CHAPTER

# 5

# Lipids

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OUTLINE



## INTRODUCTION

Lipids have critical roles in nervous system structure and function. Synaptic complexes and myelin are characterized by unique lipid compositions that contribute to the specialized properties of these nervous system structures. Multiple signaling pathways involving lipid intermediates regulate cell differentiation and synaptic transmission. Lipid modification of proteins is a key mechanism for modulating the activity of trophic factors and receptors. Since lipids constitute about one-half of brain tissue by dry weight, it is not surprising that lipid biochemistry and neurochemistry have evolved together. Like other tissues, the brain contains phospholipids, sterols and sphingolipids. Many complex lipids, including gangliosides, cerebrosides, sulfatides and phosphoinositides, were first discovered in brain, where they are highly enriched compared to other tissues. In fact it was J. L. W. Thudichum who first defined the chemical composition of the brain including a number of lipids, e.g., cerebrosides, sulfatides, sphingomyelin, and several phospholipids (Thudichum, 1884). The brain is also enriched in many other specialized phospholipids including the vinyl ether–linked phospholipids called plasmalogens.

# PROPERTIES OF BRAIN LIPIDS

## Lipids have multiple functions in brain

While in other parts of the body lipids have a major role in energy storage in the form of triacylglycerol, e.g., heart and muscle, in the brain the primary functions of lipids are formation of biomembranes and lipid-mediated signal transduction. In addition, derivatives of lipids, for example the utilization of arachidonic acid to form the bioactive eicosanoids (see Ch. 36) and the utilization of sterols to form neurosteroids, have a much broader dynamic role in brain function than previously thought. Indeed, some membrane lipids, such as polyphosphoinositides and phosphatidylcholine, which were previously believed to have only a structural role, also have important functions in signal transduction across biological membranes. Cholesterol and sphingolipids play a central role in formation of lipid rafts, which function in protein trafficking and signaling at the cell surface (see Ch. 2). The covalent modification of proteins by fatty acids and by isoprenoids has an integral role in anchoring and organizing proteins within biomembranes (see below). These discoveries established that lipids participate in both the function and the structure of neural membranes.

## Membrane lipids are amphipathic molecules

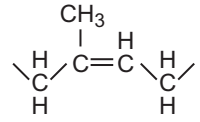
All membrane lipids are amphipathic molecules with a small polar, or hydrophilic, moiety and a large nonpolar, or hydrophobic, moiety. The hydrophilic regions of lipid molecules associate with water and water-soluble ionic compounds by hydrogen and electrostatic bonding. The hydrophobic regions cannot form such bonds and therefore associate with each other outside the aqueous phase by weak interactions such as Van der Waal forces. Because of these properties, phospholipids naturally form micelles or bilayers, with the hydrophilic portions of the molecule interacting with the aqueous phase and the hydrophobic portions interacting with each other in a "tail-to-tail" manner (see Ch. 2). The concentration at which this interaction occurs is called the critical micelle concentration (CMC). For phospholipids this is very low (nM), while for fatty acids this can range from 5μM for palmitic acid to 90μM for arachidonic acid, respectively. On the other hand, lipid molecules containing comparatively large polar groups, such as lysophospholipids, gangliosides and natural or synthetic detergents, are fairly soluble in water. However, like all lipid molecules, even these highly soluble lipids will form micelles once the solubility limit or CMC is reached.

## The hydrophobic components of many lipids consist of either isoprenoids or fatty acids and their derivatives

Lipids were originally defined operationally, on the basis of their extractability from tissues with organic solvents such as a chloroform/methanol mixture, but this is no longer the sole criterion. For example, the protein component of myelin proteolipid is extractable into lipid solvents but, nevertheless, is not considered to be a lipid since its structure is that of a highly hydrophobic polypeptide. In fact, many integral membrane proteins contain 'hydrophobic' membrane-spanning regions (see Ch. 2). Conversely, gangliosides are considered to be lipids on the basis of their structure, even though they are water soluble. It is apparent, then, that lipids are defined not only by their physical properties but also on the basis of their chemical structure. Chemically, lipids can be defined as compounds containing long-chain fatty acids and their derivatives or linked isoprenoid units. Fatty acids in lipids are either esterified to the trihydroxy alcohol glycerol or are present as amides of sphingosine, a long-chain dihydroxyamine. The isoprenoids are made up of branched-chain units and include sterols, primarily cholesterol.

## Isoprenoids have the unit structure of a five-carbon branched chain

Isoprenoid units have the formula $C_5H_8$ and the structure:




These isoprenoid units are used as the building blocks for the most abundant sterols in the brain and this synthesis occurs in both neurons and glia, especially in oligodendrocytes, in the developing brain, but almost exclusively in astrocytes in the adult brain. It is important to note that cholesterol found in the blood does not enter into the brain and that the brain relies on synthesis of cholesterol *de novo* (see Ch. 2 for additional details). Unlike other tissues, normal adult brain contains virtually no cholesteryl esters, except in some demyelinating diseases such as X-linked adrenoleukodystrophy. Cholesteryl esters and desmosterol, the immediate biosynthetic precursor of cholesterol, are found in developing brain and in some brain tumors but not in normal adult brain. Other isoprenoid substances are also present in brain: dolichols, very long (up to $C_{100}$) branched-chain-alcohols that are cofactors for glycoprotein biosynthesis; squalene, which is the linear $C_{30}$ precursor of all steroids; and the carotenoids, including retinal and retinoic acid. Some isoprene units, such as farnesyl ($C_{15}$) and geranyl-geranyl ($C_{20}$), are found covalently linked via thioether bonds to membrane proteins (see below for structures of some of these compounds and for the numbering system for cholesterol).

## Brain fatty acids are long-chain carboxylic acids that may contain one or more double bonds

The brain contains a variety of straight-chain monocarboxylic acids, with an even number of carbon atoms ranging from $C_{12}$ to $C_{26}$. The hydrocarbon chain may be saturated or may contain one or more double bonds, all in *cis* (Z) configuration. When multiple double bonds are present, they are nonconjugated and almost always three carbons apart. The unsaturated fatty acids are classified by the location of the first carbon of the first double bond closest to the methyl end and are defined by the n nomenclature. For instance, for linoleic acid, there are 18 carbons and two double bonds with the first double bond found on the 6th carbon from the

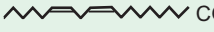
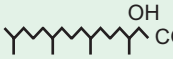
| Structure | Chemical name | Trivial name | Abv. |
|---|---|---|---|
|  | Dodecanoic acid | Lauric acid | 12:0 |
|  | Tetradecanoic acid | Myristic acid | 14:0 |
|  | Hexadecanoic acid | Palmitic acid | 16:0 |
|  | Octadecanoic acid | Stearic acid | 18:0 |
|  | 9-Octadecenoic acid | Oleic acid | 18:1n-9 |
|  | 9,12-Octadecadienoic acid | Linoleic acid | 18:2n-6 |
|  | 9,12,15-Octadecatrienoic acid | α-Linolenic acid | 18:3n-3 |
|  | 5,8,11,14-Eicosatetraenoic acid | Arachidonic acid | 20:4n-6 |
|  | 5,8,11,14,17-Eicosapentaenoic acid | Eicosapentaenoic acid | 20:5n-3 |
|  | 4,7,10,13,16,19-Docosahexaenoic acid | Docosahexaenoic acid | 22:6n-3 |
|  | Tetracosanoic acid | Lignoceric acid | 24:0 |
|  | 15-Tetracosenoic acid | Nervonic acid | 24:1n-9 |
|  | 2-Hydroxytetracosanoic acid | Cerebronic acid | 24h:0 |
|  | 3,7,11,15-Tetramethylhexadecanoic acid | Phytanic acid | |

FIGURE 5-1 **Structures of some fatty acids of neurochemical interest (see also Fig. 5-7 and text)**. The 'n minus' nomenclature for the position of the double bond(s) is given here. Note that the position of the double bond from the carboxyl end can also be indicated by the symbol Δ, so that linoleic acid may be also be designated as $18{:}2^{\Delta 9,12}$. The linolenic acid shown is the α isomer.

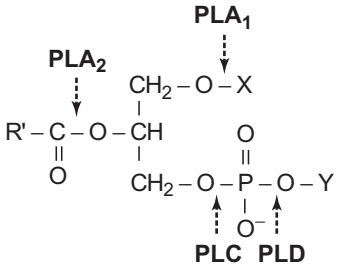
methyl end, and is thus 18:2n-6 (Fig. 5-1). The major unsaturated fatty acid families are n-3, n-6 and n-9; these families are not interconvertable, e.g., n-3 cannot become an n-6 (for review, see Barceló-Coblijn & Murphy, 2009). These nomenclature conventions are convenient from both the biochemical and the nutritional points of view because fatty acids are elongated and degraded *in vivo* by two carbon units from the carboxyl end. Animals need certain polyunsaturated fatty acids (PUFAs), termed essential fatty acids (linoleic acid [LNA or 18:2n-6], and alpha-linolenic acid [ALA or 18:3n-3]), in their diet, as discussed below and in Ch. 36. A similar, widely used but outdated nomenclature uses the omega (ω) designation, indicating the position of the first double bond counting from the methyl (ω−carbon) end. The brain contains some unusual fatty acids, such as very long (20–26 carbons), odd-numbered and 2-hydroxy fatty acids, prevalent in the cerebrosides. A list of major brain fatty acids with their common names and structures is given in Figure 5-1.

