCDP type-1 is caused by mutations in the PEX7 gene and is the most frequent type of RCDP. RCDP type-2 is caused by mutations in the DHAP-S gene [27], and RCDP type-3 is caused by mutation in the ADHAP-S gene [26]. This genetic heterogeneity is reflected in the biochemical features of the different types of RCDP. RCDP type-1 is characterised by impaired plasmalogen biosynthesis and deficient α-oxidation of phytic acid [96]. A third defect, in the β-oxidation of VLCFA, may be present as blood cells show an accumulation of VLCFA, albeit mild [97]. RCDP type-2 and type-3 are both characterised by an isolated impairment in plasmalogen biosynthesis.

The severe depletion of plasmalogens in RCDP patients causes defects in bone and brain development. Although not extensively studied, it has been proposed that in RCDP, the coronal clefts in vertebrae are due to retardation and disruption in the process of bone replacement, possibly due to delayed chondrocyte maturation [98]. This delay in the process of bone replacement may be the cause of the shortening of proximal bones. Although there is no doubt that it is the defect in plasmalogen biosynthesis that causes these bone abnormalities, there is still no mechanistic explanation correlating the impairment in bone ossification to the lack of plasmalogens. Brain developmental defects have also been identified in RCDP patients. If there is a neuronal migration defect in RCDP patients, it is not as severe as the defect observed in Zellweger patients. Nevertheless, post-mortem studies of three RCDP patients have shown that the brains display dysplastic olives that may be indicative of a neuronal migration defect [99–101]. Cerebellar atrophy due to loss of Purkinje and granular neurons [102] and abnormal myelination [103–106] has also been observed in RCDP patients.

The study of Perichon et al. [85] revealed that fibroblasts derived from RCDP type-1 patients have, when compared to Zellweger fibroblasts, a greater deficiency in muscarinic cholineric signal transduction and APP secretion. Normal muscarinic cholineric signal transduction was found in fibroblasts derived from X-linked adrenoleukodystrophy and peroxisomal bifunctional enzyme-deficient patients, although in the latter an increase in APP secretion was observed. When taken together, these results suggest that the defect in signal transduction is related to a defect in plasmalogens.

A link between the role of plasmalogens in signal transduction and modulation of membrane dynamics is also suggested by the finding of abnormal caveolae and clathrin-coated pits in fibroblasts of RCDP type-2 and type-3 patients [107]. In these fibroblasts caveolae were found to be scarce and with abnormal morphologies and the level of caveolin-1 (a structural component of caveolae) was also found reduced. In contrast, clathrin-coated pits were flattened and possessed increased levels of clathrin. Moreover, cholesterol was found to accumulate in perinuclear structures and there was an impairment in the rate of endocytosis. Both findings may be related to the defects observed in caveolae and clathrin-coated pits, respectively, and indicate that the role of plasmalogens as mediators of membrane dynamics may alter intracellular transport and signal transduction.

7.3. Alzheimer’s disease (AD)

Patients suffering from AD have been shown to have reduced levels of plasmalogens in brain areas that show active degeneration [108]. The selectivity of the deficiency for affected brain areas may be due to alterations in membrane stability [109]. Nevertheless, the plasmalogen deficiency in AD seems to be relevant and specific as other neurodegenerative disorders, such as Huntington’s disease and Parkinson’s disease, do not display reductions in the plasmalogen content of the corresponding affected brain regions. Recently, Han et al. [110] correlated the levels of plasmalogens with different clinical dementia ratings (CDR) of AD patients. Even at initial stages of AD (lower CDR score), a reduction of plasmalogens was observed and patients with successive higher CDR scores had further reductions in the level of plasmalogens.

Two putative scenarios have been put forward to explain the deficiency of plasmalogens in AD brain. The first involves the putative antioxidant nature of plasmalogens: the increased oxidative status in AD brains and the inherent propensity of the vinyl–ether bond of plasmalogens to be attacked by radicals would lead to a decrease in plasmalogen content. The second scenario predicts the reduction of plasmalogen levels by the stimulation of psPLA2, a process that may be receptor-mediated [111]. In both cases, it will be important to measure in AD brains the by-products obtained from either radical oxidation (e.g. long-chain aldehydes) or from psPLA2 action (e.g. lysoplasmalogens) in order to discriminate between the two possibilities.

