Biological Functions of Plasmalogens 13

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Abstract

Plasmalogens (Pls) are one kind of phospholipids enriched in the brain and other organs. These lipids were thought to be involved in the membrane bilayer formation and anti-oxidant function. However, extensive studies revealed that Pls exhibit various beneficial biological activities including prevention of neuroinflammation, improvement of cognitive function, and inhibition of neuronal cell death. The biological activities of Pls were associated with the changes in cellular signaling and gene expression. Membrane-bound GPCRs were identified as possible receptors of Pls, suggesting that Pls might function as ligands or hormones. Aging, stress, and inflammatory stimuli reduced the Pls contents in cells, and addition of Pls inhibited inflammatory processes, which could suggest that reduction of Pls might be one of the risk factors for the diseases associated with inflammation. Oral ingestion of Pls showed promising health benefits among Alzheimer's disease (AD) patients, suggesting that Pls might have therapeutic potential in other neurodegenerative diseases.

Keywords

Plasmalogens · Neuroinflammation · Neurodegenerative diseases · Aging · Signaling mechanism

13.1 What Are Plasmalogens (Pls)?

Pls are one kind of ether phospholipids found in mammals, invertebrate organisms, and in anaerobic bacteria. Pls are the major phospholipids in mammals, because they constitute 20% of total phospholipids. Pls are plasmenyl type ether phospholipids having vinyl ether bond at their sn-1 position, different from other plasmanyl phospholipids having only the ether bond at sn-1 (Fig. 13.1). The ether phospholipid and plasmanyl are often called as alkyl phospholipids and plasmenyl as alkenyl phospholipids. The expressions of ether phospholipids including Pls are ubiquitous. Ether phospholipids are important structural components of cell membranes in mammals. It has been known that in mammals the fatty acid at the sn-1 position can be derived from the three kinds of fatty alcohols (16:0, 18:0, and 18:1). The sn-2 position is mainly occupied by polyunsaturated fatty acids (PUFA), but monounsaturated fatty acid, such as oleic acid

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(18:1), is also common. The head group of Pls is mainly of two kinds, ethanolamine and choline, but the other head group is also present including serine-Pls found in the human retina. Ethanolamine Pls (Pls-Etn) are highly enriched in the brain and nervous tissues. Choline Pls (Pls-Cho) are enriched in other peripherals organs including the heart, kidney, and intestinal tissues. It is believed that Pls-Etn can form rigid and condensed lipid bilayer compared to Pls-Cho, which can form relatively flexible lipid bilayer. Because of the smaller head group, the Pls-Etn and serine-Pls are believed to be enriched in the inner leaflet of lipid bilayer, whereas the Pls-Cho are present in the outer leaflet. The scientists, Feulgen and Volt, first described Pls in their studies of tissue section where they reported that these special lipids are present inside of the cells [1]. Pls are ubiquitous and enriched in the cell membranes including the plasma membrane, mitochondrial membrane, and nuclear membrane of the mammalian cells [1, 2]. Pls are also found to be enriched in lipid raft domains of cell membrane, suggesting that they might regulate cellular signaling events. Besides their enrichment in cell membrane, Pls are found to be secreted from the cells including glial and neurons. The normal concentration of Pls in the human blood is about 5 mg/dl. It is still unknown whether secreted Pls exist as free lipids or lipoproteins. By localizing in the inner leaflet of the plasma membrane, Pls-Etn might bind with membrane proteins to modulate their intracellular signaling. Pls-Etn were shown to activate cellular signaling, but it remained unknown whether Pls-Cho could induce cellular signaling. The Pls-Etn-mediated cellular signaling events will be discussed in the latter part of this chapter. Like other phospholipids, Pls can be degraded to various other components which could induce several cellular events. Among the secondary products, the most common are lyso-Pls, lyso-PAF (platelet-activating factor), PAF, and alkyl LPA (Fig. 13.2). Pls in the cell membrane can be degraded by reactive phospholipase A2 (PLA2), resulting in the formation of lyso-Pls and free fatty acids including arachidonic acid (AA) and DHA (Fig. 13.2). It has been known that AA shows various beneficial effects in brain protective functions in the cells. The activation of the nuclear receptor peroxisome proliferator-activated receptor gamma (PPARγ) is also shown to be triggered by AA [3]. The lyso-Pls are mainly enriched in the cell membrane and known to be functionally important.

13.2 Extraction and Purification of Pls

Although it has been known that mammalian tissues contained enriched amount of Pls, it was not known how to extract these intact Pls, aiming at studying the biological significances of these lipids in health. Mawatari et al. succeeded in separating purified Pls from various cells and tissues by high-performance liquid chromatographic (HPLC) method [4], which enabled them to examine biological activities of Pls. On the basic concept of acid hydrolysis of Pls, which could yield lysophospholipid and a fatty aldehyde, they analyzed the purity of extracted Pls from the sources. To purify Pls by the single chromatographic (HPLC) run, they first collected the total chloroform lipid extracts. The tissue samples were subjected to lipid extraction using the method of Bligh and Dyer [5]. To put it simply, the tissues were homogenized in 3 ml of methanol-chloroform (2:1) mixture followed by addition of 1 ml chloroform. After addition of 2 ml of 0.88% KCl solution followed by a brief centrifugation, the lower chloroform lipid portion was collected and dried under a stream of nitrogen gas. It is to be noted that all the solvents used for lipid extraction contained 1.2 mM butylhydroxytoluene (BHT). These lipid extracts were then subjected to the single chromatographic run by the HPLC system aimed at getting purified Pls [4]. To our knowledge, it is the first report of successful extraction of intact Pls from animal tissues. Both the ethanolamine Pls (Pls-Etn) and the choline Pls (Pls-Cho) could be separated by the HPLC technique described by the Fujino's group [4]. Mawatari et al. also identified novel approaches to extract and purify plasmalogens by hydrolyzing diacylphospholipids with

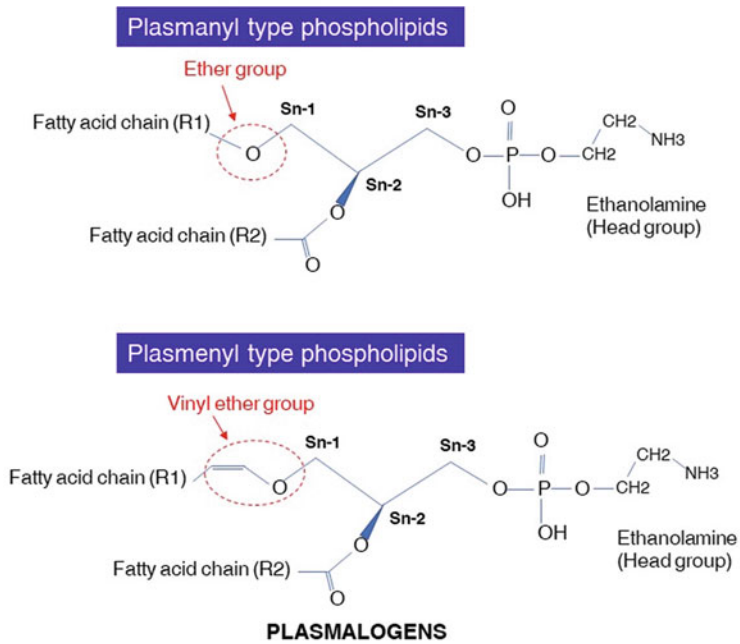

Fig. 13.1 Schematic diagram. General structures of plasmanyl- and plasmenyl- phohspholipids. Plasmalogens (Pls) are plasmenyl type phospholipids. Pls contain vinyl ether group at sn-1 position compared to the ether group of plasmanyl type phospholipids. Fatty acids at sn1 position of Pls are mainly palmitic acid (16:0), stearic acid (18:0), and oleic acid (18:1). The fatty acids at sn-2 may contain polyunsaturated (PUFA) and monounsaturated fatty acids. The head groups might be ethanolamine, choline, serine, etc

phospholipase A_1 (PLA_1) [6]. In this method, PLA_1 treatments degraded diacylphospholipids including the phosphatidylethanolamine (PE) and phosphatidylcholine (PC) in the crude plasmalogens mixture which was obtained from the hexane/acetone solvent extraction of total lipids. The PLA_1 treatments yielded a large amount of lysophospholipids and other substances which were removed from the plasmalogens enriched with hexane portion by making the Na_2SO_4 partition in the hexane/2-propanol (3:2) solvent layers [6]. This process yielded 92% pure Pls which were then subjected to hexane/acetone (1:1) solvent extraction, leading to the highly pure Pls (97% of phospholipids). To purify further, the HPLC separation was conducted. Here, we describe the relative contents of Pls obtained from the chicken breast meat and scallop (Table 13.1). The scallop-derived Pls were enriched in EPA- and DHA-containing Pls compared with the chicken breast meat-derived Pls which were found to be enriched in arachidonic acid- and DHA-containing Pls (Table 13.1). Both the chicken- and scallop-derived plasmalogens were found to be effective in inhibiting neuroinflammation, and there were no comparative studies. Additional experiments are needed to examine the differences in their biological activities to address whether scallop-Pls, which are enriched in EPA-containing Pls, have better function than the chicken breast meat-derived Pls.