## COMPLEX LIPIDS

### Glycerolipids are derivatives of glycerol and fatty acids

Most brain glycerolipids are produced using phosphatidic acid (PtdOH) as a central precursor, which is 1-acyl, 2-acyl-*sn*-glycerol-3-phosphate. The notation *sn* refers to stereochemical numbering, with the secondary hydroxyl group of glycerol at C-2 shown on the left, that is, the l-configuration of Fischer's projection, and the phosphate at C-3. This special nomenclature is employed because, unlike the trioses or other carbohydrates, glycerol does not have a reporter carbonyl group to assign an absolute D- or L-configuration. As shown in Figure 5-2, the hydroxyl groups on C-1 and C-2 of glycerolipids are esterified with fatty acids. The fatty acid at *sn*-1 is usually saturated or monounsaturated, whereas that at *sn*-2 is generally polyunsaturated. In addition, there are lipid species in which *sn*-1 is ether-linked either to an aliphatic alcohol, termed an alkyl, or to an αβ-unsaturated alcohol, alk-1-enyl, which are referred to as plasmalogens (Fig. 5-2). While diacylglycerophospholipids contain an alkali-labile, acid-stable ester linkage that can be saponified, the alkyl ether linkage is alkali- and acid-stable whereas the alkenyl ethers are alkali-stable and acid-labile (see Barceló-Coblijn & Murphy, 2009). A useful general term that includes all of these various aliphatic substituents, acyl, alkenyl and alkyl, is 'radyl.' For example, 1,2-diradyl-*sn*-glycerol-3-phosphorylethanolamine is a term that includes phosphatidylethanolamine (PtdEtn) as well as its plasmalogen analogs (PlsEtn) and the alkyl analogs (PakCho). More recently these have been referred to as ethanolamine glycerophospholipids or EtnGpl.

If positions 1 and 2 are acylated and the *sn*-3 hydroxyl group is free, the lipid is a 1,2-diacyl-*sn*-glycerol (DAG).



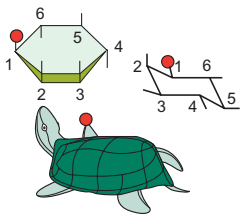

| Y | Lipid | Abv. |
|---|---|---|
| H | Phosphatidate | PtdOH |
| $CH_2-CH_2-\overset{+}{N}H_3$ | Phosphatidylethanolamine | PtdEtn |
| $CH_2-CH_2-\overset{+}{N}(CH_3)_3$ | Phosphatidylcholine | PtdCho |
| $CH_2-\underset{H}{\overset{NH_3^+}{C}}-COO^-$ | Phosphatidylserine | PtdSer |
|  | Phosphatidylinositol | PtdIns |
| $CH_2-CH(OH)-CH_2HO$ | Phosphatidylglycerol | PtdGro |
| Phosphatidylglycerol | Cardiolipin | $Ptd_2Gro$ |

FIGURE 5-2 **The structure of phosphoglycerides**. In most lipids, X is acyl, that is, R–(C = O). In alkyl ethers, present mainly in brain ethanolamine glycerophospholipids (2–3%), X is a long-chain hydrocarbon ($C_{16}$, $C_{18}$). For plasmalogens, which constitute about 60% of adult human brain ethanolamine glycerophospholipids, X is 1-alk-1′enyl (i.e., –C = CH–R). *Arrows* indicate sites of enzymatic hydrolysis of the phosphoglycerides. *$PLA_1$*, phospholipase $A_1$; *$PLA_2$*, phospholipase $A_2$; *PLC*, phospholipase C; *PLD*, phospholipase D. Note that *myo*-inositol is written in the D-configuration, where the 1′ position is linked to the PtdOH moiety. For polyphosphoinositides, additional phosphate groups are present in the 3, 4 or 5 positions. See Chapter 23 for further detail regarding the stereochemistry of inositol and the use of the turtle representation.

The DAGs play both a biosynthetic (see later) and a lipid-mediated signaling role in that they activate protein kinase C (PKC) (see Chs. 23 and 25). In addition, DAGs can be fusogenic and have been proposed to play a role in altering cell morphology, for example, in fusion of synaptic vesicles (see Ch. 7). Other non–phosphorus-containing glycerides of interest are DAG-galactoside and its sulfate. These minor glycolipids are found primarily in white matter and appear to be analogous to their sphingosine-containing counterparts, the cerebrosides, described below.

Glycerophospholipid classes are defined on the basis of the substituent base at *sn*-3 of the diacylglycerophosphoryl (phosphatidyl) function (Fig. 5-2). The bases are short-chain polar alcohols phosphodiester-linked to PtdOH. The amount and distribution of these lipids vary with brain regions, age, and disease states (Sastry, 1985; Wells & Dittmer, 1967). See also Fig 5-3. In quantitatively decreasing order in adult human brain, they are ethanolamine glycerophospholipids which include the plasmalogens (PlsEtn) and phosphatidyl ethanolamine (PtdEtn), phosphatidylcholine (PtdCho, historically called 'lecithin'); and phosphatidylserine (PtdSer). The phosphoinositides include phosphatidylinositol (PtdIns), phosphatidylinositol-4-phosphate (PtdIns-4P) and phosphatidylinositol 4,5-bisphosphate (PtdIns-4,5$P_2$), which are quantitatively minor phospholipids but play an important role in lipid-mediated signal transduction and membrane trafficking. They are also commonly found abbreviated as PI, PIP and $PIP_2$ respectively, and are discussed in more detail, as are the phosphatidylinositide-3-phosphate (PtdIns-3-P) family of inositides, in Ch. 23. The phosphatidylglycerol (PtdGro) in brain, as in other tissues, are present in mitochondrial membranes. Of these, cardiolipin ($Ptd_2Gro$) is the most prevalent (see Box 5-1).

Each phospholipid class in a given tissue has a characteristic fatty acid composition. Though the same fatty acid may be present in a number of lipids, the quantitative fatty acid composition is different for each class of lipids and remains fairly constant during the growth and development of the brain. A typical distribution profile of the major fatty acids in rat brain phospholipids is given in Table 5-1. Not only do the phospholipids differ in the structure of the polar head groups, or phospholipid classes, but within each class there are a variety of combinations of pairs of fatty acids, giving rise to molecular species that differ in the nature and positional distribution of fatty acids esterified to the glycerol backbone. For example, the 1-stearoyl, 2-arachidonyl (18:0-20:4) species is predominant in PtdIns, while 22:6 acids are enriched in PtdEtn and PtdSer. The fatty acid substituents for a given phospholipid class isolated from white and gray matter may differ dramatically. Thus, white matter PtdEtn contains 42% 18:1 and 3% 22:6, while gray matter PtdEtn contains only 12% 18:1 and 24% 22:6 (Lee & Hajra, 1991). As noted below, brain lipids contain some unusually long and polyunsaturated fatty acids from both the n-3 and n-6 families of essential fatty acids, which cannot be

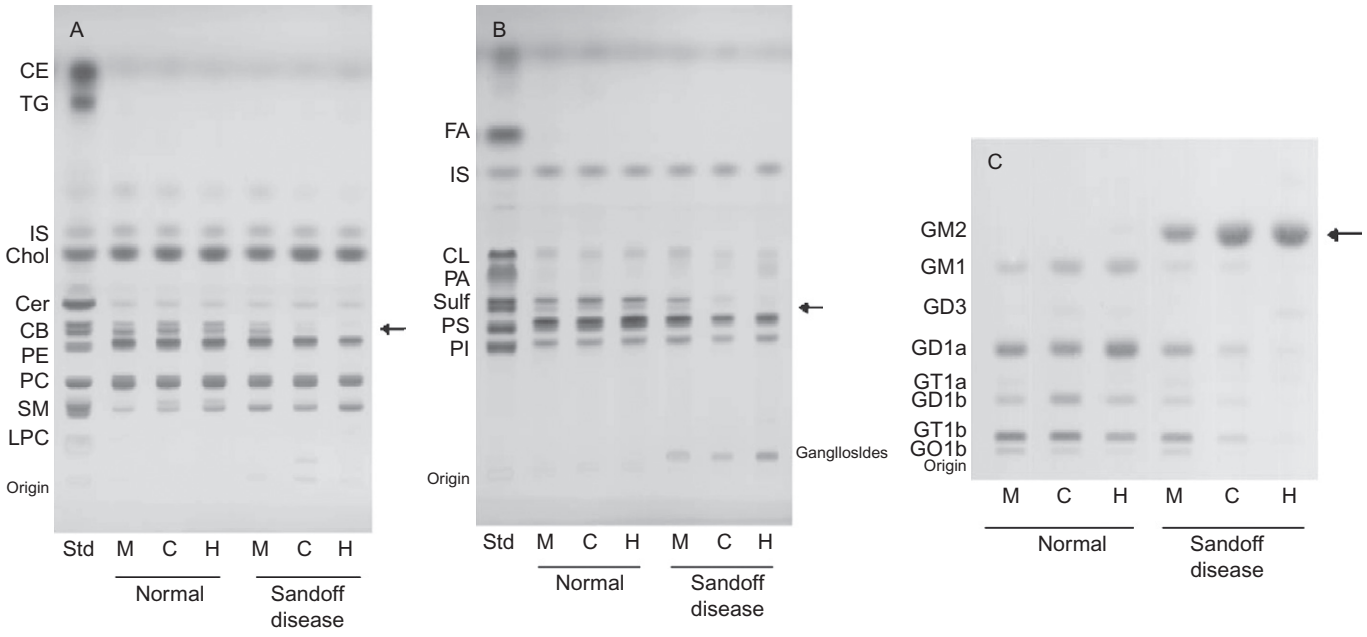



**FIGURE 5-3 High-performance thin-layer chromatogram (HPTLC) of cerebral cortex neutral lipids (A), acidic lipids (B), and gangliosides (C) in normal and Sandhoff disease mice (M), cats (C), and humans (H).** The amount of neutral lipids and acidic lipids spotted per lane was equivalent to approximately 70 μg and 200 μg brain dry weight, respectively. The neutral lipid and the acidic lipid plates were developed to a height of 4.5 cm and 6.0 cm, respectively, with chloroform: methanol: acetic acid: formic acid: water (35:15:6:2:1 by volume), and were then developed to the top with hexane: diisopropyl ether: acetic acid (65:35:2 by volume). The bands were visualized by charring with 3% cupric acetate in 8% phosphoric acid solution. The amount of ganglioside spotted per lane was equivalent to 1.5 μg sialic acid. The plate was developed in one ascending run with chloroform: methanol: 0.02% aqueous calcium chloride (55:45:10 by volume). Gangliosides were visualized with the resorcinol-HCl spray and are named according to Svennerholm's nomenclature (see Fig. 5-5). CE, cholesteryl esters; TG, triacylglycerols; IS, internal standard; Chol, cholesterol; CM, ceramide; CB, cerebroside (doublet, indicated by arrow); PE, phosphatidylethanolamine; PC, phosphatidylcholine; SM, sphingomyelin; GA2, asialo GM2; LPC, lyso-phosphatidylcholine, FA, fatty acids; CL, cardiolipin; PA, phosphatidic acid; Sulf, sulfatides (doublet, indicated by arrow); PS, phosphatidylserine; PI, phosphatidylinositol. The arrows identify reductions in galactocerebrosides **(A)**, sulfatides **(B)**, and elevation of GM2 **(C)**. (From Baek et al., 2009 and Lipids, with permission.)

biosynthesized in the animal body *de novo* (see also Ch. 36). This implies the existence of a mechanism for transporting essential fatty acids across the blood–brain barrier, which is driven both by diffusion of the fatty acids as well as protein facilitated uptake. The formation of an acyl-CoA by long chain acyl-CoA synthetases and utilization of fatty acids to form lipids is also a key driving force for fatty acid uptake. In fact, acyl-CoA synthetases may have a major role in defining molecular species and trafficking fatty acids into specific metabolic pools (Barceló-Coblijn & Murphy, 2009; Milner et al., 2010) and these molecular species are also defined by specific enzyme steps during phospholipid biosynthesis (see below). There is considerable interest in the role of the polyunsaturated fatty acids and their metabolites in brain after breakdown of their parent phospholipids in conditions such as ischemia and hypoxia (see Chs. 11 and 35).