The role played by a plasmalogen deficiency in AD pathogenesis remains obscure. Reduced levels of plasmalogens may abrogate the membrane instability of AD neurons and/or it may increase the toxic effect of the accumulation of amyloid β protein (Aβ). Recent findings may lead to an interesting twist on how the plasmalogen deficiency in AD brains is perceived. The sequential cleavage of amyloid precursor protein (APP) by β- and γ-secretase results in the release of Aβ that has amyloidogenic properties. α- and γ-secretase also sequentially cleave APP but the peptides resulting from this cleavage are non-
amyloidogenic. Ebeholt et al. [112] have shown that there are two pools of APP, one inside and one outside lipid rafts. APP localized outside of lipid rafts seems to be cleaved by α-secretase whereas APP localized in lipid rafts seems to be cleaved by β-secretase. Thus, Aβ production may be influenced by the organisation of the plasma membrane and the interactions of APP with lipid rafts. As discussed above, plasmalogens are enriched in lipid rafts and a reduction of plasmalogens may alter the physical properties and protein composition of lipid rafts. This hypothesis may suggest that the receptor-mediated degradation of plasmalogens by psPLA2 in AD brains may function as a cellular response to decrease the pool of APP in lipid rafts and thus reduce the levels of Aβ produced. Nevertheless, the reduction of plasmalogen content will undoubtedly have adverse effects as concluded from the severe clinical manifestations in patients with either DHAP-AT or ADHAP-S deficiencies.

7.4. Niemann–Pick type C (NPC)

NPC is an autosomal recessive lipid storage disorder characterized by accumulation of unesterified cholesterol, neutral and acidic glycosphingolipids and sphingomyelin, which leads to progressive degeneration of the central nervous system [113,114]. A study performed in the mouse model of NPC revealed an involvement of peroxisomes in stages preceding the appearance of the disease symptoms [115]. In this study, the activities of peroxisomal β-oxidation enzymes and catalase were found to be decreased in livers of NPC mice when compared to control mice. Surprisingly, these defects were not present at the time of appearance of the disease symptoms. Analysis of brain phospholipids revealed a decrease in plasmalogen levels in the early stages of the disease. It should be mentioned that no studies in NPC patients have been reported although in literature the study of Schedin et al. is often linked to the human condition [111,116]. Studies in NPC patients and a more thorough study in the mouse model for the disease should lead to a better understanding of the role of plasmalogens and peroxisomal functioning in the development of NPC. The recent finding that deficiencies in plasmalogens biosynthesis lead to impaired cholesterol transport [73] may be in favour of the hypothesis that in NPC mice the reduction in plasmalogen levels modulates the initial stages of the disease and also exacerbates the overall cholesterol transport defect in NPC.

7.5. Down syndrome (DS)

Studies in brain samples from DS patients have revealed a reduction in the levels of plasmalogens and phosphatidylinositol [117] that may be caused by a generalised loss of membranes or an altered lipid metabolism towards plasmalogens and phosphatidylinositol. In this neurodegenerative disorder, the apparent specificity in phospholipid loss suggests an altered lipid metabolism towards plasmalogens and phosphatidylinositol rather than a generalised loss of membranes.

7.6. Neuronal ceroid lipofuscinosi (NCL)

NCL is an inherited neurodegenerative disorder characterised by the accumulation of lipopigments in the lysosomal compartment [118]. A decrease in the amount of plasmalogens and DHA has been observed in red blood cells of juvenile NCL patients [119]. In contrast, in a more recent study Granier et al. [120] reported that fibroblasts derived from late infantile NCL patients had an increase in their plasmalogen content. These conflicting results may be due to the genetic heterogeneity of the disorder, which has at least eight distinct complementation groups [121]. CLN3, encoding a lysosomal transmembrane protein, is the gene responsible for juvenile NCL, and CLN2, encoding a serine protease, is the gene responsible for late infantile CLN. Abnormal membrane phospholipids and altered signal transduction have been observed in NCL patients [122,123]. The abnormalities in plasmalogens content may be the reflection of such deficiencies.

