22 Lipids in Alzheimer's Disease Brain

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Abstract: Lipids are important biological molecules. The lipids of physiological importance for humans have four major functions: (1) structural components of biological membranes; (2) energy reserves, predominantly in the form of triacylglycerols; (3) both lipids and lipid derivatives serve as vitamins and hormones, and (4) lipophilic bile acids aid in lipid solubilization. Fatty acids fill two major roles in the body: as the components of more complex membrane lipids and as the major components of stored fat in the form of triacylglycerols. Alterations in lipid structure and/or metabolism lead to many neurodegenerative diseases, among which Alzheimer's disease (AD) is of great concern due to the increasing life-span of the world's population. Additionally, altered cholesterol metabolism, modulation in phospholipid content, and phospholipid asymmetry in plasma membranes may play pivotal role in the progression of AD. Amyloid β-peptide [Aβ (1–42)] plays a central role in the pathogenesis of AD. Aβ (1–42) is heavily deposited in the brains of Alzheimer's disease (AD) patients, and free radical oxidative stress of neuronal lipids is extensive. Research by our group and others suggests that this observation is linked to Aβ-induced oxidative stress in AD brain. This chapter summarizes current knowledge on lipid alterations in AD brain, one potential cause of the external oxidative stress in AD brain, and the consequences of Aβ-induced lipid peroxidation in this neurodegenerative disorder.

List of Abbreviations: AD, Alzheimer's disease; Aβ(1-42), amyloid beta-peptide; PtdSer, Phosphatidylserine; PtdEtn, Phosphatidylethanolamine; PtdIns, phosphatidylinositol; PtdCho, phosphatidylcholine; GEM, glycosphingolipid-enriched membranes; RBC, red blood cells; i-NOS, inducible nitric oxide synthase; GPC, glycerophosphatidylcholine; GPE, glycerophosphatidylethanolamine; $sPLA_2$, Ca^{2+} -dependent secretory phospholipase A_2; $cPLA_2$, Ca^{2+} -dependent cytosolic phospholipase A_2; $iPLA_2$, Ca^{2+} -independent phospholipase A_2; HNE, 4-hydroxy-2-nonenal; DAG, diacylglycerol; NCT, nicastrin; βaPP, β-amyloid precursor protein; TBARS, thiobarbituric acid reactive substances; IsoPs, isoprostanes; NPs, neuroprostanes; PG, prostaglandin; LV, levuglandins; NeuroKs, neuroketals; NFT, neurofibrillary tangles; SP, senile plaques; FAEE, ferulic acid ethyl ester; MCL, mild cognitive impairment; ROS, reactive oxygen species; RNS, reactive nitrogen species

1 Introduction

Alzheimer's disease (AD) is a progressive dementing disorder characterized clinically by a loss of memory and cognition, and later aphasia (Katzman and Saitoh, 1991). Pathologically AD is characterized by senile plaques (composed of aggregated amyloid β-peptide [Aβ]) and dystrophic neurites, neurofibrillary tangles (composed of hyperphosphorylation of tau), and synapse loss (Katzman and Saitoh, 1991). The AD brain is under oxidative stress, including lipid peroxidation (Butterfield and Lauderback, 2002). Aβ can induce lipid peroxidation (Butterfield et al., 1994; Mark et al., 1997; Lauderback et al., 2001).

Lipids are amphipathic molecules. Phospholipids derive from glycerol esters of two fatty acids and phosphoric acid. The latter moiety is esterified to an alcohol that gives the phospholipid its name. With numerous species of lipids expressed in eukaryotic plasma membranes and cells have the task of organizing the lateral and transverse distribution of membrane phospholipids to specific sites. Cells have developed a number of mechanisms to deal with this issue, one of which is to create a dynamic equilibrium to their site of function that ensures interaction of specific lipids with appropriate partner moieties. The targeting of phospholipids to specific membrane sites is essential for maintaining cell shape, homeostasis, and signal transduction cascades. Aminophospholipids also have been implicated in a diverse array of processes ranging from catabolism to inflammation and cell proliferation to cell death (*Table 22-1*).

The components of biological membranes are asymmetrically distributed between the membrane surfaces, and lipids are asymmetrical as they are present on both sides of the bilayer, but in different and highly variable amounts. Asymmetry is maintained by the action of a protein that requires ATP known as flippase. The choline-containing phospholipids (phosphatidylcholine and sphingomyelin) are found on the external surface of the plasma membrane, while the aminophospholipids (phosphatidylserine [PtdSer] and phosphatidylethanolamine [PtdEtn) are localized primarily on the cytoplasmic lamellae (Kurz et al., 2005). ATP-dependent processes (Pomorski et al., 2001) maintain phospholipid asymmetry, which is critical to normal cell function. An alteration in phospholipid distribution, particularly the appearance of PtdSer on the extracellular surface,

Table 22-1
Arrangement of aminophospholipids in outer leaflet of cells

Cell type	Lipid	Remarks	References
Non-pathological cells			
PC-12 cells	PtdEtn	Decrease in Outer leaflet PE	Ikemoto et al. (1999)
Macrophages	PtdSer	PtdSer expression for engulfment of apoptotic targets	Callahan et al. (2000)
Mast cells	PtdSer	PS exposure on IgE stimulation	Martin et al. (2000)
Pathologic cells			
Apoptotic cells	PtdSer	PS expression an early event	Fadok et al. (1992)
Aβ-treated cells	PtdSer	PS expression as an early event (Annexin V and NBD-PS labeling)	Mohmmad Abdul and Butterfield (2005)
Carcinoma	PtdSer	Annexin V Labeling	Rao et al. (1992)
Diabetes	PtdSer, PtdEtn	Demonstrated in vitro from patient samples	Wali et al. (1988)

occurs during apoptosis and contributes to the recognition and destruction of these cells by macrophages (Kirschnek et al., 2005).

2 Lipids in Plasma Membranes

2.1 Types of Lipids in Plasma Membranes

Plasma membranes contain a wide diversity of lipids, all of which are amphipathic. There are three main types of membrane lipids: phosphoglycerides, sphingolipids, and cholesterol. Most membrane lipids contain a phosphate group, which makes them phospholipids, and as they are built on glycerol backbone, they are called phosphoglycerides. Membrane phosphoglycerides have an additional group linked to the phosphate, most commonly choline (forming phosphatidylcholine, PtdCho), ethanolamine (forming phosphatidylethanolamine, PtdEtn), serine (forming phosphatidylserine, PtdSer), or inositol (forming phosphatidylinostol, PtdIns). Each of these groups is small and hydrophilic and together with the negatively charged phosphate to which they are attached, forms a, highly water-soluble domain at one end of the molecule, called the head group. At physiological pH, the head groups of PtdSer and PtdIns have an overall negative charge, whereas those of PtdCho and PtdEtn are zwitterions. Phosphoglycerides most often contain one saturated (α-chain) and one unsaturated fatty acyl chain (β-chain).