## In sphingolipids, the long-chain aminodiol sphingosine serves as the lipid backbone

Sphingosine resembles a monoradyl glycerol but has asymmetric carbons at both C-2 and C-3. The chiral configuration is like that of the tetrose D-erythrose. That is, the amino group at C-2 and hydroxyl group at C-3 are in *cis* configuration (2S, 3R). Unlike unsaturated fatty acids, the double bond between C-4 and C-5 in sphingosine is in the *trans* (E) configuration. In the IUPAC-IUB nomenclature, the saturated analog of sphingosine, dihydrosphingosine or D-erythro-2-amino-1,3-octadecanediol, is termed sphinganine, and sphingosine is (E-4) sphingenine. While in most sphingolipids the sphingosine is 18 carbons long, in brain gangliosides there is a significant representation of the $C_{20}$ homolog.

The amino group of sphingosine is acylated with long-chain fatty acids and the *N*-acylated product is termed a ceramide (Fig. 5-3). C-1 of ceramide is linked to different head groups to form various membrane lipids. For example, sphingomyelin is the phosphodiester of ceramide and choline. The fatty acids in sphingomyelin have a bimodal distribution: in white matter they are mostly 24 carbons long (lignoceric and nervonic) while in gray matter stearic acid (18:0) predominates (see Table 5-1). Most of the glycolipids in brain consist of ceramide glycosidically linked at C-1 with different mono- or polysaccharides. The major glycolipid of mammalian brain is galactocerebroside, in which galactose is β-glycosidically linked to ceramide; it

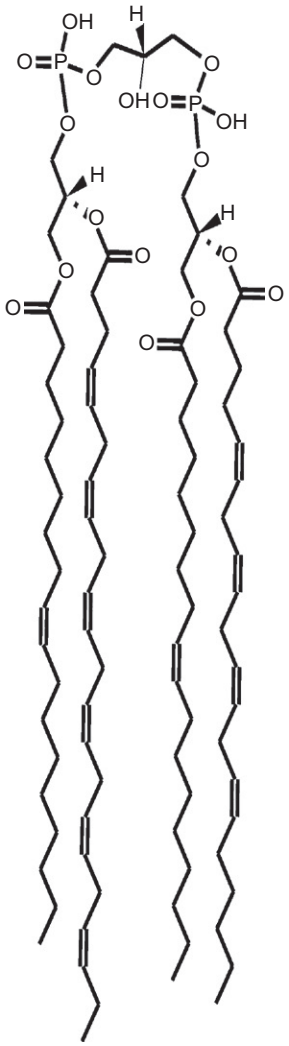
## CARDIOLIPIN AND ENERGY METABOLISM IN NORMAL BRAIN, NEURODEGENERATION AND GLIOMAS

*Thomas N. Seyfried*

Cardiolipin ($Pdt_2Gro$ or 1,3-diphosphatidyl-*sn*-glycerol, CL) is a complex mitochondrial-specific phospholipid that regulates numerous enzyme activities, especially those related to oxidative phosphorylation and coupled respiration. CL is essential for efficient oxidative energy production and mitochondrial function. CL contains two phosphate head groups, three glycerol moieties, and four fatty acyl chains and is primarily enriched in the inner mitochondrial membrane (Box Fig. 5-1). CL binds complexes I, III, IV and V and stabilizes the supercomplexes (I/III/IV, I/III and III/IV), demonstrating an absolute requirement of CL for catalytic activity of these respiratory enzyme complexes (Kiebish et al., 2008). CL restricts pumped protons within its head group domain, thus providing the structural basis for mitochondrial membrane potential and supplying protons to the ATP synthase. Respiratory-complex proteins that interact with CL have evolved to form hydrophobic grooves on their surface. These grooves accommodate the fatty acid chains of CL. While the amino acid sequence of electron transport proteins is highly conserved, considerable variability occurs in the acyl chain composition of CL across tissues and disease states.

Recent studies using multidimensional mass spectrometry–based "shotgun" lipidomics (MDMS-SL) identified almost 100 molecular species of CL in highly purified mitochondria from mammalian brain (Cheng et al., 2008; Kiebish et al., 2008). Moreover, these molecular species form a unique pattern, consisting of seven major groups, when arranged according to fatty acid chain length and degree of unsaturation (Kiebish et al., 2008) (Box Fig. 5-2). This unique fatty acid pattern is expressed in CL analyzed from synaptic mitochondria (enriched in neurons) as well as from non-synaptic (NS) mitochondria (enriched in cell bodies of neurons and glia). In contrast to the complex fatty acid molecular speciation found in brain CL, CL analyzed from non-neural tissues such as liver and heart contains mostly tetra 18:2 CL. The brain therefore appears unique among tissues in expressing a very complex distribution of CL fatty acid molecular species.

The unique distribution of molecular species in brain CL is thought essential for neural cell energy metabolism and could contribute to the metabolic compartmentation of the brain (Kiebish et al., 2008). In light of the role of CL in maintaining electron transport chain activities, disturbances in the content and composition of CL could profoundly influence energy metabolism and neural cell viability and function. Analysis of CL composition and content in models of neurodegeration will elucidate the role of CL metabolism and mitochondrial function in various neurodegenerative diseases. Recent evidence demonstrates the role of α-synuclein in regulating the CL composition in brain, thus highlighting a role of altered mitochondrial lipid metabolism and function in Parkinson's disease. Deficiencies in α-synuclein resulted in decreased remodeling of neural CL corresponding to decreased linked I/III electron transport chain activities, thus demonstrating an importance of cardiolipin maintenance in regulating neural mitochondrial functionality in neurodegeneration (Ellis et al., 2005). Additionally, changes in cardiolipin content have also been found in models of aging, traumatic brain injury, and familial amyotrophic sclerosis (Pope et al., 2008). Mutations in the Tafazzin gene, a characterized cardiolipin transacylase, is the cause of Barth syndrome, which results in severe alterations in cardiolipin remodeling and monolysocardiolipin accumulation, leading to dilated cardiomyopathy, neutropenia, muscle weakness, and a loss of mitochondrial function. The effect of Tafazzin mutations on the maintenance

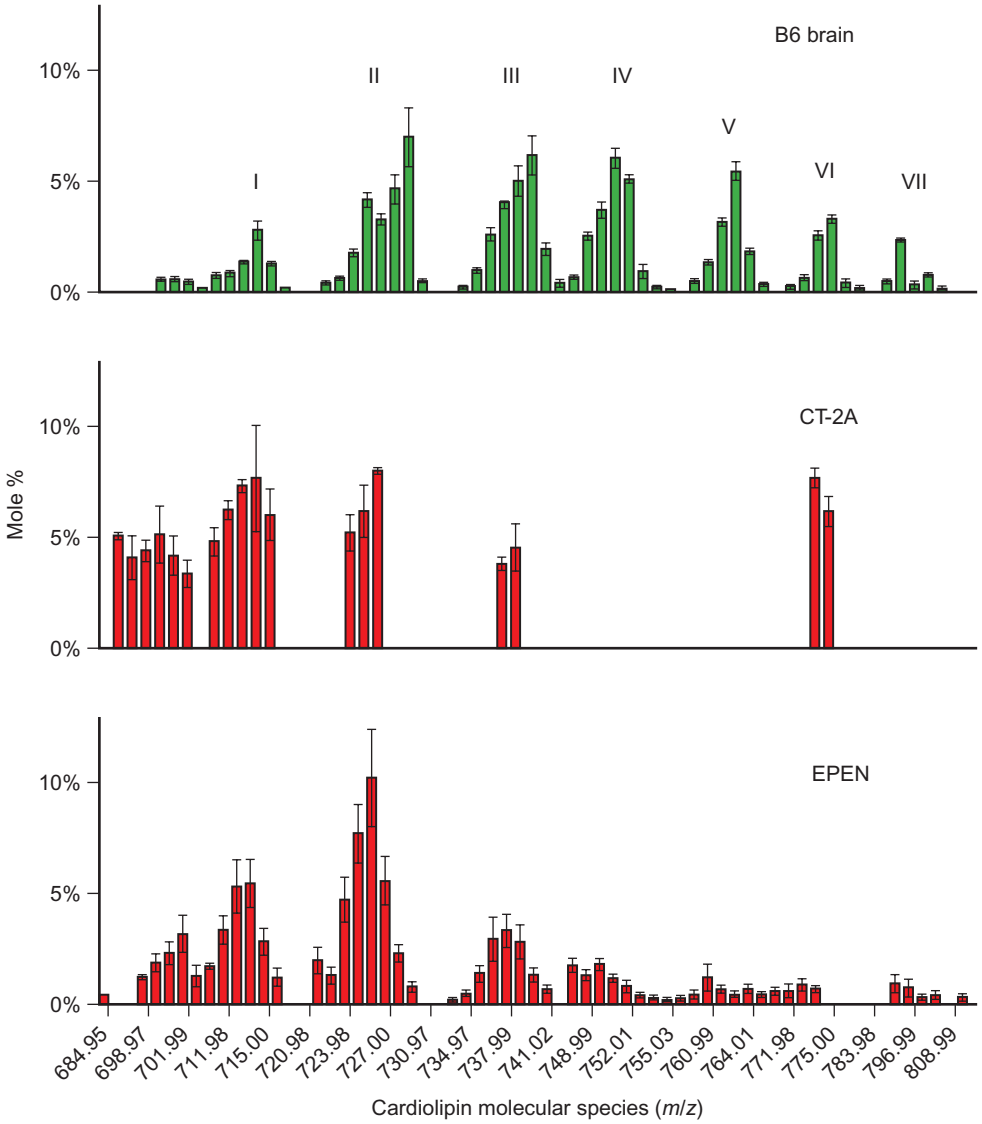


BOX FIG. 5-1 **Structure of cardiolipin (1, 1′, 2, 2′-tetraoleyl cardiolipin)**. This is one of the over 100 cardiolipin molecular species present in mouse brain mitochondria.