13.3 Antiapoptotic Function of Plasmalogens

During the development process of early mammalian life, various extracellular factors remained highly active to support the growth of neuroblast and immature neuronal cells. Interestingly, PUFA-containing Pls are found to be enriched in the mouse brain during the developmental

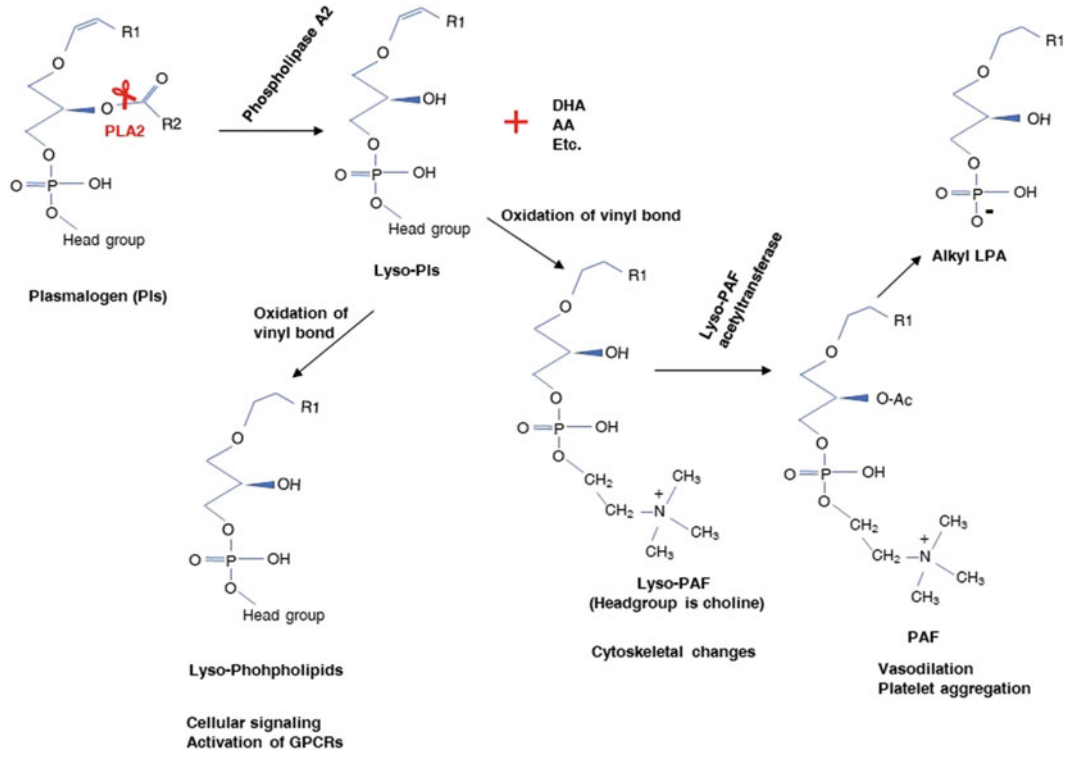

Fig. 13.2 Schematic diagram. Plasmalogens can undergo enzymatic reaction and oxidation reaction to form various bioactive substances

stages compared to the brain of older mice. The embryonic and young mouse brains are enriched with PUFA-containing Pls more than the MUFA-containing Pls such as oleic acid Pls. When we treated neuronal cells with purified Pls enriched with PUFA, we observed the attenuation of neuronal apoptosis caused by the nutrient deprivation [7]. This evidence could suggest that PUFA-Pls are crucially important for immature neurons for their survival during embryonic development and young stage of life. Neuronal apoptosis is accompanied with activation of caspase proteins. There are two major kinds of apoptosis events such as intrinsic and extrinsic apoptosis pathways. Extrinsic pathway of apoptosis is caused by membrane-bound death receptors including fas-associated death domain (FADD) and associated with the activation of caspase-8- protein. Intrinsic pathway is mediated by activation of caspase-9 protein and is associated with the failure of mitochondrial membrane integrity. A dysfunction of the mitochondrial membrane integrity is triggered by increased expression of proapoptotic Bax protein compared to the antiapoptotic factor Bcl2 which could lead to the release of cytochrome-C from the mitochondria to induce activation of caspase-9. Besides these two major pathways of apoptosis, there is another apoptosis pathway involved with the stress signaling of the endoplasmic reticulum (ER), which leads to the activation of caspase-12. These activations of caspases can further activate Caspase-3 protein which could induce apoptosis by inducing the DNA fragmentation. Our experimental evidence showed that nutrient deprivation induced th activation of caspase-9 and Pls treatments inhibited this effectively (Fig. 13.3) [7]. In the adult brain, neuronal cell death can occur by a sudden deficiency of nutrients, especially during the ischemic condition such as

Table 13.1 Relative composition of purified Pls from scallop and chicken breast meat

Types of fatty acids-containing Pls-Etn	Pls from scallop (% of Pls-Etn)	Pls from chicken breast meat (% of Pls-Etn)
Eicosapentaenoic acid (EPA) (20:5)	23.9%	–
Docosahexaenoic acid (DHA) (22:6)	38.9%	18.6%
Arachodonic acid (20:4)	9%	24.9%
α-Linolenic acid (18:3)	1%	7.2%
Linoleic acid (18:2)	1.2%	4.1%
Oleic acid (18:1)	2.6%	26.3%
Stearic acid (18:0)	2%	2.2%
Palmitic acid (16:0)	5.2%	3.6%
Others	16.2%	13.1%

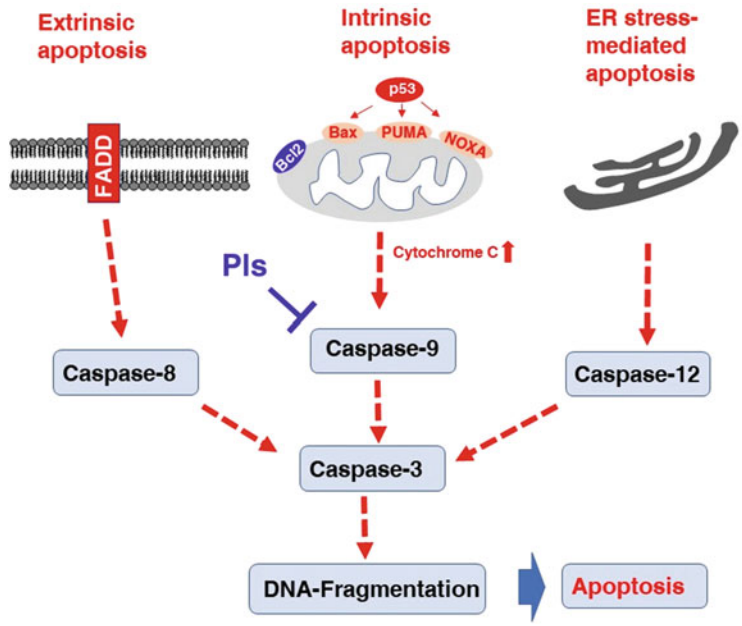

Fig. 13.3 Pls inhibit neuronal cell death by attenuating activation of Caspase-9. Neuronal cells undergo apoptosis by intrinsic pathway by nutrient deprivation. The nutrient deprivation did not activate extrinsic and endoplasmic reticulum (ER) stress-mediated apoptosis pathways. The Pls treatments inhibited intrinsic apoptosis pathways associated with inhibition of caspase-9, caspase-3 and DNA fragmentation. The intrinsic pathway is caused by mitochondria-mediated release of cytochrome-C, which is associated with the abnormal expression pattern of various mitochondrial membrane proteins including Bax, PUMA and NOXA. The expression of these proteins are known to be regulated by apoptosis inducer protein p53. (Sources: Hossain et al., *Plos One,* 2013)

following brain stroke. The stroke-induced neuronal cell death is often associated with the activation of Caspase-9, an intrinsic pathway [8]. It has been shown that Pls inhibit apoptosis of neuronal-like cells (Neuro2A) originated from adult mice, further suggesting that Pls might prevent neuronal cell death in the adult brain following stroke. Future studies are necessary to address this issue to explore a novel therapeutic strategy to reduce the brain injury following stroke.

13.4 Plasmalogens and Neuroinflammation

13.4.1 Reduction of Pls-Caused Neuroinflammation

Neuroinflammation is associated with neurodegenerative diseases including Alzheimer's disease (AD) and Parkinson's disease (PD). The reduction of Pls has been found in the postmortem brains of patients with AD. However, it was not known whether brain Pls could be reduced at the early stage of AD, especially when the neuroinflammation could be seen without massive accumulation of Aβ proteins. It has been known that neuroinflammation occurs at the early stage of AD including the people with MCI (mild cognitive impairment) [9]. Professor Fujino's group found that the Pls are reduced in serum and the red blood cells (RBC) in patients with MCI compared to the control healthy individuals (manuscript in preparation). This evidence could indicate that Pls are reduced at the early stages of AD, which could be one of the risk factors for neurodegenerative diseases. To understand what would happen when brain Pls are reduced, we knocked down the Pls-synthesizing enzyme GNPAT in the mouse brain. Surprisingly, we observed that the GNPAT knockdown-mediated reduction of brain Pls increased the activation of glial cells associated with upregulation of pro-inflammatory cytokines [10]. These findings suggest that the reduction of brain Pls is one of the causes of neuroinflammation, and it might induce the progression of AD. Various studies showed that neuroinflammation caused by LPS injection in mice could increase the accumulation of Aβ proteins and phosphorylation of tau proteins (p-Tau) in the brain. Therefore, it is likely that the reduction of brain Pls could lead the progression of AD by enhancing neuroinflammation-mediated accumulation of toxic substances in the brain such as Aβ and p-Tau. Neurofibrillary tangles (NFTs), marked by excessive accumulation of p-Tau proteins, are found in the progressive stages of AD but not in patients with MCI, suggesting that prolonged neuroinflammation could result in the formation of NFTs. Therefore, inhibition of neuroinflammation at the early stages of AD could prevent the disease progression and prolong the life span.