A less abundant class of membrane lipids, which are the derivatives of sphingosine, an amino alcohol that contains a long hydrocarbon chain, is called sphingolipids. Sphingolipids consist of sphingosine linked to a fatty acid by its amino group, called ceramide. The various sphingosine-based lipids have additional groups esterified to the terminal alcohol of the sphingosine moiety. If the substitution is phosphorylcholine, the molecule is sphingomyelin. If the substitution is a carbohydrate, the molecule is a glycolipid. If the carbohydrate is a simple sugar, the glycolipid is called cerebroside; if the carbohydrate is an oligosaccharide, the glycolipid is called a ganglioside. All the sphingolipids are amphipathic and basically similar in overall structure to the phosphoglycerides. Another lipid component of certain membranes is the sterol cholesterol, which in certain animal cells may constitute up to 50% of the lipid molecules in the plasma membrane. Cholesterol is smaller than the other lipids of the membrane and less amphipathic. Cholesterol leads to increased lipid fluidity in membranes in which unsaturated lipids occur. Cholesterol is asymmetrically distributed in the membrane (Wood et al., 1990).

Lipid rafts are specialized membrane domains enriched in certain lipids, cholesterol, and proteins (Simons and Ikonen, 1997). Caveolae are flask-shaped invaginations on the cell surface that are a type of

membrane raft; these moieties were named "caveolae intracellulare" (Yamada, 1955). It presently seems that there could be three types of lipid rafts: caveolae; glycosphingolipid-enriched membranes (GEM); and polyphosphoinositol-rich rafts. It may also be that there are inside rafts (PIP_2 rich and caveolae) and outside rafts (GEM). Lipid rafts are presumed to be signaling centers, perhaps regions of cholesterol import (Huang et al., 1999) and involved in endocytosis (Thomsen et al., 2002).

2.2 Synthesis and Transport of Phospholipids to Plasma Membrane

Assembly of the phospholipid bilayer of cellular membranes is a fundamental aspect of cell growth and proliferation. Phospholipids are concomitantly synthesized and inserted at the cytoplasmic surface of the endoplasmic reticulum. Following this asymmetric assembly, transmembrane movement to the lumenal leaflet of the endoplasmic reticulum must occur in order to ensure coordinated growth of the bilayer. For phosphatidylcholine, the predominant phospholipid of eukaryotic membranes, the latter process appears to be facilitated by a specific transport protein. There are several pathways for the synthesis of aminophospholipids in eukaryotic cells (Vance and Shiao, 1996; Voelker, 2000). Phosphatidylserine (PtdSer) is synthesized by a Ca^{2+}-dependent PtdSer synthase-catalyzed reaction in which serine is exchanged with the head group of phosphatidylcholine (PtdCho) or phosphatidylethanolamine (PtdEtn) (Kuge et al., 1986). This occurs mainly in the endoplasmic reticulum (ER) and in specialized ER-derived mitochondrial-associated membranes [MAM] (Vance and Vance, 1988) that bridge the ER to the mitochondria. The newly synthesized lipid is transported from the MAM to the mitochondrial outer membrane by an ATP-dependent mechanism (Voelker, 1989). The lipid then moves to the inner membrane where it serves as a substrate for PS decarboxylase I to generate PE. The Golgi and vacuoles also synthesize PE by decarboxylating PS with decarboxylase II. Both vesicular and cytosolic protein-mediated transfer mechanisms are involved in the transport of aminophospholipids from their sites of synthesis to the plasma membrane (Sprong et al., 2001). The delivery of lipids from extracellular sources can also alter the transverse distribution of phospholipids. HDL and LDL act as lipid transport proteins in plasma to deliver phospholipids, cholesterol, and fatty acids to the cell's outer membrane leaflet where they are utilized by the cell.

Additionally, endo- and exocytosis, which involve multiple membrane fusion events, may induce lipid intermixing between membrane leaflets. In order to maintain an appropriate asymmetric aminophospholipid distribution, cells have developed several mechanisms. Lipid asymmetry is generated primarily by selective synthesis of lipids on one side of the membrane. Because passive lipid transbilayer diffusion is slow, a number of proteins have evolved to maintain this lipid gradient. These proteins fall into three classes: (1) "flippases" are cytofacially-directed, ATP-dependent transporters (Seigneuret and Devaux, 1984); (2) "floppases" are exofacially-directed, ATP-dependent transporters (Connor et al., 1992); and (3) "scramblases" are bidirectional, ATP-independent transporters (Williamson et al., 1985). The flippase is highly selective for phosphatidylserine and functions to keep this lipid sequestered crossing from the external cell surface. Floppase activity has been associated with the ABC class of transmembrane transporters. Although they are primarily nonspecific, at least two members of this class display selectivity for their substrate lipid. Scramblases are inherently nonspecific and function to randomize the distribution of newly synthesized lipids in the endoplasmic reticulum or plasma membrane lipids in activated cells. It is the combined action of these proteins and the physical properties of the membrane bilayer that generate and maintain transbilayer lipid asymmetry.

2.3 Identification of Phospholipid Transporters

Once lipid asymmetry has been established, it is maintained by a combination of slow transbilayer diffusion, protein–lipid interactions, and protein-mediated transport. The thermodynamic barrier to passive lipid flip-flop prevents rapid spontaneous transbilayer diffusion of phospholipids. The half time for phospholipid flip-flop is approximately long and depends on the nature of the lipid and the membrane. In the human erythrocyte, flip-flop rates are dependent on the phospholipid acyl chain length and the

degree of unsaturation (Middelkoop et al., 1986). Although membrane lipid asymmetry has been known for many years, the mechanisms for maintaining or regulating the transbilayer lipid distribution are still not completely understood. Perhaps the most significant contributors to the maintenance of transbilayer lipid asymmetry are proteins that catalyze the movement of lipids across the membrane (*Table 22-2*). These activities are classified according to the substrate specificity, requirements for energy, and direction of transport. Most of the pivotal studies of membrane phospholipid asymmetry have been performed in human red blood cells (RBC). Although these cells have provided a wealth of information on the biochemical properties of aminophospholipid transporters, further progress has been impeded by the inability to identify the active protein components in these cells by molecular biology techniques. The ultimate transbilayer distribution of lipids is determined by the specificity of the lipid transporters located in the membrane. Each of the transport activities described in a following section displays a unique specificity or non-specificity that defines its function in the determination of lipid organization. The following summarizes the current state of knowledge regarding the specificity of these transport activities.