7.7. Retinitis pigmentosa (RP)

RP is genetically heterogeneous with a large number of different genetic forms, including X-linked and autosomal dominant and autosomal recessive variants [124,125]. In the early stages of the disorder, RP patients develop night blindness, followed by tunnel vision and ultimately complete loss of vision. RP primarily affects photoreceptors and other eye pathological findings are presumably the consequence of photoreceptor degeneration [126,127]. Abnormally low levels of DHA have been observed in RP patients [128]. Further studies performed in red blood cells of RP patients have shown a reduction of DHA in the PE pool of phospholipids [129] whereas the levels of plasmalogens were increased. These findings were observed in dominant, recessive and isolated forms of RP but not in the X-linked form of RP. However, a recent study evaluated the content and synthesis of DHA in the X-linked form of RP and found a lower rate of DHA synthesis and consequently reduced levels of DHA [130]. Thus, it is not fully understood if the abnormal profiles of PUFAs and plasmalogens are due to an abnormal peroxisomal metabolism or a consequence of a secondary phenomenon. Nevertheless, the decrease in DHA in retina may lead to the loss of photoreceptors in RD patients since it has been shown that DHA prevents loss of retina photoreceptors after induced oxidative stress [131].

8. Concluding remarks

The importance of plasmalogens for normal development and well-being has been recognized by the identification and characterization of human disorders in which the
biosynthesis of plasmalogens is deficient. The effect of plasmalogen depletion or reduction as observed in some human diseases or disorders still requires detailed studies in order to unravel both the biochemical and/or cellular processes that affect plasmalogen levels and the mechanism(s) behind the pathophysiology of such disorders.

Several functions have been attributed to plasmalogens, but the physiological role(s) has remained elusive. From the point of view of a “loss of function”, it will be important to investigate all of the assigned functions of plasmalogens in patients with defective plasmalogen biosynthesis and in patients with reduced levels of plasmalogens. From the phenotypic presentation of patients with RCDP type-2 and type-3, at least one major conclusion may be drawn: one of the primary roles of plasmalogens resides in regulating chondrocyte development and bone ossification, despite the fact that it is not known how the different proposed functions of plasmalogens control such multi-factorial and tightly regulated processes. From the wide variety of clinical presentations and disease progressions, it can also be postulated that a variety of factors mediate the effects of a deficiency in plasmalogens.

Although it is known that alkyl-glycerols, e.g., 1-O-octadecyl-rac-glycerol or 1-O-hexadecyl-rac-glycerol, restore plasmalogen levels in fibroblasts of Zellweger and RCDP patients, and thus appear an invaluable tool for therapy, only one study actually reported increased levels of plasmalogens in two Zellweger patients upon supplementation with these compounds [132]. Despite the absence of an obvious clinical improvement, it should be noted that Zellweger patients have a large number of peroxisomal biochemical abnormalities that may hinder a clear assessment of the therapeutical value of alkyl-glycerols. Trials in RCDP have not been reported and with the increasing number of human disorders in which a decrease in plasmalogens is observed, it may be relevant to reassess the in vivo efficacy of alkyl-glycerols in restoring plasmalogen levels.

An additional tool to investigate the role of plasmalogens has been recently generated by the creation of mutant mice with defects in plasmalogen biosynthesis. Three mouse models for the Zellweger syndrome have been produced by targeted deletion of the pex2, pex5 and pex13 genes [133–135]. A mouse model for RCDP type-1 has been generated by targeted deletion in the pex7 gene [136] and a model for RCDP type-2 has been generated by targeted deletion in the DHAP-AT gene [137]. Overall, these mutant mice have the phenotypic hallmarks of the corresponding human disorders, which include hypotonia, delayed growth, cataracts and defects in neuronal migration and bone ossification. They will undoubtedly be valuable tools to elucidate the molecular mechanism(s) behind the pathological conditions and to unravel how or if the attributed roles to plasmalogens, including plasmalogens as mediators of membrane dynamics and signalling, affect or modulate such a variety of processes like chondrocyte maturation in relation to bone ossification, myelination of the central and peripheral nervous system and signal transduction (Fig. 6).

Acknowledgements

The authors gratefully acknowledge Prof. Henk van den Bosch for the many years of fruitful collaborations, his willingness to share his extensive knowledge with us, and the friendly and pleasant atmosphere in which this took place. This work was supported by grants from the EC (QLG1-CT-2001-01277) and by the Calouste Gulbenkian Foundation with Program Praxis XXI-FCT (BD9805/96) to P.B.

References


Chapter 2


[59] J. Gootjes, P.A. Mooijer, C. Dekker, P.G. Barth, B.T. Poll-The,