## CARDIOLIPIN AND ENERGY METABOLISM IN NORMAL BRAIN, NEURODEGENERATION AND GLIOMAS (*cont'd*)

of brain cardiolipin has not yet been investigated. Additionally, Tangier's disease, caused by mutations in the ATP-binding cassette transporter 1 gene (ABCA1), also results in abnormal cardiolipin composition and accumulation in lysocardiolipin (Fobker et al., 2001); however, the effects of ABCA1 mutations on brain cardiolipin have not been investigated. Thus, the accrual of further knowledge on the role of changes in brain cardiolipin in various diseases will greatly improve our understanding of disease pathology involving altered mitochondrial metabolism in brain function.

Major changes in the content and distribution of CL molecular species have also been reported in mouse brain tumors. In marked contrast to the symmetrical distribution of CL molecular species seen in NS mitochondria of normal C57BL/6J (B6) mouse




BOX FIG. 5-2 **Distribution of cardiolipin molecular species in C57BL/6J (B6) mouse brain and in syngeneic CT-2A and EPEN brain tumor mitochondria**. Cardiolipin fatty acid molecular species are plotted on the abscissa and arranged according to the mass-to-charge ratio based on percentage distribution. The molecular species are subdivided into seven major groups (I–VII) as we previously described (Kiebish et al., 2008, 2009). All values are expressed as the mean of three independent mitochondrial preparations, where tissue from six cortexes or tumors was pooled for each preparation. It is clear that fatty acid molecular species composition differs markedly between and among tumors and their syngeneic mouse hosts. With permission from Kiebish et al (2009) and *ASN Neuro*.

## CARDIOLIPIN AND ENERGY METABOLISM IN NORMAL BRAIN, NEURODEGENERATION AND GLIOMAS (*cont'd*)

brain, the syngeneic CT-2A astrocytoma and ependymoblastoma (EPEN) tumors display a preponderance of shorter-chain molecular species, with reduced amounts of the longer-chain polyunsaturated species (Box Fig. 5-2). Additionally, the CL composition in mitochondria isolated from cultured non-neoplastic B6 astrocytes is markedly different from the CL composition of NS mitochondria from B6 brain. The CL composition of the AC is similar to that of the cultured CT-2A and EPEN tumor cells in expressing an abundance of short chain saturated or monounsaturated species characteristic of immature CL (Kiebish et al., 2009). Expression of immature CL is associated with significant reduction of complex I activity, requiring a compensatory increase in glycolysis to maintain energy balance. These findings indicate that tumorigenesis and growth environment can alter the CL composition of neural cells in different ways. As CL influences respiratory energy metabolism, alterations in CL composition can compromise mitochondrial function and neural cell physiology.

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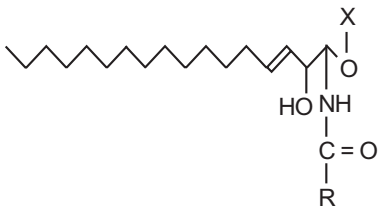
**TABLE 5-1 Distribution Profile of the Major Individual Molecular Species in the Diacylglycerol Moieties of Rat Brain Phosphoglycerides***

| Fatty acid | | | | | | |
|---|---|---|---|---|---|---|
| **C-1** | **C-2** | **PtdIns (mol %)** | **PtdIns-4,5$P_2$ (mol %)** | **PtdCho (mol %)** | **PtdEtn (mol %)** | **PtdSer (mol %)** |
| 16:0 | 22:6 | 1.4 | 0.1 | 3.3 | 4.8 | 0.8 |
| 16:0 | 20:4 | 7.8 | 9.5 | 4.4 | 2.3 | 0.6 |
| 18:1 | 20:3 | 4.1 | 1.1 | Tr | Tr | Tr |
| 18:0 | 22:6 | Tr | 1.0 | 2.5 | 17.6 | 42.4 |
| 14:0 | 16:0 | 0.6 | 0.4 | 3.1 | 1.5 | 0.8 |
| 18:0 | 22:5 | 1.0 | 0.7 | 0.4 | 0.2 | 5.3 |
| 18:0 | 20:4 | 49.5 | 66.1 | 3.8 | 22.5 | 3.8 |
| 18:1 | 18:1 | 1.7 | 2.1 | 3.4 | 11.1 | 7.0 |
| 16:0 | 18:1 | 12.7 | 6.5 | 36.2 | 15.8 | 9.1 |
| 16:0 | 16:0 | 6.9 | 1.4 | 19.2 | 0.7 | Tr |
| 18:0 | 18:1 | 7.0 | 4.6 | 14.1 | 14.8 | 23.7 |

PtdIns, phosphatidylinositol; PtdIns-4,5$P_2$, phosphatidylinositol-4,5-*bis*phosphate; PtdCho, phosphatidylcholine; PtdEtn, phosphatidylethanolamine; PtdSer, phosphatidylserine.
*Adapted from Lee & Hajra (1991).

constitutes about 16% of total adult human brain lipid (Fig. 5-3). Sulfatide is galactocerebroside esterified to sulfate at the 3 position of galactose and constitutes about 6% of brain lipid. Cerebrosides are present mainly in brain white matter, especially in myelin, and generally contain very-long-chain normal (lignoceric and nervonic), α-hydroxy (cerebronic) and odd-numbered fatty acids, such as 23:0 and 23h:0. Myelin is a specialized plasma membrane that surrounds




| X | Lipid | Abv. |
|---|---|---|
| H | Ceramide | Cer |
| $O^-$ \| $P-O-CH_2CH_2\overset{+}{N}(CH_3)_3$ \| O | Sphingomyelin | CerPCho |
| $CH_2OH$, HO, O, OH, OH | Galactocerebroside | CerGal |
| $CH_2OH$, O, OH, OH, OH | Glucocerebroside | CerGlc |
| Lactose (Glc-Gal) | Lactosylceramide | CerLac |

FIGURE 5-4 **Structure of some simple sphingolipids**. X may be a complex polysaccharide either containing sialic acid (gangliosides) or not (globosides). See also Figures 5-5 and 5-9 for the nomenclature and structure of some of the complex brain sphingolipids.

nerve processes; it is elaborated by oligodendroglial cells in the CNS and by Schwann cells in the PNS (see Chs. 1, 10 and 31). A number of neurological disorders appear to involve myelin selectively (Ch. 39). The content of myelin-enriched cerebrosides and sulfatides can also be reduced secondary to ganglioside storage (Fig. 5-3). Brain also contains many other glycolipids that are polysaccharide derivatives of glucocerebroside (Cer-Glc). Many monosaccharides, such as galactose (Gal), glucose (Glc), *N*-acetylglucosamine (GlcNAc), *N*-acetylgalactosamine (GalNAc), fucose and others, are present in various linkages in these carbohydrate head groups. One important carbohydrate is sialic acid, or *N*-acetyl (or *N*-glycolyl)-neuraminic acid (NANA), an *N*-acylated, nine-carbon amino sugar (Fig. 5-5B) containing a free carboxyl group. NANA is enzymatically formed by condensation of *N*-acetyl- (or *N*-glycolyl-) mannosamine with phosphoenolpyruvate. The sialic acid-containing glycolipids contain a free carboxylic group and are termed gangliosides. Many gangliosides have been identified in neural and other tissues, and their classification and nomenclature are somewhat complex (Yu & Ando, 1980). Svennerholm classified the gangliosides according to the number of sialic acid residues present in the molecule and its relative migration rate on thin-layer chromatograms (Fig. 5-3). IUPAC-IUB has proposed a different systematic nomenclature for both gangliosides and neutral glycolipids, or globosides. The structure and nomenclature of a major brain ganglioside are given in Fig. 5-5A (see below for other gangliosides).

## ANALYSIS OF BRAIN LIPIDS

### Chromatography and mass spectrometry are employed to analyze and classify brain lipids

The lipids from brain are generally extracted with a mixture of chloroform and methanol using variations of a method originally described by Folch et al (1957). In most procedures, the tissue or homogenate is treated with 19 volumes of a 2:1 (v/v) mixture of chloroform methanol. A single liquid phase is formed, leaving behind a residue of macromolecular material, primarily protein, with lesser amounts of DNA, RNA and polysaccharides. The subsequent addition of a small amount of water containing 0.9% KCl to the $CHCl_3$-methanol extract leads to separation into a lower chloroform phase containing the majority of lipids and an upper phase of water and methanol that contains low–molecular-weight metabolites and polar lipids, such as gangliosides. If the lower phase is evaporated to dryness and then dissolved in a lipid solvent such as chloroform, proteolipid protein remains as a precipitate and can be removed at this point. Gangliosides can be extracted from the aqueous phase by repartitioning into a nonpolar solvent. Acidic phospholipids such as the polyphosphoinositides are poorly extracted at neutral pH, so it is necessary to acidify the initial chloroform-methanol mixture for their recovery (Hajra et al., 1987). Unfortunately, the acidity leads to cleavage of plasmalogens, primarily PlsEtn and PlsCho. The lipids can be fractionated using a variety of techniques including thin-layer