2.3.1 Aminophospholipid Translocase (Flippase)

Flippase is widely distributed and is present in most plasma membranes. Aminophospholipid flippase activity is expressed in erythrocytes and has been detected in a wide variety of cell types and membranes, including platelets, lymphocytes, spermatozoa, and synaptosomes (Zachowski and Gaudry-Talarmain, 1990; Muller et al., 1994). It is likely that this transporter is essential for any membrane in which the maintenance of PtdSer asymmetry is required. The aminophospholipid flippase is perhaps the most selective of the lipid transporters. This protein prefers PtdSer to other lipids (Daleke and Huestis, 1985), and the specificity for PtdSer is defined by each of the functional groups of the lipid. Sphingolipids (Morrot et al., 1989) and diether analogs of PtdSer are also recognized as transport substrates, but transport rates are reduced compared with diacylglycerophosphoserine. The glycerol backbone is another important recognition element. There is some flexibility in lipid backbone recognition by the enzyme. However, the enzyme displays an absolute requirement for the stereochemistry of the glycerol backbone; the *sn*-2,3-glycerol analog of the naturally occurring *sn*-1,2-glycero-lipid is not a substrate for transport (Martin and Pagano, 1987). In contrast to the polar headgroup specificity, the flippase is capable of accepting PtdSer molecules containing fatty acids of various lengths and composition, including those modified by spin, fluorescent, and photoactivatable groups (Colleau et al., 1991; Demaurex et al., 1997). This activity is associated with an ATP-dependent rapid movement (within seconds to several minutes) of both PtdSer and PtdEtn from the outer to inner membrane leaflet in virtually all eukaryotic cells. Based on its ATP dependence and sensitivity to fluoride and vanadate, Seigneuret and Devaux (Seigneuret and Devaux, 1984) postulated the activity to be associated with an aminophospholipid-specific transport protein. Thus, the aminophospholipid transporter requires the participation of a Mg^{2+}-ATPase, whereas the varied and sometimes conflicting results raise the possibility that several distinct proteins cooperate to form a functional aminophospholipid transport complex.

Table 22-2
Lipid transporters

Class	Protein	Specificity	References
Flippases	Erythrocyte Mg^{2+}-ATPase	PS	Daleke and Lyles (2000)
	ABCR	PE	Weng et al. (1999)
	P_4-ATPases	Amphipaths	Halleck et al. (1998)
Floppases	ABCA1	Cholesterol	Rust et al. (1999)
	ABCB4	PC	van Helvoort et al. (1996)
	ABCC1	Short-chain phospholipids	Kamp and Haest (1998)
Scramblases	ER flippase	None	Buton et al. (1996)

ATPase II is another flippase that has been purified and cloned from bovine chromaffin granules (Moriyama and Nelson, 1988), the close homologs of which have been identified from bovine brain and human sources. These proteins are members of a new class of ATPases, the P4-ATPases (Tang et al., 1996). Defects in genes of this family produce alterations in ribosomal assembly, and familial intrahepatic cholestasis. Like the erythrocyte Mg^{2+}-ATPase, the ATPase activity of these enzymes is selectively activated by PtdSer (Moriyama et al., 1991). Although no direct evidence for transbilayer phospholipid transport has been reported, it is likely that the P_4-ATPases are involved either directly or indirectly in amphipath transport.

2.3.2 Multidrug Resistance Proteins

The second class of ATP-dependent lipid transporters is the exofacially-directed floppases. Early studies in red blood cells revealed a nonspecific outward flux pathway for NBD [1-palmitoyl-2-[6(7-nitrobenz-2-oxa-1, 3-diazol-4-yl)amino]caproyl-*sn*-glycero-3-phosphoserine] and spin-labeled lipids (Connor et al., 1992). It was recognized subsequently that some members of the ABC transporter superfamily are also capable of transporting lipids (Borst et al., 2000). ABC transporters are a diverse group of proteins that, in general, are responsible for the ATP-dependent export of amphipathic compounds. These include the multidrug resistance proteins, which export cytotoxic xenobiotics and were first discovered in drug-resistant tumor cells. Consistent with their role in general xenobiotic amphipath export, ABC proteins are, for the most part, nonspecific. However, some members of this class demonstrate a unique specificity for their respective substrate. The most well-characterized lipid floppase activities are those catalyzed by ABCA1, ABCB1, ABCB4, and ABCC1. They are broadly classified into P-glycoprotein (MDR), multidrug resistance-associated protein (MRP), and mitoxantrone resistance protein (MXR) by their size and the number of transmembrane loops (Gottesman and Pastan, 1993). The substrates for MDR and MXR are largely hydrophobic or amphiphilic, whereas those recognized by MRP are principally anionic and likely require cotransport of glutathione disulfide (GSSG) (and/or GSSX) to be operative. Experiments using fluorescent and spin-labeled phospholipid analogs provided evidence for the existence of an ATP- and protein-dependent nonspecific floppase that transports lipid from the inner to outer membrane leaflet (Connor et al., 1992). More recent studies, however, indicate that transport is a result of MRP1 activity that expels lipids out of the cells, because their fluorescent and spin-labeled reporter tags imbued the lipid with drug-like properties (Kamp and Haest, 1998). Although these results seem to eliminate a role for the purported nonspecific floppases in the externalization of authentic phospholipids, several lipid-specific floppases have been identified that include human MDR3 (Ruetz and Gros, 1994) and ABC1 (Williamson et al., 1992), which function as true PtdCho- and PtdSer-specific floppases, respectively. Taken together, these results suggest that the steady-state equilibrium distribution of membrane phospholipids is maintained by joint activities of an aminophospholipid-specific flippase and cholinephospholipid-specific floppase (MDR family) and that acquisition of the PS-expressing apoptotic phenotype requires activation of ABC1 coincident with Ca^{2+}-mediated inhibition of the aminophospholipid translocase (Williamson et al., 1992). It is interesting to note that not all ABC lipid transporters are floppases. ABCR is another ABC protein with lipid transport activity although it is a flippase, rather than floppase.

2.3.3 Scramblase

Studies with platelet and red cell membranes revealed the existence of a Ca^{2+}-dependent scrambling activity that seemed to result in complete intermixing of lipids between bilayer leaflets irrespective of headgroup specificity (Hamon et al., 2000). Three scramblase activities have been reported; two are involved in dissipating lipid gradients in biological membranes and the third is activated by Ca^{2+} in the plasma membrane of stimulated cells. The ER scramblase is known to be relatively nonspecific and was first described as a bidirectional transporter of PC and its metabolites (Chapman and Trelease, 1991). Evidence has not been found for the activity of these transporters in the plasma membrane. Thus, they may serve only to redistribute newly synthesized lipids or lipid precursors in ER and Golgi membranes. Albeit less extensive

than for platelets, lipid scrambling has also been demonstrated in a variety of other cells such as lymphocytes, endothelial cells, red blood cells, smooth muscle cells and tumorigenic cells. An increase in the intracellular calcium concentration, for instance, evoked by cellular activation, complement pore formation, or induction of apoptosis, is an essential requisite for the onset of the scrambling process. Increased intracellular Ca^{2+} can also involve activity of aminophospholipid translocase activity, indicating that the loss of lipid asymmetry does not result from mere inhibition of aminophospholipid translocase activity (Kuypers et al., 1996). Scrambling requires the continuous presence of Ca^{2+}. Removal of intracellular calcium causes an arrest of the scrambling process and may even restore aminophospholipid translocase activity, provided that its irreversible degradation by calpain is prevented. The scrambling process is bidirectional and involves all major phospholipid classes, which move at comparable rates except for sphingomyelin for which the inward movement tends to be somewhat less in comparison with the glycerophospholipids. Using fluorescent probes, it was demonstrated that lipids with unusual polar headgroups, such as the disomer form of serine (Smeets et al., 1994), or glucosyl- and galactosyl ceramides participate in the scrambling process. Lipid scrambling is not coupled to ATP hydrolysis and even occurs in resealed erythrocyte ghosts when challenged with Ca^{2+} (Williamson et al., 1985). Lipid scrambling is sensitive to the sulfhydryl modifications (Williamson et al., 1985).