13.4.2 Inflammation Can Reduce Pls

13.4.2.1 Pls Biosynthesis

The schematic diagram (Fig. 13.4) shows the enzymes involved in Pls biosynthesis pathway in cells [11, 12]. Two known peroxisome-bound enzymes involved in Pls synthesis are glyceronephosphate O-acyltransferase (GNPAT) and alkyl glyceronephosphate synthase (AGPS). The fatty acyl-CoA reductase 1 (FAR1), present in the peroxisomal membrane, catalytically converts lipogenesis-derived acyl-CoA to fatty alcohols necessary for Pls synthesis in the cells. It is known that the catalytic N-terminal domain of FAR1 faces outside of peroxisome membrane and the C-terminal is inside of peroxisome. In peroxisome, the intermediate product of glycolytic pathway, dihydroxyacetone phosphate (DHAP), and fatty alcohols undergo enzymatic reactions by GNPAT and AGPS to produce 1-0-alkyl-DHAP. The peroxisome-derived product, 1-0-alkyl-DHAP, can then undergo various enzymatic reactions in the endoplasmic reticulum (ER) to form Pls. Depending on the cell types, the head group of Pls can be ethanolamine, choline, or serine. In the brain it is mostly ethanolamine, whereas choline-type Pls are enriched in the heart tissue and the serine-type Pls are enriched in the retina. Although GNPAT and AGPS catalyze two different reactions of Pls synthesis, they can physically interact with each other for their activation [13], suggesting that the reduction of either protein, GNPAT, or AGPS can cause a reduction of Pls biosynthesis. This can be suggested further by the findings that mutation of either of these genes resulted in the reduction of Pls [14].

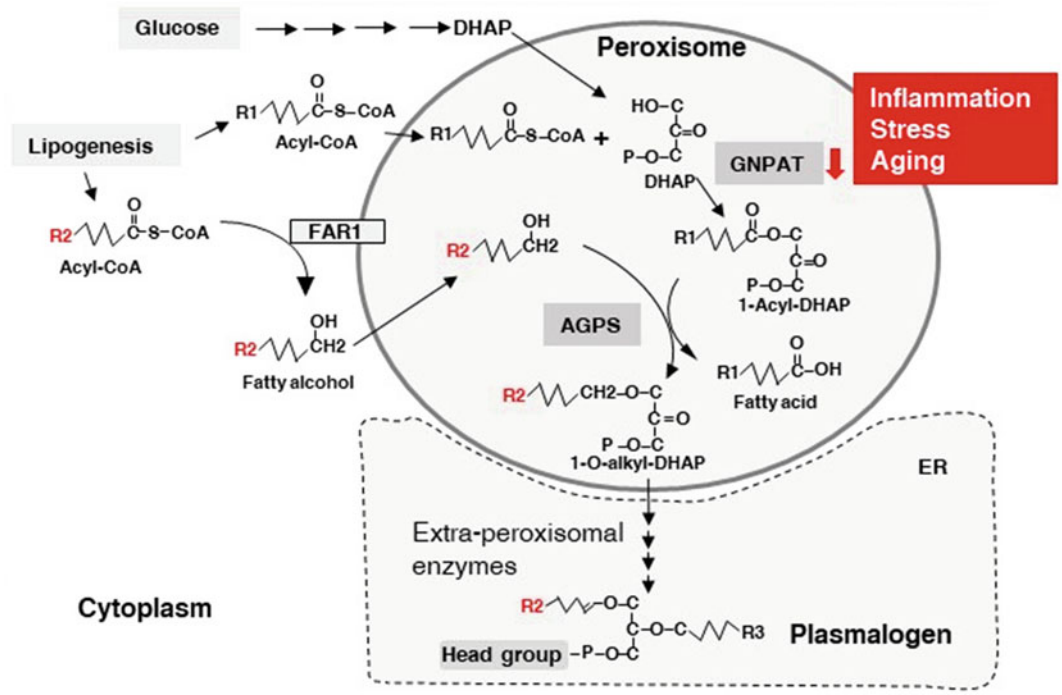

Fig. 13.4 Schematic diagram shows the plasmalogen biosynthesis pathway in cells. Pls were reduced by inflammation, stress and aging associated with the reduction of Pls synthesizing enzyme GNPAT. *DHAP* Dihydroxyacetone phosphate, *FAR1* Fatty acyl-CoA reductase 1, *GNPAT* Glyceronephosphate O-acyltransferase, *AGPS* Alkyl glyceronephosphate synthase, *ER* Endoplasmic reticulum, *R1,R2* Fatty acid chains, *Head group* Ethanolamine, choline, serine, etc

13.4.2.2 Inflammation in Various Diseases and Reduction of Plasmalogens

Inflammation is an event to fight against infection or pathogenic substances that are harmful to our health. Although inflammation is one kind of defensive events of our body, chronic inflammation could cause various disorders. The brain inflammation, marked with the increased number of activated glial cells, is often found among neurodegenerative diseases including AD [15]. It has been known that Pls are reduced among the patients with AD and PD (Table 13.2). Inflammation is also found to be associated with some cancers including colon cancer, where chronic bowel inflammation is one of the main pathogenic factors. It is unknown whether the reduction of Pls could be associated with cancers including colon cancer. However, it is likely that a reduction of Pls could accelerate inflammation of macrophages underlying the colon tissues, which might cause the formation of colon cancer. Inflammation of macrophages is known to be associated with the ulcerative colitis and in inflammatory bowel syndrome. It has not been known whether Pls are reduced by ulcerative colitis or by inflammatory bowel syndrome. However, we detected the reduction of Pls contents in colon tissues of DSS-mediated Inflammatory bowel disease (IBD) model mice (manuscript in preparation), which could suggest that Pls might be reduced in colon tissues during inflammation by the similar mechanism found in glial cells. We previously found the inflammation reduced Pls in microglial cells which are one kind of macrophages present in the central nervous system [10]. The adipose tissue inflammation, marked by the increased number of activated macrophages within adipose tissue, is reported in patients with diabetes. The activated

Table 13.2 Reduction of plasmalogens in various diseases and pathological conditions

Diseases/pathological conditions	Reduction of plasmalogen	References
Rhizomelic chondrodysplasia punctate (RCDP)	Reduction of PlsEtn in serum	(18)
Alzheimer's disease (AD)	Reduction of blood and brain PlsEtn	(14,19,20)
Parkinson's disease (PD)	Reduction of PlsEtn in lipid rafts of the brain tissue	(21,30)
Chronic heart disease (CHD)	Reduction of PlsEtn in plasma and erythrocytes	DOI:https://doi.org/10.9734/CA/2018/44602
Down syndrome	Reduction in PlsEtn in frontal cortex and cerebellum	(22)
Schizophrenia	Reduced PlsEtn and PlsChoin plasma	(23,24)
Autism	Reduced PlsEtn in plasma and in erythrocytes	(25)
Diabetes	Reduction of Pls in serum and red blood cells (RBC)	Endocrine Abstracts (2019) 63 P572I DOI: 10.1530/endoabs.63.P572
Multiple sclerosis (MS)	Reduction of PlsEtn in cerebral cortex	(26,27)
	Reduction of DHA-containing PlsEtn in serum	
Ischemia/lschemic stroke	Reduction of Pls in spinal cord ischemia in rabbits	(28,29)
	Reduction of PlsEtn in the rat model of ischemic stroke	
Neuroinflammation and stress	Reduction of PlsEtn in the mice brain	(10)
	Reduction of PlsEtn in murine glial cells by treatments with LPS and IL-1β	

macrophages release cytokines which could reduce insulin sensitivity of surrounding adipocyte cells resulting in the type 2 diabetes (T2DM). In type 1 diabetes, the chronic inflammation increased reactive T cells, inflammatory cytokines, and monocytic cells that could destroy the insulin-producing pancreatic β cells [16]. Therefore, inflammation of pancreatic tissue is believed to be one of the causes of diabetes. Increased pro-inflammatory cytokines, including TNF-α and IL-1β, were also reported among diabetic patients [17], suggesting further that inflammation is closely linked to metabolic disorders. It has been reported that Pls are reduced in RBC cell membrane and in blood plasma among the diabetic individuals when compared with healthy individuals (*Endocrine Abstracts (2019) 63 P572 | DOI:* https://doi.org/10.1530/endoabs.63.P572). The reduction of Pls in various diseases is shown in Table 13.2 [10, 14, 18–30]. Although Pls are found to be reduced in serum of diabetes patients, it remained unknown whether Pls are also reduced in the activated macrophages within the adipose tissue. Further studies will be necessary to address this issue to suggest that the reduction of Pls in macrophages is the pathogenic marker for diabetes. Atherosclerosis is the main cause of cardiovascular diseases and is caused by inflammation. In atherosclerosis, cytokines released from activated macrophages in the blood vessels can cause the inflammation resulting in the formation of plaques by foam cells. The foam cells are converted from the activated macrophages in the atherosclerosis plaques. Clinical studies showed that Pls are reduced among patients with chronic heart diseases (CHD) compared to the control age-matched healthy individuals (Cardiology