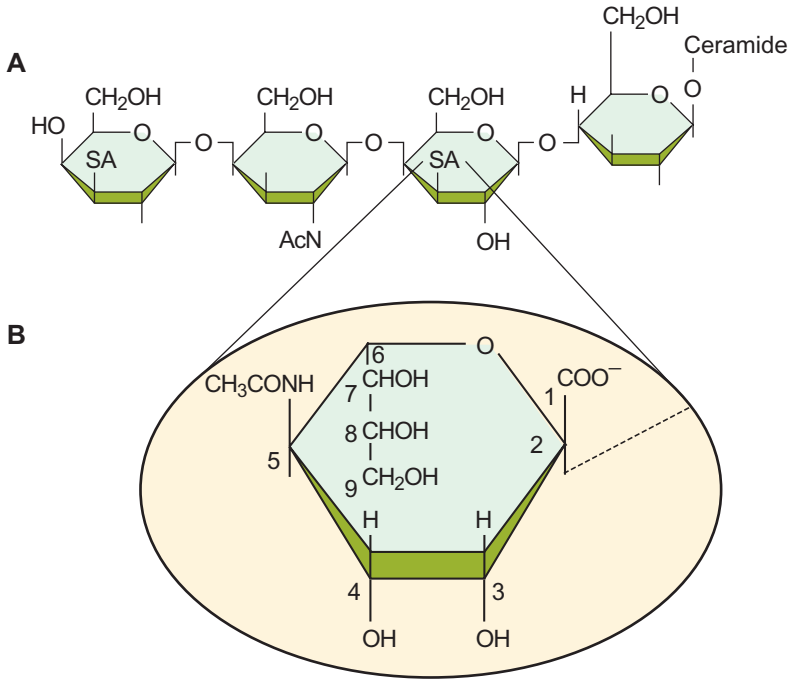



**FIGURE 5-5** **(A) The structure of a major brain ganglioside, which is termed GD1a according to the nomenclature of Svennerholm**. G denotes ganglioside, D indicates disialo, 1 refers to the tetrasaccharide (Gal-GalNac-GalGlc-) backbone and *a* distinguishes positional isomers in terms of the location of the sialic acid residues (see also Fig. 5-9). In IUPAC-IUB nomenclature, this ganglioside is termed IV$^3$NeuAc,II$^3$NeuAc-Gg$_4$Cer, where the Roman numerals indicate the sugar moiety (from ceramide) to which the sialic acids (NeuAC) are attached and the Arabic numeral superscript denotes the position in the sugar moiety where NeuAC are attached; Gg refers to the ganglio (Gal-GalNAc-Gal-Glc) series and the subscript 4 to the four-carbohydrate backbone for the 'ganglio' series. **(B)** The structure of sialic acid, also called *N*-acetyl neuraminic acid (NeuAc or NANA). Human brain gangliosides are all *N*-acetyl derivatives; however, some other mammalian brains, such as bovine, may contain the *N*-glycolyl derivatives. The metabolic biosynthetic precursor for sialylation of glycoconjugates is CMP-sialic acid, forming the phosphodiester of the 5′OH of cytidine and the 2-position of neuraminic acid.

chromatography (TLC), high-performance liquid chromatography (HPLC) and liquid chromatography coupled with mass spectral (LC-MS) analysis. In addition, ion exchange column chromatography coupled with high-performance thin-layer chromatography (HPTLC) can be used to separate all major brain lipid groups including gangliosides, as shown in Figure 5-3. A typical lipid composition of adult human brain is given in Table 5-2, and HPTLC comparison of lipid distribution in adult mouse, cat, and human normal and GM2 ganglioside storage disease is shown in Fig. 5-3. HPLC using silicic acid as the stationary phase can also be used to separate various lipid classes (Barceló-Coblijn et al., 2010).

For analysis of individual esterified fatty acids in a given lipid class, methyl esters are prepared directly by alkaline methanolysis, and the lipids separated by TLC or by HPLC as described above (Barceló-Coblijn et al., 2010). The amide-linked fatty acids of the sphingolipids require more vigorous conditions of methanolysis, such as treatment with HCl methanol. The methyl esters are then separated by gas–liquid chromatography (GLC). The molecular species can also be separated by reverse-phase HPLC; in this method the phospholipids are converted to DAG via phospholipase C and then derivatized with an ultraviolet-absorbing benzoyl group (Lee & Hajra, 1991). Subsequent separation of the derivatized DAG is achieved on the basis of differences in hydrophobicity and separated by HPLC using a C-18 column. While this technique is very useful for studies involving metabolism, a more rapid and modern technique is the use of shotgun lipidomics to analyze underivatized intact phospholipids using quantitative electrospray ionization/mass spectrometry method (ESI/MS). This method rapidly and directly analyzes the molecular profile, or 'lipidome,' of different lipid classes in very small samples, using total lipid extracts from tissues or cultured cells. By manipulating the ionization method, the mass spectrographs of polar or non-polar lipids can be obtained (Han et al., 2003). This method and the use of lipid arrays allow precise and quantitative identification of the lipid profile of a given tissue, and map functional changes that occur (Box 5-1).

## BRAIN LIPID BIOSYNTHESIS

### Acetyl coenzyme A is the precursor of both cholesterol and fatty acids

The hydrophobic chains of lipids, that is, fatty acids and isoprenoids, are biosynthesized from the same two-carbon

**TABLE 5-2 Lipid Composition of Normal Adult Human Brain***

| | Gray matter (%) | | | White matter (%) | | |
|---|---|---|---|---|---|---|
| **Constituent** | **Fresh wt** | **Dry wt** | **Lipid** | **Fresh wt** | **Dry wt** | **Lipid** |
| Water | 81.9 | – | – | 71.6 | – | – |
| Chloroform–methanol-insoluble residue | 9.5 | 52.6 | – | 8.7 | 30.6 | – |
| Proteolipid protein | 0.5 | 2.7 | – | 2.4 | 8.4 | – |
| Total lipid | 5.9 | 32.7 | 100 | 15.6 | 54.9 | 100 |
| Upper-phase solids | 2.2 | 12.1 | – | 1.7 | 6.0 | – |
| Cholesterol | 1.3 | 7.2 | 22.0 | 4.3 | 15.1 | 27.5 |
| Phospholipid, total | 4.1 | 22.7 | 69.5 | 7.2 | 25.2 | 45.9 |
| PtdEtn | 1.7 | 9.2 | 27.1 | 3.7 | 13.2 | 23.9 |
| PtdCho | 1.9 | 10.7 | 30.1 | 2.4 | 8.4 | 15.0 |
| Sphingomyelin | 0.4 | 2.3 | 6.9 | 1.2 | 4.2 | 7.7 |
| Phosphoinositides | 0.16 | 0.9 | 2.7 | 0.14 | 0.5 | 0.9 |
| PtdSer | 0.5 | 2.8 | 8.7 | 1.2 | 4.3 | 7.9 |
| Galactocerebroside | 0.3 | 1.8 | 5.4 | 3.1 | 10.9 | 19.8 |
| Galactocerebroside sulfate | 0.1 | 0.6 | 1.7 | 0.9 | 3.0 | 5.4 |
| Ganglioside, total† | 0.3 | 1.7 | – | 0.05 | 0.18 | – |

*Modified from Suzuki (1981).
†Phospholipid fractions include plasmalogen, assuming that all plasmalogen is present as PtdEtn. Ratios of PtdEtn to PtdCho are 4:1 in white matter and 1:1 in gray matter. In intact brain (based on analysis of rapidly microwaved rat brain), phosphoinositides are present in both white and gray matter in the ratio of 5:0.3:1 for phosphatidylinositol (PtdIns) phosphatidylinositol-4-phosphate (PtdIns-4P); phosphatidylinositol-4,5-bisphosphate (PtdIns-4,5$P_2$). Gangliosides are calculated on the basis of total sialic acid, assuming that sialic acid constitutes 30% of the weight of a typical ganglioside; $GD_{1a}$ is the major ganglioside of both gray and white matter. PtdEtn, phosphatidylethanolamine; PtdCho, phosphatidylcholine; PtdSer, phosphatidylserine.

donor, acetyl coenzyme A (acetyl-CoA), with differences in condensation leading to different products. In cholesterol biosynthesis, two acetyl-CoA molecules are condensed to form acetoacetyl-CoA, which can be further condensed with a third acetyl-CoA to form a $C_6$ branched-chain dicarboxylic acyl-CoA, termed β-hydroxy-β-methylglutaryl (HMG)-CoA. HMG-CoA is reduced by 2 $NADPH^+$ to form mevalonic acid, and this reduction is catalyzed by the enzyme HMG-CoA reductase, the principal regulatory enzyme for the biosynthesis of isoprenoids (Brown & Goldstein, 1986). Mevalonic acid undergoes pyrophosphorylation by two consecutive reactions with ATP, and the product is decarboxylated to form isopentenyl pyrophosphate. This $C_5H_8$ isoprene unit is the building block of all isoprenoids. Two isoprene units, isopentenyl pyrophosphate and dimethyl allyl pyrophosphate, condense to form geranyl pyrophosphate ($C_{10}$), which then condenses with another $C_5$ unit to form farnesyl pyrophosphate ($C_{15}$), the precursor of many different isoprenoids, such as dolichol, a very-long-chain (up to $C_{100}$) alcohol; a redox coenzyme, ubiquinone; and cholesterol. Polyisoprenyl pyrophosphates also alkylate some proteins via a thioether bond, e.g., farnesyl pyrophosphate, which anchors them in biomembranes (see below). During cholesterol biosynthesis, two farnesyl pyrophosphate molecules reductively condense in a head-to-head manner to form squalene, a $C_{30}$ hydrocarbon. Squalene is oxidatively cyclized to form lanosterol, a $C_{30}$ hydroxysteroid. After three demethylations, lanosterol is converted to cholesterol ($C_{27}$). An outline of the pathway of biosynthesis of cholesterol is shown in Fig. 5-5. Once formed, brain cholesterol turns over very slowly, and there is both metabolic and analytic evidence to indicate an accretion of brain cholesterol with age (see Ch. 2 and Dietschy, 2009).