3 Lipid Alterations in AD

3.1 Altered Composition

The pathogenic mechanisms in AD have been hypothesized to involve alterations in the concentrations of phospholipids (Hazel and Williams, 1990; Prasad et al., 1998). This hypothesis is supported by the presence of high concentrations of phospholipids in the brain that contain highly oxidizable polyunsaturated free fatty acids, such as arachidonic and docosohexanoic acids (Dhillon et al., 1994), and that Aβ, implicated in the pathogenesis of AD (Selkoe, 2001), prefers to localize in the phospholipid membrane core (Mason et al., 1996; Mark et al., 1997; Lauderback et al., 2001).

The loss of membrane phospholipids may be an early metabolic event in the formation of SP and NFT (Pettegrew et al., 1988) and in the loss of synapses and neurons (Svennerholm and Gottfries, 1994). Because phospholipids contain unsaturated fatty acids, which are targets of free radicals, this hypothesis is linked to the oxidative stress hypothesis of neurodegeneration in AD (Markesbery and Lovell, 1998; Butterfield, 2002). In the central nervous system (CNS), the loss of membrane phospholipids occurs by at least three different mechanisms: decreased biosynthesis, increased degradation, and increased lipid peroxidation. Experimental evidence in AD suggests that phospholipase-catalyzed degradation of phospholipids is likely to decrease the concentrations of membrane phospholipids in the brain in AD (Barany et al., 1985). Increased free radical-mediated lipid peroxidation occurs in the brain in AD (Lyras et al., 1997; Markesbery and Lovell, 1998; Lauderback et al., 2001; Montine et al., 2002; Pratico and Sung, 2004; Sultana and Butterfield, 2004) and can contribute to decreases in the concentrations of phospholipids in this disorder. Furthermore, phospholipases may enhance phospholipid degradation after free radical attack, forming lipid peroxides and lipid hydroperoxides (Hall et al., 1994; Francescangeli et al., 2000, 2002; Sun et al., 2001). Studies showing the loss of membrane phospholipids have demonstrated either decreased levels of lipid phosphorus (Nitsch et al., 1992; Svennerholm and Gottfries, 1994) or increased levels of the phospholipid catabolites, glycerophosphatidylcholine (GPC) and glycerophosphatidylethanolamine (GPE) in the AD brain (Blusztajn et al., 1990).

Ceramide is not only structurally, but also functionally, a key molecule in diverse kinds of sphingolipids (Sawai et al., 2005). In the past decade, ceramide has been shown to be of crucial significance in several cell functions including apoptosis, cell growth, senescence, and cell cycle control (Flores et al., 2000; Thon et al., 2005). Among these roles, the role of ceramide in apoptosis induction has extensively been studied, and ceramide-targeting molecular medicine for apoptosis-based diseases such as malignant tumors, atherosclerosis and neurodegenerative disorders is under extensive investigation (Mattson et al., 1997; Sawai et al., 2005). The recent advances in the research on ceramide-mediated apoptosis signaling show the relation of

ceramide level through regulation of ceramide-related enzymes with diseases such as cancer, leukemia, bacterial infections, AIDS, Alzheimer's disease, atherosclerosis, diabetes mellitus, and atopic dermatitis (Sawai et al., 2005). Amyloid beta-peptide enhances tumor necrosis factor-alpha-induced iNOS through the neutral sphingomyelinase/ceramide pathway in oligodendrocytes (Zeng et al., 2005).

Cholesterol may play a pivotal role on the production of the putative AD neurotoxin, Aβ (Sjogren et al., 2005). More importantly, this relationship has consistently been identified in both in vivo and in vitro studies (Sparks et al., 2003). Cholesterol-lowering drugs have been shown to cause a beneficial effect of lowering Aβ levels in animal models, and epidemiological data indicate a beneficial effect on the risk of AD with prior statin use (Sparks et al., 2003). However, the results using statins may not be simply due to cholesterol lowering, but may involve other statin-sensitive pathways (Laufs et al., 2002; Mohmmad Abdul et al., 2004).

Increased risk of developing late-onset AD is related to the apolipoprotein E gene found on chromosome 19 (Poirier, 2005). This gene codes for a protein that helps to carry cholesterol in the bloodstream. The APOE gene comes in several different forms, or alleles, but three occur most commonly: APOE e2, APOE e3, and APOE e4. Having one or two copies of the e4 allele increases the risk of developing AD. The e3 allele is the most common form found in the general population and may play a neutral role in AD. The e2 allele appears to be associated with a lower risk of AD. The exact degree of risk of AD for any given person cannot be determined based on APOE status. Therefore, the APOE e4 gene is called a risk factor gene for late-onset AD (Craig et al., 2004), and, consistent with the importance of lipids in AD, there are increased levels of CSF phosphorylated tau in ApoE4 carriers with mild cognitive impairment, arguably the earliest form of AD (Buerger et al., 2005).

Numerous signal transduction processes involve lipids as signaling molecules. Many of these molecules are generated by phospholipases such as phospholipase A2 that releases fatty acids such as arachidonic acid and lysophospholipids. Each of these products is implicated in signal transduction processes by itself (Farooqui and Horrocks, 2005), but also serves as a precursor for eicosanoids including the prostaglandins, leukotrienes, and lipoxins or platelet-activating factor (PAF). Phospholipase A2 is a member of the class of heat-stable, calcium-dependent enzymes catalyzing the hydrolysis of the 2-acyl bond of 3-n-phosphoglycerides. The enzyme has a molecular weight of 30,000 Da. Phospholipase A2 is activated by Ca^{2+} and inhibited by zinc, barium, and manganese ions (Kabre et al., 1999).