and Angiology: An International Journal, DOI: https://doi.org/10.9734/CA/2018/44602)
(Table 13.2). The reduction of Pls was found in the blood plasma and cell membrane of the RBC among the patients with CHD, suggesting a possibility that reduction of Pls might be linked with the activation of the macrophages and formation of atherosclerosis plaques. Inflammation is also reported to be associated with the severity of chronic fatigue syndrome (CFS) [31]. CFS is linked to anxiety- and depression-like symptoms. In animal experiments, systemic inflammation by injection with LPS often causes behavioral changes related to major depression and fatigue [32]. Inflammation-mediated increase of cytokines in the brain and peripheral organs might be directly linked with pathogenesis of depression-like behaviors. During the systemic inflammation by LPS injection in mice, we observed a reduction of brain Pls associated with neuroinflammation. This evidence could suggest that reduction of brain Pls could induce CFS and depression-like behaviors. It has been reported that there is an elevated level of pro-inflammatory cytokines in blood samples of major depression patients [33]. Although it remained unknown whether Pls are reduced in patients with major depression- or anxiety-related disorders, it could be likely that oral ingestion of Pls could reduce systemic inflammation in these patients and improve their health condition. It remained mostly unknown how the peripheral cytokines could affect brain function to elicit depression-like behaviors. Future studies will be necessary to address this issue. Oral ingestion of Pls improved cognitive function in AD patients [34], suggesting that a therapeutic approach by Pls supplement might be beneficial in other diseases marked with the Pls reduction.

13.4.3 Mechanism of Pls Reduction

Reduction of Pls is observed in many disease conditions, but the mechanism remained mostly elusive. There are two most possible causes of Pls reduction such as (1) genetic causes and (2) enzymatic degradation.

13.4.3.1 Reduction of Pls by Gene Mutations

It has been well known that mutation of the genes, encoding Pls biosynthetic enzymes, is associated with the reduction of Pls. One of the common examples is the mutations of GNPAT gene in RCDP patients [35]. Interestingly, the GNPAT knockout mice showed RCDP-like phenotypes with the reduction of blood Pls, suggesting that inhibiting GNPAT can induce the disease progression. Mutation in AGPS gene was also reported in RCDP patients associated with the reduction of Pls [35]. Mutation in the FAR1 gene has also been reported in RCDP patients [36]. Genetic mutations of these genes in other diseases associated with Pls deficiency are poorly studied. Besides the genetic mutation, downregulation of these genes could also reduce Pls.

13.4.3.2 Reduction of Pls by the Enzymatic Degradation

The Pls, like other glycerophospholipids, could be degraded by various enzymes including the phospholipase A2 (PLA2) [37]. PLA2 catalyzes the degradation of phospholipids at sn-2 position, and activation of this enzyme was found in AD [38]. Therefore, it could be possible that an increased activity of PLA2 may be one of the causes of Pls reduction in the brain. There are two kinds of PLA2: cytosolic and extracellular. PLA2 activity could produce free fatty acids and the lyso-Pls from Pls. The lyso-Pls might also have Pls-like activity because they contain the vinyl ether bond at the sn-1 position. Further studies are necessary to address whether lyso-Pls and free fatty acid could have biological function like Pls.

13.4.3.3 Reduction of Pls in the Brain by Inflammation, Stress, and Aging

It has already been argued before that inflammation is associated with many diseases and some of them were also characterized with the reduction of Pls. This evidence suggested that there might be some common mechanisms of Pls reduction in cells, especially in macrophages. Glial cells are

one kind of macrophages present in the central nervous system, and it has been found that inflammation reduced Pls in these cells. Activation of Glial cells, characterized by the increased number in reactive glial cells, are one of the markers of neuroinflammation in the brain. Resting glial cells can transform to reactive glial cells by the treatments of bacterial toxin called lipopolysaccharide (LPS), which has been used as the model of neuroinflammation. Glial cells could induce expression of pro-inflammatory cytokines including TNF-α and IL-1β by the LPS treatments. The intraperitoneal injection (i.p.) of LPS can cause peripheral and brain inflammation marked with the increased number of reactive glial cells in the brain. In these model mice of neuroinflammation, we found a significant reduction of Pls in the brain. The LPS treatments also reduced cellular Pls in murine glial cells including microglia and astrocytes. To examine the mechanism how Pls are reduced by neuroinflammation, we studied genetics of GNPAT promoter (Fig. 13.5). Bioinformatics studies showed that GNPAT promoter has two c-Myc binding sites near the transcriptional start site. By chromatin immunoprecipitation (ChIP) studies, we found that inflammatory signals increased recruitment of c-Myc transcription factor onto the *GNPAT* transcription start site (Fig. 13.5). The c-Myc is a transcription factor which has various beneficial roles in cells including DNA replication during cell division. However, excess activation or amplification of c-Myc gene is often associated with bad prognosis of cancers. The c-Myc can transcriptionally activate or suppress various gene expressions, which depend on the co-factors that bind with c-Myc. The c-Myc-mediated transcriptional suppression of target gene is known to be mediated by binding with Miz1 protein. The c-Myc and Miz1 protein complex could displace p300 co-activator from the promoter region of target genes, resulting in the transcriptional inhibition from the nearby gene promoter. It is known that the recruitment of c-Myc protein onto the *GNPAT* promoter was prerequisite for downregulation of transcription from *GNPAT* promoter. Therefore, inflammation could reduce *GNPAT* expression by increasing recruitment of c-Myc proteins onto the *GNPAT* promoter. This is the genetic mechanism of Pls reduction in glial cells, and similar phenomena were also found in the murine brain by neuroinflammation, stress, and aging. The chronic restrain stress in mice also reduced Pls in the murine brain. Pls were also found to be reduced in the brain of old mice. Interestingly, increased expression of c-Myc and reduction of *GNPAT* were observed in AD model mice and in the postmortem AD brain tissues [10], which suggests that c-Myc-mediated downregulation of *GNPAT* could be one of the common causes of Pls reduction by neuroinflammation, stress, and aging. In our study, the reduction of Pls by LPS treatments was observed among glial cells, but not in neuronal cells, which could be because neuronal cells have very low expression of TLR4 proteins. Interestingly, we observed the upregulation of c-Myc protein also in neuronal cells in by LPS-mediated neuroinflammation [10]. This could be explained by the fact that during neuroinflammation various cytokines can be released from the glial cells which could induce activation of NF-kB in neuronal cells to induce c-Myc protein, which could reduce Pls synthesis in neuronal cells. Therefore, we could propose that neuroinflammation might reduce Pls in the brain not only in glial cells but also in neuronal cells probably by the increased activity of c-Myc protein.

The c-Myc could be increased by NF-kB, because of the presence of NF-kB binding consensus sequences onto the c-Myc promoter (Fig. 13.5). Therefore, during inflammation processes, the NF-kB is recruited onto the c-Myc promoter and induces its transcription, resulting in upregulation of c-Myc gene expression. Expression of c-Myc gene is increased by aging and is believed to be a risk factor for cancer progression among older people. The increased expression of c-Myc among cancer patients is often associated with amplification of c-Myc genomic region. In neurodegenerative brains, the c-Myc expression is also found to be increased but is not associated with the amplification of the genome. This increased expression of c-Myc could be due to the increased level of inflammatory events in the brain by aging

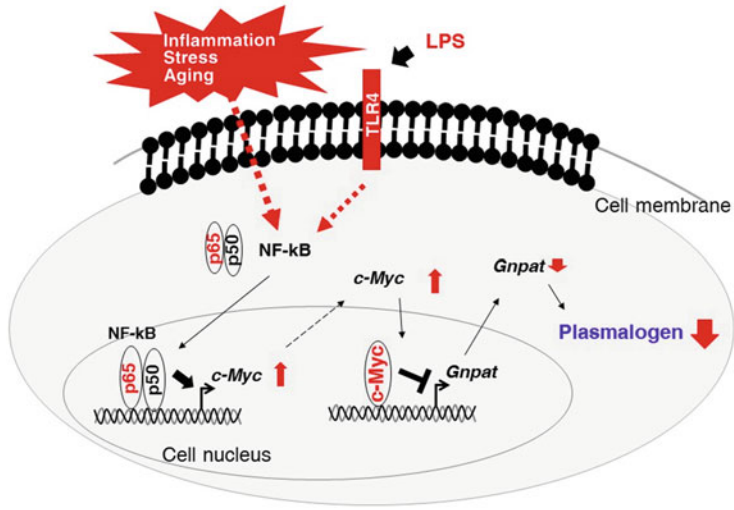

Fig. 13.5 Schematic diagram showing reduction of Pls in glial cells by inflammation. Inflammation, stress and aging enhanced NF*k*B (heteromeric complex of p65 and p50) activity to reduce Pls. LPS is the bacterial toxin that activates TLR4 to induce inflammation, a model for neuroinflammation. Activation of NF-kB increased transcription from c-Myc promoter, resulting in an increase in expression of c-Myc protein in cells. The c-Myc transcription factor recruitment onto the *Gnpat* promoter reduced the transcription, resulting in downregulation of *Gnpat* expression. Downregulation of *Gnpat* by inflammation could reduce plasmalogen synthesis in glial cells. (Source: Hossain et al., *J. Neuroscience,* 2017)

processes. The c-Myc expression is increased in astrocytes of the brain tissues of patients with AD and Parkinson's disease (PD) [39]. Myc haploinsufficient mice ($Myc^{-/+}$) live longer than the wild-type mice, suggesting that reduction of Myc protein could enhance life span [40]. Therefore, the increase of c-Myc in older people could accelerate aging process and could reduce life span, which could be due to the reduction of Pls contents. We can suggest that the increased level of Pls and reduced level of c-Myc protein could reduce the risk of cancer and increase life span (Fig. 13.6). It is still unknown whether Pls can reduce c-Myc expression and inhibit cancer progression. More studies will be necessary to address this issue. It will be very important to know the contents of Pls in the c-Myc-amplified tumors to examine the role of Pls in tumor growth and regression. Chronic inflammation is often associated with cancers such as colitis, pancreatitis, and hepatitis which are linked to colon, pancreatic, and liver cancers, respectively. The Pls could be reduced by those chronic inflammation processes, resulting in the increase of risk of having cancers. Therefore, any therapeutic approach to increase Pls in the affected tissues might have promising anti-inflammation and anticancer activities. Additional studies will be necessary to address these issues.