Fatty acids are biosynthesized via elongation of $C_2$ units. Here, acetyl-CoA is carboxylated by bicarbonate to form malonyl-CoA, which then condenses with an acetyl-CoA to form a β-ketoacyl-CoA and $CO_2$. This release of $CO_2$ ($HCO_3^-$) drives the reaction forward and elongates the chain by acetyl units. The ketone group is then enzymatically reduced, dehydrated and hydrogenated, resulting in an acyl-CoA that is two carbons longer than the parent acyl-CoA. NADPH acts as the reducing agent for the reduction of both the ketone group and the double bond. All four reactions, condensation, reduction, dehydration and hydrogenation, are carried out by fatty acid synthase, a large, multifunctional, dimeric enzyme. This cycle is repeated until the proper chain length ($>C_{12}$) is attained, after which the

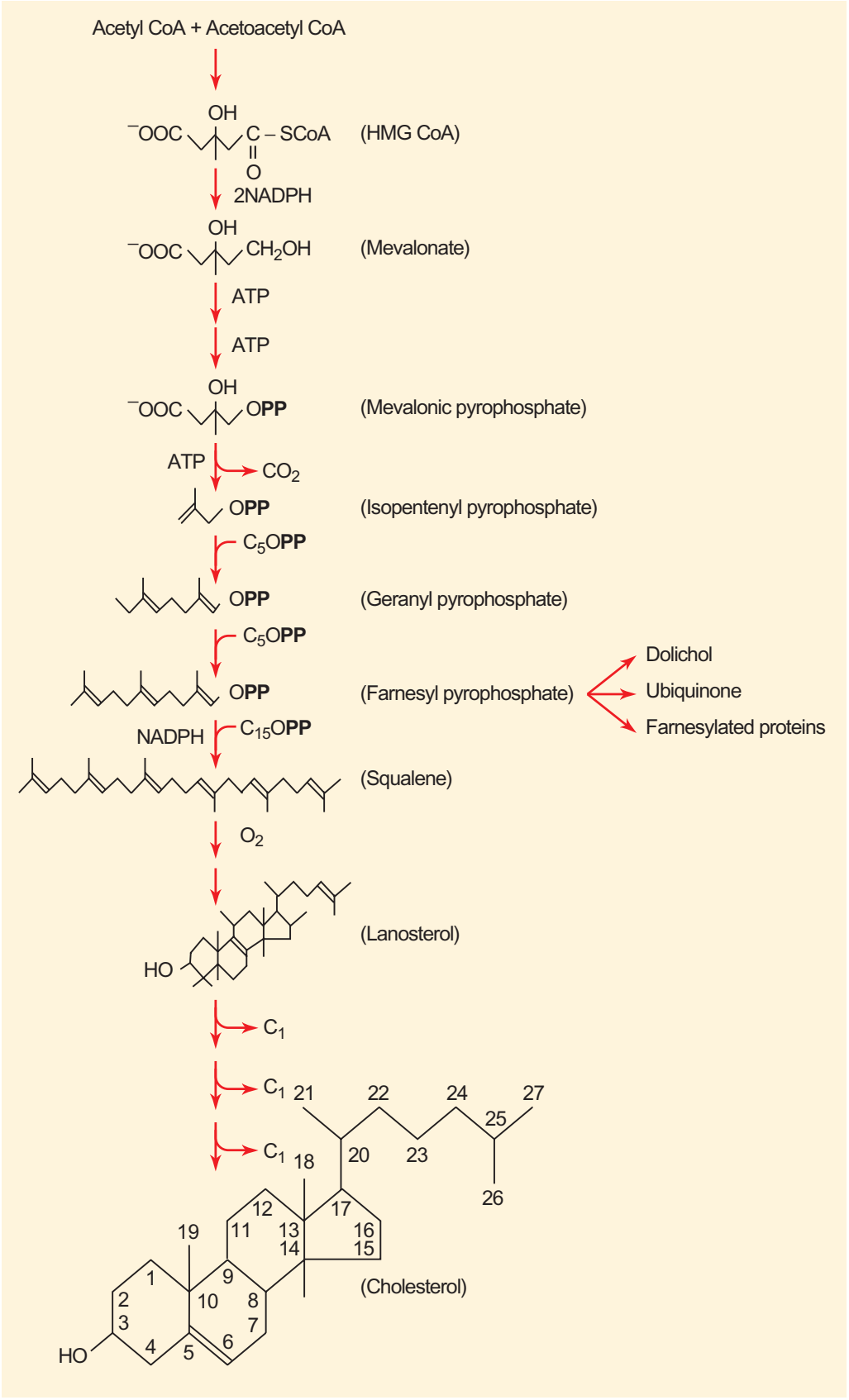



FIGURE 5-6 **Pathways of biosynthesis of isoprenoids.**

fatty acid is hydrolyzed from its thioester link with the enzyme.

Preformed or exogenous fatty acids are extended by a similar mechanism and catalyzed by enzyme(s) called elongases that are present in the endoplasmic reticulum (ER) (Cinti et al., 1992). There is also a minor mitochondrial chain-elongation system in which acetyl-CoA rather than malonyl-CoA is utilized to lengthen the fatty acid chain. Fatty acids are converted to unsaturated fatty acids mainly in the endoplasmic reticulum by desaturases. Fatty acyl-CoA desaturases, of which $\Delta^9$-desaturases are most characterized, remove two hydrogens from the CH2-CH2 groups of long-chain intermediates, such as 18:0-CoA by oxidizing them with molecular oxygen. In brain, this enzyme is responsible for the conversion of stearic acid (18:0) to oleic acid (18:1n-9) and palmitic acid (16:0) to palmitoleic acid (16:1n-7) (see Fig. 5-1). The electrons are transferred via cytochrome $b_5$ which in turn is reduced to NADH via cytochrome $b_5$ reductase. In brain, the major PUFAs contained in phospholipids are arachidonic acid (ARA or 20:4n-6) and docosahexaenoic acid (DHA or 22:6n-3), are major phospholipid components, although other major PUFAs in the brain include 22:4n-6 and 22:5n-3. While these fatty acids are taken up from the plasma (Barceló-Coblijn & Murphy, 2009), the astrocytes in the brain fully express the complement of enzymes to elongate and desaturate the essential fatty acid precursors 18:2n-6 and 18:3n-3 to longer-chain n-6 and n-3 family members. These longer-chain fatty acids are formed by chain elongation and desaturation of shorter-chain fatty acids (Fig. 5-7). The additional double bonds are introduced between an existing double bond and the fatty acid carboxyl group. For example, stearic acid (18:0) is converted to oleic acid (18:1n-9) in brain but cannot be interconverted to linoleic acid (18:2n-6). Thus, fatty acids of the n-3 and n-6 series are considered "essential fatty acids" because they can be only obtained from dietary sources, mainly from plants. In instances where n-6 fatty acids are not available in the diet, then n-9 fatty acids are further chain-elongated and desaturated to form abnormal fatty acids in an effort to compensate for a loss of n-6 fatty acids and form 20:3n-9 (Fig. 5-7). This fatty acid is named 'Mead acid' after its discovery by James Mead in the tissues of animals fed a fat-free diet over extended periods. Mead acid is a structural substitute for arachidonic acid and, like ARA in normal animals, is enriched in the PtdIns of essential-fatty-acid–deficient animals. However, it does not form bioactive molecules such as the eicosanoids. Another example is in n-3 dietary deficiency; arachidonic acid (ARA or 20:4n-6) is elongated and desaturated to form docosapentaenoic acid (DPAn-6 or 22:5n-6), which is a structural

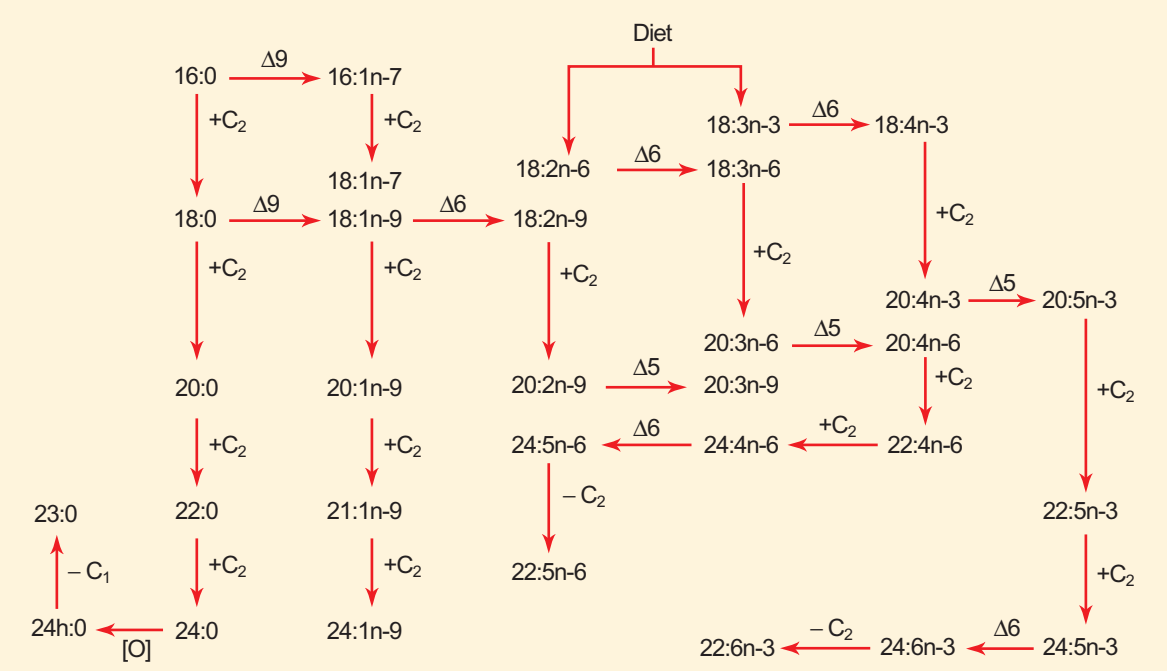



FIGURE 5-7 **Pathways for the interconversion of brain fatty acids**. Palmitic acid (16:0) is the main end product of brain fatty acid synthesis. It may then be elongated, desaturated and/or β-oxidized with the carbons recycled to form different long chain fatty acids. The monoenes (18:1n-7, 18:1n-9, 24:1n-9) are the main unsaturated fatty acids formed de novo by $\Delta^9$ desaturation and chain elongation. As shown, the very-long-chain fatty acids are α-oxidized to form α-hydroxy and odd-numbered fatty acids. The polyunsaturated fatty acids (PUFAs) are formed mainly from exogenous dietary fatty acids, such as linoleic (18:2n-6) and α-linolenic (18:3n-3) acids, by chain elongation and desaturation at $\Delta^5$ and $\Delta^6$, as shown. The accepted Sprecher pathway is shown for the formation of 22:6n-3 by elongation and $\Delta^6$ unsaturation of 22:5n-3 in the endoplasmic reticulum to form 24:6n-3, followed by one cycle of β-oxidation (–C2) in peroxisomes (Sprecher, 2000). This is illustrated in the biosynthesis of DHA (22:6n-3) above. In severe essential fatty acid deficiency, the abnormal PUFAs, such as 20:3n-9 and 22:5n-6 are also synthesized *de novo* to substitute for the normal PUFAs.

substitute for docosahexaenoic acid (DHA or 22:6n-3), and like DHA is found in DHA enriched phospholipids such as PtdEtn and PtdSer.