There are more than 19 different isoforms of PLA_2 in the mammalian system, but recent studies have focused on three major groups, namely Ca^{2+}-dependent secretory enzymes (sPLA2), the Ca^{2+}-dependent cytosolic enzyme (cPLA2), and a Ca^{2+}-independent PLA2 (iPLA2) (Balsinde and Dennis, 1997). There is accumulating evidence for the involvement of specific PLA_2s in AD brain pathology and neurodegeneration (Macchioni et al., 2004). In two separate studies, a decrease in PLA_2 activity was found in the parietal and temporal cortex (Ross et al., 1998), as well as the prefrontal cortex, of the AD brain (Talbot et al., 2000). On the other hand, immunohistochemical studies showed an increase in $cPLA_2$ immunoreactivity associated with the glial fibrillary acidic protein-positive astrocytes in the AD brain (Stephenson et al., 1996). In a recent gene array study, profiling of 12,633 genes in the hippocampal CA1 area of AD patients indicated an increase in $cPLA_2$ and COX-2 expression, as well as upregulation of a number of apoptotic and proinflammatory genes (Colangelo et al., 2002). Additionally, cytosolic phospholipase A2 mediates neuronal apoptosis induced by soluble oligomers of the Aβ peptide (Sun et al., 2001; Kriem et al., 2005), and the brain and platelet PLA2 activity was significantly lower in AD than in controls. Mean PLA2 activity in MCI individuals was between the values of AD patients and controls, with a subgroup showing PLA as low as the lowest AD patients, but the differences from MCI were not significant from AD and control groups (Gattaz et al., 2004). These findings are in agreement with the increased oxidative and inflammatory responses and decrease in PLA_2 activity associated with AD pathology.

Other important phospholipases include phospholipase C, which controls the production of inositol-1,4,5-trisphosphate (IP3), which, in turn, induces cytosolic Ca^{2+} release and diacylglycerol (DAG). The latter moiety activates protein kinase C. Phospholipase D generates phosphatidic acid (PA), which subsequently can be metabolized either by PLA2 generating lysophosphophatidic acid (Lyso PA), a potent cellular mitogen, or by phosphatidate phosphohydrolase (PAP) yielding DAG. Sphingomyelinase, a phospholipase C type enzyme, and related enzymes of sphingolipid metabolism are implicated in apoptosis and other signaling processes (Mattson et al., 1997).

Several lines of evidence suggest the role of Aβ (1–42)-induced lipid peroxidation in AD. D609, a tricyclodecanol derivative of xanthic acid, scavenges hydroxyl radicals and reacts with electrophilic products of lipid oxidation (HNE and acrolein) in a manner similar to GSH (Lauderback et al., 2003). Synaptosomes isolated from rodents, previously injected intraperitoneally with D609, followed by treatments with the oxidants Fe^{2+}/H_2O_2, 2,2-azobis-(2-amidinopropane) dihydrochloride (AAPH), showed a significant reduction in 4-hydroxy-2-nonenal (HNE) [a lipid peroxidation product] (Joshi et al., 2005). Other studies suggest that D609 significantly attenuated Aβ (1–42)-induced cytotoxicity and lipid peroxidation in vitro (Sultana et al., 2004) and in vivo (Joshi et al., 2005; Perluigi et al., 2006). The antioxidant property of D609 is associated with the free thiol group of xanthate. D609 is capable of detoxifying aldehydic products of lipid peroxidation by a mechanism similar to GSH (Lauderback et al., 2003).

Lipid rafts are membrane microdomains enriched in cholesterol and sphingolipids, and proteins (Simons and Ikonen, 1997). Several lines of evidence suggest the involvement of lipid rafts in β- and γ-cleavage of βAPP (Ehehalt et al., 2003). It has been reported that the proteins relevant to Aβ generation, including presenilin, nicastrin, and a small portion of βAPP, localize in lipid rafts (Lee et al., 1998). Additionally, β-secretase localizes in lipid rafts and cholesterol depletion abrogates this localization (Riddell et al., 2001). Researchers (Ehehalt et al., 2003) have reported that βAPP exists in two pools; one associated with lipid rafts, in which β-cleavage occurs, and the other outside of lipid rafts, where α-cleavage occurs. The gamma-secretase complex is responsible for the final cleavage event in the processing of beta-amyloid precursor protein (βAPP), resulting in Aβ generation. The gamma-secretase complex is a multiprotein complex composed of presenilin, nicastrin (NCT), APH-1, and PEN-2. Recent reports have suggested that γ-secretase activity is predominantly localized in lipid rafts and that cholesterol can directly regulate the γ-secretase activity in isolated lipid rafts (Wahrle et al., 2002). Presenilin and NCT have been reported to be localized in lipid rafts. Thus, lipid rafts offer a structural platform for examining the effect of cholesterol on Aβ generation.

Compositional alterations in brain phospholipids, due in part to lipid peroxidation, have been reported for AD brain (Prasad et al., 1998). Polyunsaturated fatty acids (PUFA), including arachidonic (AA), and docosohexanoic acid (DCH), are abundant in brain and highly oxidizable. Consequently, AA and DCH are vulnerable to free radical attack, and PUFA are predicted to decrease in AD brain if lipid peroxidation were increased. In an analysis, regional levels of membrane phospholipids PtdEtn, PtdIns, and PtdCho were measured in the brain of AD and control subjects and the results suggest that the levels of PtdEtn-derived and PtdIns-derived total fatty acids were significantly decreased in the hippocampus of AD subjects (Prasad et al., 1998). Additionally, significant decreases were found in PtdEtn-derived stearic, oleic and arachidonic and DCH, and in PtdIns-derived oleic and arachidonic acids (Prasad et al., 1998). In the inferior parietal lobule of AD subjects, significant decreases were found only in PtdEtn and those decreases were contributed by stearic, oleic and arachidonic acids. In the superior and middle temporal gyri and the cerebellum of AD subjects, no significant decreases were found in PtdCho-, PtdEtn- , and PtdIns-derived fatty acids. The decrease of PtdEtn and PtdIns, which are rich in oxidizable AA and DCH, but not of PtdCho, which contains lesser amounts of these fatty acids, suggests a role for oxidative stress in the increased degradation of brain phospholipids in AD (Prasad et al., 1998). Analyses using high-performance liquid chromatography (HPLC) and gas chromatography (GC) also describe a decrease in PtdEtn-plasmalogen in AD brain, confirming PtdEtn as a phospholipid exhibiting major structural modifications (Guan et al., 1999). One mechanism for decreased free fatty acids is oxidative stress-induced stimulation of phospholipase A_2, and Aβ added to synaptosomes led to free fatty acid release, primarily in the PtdEtn fraction, an effect blocked by the free radical scavenger vitamin E (Koppal et al., 1998).

3.2 Formation of Reactive Aldehydes: HNE and Acrolein

Free radical attack on phospholipid PUFA can ultimately lead to multiple aldehydes with different carbon chain lengths, including acrolein and HNE (Esterbauer et al., 1991). These alkenals have different reactivity than free radicals due to their half-life ranging from minutes to hours. In particular, HNE is able to diffuse to sites distant from that of its formation (Butterfield and Stadtman, 1997; Butterfield, 1997).

HNE, an α,β-unsaturated aldehyde, is one of the major products of lipid peroxidation. HNE reacts with proteins, forming stable covalent adducts to histidine, lysine, and cysteine residues through Michael addition, thereby introducing carbonyl functionalities into proteins following oxidative damage, e.g., lipid peroxidation (Esterbauer et al., 1991; Uchida and Stadtman, 1992; Subramaniam et al., 1997; Butterfield and Lauderback, 2002). HNE can inhibit synthesis of DNA, RNA, and proteins and alter activity of glycolytic, degradative, and transport proteins (Esterbauer et al., 1991).