13.4.4 Pls Inhibit Neuroinflammation

It has been known that inflammation signals reduce cellular Pls by downregulating the Pls-synthesizing enzyme called GNPAT via activation of NF-*k*B and c-Myc. Therefore, the next question which remained unknown was whether Pls could prevent neuroinflammation. Interestingly, i.p. injection of purified ethanolamine Pls (Pls-Etn) effectively inhibited neuroinflammation in the murine brain [41]. The i.p. injection of Pls-Etn attenuated the downregulation of Pls contents during neuroinflammation caused by LPS. These findings suggest that peripheral injection of Pls could prevent the reduction of Pls

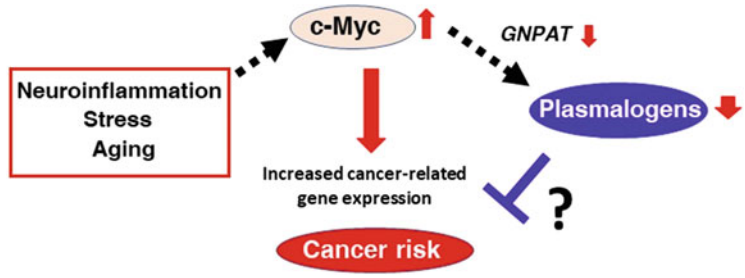

Fig. 13.6 Schematic diagram shows the c-Myc-mediated reduction of Pls might increase the risk of cancer. Neuroinflammation, stress and aging increased c-Myc. The c-Myc could increase cancer risk by inducing proto-oncogene expression. c-Myc could also reduce Pls by downregulating GNPAT expression. Reduction of Pls by c-Myc might accelerate cancer progression, suggesting that Pls might have inhibitory effects on cancer progression

during brain inflammation. Therefore, it is possible that Pls intake can prevent brain inflammation. Here, the brain inflammation means the activation of glial cells in the murine brain by i.p. injection of LPS. In addition to the i.p. injection of Pls, the oral intake of Pls could also prevent neuroinflammation and reduce accumulation of Aβ in the brain cells including neurons and astrocytes [42]. It is still unknown how the peripherally injected Pls prevented neuroinflammation and attenuated the reduction of Pls in the brain. Further studies are necessary to address this issue. Direct application of Pls to the cultured microglial cells prevented the LPS-mediated inflammatory signals in microglial cells and reduced expression of pro-inflammatory cytokines including IL-1β, TNF-α, and MCP-1 [41–44]. This evidence suggests that Pls could have direct effects on the glial cells to protect from inflammation. To examine the mechanism how Pls could inhibit neuroinflammation, we investigated the inflammatory processes in microglial cells by in vitro experiments. It has been known that endocytosis of TLR4 is one of the key events to induce inflammatory signal in microglial cells. We found that the inhibition of TLR4 endocytosis by dynasore, a GTPase inhibitor, in microglial cells effectively inhibited LPS-mediated inflammatory signal [43]. In addition, inhibition of caspase-3 also prevented the LPS-induced inflammatory signal. These results suggest that in microglial cells, LPS-mediated inflammatory signal is effectively controlled by two events: the first is the endocytosis of TLR4 and the second is the activation of caspase-3. Activation of caspase-3 is found to be associated with induction of NF-kB-mediated transcriptional regulation of pro-inflammatory genes (Fig. 13.7). Treatments of microglial cells by purified Pls extracted from a scallop, which are rich in the DHA-containing Pls, inhibited endocytosis of TLR4 (Fig. 13.7) [43]. The endocytosis process of TLR4 is influenced by various factors. It is known that LPS treatments could enhance endocytosis of TLR4 by increasing its recruitment with the adaptor proteins such as CD14 and MD2. LPS treatments enhance the formation of the complex, TLR4-CD14-MD2, which is necessary for the endocytosis of TLR4 to induce inflammatory signal in glial cells. Pro-inflammatory cytokines are not always bad for the health, because they also have defensive roles against infection and other minor inflammation. However, nitric oxide (NO) is a signal molecule which is well known to be involved in the pathogenesis of inflammation-related diseases. The increased level of NO is produced by overactivity of nitric oxide synthase-2 (NOS2) gene in cells. Overproduction of NOS2 could induce NO which is able to maintain the inflammatory signals in various diseases including rheumatoid arthritis and lung cancers. Macrophage-inducible NO production could initiate tumor cell growth such as in squamous cell carcinoma (SCC)

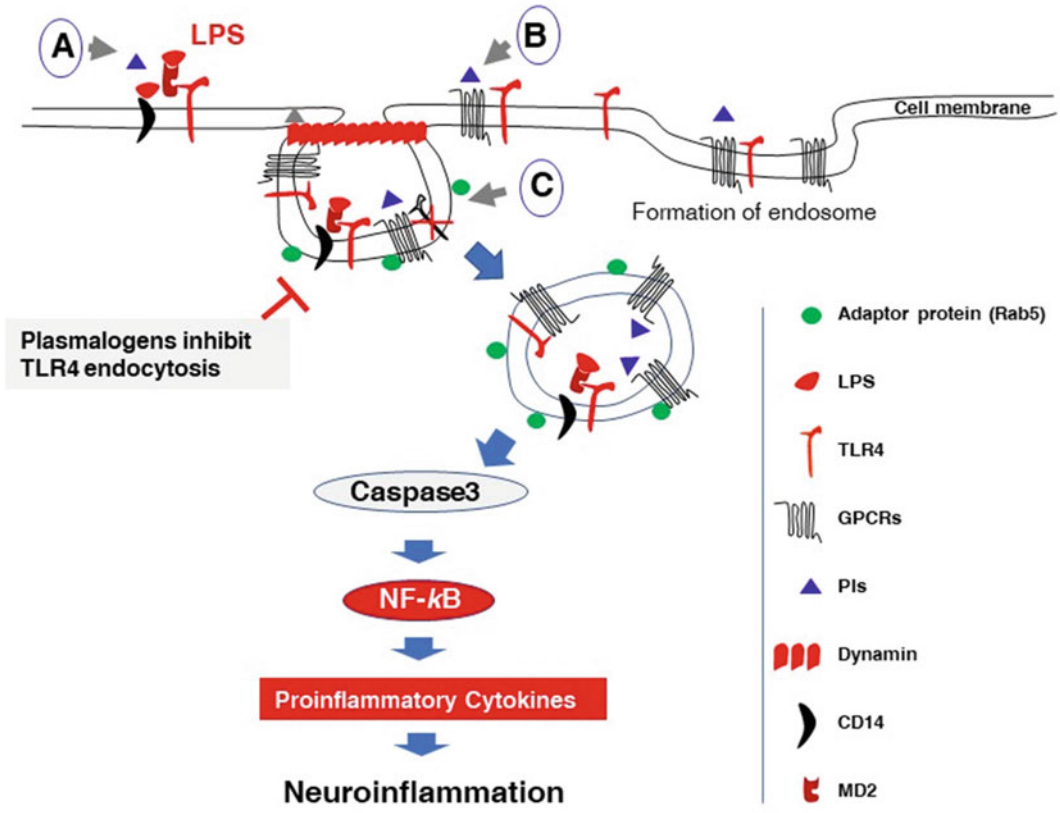

Fig. 13.7 Schematic diagram of Endocytosis of TLR4 and the inhibitory mechanism of plasmalogens. (**a**) Pls might function as ligands for CD14 or MD2 to block the LPS effects on TLR4 activation. (**b**) Pls-mediated activation of the GPCRs (possible ligands for Pls) might inhibit recruitment of CD14 and MD2 to TLR4. (**c**) Pls-mediated activation of the GPCRs might reduce TLR4 recruitment to endosomal vesicles. (Source: Fatma A. et al., Mol. Neurobiol., 2018)