Fatty acids are degraded by two-carbon units in a reverse manner analogous to their biosynthesis. The acyl-CoA formed is first dehydrogenated to αβ-unsaturated acyl-CoA, and then hydrated to β-hydroxyacyl-CoA, followed by oxidation to β-ketoacyl-CoA. The C–C bond between C-2 and C-3 of the latter compound is broken by a free CoA molecule via thiolysis to form an acyl-CoA that is two carbons shorter and acetyl-CoA. Unlike fatty acid biosynthesis, each step of the β-oxidation of fatty acids is catalyzed by a distinct enzyme. These are present both in mitochondria and in peroxisomes. Though the biochemical steps are similar in the two cellular compartments, there are some differences between the peroxisomal and mitochondrial β-oxidation pathways. In mitochondria, the first dehydrogenation is carried out by an FAD-containing enzyme, which is coupled to oxidative phosphorylation, thus generating ATP. In peroxisomes, however, this dehydrogenation is carried out by a flavin-containing oxidase, which reacts directly with molecular oxygen to form $H_2O_2$, which is further decomposed by peroxisomal catalase to $H_2O$ and $O_2$, thus wasting the chemical energy. Two separate mitochondrial enzymes, enoyl-CoA hydratase and β-hydroxy acyl-CoA dehydrogenase, catalyze the next two reaction steps, while in peroxisomes both the reactions are catalyzed by a single multifunctional enzyme protein. The mitochondrial β-oxidation pathway is most efficient for long-chain fatty acids (<C20) and these fatty acids are used for energy and for carbons essential for the synthesis of amino acid neurotransmitters and cholesterol (Ch. 11; Barceló-Coblijn et al., 2009), while the peroxisomal β-oxidation pathway is probably responsible for the oxidation of very-long-chain fatty acids (>$C_{20}$). In addition, the peroxisomal β-oxidation pathway is critical in the Sprecher pathway (Voss et al., 1991) for the formation of DHA from DPAn-3 and is responsible for converting 24:6n-3 to 22:6n-3 (DHA) (Voss et al., 1991). A number of genetic diseases involve peroxisomal disorders, such as Zellweger's cerebrohepatorenal syndrome and X-linked adrenoleukodystrophy, in which there is an accumulation of these very-long-chain saturated fatty acids (Moser et al., 2001), especially in neural tissues (see Ch. 43), illustrating the importance of this system in maintaining proper brain fatty acid composition.

In addition to the classical β-oxidation of fatty acids, known to occur in all tissues, significant α-oxidation, especially of the fatty acids of galactocerebroside, occurs in brain. In this reaction, carbon 2, termed the α carbon, of a long-chain fatty acid is hydroxylated, then oxidized and decarboxylated to form a fatty acid one carbon shorter than the parent fatty acid. This quantitatively minor α-hydroxylation pathway may explain the origins of both the comparatively large amounts of odd carbon fatty acids and of 2-hydroxy fatty acids in brain galactocerebrosides. Another α-oxidation pathway normally present in liver and other tissues is defective in the genetic disorder Refsum's disease. This results in the failure to metabolize the dietary branched-chain fatty acid phytanic acid (see Fig. 5-1), which can be initially metabolized only by ω-oxidation in these patients (Wanders et al., 2001). In Refsum's disease, this branched-chain fatty acid accumulates in nervous tissues, resulting in severe neuropathy (see Ch. 38)

## Phosphatidic acid is the precursor of all glycerolipids

Phospholipid biosynthesis is linked to glycolysis through the use of dihydroxyacetone phosphate (DHAP), formed from *sn*-glycerol-3-phosphate (Gro-3-P) via its reduction by NADH, catalyzed by glycerophosphate dehydrogenase. Gro-3-P is subsequently acylated by the consecutive action of glycerophosphate acyl transferase (GPAT) to from lysophosphatidic acid (lysoPtdOH). Two forms of GPAT are found in the brain: one in the mitochondria and one in the endoplasmic reticulum (ER). This step requires an acyl-CoA, and this enzyme acylates the *sn*-1 position with a saturated or monounsaturated fatty acid. LysoPtdOH is thus acylated in the ER by lysophosphatidic acid acyltransferase (LAT) to form the central precursor phosphatidic acid (PtdOH).

For PtdOH to form more complex phospholipids, it is hydrolyzed to 1,2-diacyl-*sn*-glycerol (DAG) by the action of phosphatidic acid phosphatase (PAP). This DAG is then a central precursor for the Kennedy pathway reactions that form PtdEtn and PtdCho. For PtdEtn biosynthesis, CDP-Etn is condensed with the DAG by ethanolamine phosphotransferase, forming PtdEtn. This enzyme prefers a PUFA at the *sn*-2 position of the DAG, possibly accounting for the large amount of 20:4n-6 and 22:6n-3 associated with PtdEtn. For PtdCho, CDP-Cho is condensed with the DAG by choline phosphotransferase. PtdCho can also be formed by the sequential methylation of PtdEtn by phosphatidylethanolamine methyltransferase (PEMT). Although this is a minor route, it may account for formation of PUFA-enriched PtdCho, as this enzyme has a preference for a PtdEtn with a 22:6n-3 in the *sn*-2 position. Phosphatidylserine (PtdSer) is formed by base exchange using PtdCho and PtdEtn as substrates, linking its formation to the Kennedy pathway. PtdSer synthase I and PtdSer synthase II catalyze these reactions, with PtdSer synthase II using only PtdEtn as a substrate, thereby accounting for the enrichment of PtdSer in 22:6n-3. PtdSer can be decarboxylated in the mitochondrion by PtdSer decarboxylase to form mitochondrial PtdEtn (Kennedy, 1986).

To synthesize phosphatidylinositol (PtdIns) and cardiolipin ($Ptd_2Gro$), PtdOH is converted to CDP-DAG by CDP-DAG synthase, an enzyme with very high activity in the brain and a preference for a PtdOH containing an *sn*-1 18:0 and an *sn*-2 20:4n-6 (Fig. 5-7). Using this CDP-DAG, PtdIns is formed by the condensation of myo-inositol to the CDP-DAG by PtdIns synthase. PtdIns is sequentially phosphorylated on the inositol moiety by specific kinases to form a variety of products key to lipid-mediated signal transduction in the brain (see Ch. 23). The CDP-DAG is also used to form $Ptd_2Gro$ via the sequential actions of three enzymes located in the inner mitochondrial membrane. These enzymes are glycerol phosphate transferase, which forms PtdGroP, and phosphatidylglycerol phosphatase, which in turn dephosphorylates PtdGroP to form PtdGro, the direct precursor for $Ptd_2Gro$. Cardiolipin synthase condenses a CDP-DAG with PtdGro to form cardiolipin ($Ptd_2Gro$), a crucial phospholipid required

for mitochondrial function (see Box 5-1). The pathways from brain phospholipid biosynthesis, including the enzymes that catalyze each step, are summarized in Figure 5-8.

The newly biosynthesized phospholipids undergo deacylation to the corresponding lysophospholipids, which can be further degraded or reconverted to the parent lipids by reacylation, often with a different fatty acyl substitute. The reacylation of lysophospholipids occurs by transferring acyl groups from acyl-CoAs or from other phospholipids either by CoA-dependent or CoA-independent acyltransferase. The acyltransferase(s) catalyzing the reacylation reactions is very specific toward the acyl donor and lysophospholipid substrates. These preferences, combined with selectivity during the biosynthesis of phospholipids highlighted above, are important processes by which the specific distribution of fatty acids in each individual class of membrane phosphoslipid is regulated. Thus, the initial fatty acid composition of a biosynthesized lipid may not reflect its ultimate composition.

To synthesize ether phospholipids, the first two reactions occur in the peroxisome (Hajra, 1995). DHAP is used as the precursor from glycolysis and is first acylated by DHAP acyltransferase in the peroxisome, forming 1-acyl DHAP,