One mechanism for increasing HNE in AD may be the oxidative stress associated with Aβ (Varadarajan et al., 2000; Lauderback et al., 2001). Aβ is widely reported to cause lipid peroxidation in brain cell membranes, which is inhibited by free radical antioxidants (Butterfield et al., 1994; Avdulov et al., 1997; Gridley et al., 1997; Mark et al., 1997, 1999; Bruce-Keller et al., 1998; Daniels et al., 1998; Yatin et al., 2000; Boyd-Kimball et al., 2004). Further, Aβ leads to HNE and 2-propen-1-al (acrolein) formation (Mark et al., 1997), and these alkenals alter the conformation of membrane proteins (Subramaniam et al., 1997; Pocernich et al., 2001). Moreover, these reactive aldehydes are toxic to neurons (Mark et al., 1997; Subramaniam et al., 1997; Lovell et al., 2001). Indeed, an increase in free- and protein-bound HNE was found in rat hippocampal neurons or synaptosomes exposed to Aβ (Mark et al., 1997; Lauderback et al., 2001; Boyd-Kimball et al., 2004; Sultana et al., 2005). HNE induced apoptosis in PC12 cells or neurons (Kruman et al., 1997) suggesting that Aβ-induced HNE production may contribute to an indirect mechanism of neuronal death. The concentration of free HNE is elevated in multiple brain regions and in ventricular cerebrospinal fluid (CSF) in AD (Markesbery and Lovell, 1998).

Protein-bound HNE also is elevated in AD (Sayre et al., 1997; Lauderback et al., 2001; Sultana and Butterfield, 2004), and may relate to apolipoprotein E allele (Montine et al., 1997). As already sited, ApoE e4 allele is a risk factor for AD (Craig et al., 2004), suggesting that the degree of expression the lipid transporter ApoE in brain might be associated with HNE production in AD. Stereotaxic injection of HNE in rat forebrain selectively inhibited cholineacetyltransferase (ChAT) (Bruce-Keller et al., 1998), the activity of which is greatly diminished in AD. Consistent with this observation, synaptosomes treated with Aβ (1–42) result in HNE being bound to ChAT (Butterfield and Lauderback, 2002), which may possibly correlate with the diminished ChAT activity in AD. HNE also may play a role in glutamate-induced neurotoxicity in AD. Glutamate, an excitotoxin that exerts its effects via stimulation of *N*-methyl-D-aspartate (NMDA) receptors, thereby increasing intracellular free radicals (Lafon-Cazal et al., 1993), is removed from the synapse by glutatmate transporters, particularly, the glial glutamate transporter, GLT-1 [also called as EAAT2] (Maragakis and Rothstein, 2001). Aβ inhibits glutamate uptake (Harris et al., 1995; Harris et al., 1996) possibly by a mechanism that involves Aβ-induced lipid peroxidation and subsequent HNE modification to glutamate transporters (Keller et al., 1997; Lauderback et al., 2001). In AD brain GLT-1 has significantly more HNE bound to it than does this transporter in aged-matched neurologically normal controls (Lauderback et al., 2001), and full-length Aβ added to rodent synaptosomes induced HNE binding to GLT-1 (Lauderback et al., 2001), suggesting the possibility that these two observations are related. The activity of glutathione S-transferases (GST), an enzyme that shows high catalytic (and thus detoxifying) activity against HNE (Bruns et al., 1999), was significantly decreased in AD brain (Lovell et al., 1998). Taken together with the increased levels of lipid peroxidation in AD brain, these findings may in part account for neurodegeneration in AD brain. Additionally, the alpha class of glutathione S-transferase (GST) can detoxify HNE and plays an important role in cellular protection against oxidative stress. GST and MRP1 are themselves oxidatively modified by HNE in AD brain (Sultana and Butterfield, 2004), which, since oxidatively modified proteins are generally dysfunctional (Butterfield and Boyd-Kimball, 2004), may account in part for the elevated HNE accumulation in this disorder (Markesbery and Lovell, 1998). Protein-bound HNE is also elevated in brain from persons with MCI, suggesting that lipid peroxidation is an early event in the progression of AD (Butterfield et al., 2006).

Acrolein is the most reactive of the α,β-unsaturated aldehydes produced by lipid peroxidation because of its electrophilic properties (Esterbauer et al., 1991). Even at low concentrations acrolein can structurally change transmembrane and cytoskeletal proteins (Pocernich et al., 2001). Acrolein adducts were reported in NFT in AD but not in control brain (Calingasan et al., 1999), and an increase in protein-bound acrolein in AD compared with age-matched controls was described (Lovell et al., 2001). Further, acrolein is toxic to primary hippocampal cultures (Lovell et al., 2001), and acrolein is reportedly bound in excess to

alpha-ketoglutarate dehydrogenase in AD brain (Calingasan et al., 1999). This may suggest that acrolein can disrupt mitochondrial function (Pocernich and Butterfield, 2003).

3.3 TBA Reactive Substances

One measure of lipid peroxidation is elevation of thiobarbituric acid reactive substances (TBARS). Unfortunately, reaction of nonlipid molecules with thiobarbituric acid makes TBARS a nonspecific marker of membrane lipid peroxidation, possibly accounting for disagreements in reports about TBARS levels in different brain regions in AD. An increase in TBARS in AD frontal lobe but not in the cerebellum was reported (Subbarao et al., 1990), while others (Balazs and Leon, 1994) described a significant TBARS increase in sensory and occipital cortices of AD. Only the inferior parietal lobe seemed to be affected by lipoxidation in one study (Palmer and Burns, 1994), while others (Lovell et al., 1995) showed statistically significant elevations in TBARS in the hippocampus and the cortex. A TBARS increase in all the regions of AD brain, with higher statistical significance in the temporal cortex, was described (Marcus et al., 1998):, a result confirmed by others (Tamaoka et al., 2000). One mechanism of increasing lipid peroxidation measured by TBARS is the addition of a free radical initiating source, and Aβ (1–40) was shown to increase TBARS in synaptosomal membranes (Lauderback et al., 2001).

3.4 Isoprostanes and Neuroprostanes

Isoprostanes (IsoPs) are prostaglandin (PG)-like compounds that are formed nonenzymatically in vivo by free radical-induced peroxidation of arachidonic acid. IsoP formation proceeds through bicyclic endoperoxide PGH_2-like intermediates. The endo-peroxide intermediates are reduced to form PGF_2-like compounds (F_2-IsoPs) (Morrow et al., 1990) or undergo rearrangement to form E-ring and D-ring compounds (E_2/D_2-IsoPs) (Morrow et al., 1994) and thromboxane-like compounds [isothromboxanes] (Morrow et al., 1996). An increase in CSF levels of the isoprostane 8,12-iso-iPF2alpha-VI, a specific marker of in vivo lipid peroxidation, was demonstrated for CSF in AD patients (Pratico et al., 2004). Poor cerebral clearance of end products of oxidative reactions via CSF circulation may contribute to and sustain ongoing stress. CSF drainage via a low-flow ventriculoperitoneal shunt may improve removal of these products, reducing oxidative stress (Pratico and Sung, 2004). A novel aspect of the formation of IsoPs is that, unlike cyclooxygenase-derived prostaglandins, IsoPs are formed in situ esterified to phospholipids and subsequently released (Morrow et al., 1992). The quantification of F_2-IsoPs has emerged as one of the most accurate approaches to assessing oxidant injury in vivo (Roberts and Morrow, 1997). Furthermore, IsoPs are capable of exerting potent biological activity (Roberts and Morrow, 1997).