[45]. Elevated NO in serum could be a marker for systemic inflammation. Macrophages are the prime sources of NO, and we examined whether Pls could inhibit NO production during the inflammation. We used the brain macrophage-like cells, microglia, and found that treatments with scallop-Pls inhibited NO production and also inhibited the NOS2 gene expression during the LPS-mediated inflammation (Fig. 13.8) [44]. It has been known that there are two LPS-induced inflammatory signals, MyD88-dependent and MyD88-independent, which can induce NOS2 expression in macrophage-like cells (Fig. 13.8). The scallop-Pls, which are high in PUFA-containing Pls, inhibited both the pathways to attenuate upregulation of NOS2 and NO. The LPS-mediated inflammation also reduced the Pls synthesis by downregulation of two key enzymes of Pls synthesis, GNPAT and AGPS (Fig. 13.8). Pls inhibited not only the NF-kB but also the p38MAPK activity in glial cells [44]. The c-Fos/c-Jun transcriptional activity was involved in upregulation of NOS2 expression in microglial cells, and this was inhibited by DHA-Pls but not by oleic acid Pls [44], suggesting that PUFA-containing Pls are effective in inhibiting inflammation signals in microglial cells. It is still unknown whether the DHA-containing Pls-Etn, which showed the inflammation inhibitory effects in the microglial

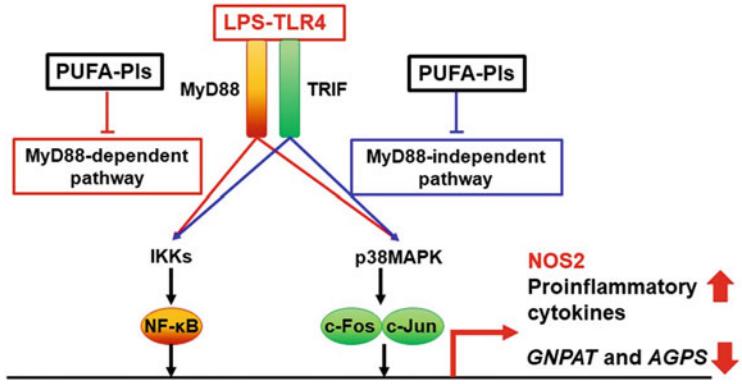

Fig. 13.8 PUFA-Pls inhibit LPS-mediated inflammatory processes and *NOS2* expression in microglial cells. PUFA-Pls could inhibit both MyD88-dependent and independent pathways of inflammatory signals in microglial cells. Activation of NF-kB and p38MAPK were inhibited by Pls. LPS-mediated inflammatory signals induced NOS2 expression and at the same time reduced the Pls synthesizing enzymes GNPAT and AGPS. (Source: Youssef et al., Neuroscience, 2019)

cells, is the main Pls component to show anti-inflammatory activities. Additional experiments will be necessary to address this issue by using the other PUFA-containing Pls inducing EPA and arachidonic acids.

13.5 Plasmalogens and Cognitive Function

Pls have long been known to be reduced among AD patients especially in the brain and serum, but it remained unknown whether Pls had any influences on cognitive function. In experimental mice, and in human subjects, the deficiency of cognitive function has been linked with brain inflammation. Although the ethanolamine Pls were found to be reduced in the postmortem brains of AD, it is likely that the downregulation of Pls can occur at the early stage of disease progression especially before the excessive accumulation of amyloid beta in the brain. This can be suggested by the findings that Pls-Etn are reduced in serum among the patients with MCI. MCI is the early stage of AD and the excessive accumulation of Aβ is not seen in these brains.

13.5.1 Plasmalogens Enhance Memory

In the clinical trial, oral ingestion of Pls showed beneficial effects to improve memory [34]. The oral ingestion of Pls for 6 months improved cognitive function among patients with AD and MCI. In this clinical study, the scallop-derived Pls which are high with DHA- and EPA-containing Pls were used. We still do not know whether the Pls, extracted from chicken, which are high with arachidonic acid-containing Pls, are also effective to enhance cognitive function of patients with AD. In Morris water maze memory test, it has been found that oral ingestion of scallop-Pls could improve learning and memory (manuscript in preparation). Therefore, oral intake of Pls has cognitive improvement function in mice and in human. These findings are very interesting especially in the aspect of therapeutic potential to improve cognitive function. The clinical studies were carried out among the patients with cognitive impairments. Therefore, it is still unknown whether oral intake of Pls could prevent the onset of AD. Additional experiments will be necessary to address this issue. Pls were found to be reduced at the early stage of AD and among the aged mice, which suggests that a reduction of Pls might initiate the progression of AD. Therefore, it

could be likely that oral ingestion of Pls might prevent the onset of AD. This hypothesis could be supported by our recent findings that daily intake of Pls prevented the onset of AD-like pathologies such as accumulation of Aβ in the brain of triple transgenic AD (3xTg-AD) model mice (manuscript in preparation). It is still not known how the oral intake of Pls prevented the accumulation of Aβ proteins in the brain of AD mice. Additional studies could reveal the mechanism how the orally ingested Pls could prevent the onset of AD. We previously found that brain inflammation reduced learning and memory among experimental mice and the oral intake of Pls attenuated the learning and memory processes associated with attenuation of glial activation in the brain [42]. The changes in the brain to boost cognitive function among patients with AD who underwent clinical studies of oral intake of Pls remained largely unknown. To examine the memory-related changes in the brain by Pls diet, we performed mice experiment.

13.5.2 Plasmalogens Induce Memory-Related Gene Expression in Neuronal Cells

It remained unknown how the oral intake of Pls improved cognitive function among the AD patients. To examine the possible memory-boosting role of plasmalogens, we performed mice study and in vitro studies with cultured neuronal cells. Oral ingestion of scallop-Pls for 2 months improved learning and memory tasks in Morris water maze test. This memory is called hippocampal-dependent spatial memory. Spatial memory is also found to be reduced in patients with AD and even in older population. The brain-derived neurotrophic factor (BDNF) is a well-known neuropeptide that plays a crucial role in maintaining spatial memory in mice and in human. The reduction of hippocampal BDNF might reduce spatial learning and memory. BDNF could play one of the key roles in memory process because of their ability to regulate various events related to memory such as increasing the dendritic spines and enhancing the synaptic transmission (Fig. 13.9). Interestingly, the oral ingestion of Pls increased expression of BDNF in the hippocampus of adult mice and associated with the improvement of learning and memory tasks. These findings could suggest a possible mechanism of Pls-mediated improvement of cognitive function in patients with AD. BDNF could be secreted from neuronal cells and astrocytes in the brain. The Pls treatments in cultured neuronal cells induced BDNF expression and associated with the increased phosphorylation of AKT, ERK, and the transcriptional factor cAMP response element-binding (CREB) proteins (Fig. 13.9). Bioinformatic studies revealed that there are various CREB bindings sites onto the BDNF promoter and the Pls treatments increased the recruitment of p-CREB protein onto those binding sites, resulting in upregulation of *BDNF* transcription (Fig. 13.9). We previously found that Pls treatments increased phosphorylation of ERK and AKT in neuronal cells via the membrane-bound GPCR proteins [7, 46]. Activation of ERK and AKT could increase phosphorylation of CREB and could enhance their localization into the nucleus, resulting in the recruitments onto the BDNF promoter. The increased BDNF in extracellular fluids could increase translocation of the kinase receptor TrkB (receptor for BDNF) into lipid rafts (Fig. 13.9). The recruitment of TrkB or recruitment of GPCR in the lipid rafts could induce cellular signaling to enhance gene expression related to synaptic function and memory processes (Fig. 13.9). The Pls-mediated memory signaling is positively regulated by BDNF, and it is also known that BDNF stimulation could also induce Pls in neuronal cells. Therefore, Pls could have a very vital role in the memory processes of humans via their potentials to regulate BDNF in the hippocampus. Additional experiments are necessary to address this in the future.

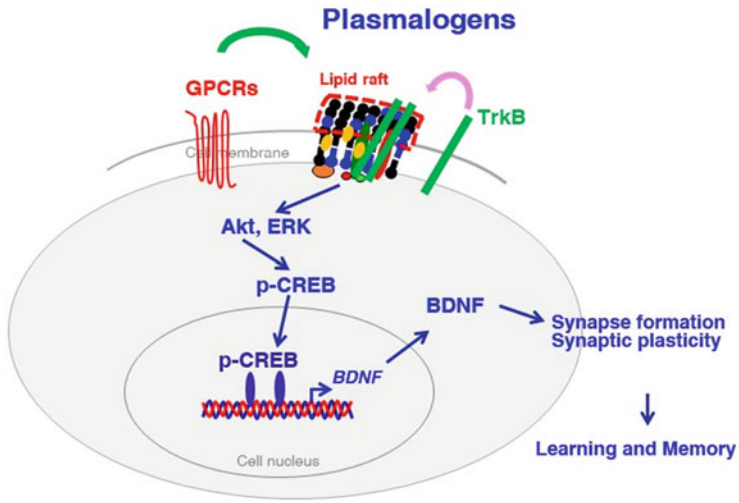

Fig. 13.9 Schematic diagram showing the possible mechanism of Pls-mediated memory signaling in neuronal cells. Lipid raft localization of TrkB and GPCRs (possible receptors for Pls) could induce activation of AKT and ERK proteins. AKT and ERK could induce phosphorylation of CREB transcription factor which could be recruited onto the *BDNF* promoter to induce its transcription. Pls-mediated BDNF induction in neuronal cells could play one of the major roles in improving cognitive function by increasing dendritic spines and synaptic plasticity

13.5.3 Reduction of Brain Pls Inhibits Learning and Memory Performance

It has been known that oral intake of Pls improved cognitive function among AD patients and in mice. However, it was mostly unknown whether a reduction of brain Pls itself could reduce cognitive function. The hippocampus is the key region of the brain controlling spatial memory. Spatial memory can be analyzed in mice by Morris water maze (MWM) tests. The MWM tests are one kind of navigation tasks which is often used to check hippocampal-mediated learning and memory process. We found a significant reduction of learning and memory tasks in mice when Pls concentration was reduced in the hippocampus by knockdown of Pls-synthesizing enzyme GNPAT (manuscript in submission). This finding suggests that reduction of brain Pls has negative effects on cognitive function in mice and probably also in humans. In mice, Pls reduction in the hippocampus caused the downregulation of *BDNF* gene associated with reduced expression of phosphorylated AKT and ERK proteins (Fig. 13.9). The Pls reduction in neuronal cells reduced recruitment of CREB transcriptional factor onto the *BDNF* promoter (manuscript in submission). In addition, the reduction of brain Pls showed a reduction of dendritic spines of the hippocampal neurons. Direct application of Pls in cultured neurons increased the dendritic spines, suggesting that Pls could induce BDNF expression to regulate dendritic function in maintaining memory processes in the hippocampus. Therefore, a reduction of hippocampal Pls content might impair cognitive function because of the reduction of BDNF. It is still unknown whether BDNF is reduced in the hippocampus of the AD patient's brain especially when Pls contents were decreased.

13.5.4 Plasmalogens Might Inhibit Systemic Inflammation to Enhance Cognitive Function

Besides the direct effect of Pls in the brain cells, it might also be possible that orally ingested Pls

could inhibit systemic inflammation to reduce neuroinflammation in the brain. Oral ingestion of Pls might inhibit the production of peripheral pro-inflammatory cytokines. In the gut, pathogenic bacteria can produce LPS that might induce systemic inflammation in older people. This systemic inflammation could have negative effects in cognitive function. In our previous study, we found that LPS injection reduced learning and memory in mice associated with the increased neuroinflammation in the brain. The oral ingestion of Pls inhibited this neuroinflammation in those mice, suggesting that Pls could prevent inflammatory signals to improve cognitive function [42]. Therefore, it is also likely that Pls-mediated improvement of cognitive function among the AD patients might be associated with the reduction of serum pro-inflammatory cytokines. Additional studies will be necessary to address whether Pls drinking could inhibit systemic inflammation or inflammation in gut macrophages. It has been known that systemic inflammation is increased among older people compared to young adult, suggesting that the anti-inflammatory role of Pls might be involved in part to improve the cognition.

13.5.5 Reduction of Brain Pls Is One of the Key Pathogenic Factors in Neurodegenerative Diseases

We recently identified that glial cells could secrete ethanolamine Pls (Pls-Etn) in extracellular medium, suggesting that microglia and astrocytes might also secrete Pls in the brain which could act on neuronal cells to modulate the brain function including cognitive function (Fig. 13.10). We previously argued that Pls synthesis in the glial cells could be reduced by aging, stress, and inflammation. The reduction of Pls in the brain might be one of the key pathogenic factors for neurodegenerative diseases associated with neuroinflammation. There are about 85 billion glial cells in the human brain, and they occupy about half the volume of the brain and spinal cord. The enrichment of glial cells in the brain gives rise to a possible hypothesis that a constant supply of Pls by glial cells could have tremendous effects on the neurons (Fig. 13.10). Pls are shown to have various beneficial effects related to neuronal function, which strongly suggests that a reduction of these lipids might have worse effects in the brain which could enhance the chances to have neurodegenerative diseases. These lipids are also reduced in neurodegenerative diseases including AD and PD. We also found that reactive glial cells in the AD brain have increased c-Myc expression that could reduce Pls synthesis by downregulating GNPAT. It is still unknown how Pls are reduced in the patient's brain with PD, but it could be due to neuroinflammation. Apoptosis or the shrinkages of healthy neurons is associated with neurodegenerative diseases. Because of antiapoptotic role, Pls in the adult brain could prevent neuronal cell death, suggesting that a reduction of brain Pls might lead to the neuronal cell death. Therefore, the reduction of Pls could be one of the major causes for neuronal damages associated with neurodegenerative diseases. A possible therapeutic approach to recover normal content of Pls in the neurodegenerative brains could have tremendous beneficial effects in attenuating the disease progression.

13.6 Plasmalogens and Aging

Many diseases including AD, diabetes, and immune dysfunction are related to aging because they appear to be common among older people compared to young individuals. Here, we discuss some aging-related phenotypes observed in *C. elegans* associated with the deficiency of Pls. It has been known that ethanolamine Pls are also enriched in *C. elegans*.

13.6.1 Deficiency of Pls Reduces Life Span

Interestingly, *C. elegans* has most of the Pls-synthesizing enzymes including *acl-7* (human analog of GNPAT), *fard-1* (human analog of FAR1), and *ads-1* (human analog of

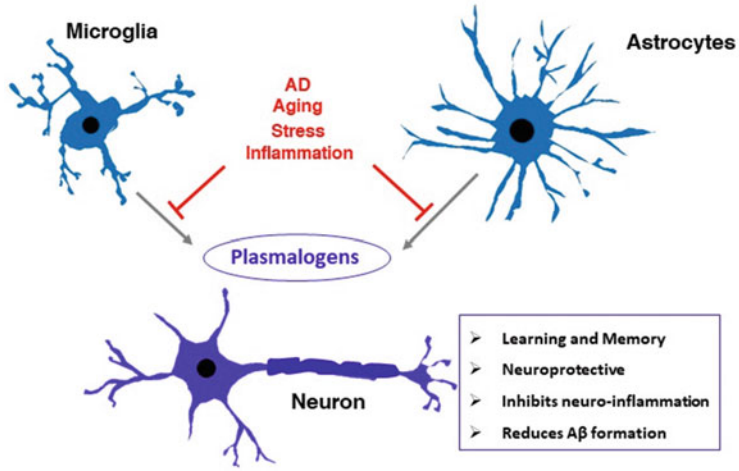

Fig. 13.10 Schematic diagram of biological effects of Plasmalogen in brain. Astrocytes and microglial cells could release plasmalogens in brain aiming to protect neuronal cells from various damages including neuroinflammation. Pls enhance learning and memory processes of brain. Pls reduce formation of toxic amyloid beta proteins in brain. Reduction of Pls in glial cells can be caused by aging, stress, and inflammation. This reduction is also found in patient's brain with Alzheimer's disease (AD)

AGPS). All these mutant (acl-7, ads-1 and fard-1) *C. elegans* showed a reduction of life span (manuscript in preparation). The contents of Pls-Etn were found to be reduced in these mutant *C. elegans* comparted to the wild-type *C. elegans* (N2). Primary evidence showed that reduction of Pls in these mutants was associated with the reduction of sir-2.1 gene (manuscript in preparation). In mouse cell line, Pls treatments induced protein expression of several analogs of sirtuins including Sirt1 (manuscript in preparation). These findings could suggest that Pls might enhance life span by regulating protein expression of sirtuins. Additional experiments will be necessary to address this.

13.6.2 Deficiency of Pls Shows Metabolic Syndrome-like Phenotype

The Pls-deficient *C. elegans* mutants (acl-7, ads-1, and fard-1) showed an increase in fat deposition compared to wild-type *C. elegans* when treated with high concentration of glucose (manuscript in preparation). It has been known that older people are more susceptible to getting fat compared to young individual. The glucose ingestion-mediated fat deposition in *C. elegans* could be a model of obesity. The mechanism of obesity in *C. elegans* could be different from humans. However, the increase in fat deposition among the Pls-deficient *C. elegans* could suggest that reduction of Pls among humans could have worse effects among diabetes patients. It has already been known that Pls are reduced among patients with diabetes (discussed earlier), suggesting that the Pls reduction might accelerate fat deposition among the patients with metabolic syndromes. Additional studies will be necessary to address this issue.

13.6.3 Deficiency of Pls Reduces Immune Function to Fight Against Pathogenic Bacteria

The reduction of immune defense is common among aged people, and they usually get worse effects when infected with bacteria or virus compared to younger people. We observed the increase in mortality of Pls-deficient *C. elegans*

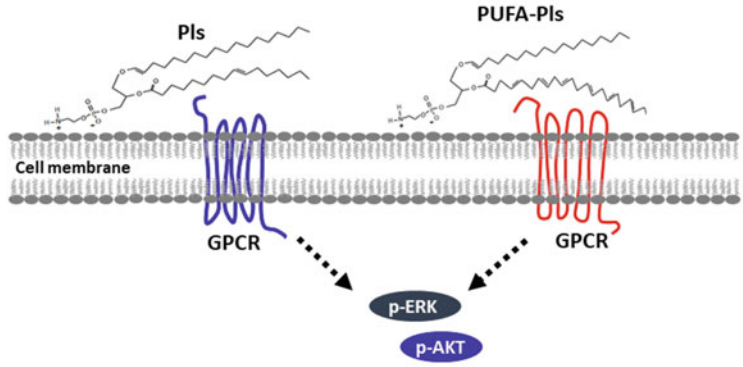

Fig. 13.11 Plasmalogens might function as hormones or ligands to elicit biological effects. Extracellular addition of Pls induces cellular signaling (phosphorylation of AKT and ERK) in a short period of time. Pls induce signaling events via the GPCRs (GPR1, GPR19, GPR21, GPR27, and GPR61). These GPCRs are found to be present not only in the brain but also found in gut epithelial cells, suggesting that Pls might have wider biological effects from brain to gut

mutants (acl-7, ads-1, and fard-1) compared to the wild type when subjected to infection with the pathogenic bacteria *Staphylococcus aureus* (manuscript in preparation). These findings suggest that Pls deficiency could reduce immune function to fight against the infection in *C. elegans*. It could be likely that reduction of Pls among older people might be linked to the reduction of their immune function to fight against bacterial infection or even viral infection. It is still unknown whether the Pls deficiency could be linked with the immune deficiency in human.