Isoketals (IsoKs) are highly reactive products of the isoprostane pathway of free radical-induced lipid peroxidation that rapidly form covalent protein adducts and form protein cross links in vitro. Isoketal adducts from an animal model of oxidative injury revealed that initial adducts were formed by isoketals esterified in phospholipids, representing a novel oxidative injury-associated modification of proteins by phospholipids (Brame et al., 2004). Studies on potassium channels revealed that isoketal adduction profoundly altered protein function, inhibiting potassium current in a dose-dependent manner (Brame et al., 2004). This result suggests that phospholipid-esterified isoketals rapidly adduct membrane proteins and that such modification can alter protein function, suggesting a generalized cellular mechanism for alteration of membrane function as a consequence of oxidative stress (Brame et al., 2004).

Neuroketals are the highly reactive γ-ketoaldehydes formed by the neuroprostane pathway of free radical-induced lipid peroxidation that is analogous to cyclooxygenase-derived levuglandins (LGs). Neuroketals (NeuroKs) are formed from the oxidation of DCH, which is enriched in the brain, and measurement of neuroprostanes may provide a unique marker of oxidative neuronal injury (Roberts and Morrow, 2002). Adduction of IsoK to model proteasome substrates significantly reduced their rate of degradation by the 20S proteosome (Davies et al., 2002). At lower concentrations, an IsoK-adducted protein (ovalbumin)

and peptide (Aβ1–40) significantly inhibited chymotrypsin-like activity of the 20S proteosome (Davies et al., 2002). Additionally, incubation of IsoK with P19 neuroglial cultures dose-dependently inhibited proteasome activity and induced cell death suggesting that IsoKs/NeuroKs can inhibit proteasome activity and may have relevance to the pathogenesis of neurodegenerative diseases, if overproduced (Davies et al., 2002).

As noted, free radical-induced oxidation of AA results in the formation of F_2- and D_2/E_2- isoprostanes; in contrast, analogous oxidation of gray matter-resident DCH leads to F_4- and D_4/E_4-isoprostanes [neuroprostanes (NPs)] (Roberts et al., 1998). The level of IP, the quantitation of which directly relates to lipid peroxidation in vivo (Morrow, 2000), is increased in AD CSF (Montine et al., 1998). Consistent with Aβ-associated free radical-induced lipid peroxidation (Butterfield et al., 1994; Butterfield and Lauderback, 2002), IP is significantly elevated in rat hippocampal culture after Aβ addition (Mark et al., 1999). The levels of F_2-IP in AD lateral ventricular fluid also were significantly elevated, and the increase was related with the extent of degeneration but independent of the distribution of NFT or the ApoE genotype (Montine et al., 1999). AD brain IP and NP levels in vivo were quantified, showing an increase in total NP level, but not total IP (Reich et al., 2001). In aggregate, these results suggest that lipid peroxidation, resulting in neurotoxic reactive aldehydes, may be important in the neurodegeneration observed in AD brain. Further, the extensive lipid peroxidation in AD brain suggests that brain-accessible, lipid-resident antioxidants that can block free radical-induced lipid peroxidation, or raising the in vivo level of glutathione, that is able to protect neurons from HNE and acrolein (Subramaniam et al., 1997; Pocernich et al., 2001) may be promising therapeutic strategies for this disorder (Drake et al., 2002).

Quantification of NPs might provide a unique marker of oxidative injury in the brain. Furthermore, these compounds, like IsoPs, could potentially exert biological activity. This possibility is supported by the finding that $PGF_{4\alpha}$, the four series F-prostaglandin corresponding to the structure expected from cyclooxygenase action on C22:6, is approximately equipotent with cyclooxygenase-derived $PGF_{2\alpha}$ in contracting gerbil colonic smooth muscle strips (Markesbery, 1997). In addition, the formation of NPs esterified in lipids might be expected to have significant effects on the biophysical properties of neuronal membranes, which might impair normal neuronal function. This may be particularly relevant to AD, since it has been suggested that one of the physiological functions of DCH may be to maintain a certain state of membrane fluidity and promote interactions with membrane proteins that are optimum for neuronal function (Salem and Niebylski, 1995).

Neuroprostanes are readily detected esterified to lipids in the brain. However, NP measurements in humans would be limited to samples of brain removed surgically or postmortem samples of human brain. Measurements of F_4-NPs made in human brain samples obtained after death could be quite problematic because of the possibility of artifactual generation of NPs by autoxidation of DCH during the time interval between death and sample procurement. Although invasive, cerebrospinal fluid is frequently obtained for diagnostic purposes in patients with suspected neurological disorders. Thus, the availability of a marker of oxidative injury in the brain that could be measured in CSF intra vitam would be an important advance. Hence, the finding that F_4-NPs could be detected in human CSF clearly has potentially important clinical applications. Markers of lipid peroxidation are increased in CSF of patients with AD (Lovell et al., 1997; Montine et al., 1997). However, these assays have shortcomings related to measurement of reactive molecules, i.e., 4-hydroxynonenal, and require large volumes of fluid. However, F_4-NPs were detectable using negative ion chemical ionization mass spectrometry in 1–2 ml of CSF from normal subjects, an amount that can usually be obtained safely from patients for diagnostic purposes. Although it was a limited study, the finding that F_4-NP concentrations in cerebrospinal fluid from patients with AD were significantly higher than levels in age-matched control subjects highlights the potential of this approach to provide insights into the role of free radicals in the pathogenesis of neurological disorders. Another potentially important aspect of this finding is that serial measurements of F_4-NPs in CSF might provide a biochemical assessment of disease progression as well as a means to monitor efficacy of therapeutic intervention, e.g., with antioxidants, during life. Elevation of tissue and urinary isoprostanes is characteristic of human atherosclerosis. Deficiency in apolipoprotein E in the mouse (apoE−/−) resulted in atherogenesis and an increase in iPF2alpha-VI, an F2-isoprostane, in urine, plasma, and vascular tissue (Pratico et al., 1998). Supplementation with vitamin E significantly reduced isoprostane generation, but had no effect on plasma cholesterol levels in apoE−/− mice (Pratico et al., 1998).

Therefore, there may be distinct advantages associated with measuring either IsoPs or NPs to assess oxidant injury in the brain. NPs possess important biological actions that may be relevant to the pathophysiology of oxidant injury to the brain. As mentioned, this possibility is greatly supported by the finding that C22-$PGF_{4\alpha}$ is bioactive. This compound is one of the F_4-NPs that would be formed, although, analogous to IsoPs, compounds in which the side chains are oriented *cis* likely predominate over compounds in which the side chains are oriented *trans* in relation to the cyclopentane ring (Morrow et al., 1990). However, in the case of the IsoPs, inversion of the stereochemistry of the upper side chain of $PGF_{2\alpha}$ and PGE_2 affords different and/or more potent biological actions (Roberts and Morrow, 1997).

In addition, phospholipids containing esterified NPs are unnatural and unusual molecules. Thus, enhanced formation of these unusual phospholipids in neuronal membranes in settings of oxidant injury to the brain might lead to profound alterations in the biophysical properties of the membrane, e.g., degree of fluidity, which in turn might greatly impair normal neuronal function. Future studies using synthetic NP-containing aminophospholipids in model membranes to assess the extent to which these unique phospholipids alter membrane properties should provide valuable insight into the potential relevance of the formation of these phospholipids in settings of oxidative neuronal injury.

4 Modulation in Phospholipid Asymmetry in AD by Aβ (1–42)

The maintenance of membrane lipid asymmetry is a dynamic process that influences many events over the lifespan of the cell. As already described, most cells restrict the bulk of the aminophospholipids to the inner membrane leaflet by means of specific transporters. For the major glycerophospholipids (PtdSer, PtdEtn, PtdCho, and PtdIns), de novo synthesis occurs on the cytosolic side of the endoplasmic reticulum [ER] (Bell et al., 1981). With the exception of PtdCho, this places the newly synthesized lipids on the side of the membrane in which they are ultimately enriched in the plasma membrane. Because of the thermodynamic barrier to spontaneous transbilayer movements, these lipids should remain enriched on the cytoplasmic side of the membrane, provided that there is no perturbation to the membrane. However, the asymmetric addition of newly synthesized phospholipids to one leaflet of the bilayer generates an unstable membrane. Accumulation of lipid on one side of the membrane can induce membrane bending and consequent membrane shape changes (Daleke and Huestis, 1985). The asymmetric distribution of PS over the cellular membrane requires much of the cell's energy and requires the involvement of an ATP-dependent enzymatic activity (flippase) for its maintenance (Dolis et al., 1997). Indeed, if the cell fails to engage mechanisms to maintain asymmetry, aminophospholipids appear at the external cell surface. One of the important consequences of altered membrane asymmetry is the recognition and engulfment of phosphatidylserine bearing vesicles and cells by mononuclear phagocytes (Schroit et al., 1985). Loss of phospholipid asymmetry, measured by the exposure of PtdSer on the outer leaflet of the membrane bilayer, is a typical early event that follows apoptotic insult (Fadok et al., 1992). A large body of evidence indicates that lipid peroxidation is directly responsible for the generation of the apoptotic phenotype (Kagan et al., 2000).

4.1 In Vitro and In Vivo Studies

An aminophospholipid, PtdSer is sequestered in the inner leaflet of the plasma membrane in nonstimulated cells. An early signal of synaptosomal apoptosis is the loss of phospholipid asymmetry and the appearance of phosphatidylserine in the outer leaflet of the membrane (Balasubramanian and Schroit, 2003). The inactivation of the transmembrane enzyme aminophospholipid-translocase (or flippase) by Aβ (1–42) has been investigated (Mohmmad Abdul and Butterfield, 2005). Flippase activity depends on a critical cysteine residue, a putative site of covalent modification by the Aβ (1–42)-induced lipid peroxidation products, HNE or acrolein. Pretreatment of synaptosomes with D609 (already discussed) and ferulic acid ethyl ester (FAEE), a potent antioxidant (Sultana et al., 2005) significantly protected against Aβ (1–42)-induced loss of phospholipid asymmetry in synaptosomal membranes (Mohmmad Abdul and Butterfield, 2005). Annexin

V binding and NBD-PS [1-palmitoyl-2-[6(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]caproyl-*sn*-glycero-3-phosphoserine] binding assays were used to study the externalization of PS. The results suggest that D609 and FAEE exert protective effects against Aβ (1–42)-induced apoptosis in synaptosomal membranes (Mohmmad Abdul and Butterfield, 2005). As already noted, Aβ interaction with membrane produces HNE (Markesbery, 1997; Lauderback et al., 2001). Additionally, HNE, too, was reported to lead to loss of phospholipid asymmetry in synaptosomes (Castegna et al., 2004). In vivo studies carried out at our lab recently with purified synaptosomes from AD and mild cognitive impairment (MCI) brain samples suggest a significant increase the modulation in phospholipid asymmetry (externalization of PS) compared with the aged matched controls (Bader Lange et al., 2008), consonant with the notion that oxidative stress-mediated lipid peroxidation and subsequent apoptosis are early events in the progression of AD.

5 Concluding Remarks

The AD brain is under intense oxidative stress. Aβ plays a central role in the pathogenesis of AD. Soluble, oligomeric Aβ is postulated to insert into neuronal membranes, where, in processes that are inhibited by the chain-breaking antioxidant vitamin E and other antioxidants, Aβ-induced lipid peroxidation, protein oxidation, ROS and reactive nitrogen species (RNS) formation occur (Butterfield, 1997; Varadarajan et al., 2000; Butterfield, 2002; Butterfield and Boyd-Kimball, 2004). This oxidative damage or membrane modification, resulting from the reaction of the lipid peroxidation products HNE and/or acrolein with enzymatic, transport, or structural proteins, alters synaptic membranes and leads eventually to the death of the neuron. HNE can diffuse from the site of its production, potentially modifying neuronal organelles and changing their function (Butterfield and Stadtman, 1997). Aβ inhibits the function of several neuronal and glial transmembrane transport systems, including ion-motive ATPases, glutamate transporters, the glucose transporter, guanosine triphosphate (GTP)-coupled transmembrane signaling proteins, MRP-1, and the polyamine transporter (Butterfield and Lauderback, 2002). Additionally, Aβ plays a pivotal role in phospholipid asymmetry in AD (both in vitro and in vivo) and compositional alterations in brain phospholipids. Each of these has functional consequences that are deleterious to neurons, such as loss of cell potential to accumulation of excitotoxic glutamate, decreased glucose availability, decreased intracellular communication, and increased neurotoxicity. Genetic mutations and other mechanisms (e.g., apolipoprotein genotype, redox metal ions, etc.) that potentially lead to an increased Aβ deposition may contribute to this Aβ-induced lipid peroxidation and neurotoxicity. Continued investigation of the role of Aβ in oxidative stress in lipid peroxidation in AD and animal models relevant to AD, as well as studies employing therapeutic agents to block Aβ-associated oxidative stress, cholesterol lowering drugs, and association of the β-secretase/active γ-secretase complex with lipid rafts and its relation to cholesterol metabolism should provide greater insight into the relationships among Aβ (1–42), lipid peroxidation, oxidative stress, and neurodegeneration in AD brain.

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