13.7 Mechanism of Action of Pls in Inducing Cellular Signaling

13.7.1 Plasmalogens Might Function as Ligands or Hormones to Elicit Biological Effects

Extracellular addition of Pls induces cellular signaling by GPCRs, which suggests that Pls might function as ligands or as hormones. We found that glial cells readily secret plasmalogens in extracellular space and the extracellular Pls can activate signaling molecules in neuronal cells, suggesting that Pls might function as paracrine hormones in the brain. In the periphery, Pls are detected in serum. Therefore, Pls could reach to various target organs and function as endocrine hormones to activate cells having the GPCR (possible receptor for Pls) expression. However, further studies will be necessary to address these issues. The oral ingestion of a very low dose of Pls (1 mg/day) has been shown to improve cognitive function in patients with AD, suggesting that Pls might function as potent bioactive compounds or as hormones. The in vitro studies showed that some membrane-bound GPCRs such as GPR1, GPR19, GPR21, GPR27, and GPR61 could regulate the Pls-mediated induction of cellular signaling. It is likely that Pls could modulate cellular function in any tissues of mammal if they expressed either of these receptors. These GPCRS are highly expressed in neuronal cells, suggesting that glial cell-mediated release of these bioactive phospholipids could stimulate the surrounding neurons by activating these GPCRs (Fig. 13.11). Interestingly, our recent evidence showed that GPR19 and GPR61 are expressed in the gut epithelial cells of mouse. Our study showed the presence of GPR19 and GPR61 in the gut epithelial cells, suggesting further that the low dose of Pls (1 mg/day) could elicit biological effects via activating these gut

GPCRs. In the clinical studies, 6-month oral ingestion of Pls at the low dose (1 mg/day) produced significant effect in improving cognitive function among AD patients. The blood plasma concentration of Pls is about 5 mg/dl, which raised a question on how a low dose of Pls, when ingested orally, could evoke physiological functions including the cognitive function. To answer this, we could suggest that the purified Pls, when ingested orally, could activate the GPCRs in the gut epithelial cells to induce biologically active substances which might affect the brain function either directly or indirectly. Pls are lipid soluble, and it is likely that they can be present in physiological system either as free or as lipoprotein. Lipoprotein formation of Pls has not been known, and it is still not known whether any soluble proteins could bind with free Pls. However, in physiological system like other lipids, Pls could exist as protein bond complexes called lipoproteins. However, when we treated the cells with purified Pls, which were free from any proteins, they could induce cellular signaling within 5 minutes. This evidence could suggest that free Pls are functionally active to induce cellular signaling via the membrane-bound GPCRs. However, the Pls could form lipoproteins inside the cells. The orally administered purified Pls used for the experiments and for the clinical trial were extracted from scallop and free of proteins, which suggests that the free-form of Pls are functionally active even when administered at a very low dose. This evidence could suggest that these special lipids could function as ligands or hormones to activate the GPCRs inside the gut epithelium or other tissues to elicit their biological activities. Pls have the polar hydrophilic group, ethanolamine, which could bind with polar groups on the cell membrane and could interact with surrounding GPCRs (Fig. 13.11). Additional experiments are necessary to address whether Pls could function as ligands or hormones to activate membrane-bound GPCR from outside of the cells.

13.7.2 Plasmalogens Might Change Membrane Dynamics to Induce Cellular Signaling

In addition to the hypothesis that Pls could function from outside of the cells as hormones or ligands, it could also be possible that these lipids can change the membrane dynamics. It has been known that phospholipids have a propensity to form liposome. The property to form liposome is the basis of their use as carriers for liposome-derived drug delivery systems. It is possible that Pls in extracellular space might form liposome because they could form hexagonal structure. Pls with longer tails could form bilayer of the liposome vesicles. These liposomes could integrate on the plasma membrane of live cells called fusion. The fusion of liposome is a well-known event in biological systems. This could change the membrane dynamics and could form lipid raft domains to induce the cellular signaling by recruiting the GPCRs or other membrane-bound receptors (Fig. 13.12). This hypothesis could be supported by the findings that Pls are highly enriched in lipid rafts of hippocampal tissues (manuscript in preparation). We noticed that glial cells can secret Pls-Etn in extracellular space, suggesting that these Pls could form liposome. In the brain, the glial cell-mediated secretion of Pls might lead to the formation of Pls-liposome. The Pls-liposome might fuse on the plasma membrane of the surrounding neurons to induce cellular signaling. It might also be possible that neuronal cells could release neurotransmitter by forming the Pls-liposome in the synaptic clefts. The glial cell-mediated formation of Pls-liposome could also enter in the synaptic cleft to modulate synaptic activity by integrating on axonal terminals or dendritic spines. Therefore, the existence of Pls-liposomes in the brain could have various biological effects probably by activating the membrane-bound GPCRs. Additional experiments will be necessary to address whether the GPCRs, which are the possible receptors of Pls, are present in neuronal synapses.

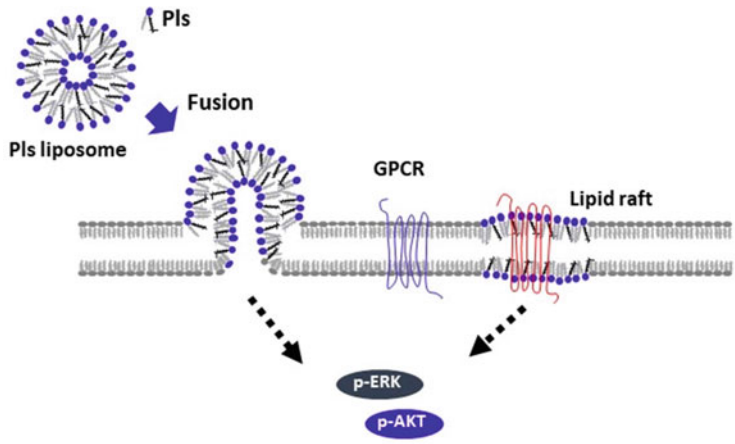

Fig. 13.12 Pls might induce cellular signaling by changing the membrane structure. Extracellular addition of Pls, which could exist as liposome, might integrate into cell membrane to induce cellular signaling by allowing activation of membrane protein(s) including GPCRs. Pls were found to be enriched in lipid raft and their enrichment might enhance localization of GPCRs in these compartments to induce cellular signaling

13.7.3 Other Possible Mode of Action of Plasmalogens

Like other phospholipids, Pls could be degraded to form several bioactive components including free fatty acids and lyso-Pls. These bioactive substances could also play a role to elicit physiological function. In addition to that, Pls could also maintain membrane structures in other intracellular organelles such as the mitochondrial and nuclear membrane. The membrane dynamics of these intracellular organelles could be strictly maintained by Pls, and a physiological reduction of Pls could have detrimental effects. Extracellular addition of Pls showed an increase in nuclear Pls, which suggests that because of their lipid solubility nature, Pls could penetrate the outer cell membrane to enter into the nucleus. The role of Pls in the nucleus is mostly unknown and mysterious. We found that Pls treatments increased transcriptional activity of the nuclear receptor, peroxisome proliferator-activated receptors (PPAR). However, we still do not know whether Pls can be integrated to nuclear membrane to modulate the PPAR activity or Pls could bind directly to PPARs transcription factor to increase their translocation onto genomic DNA. Further studies will be necessary to address these issues.

13.8 Conclusion and Future Direction

The deficiency of Pls in neurodegenerative diseases has been reported a long time ago, but until recently the function of Pls remained elusive. Recent studies showed that these lipids have various biological functions including their ability to inhibit neuroinflammation, increase neuronal survival, and enhance cognitive function. Direct application of these lipids to the cells showed promising effects in inhibiting inflammatory signals and in inducing activation of signaling molecules such as AKT and ERK. Pls also induced various gene expressions in neuronal cells related to memory including the neuropeptide BDNF. Surprisingly, Pls treatments for 5 minutes could increase phosphorylation of AKT and ERK proteins, suggesting that these lipids might function as hormones or ligands. Several GPCRs were also identified as possible receptors for these lipids. The Pls concentration of Pls in the human blood is about 5 mg/dl, but the oral ingestion of Pls at low dose (1 mg/day)

improved cognitive function in patients with AD, suggesting further that these lipids might work as hormones. We need further studies to elucidate the detailed mode of function of these lipids to explore novel therapeutics to prevent or even to cure various diseases which are associated with a reduction of Pls and systemic inflammation. To precisely understand the mode of function of Pls, we need to investigate the following: (1) What type of Pls is biologically active? (2) Do Pls work as hormone or ligands to activate membrane protein? (3) How did the extracellular Pls activate membrane receptors? (4) Do Pls activate nuclear receptor by a direct interaction? (5) Do Pls cross the blood-brain barrier? (6) Do the orally ingested Pls have any peripheral effects to enhance cognitive function? Therefore, a lot of studies will be necessary to understand the mode of function of Pls in our health. The outcomes of these studies could reveal novel therapeutic strategies to improve our health.

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