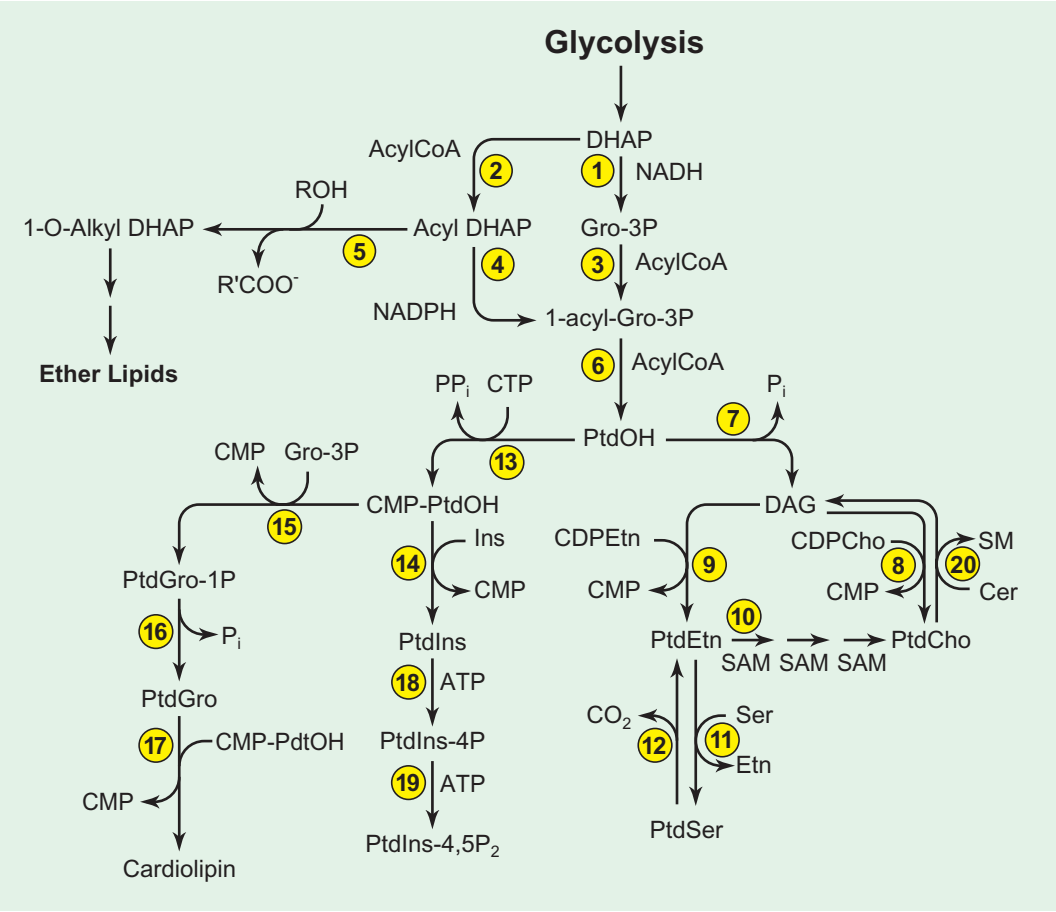



FIGURE 5-8 **Schematic representation of glycerophospholipid biosynthesis**. Note that dihydroxyacetone phosphate (*DHAP*) may be reduced to glycerophosphate or may be first acylated and then serve as a precursor of ether lipid. The alkyl analog of phosphatidic acid (i.e., 1-*O*-alkyl,2-acyl-*sn*-glycerol-3-P, PakOH) is converted to the alkyl analog of phosphatidylethanolamine (*PtdEtn*) by the same diacylglycerol (*DAG*) pathway as shown for the diacyl lipids, and the alkyl analog of PtdEtn is dehydrogenated to form the 1-alk-1′enyl analog of PtdEtn, ethanolamine plasmalogen (PlsEtn, not shown). As mentioned in the text, phosphatidic acid (*PtdOH*) is converted to DAG, which is converted to the major brain lipids phosphatidylcholine (*PtdCho*) and PtdEtn. The acidic lipids are formed via the conversion of PtdOH to CDP-DAG. PtdEtn is converted to PtdCho via methylation while both PtdEtn and PtdCho are converted to PtdSer via base exchange. Not only PtdEtn (as shown) but also PtdCho is converted to PtdSer by base-exchange reaction. Exchange of the head group of PtdCho with ceramide to form sphingomyelin is also shown. The enzymes catalyzing lipid biosynthesis are as follows: *1*, glycerophosphate dehydrogenase; *2*, dihydroxyacetone phosphate acyltransferase; *3*, *sn*-glycerol-3-phosphate acyltransferase; *4*, acyl/alkyl dihydroxyacetone phosphate reductase; *5*, alkyl dihydroxyacetone phosphate synthase; *6*, 1-acyl glycerol-3-phosphate acyltransferase; *7*, phosphatidate phosphohydrolase; *8*, diacylglycerol cholinephosphotransferase; *9*, diacylglycerol ethanolaminephosphotransferase; *10*, phosphatidylethanolamine *N*-methyl transferase and phosphatidyl-*N*-methylethanolamine *N*-methyl transferase; *11*, phosphatidylethanolamine:serine transferase; *12*, phosphatidylserine decarboxylase; *13*, phosphatidate cytidyltransferase; *14*, phosphatidylinositol synthase; *15*, CDP-DAG:glycerol-3-phosphate phosphatidyltransferase; *16*, phosphatidylglycerol phosphatase; *17*, cardiolipin synthase; *18*, phosphatidylinositol-4-kinase; *19*, phosphatidylinositol-4-phosphate 5-kinase; *20*, phosphatidylcholine:ceramide cholinephosphotransferase.

which is then converted to an ether lipid by alkyl DHAP synthase, which exchanges the acyl group in the *sn*-1 position with a fatty alcohol, generally 16:0alc, 18:0alc, or 18:1alc. The enzymes catalyzing these first two steps have peroxisomal targeting sequences. Two additional steps occur in the ER. The ketone at the *sn*-2 position is reduced to an OH group using NADPH and is catalyzed by alkyl/acyl DHAP reductase, thereby forming a 1-*O*-alkylGro, which is a substrate for LAT to synthesize an ether linked form of PtdOH referred to as PakOH. Similar to other pathways, PakOH is a substrate for PAP to form a 1-*O*-alkyl, 2-acyl glycerol product which is used by ethanolamine or choline phosphotransferase in the presence of CDP-Etn or CDP-Cho to form PakEtn or PakCho, respectively. The PakEtn can then be desaturated between the $\alpha$ and $\beta$ carbons by delta-1-desaturase, forming the vinyl ether linkage that characterizes the plasmalogens; in turn, this enzyme produces ethanolamine plasmalogen (PlsEtn). PlsEtn is the direct precursor for PlsCho and may be converted to by the action of PEMT, which can use a vinyl ether–linked substrate. Note that delta-1-desaturase cannot use PakCho as a substrate, although this important lipid is the precursor for platelet activating factor (Ch. 36) (Fig. 5-8).

The phospholipids are hydrolyzed by specific phospholipases, as indicated in Figure 5-2. The acyl groups at *sn*-1 and *sn*-2 are hydrolyzed by phospholipases $A_1$ and $A_2$ ($PLA_1$, $PLA_2$), respectively. The presence of $PLA_1$ in brain is inferential. The head groups are hydrolyzed by class-specific phospholipases. Thus, PtdCho and PtdIns-4,5$P_2$ are cleaved by different phospholipases. The bond between DAG and phosphate is hydrolyzed by phospholipase C, generating a DAG and inositol 1,4,5-triphosphate ($IP_3$), which act as downstream activators of PKC family kinases. The bond between the phosphate and the polar alcohol is hydrolyzed by phospholipase D and releases a PtdOH that is further converted via phosphatidic acid phosphatase (PAP) to a DAG. Note that this system does not release $IP_3$ and is operative in the ER and elsewhere, while the PLC system is operative at the level of the nuclear and plasma membranes. These enzymes are important not only for the catabolism of these lipids but also for the generation of biological signal-transduction-messenger lipid products such as DAG and the formation of 2-arachidonyl glycerol, a potent endocannabinoid (Ch. 61), by the hydrolysis of the *sn*-1 fatty acid on DAG by DAG lipase. Many of these enzymes are regulated, indirectly or directly, by cell-surface receptors. The brain also contains specific hydrolases—a plasmalogen-selective $PLA_2$ that releases arachidonic acid, with the lysoplasmalogen further hydrolyzed by the action of lysoplasmalogenase, which catalyzes the hydrolysis of the alkenyl-ether bond to form long-chain aldehydes and lysolipids or glycerophosphorylethanolamine, respectively.

## Sphingolipids are biosynthesized by adding head groups to the ceramide moiety

Sphinganine, also termed dihydrosphingosine, is biosynthesized by a decarboxylating condensation of serine with palmitoyl-CoA to form a keto intermediate, which is then reduced by NADPH (Fig. 5-9). Sphinganine is acylated, then dehydrogenated to form ceramide. Free sphingosine 'salvaged' from sphingolipid breakdown, also termed sphingenine, can be enzymatically acylated with acyl-CoA to form ceramide.

Ceramide is the precursor of all sphingolipids; sphingomyelin is formed by a reaction that transfers the head group of PtdCho to ceramide to form sphingomyelin and DAG (Figs. 5-8 and 5-9), while sphingosine-containing glycolipids are formed from consecutive glycosylation of ceramide by various nucleotide carbohydrate derivatives. For example, galactocerebroside is formed by glycosylation of ceramide with UDPGal, whereas glucocerebroside is formed by glycosylation of ceramide with UDPGlc (Radin, 1984). The latter, Cer-Glc, is the precursor of neutral glycolipids, also termed globosides, and acidic glycolipids, also termed gangliosides. The CMP derivative of the *N*-acetyl (or *N*-glycolyl) neuraminic acid NANA, or NeuAc, is the donor of this moiety to form gangliosides. Some of the reactions forming these complex glycolipids are shown in Figure 5-9. The specificity of these membrane-bound glycosyl transferases toward the lipid substrate and to the water-soluble nucleotide derivatives determines the structures of the products.

These same glycolipids are broken down by specific hydrolases present in lysosomes and stimulated by noncatalytic lysosomal proteins. A congenital deficiency of either one of the hydrolases or in the helper proteins results in the accumulation of lipid intermediates in lysosomes, leading to a lysosomal storage disease. For example, in Gaucher's disease, Cer-Glc accumulates because of a defect in its hydrolysis, whereas in Tay–Sachs disease, the GM2-ganglioside concentration is increased because of a deficiency in hexosaminidase A, the hydrolase releasing *N*-acetylgalactosamine (see Ch. 43).

# GENES FOR ENZYMES CATALYZING SYNTHESIS AND DEGRADATION OF LIPIDS

Because of interest in genetic lysosomal lipid storage diseases (Ch. 43), genes coding for a number of lipid hydrolytic enzymes have been identified and cloned. Progress has also been made in the elucidation of genes coding for biosynthetic enzymes, including transferases in the pathway for ganglioside formation and the UDP-glycosyltransferases that lead to cerebroside formation. In recent years, dozens of genes coding for enzymes catalyzing synthesis of cholesterol, phospholipids, galactolipids and gangliosides have been identified. Mutant or knockout mice defective in specific enzymes involved in lipid synthesis have provided powerful tools for genetic analysis of lipid function in the nervous system. For example, disruption of the genes for ceramide galactosyl transferase or galactosyl ceramide sulfotransferase, enzymes synthesizing galactocerebroside and sulfatide, both major sphingolipid components of myelin, gave unexpected results. While myelination and compaction of myelin was not affected initially, the mice displayed abnormal paranodal junctions and later disruption of myelin stability. Comparison of the two kinds of knockout mice showed

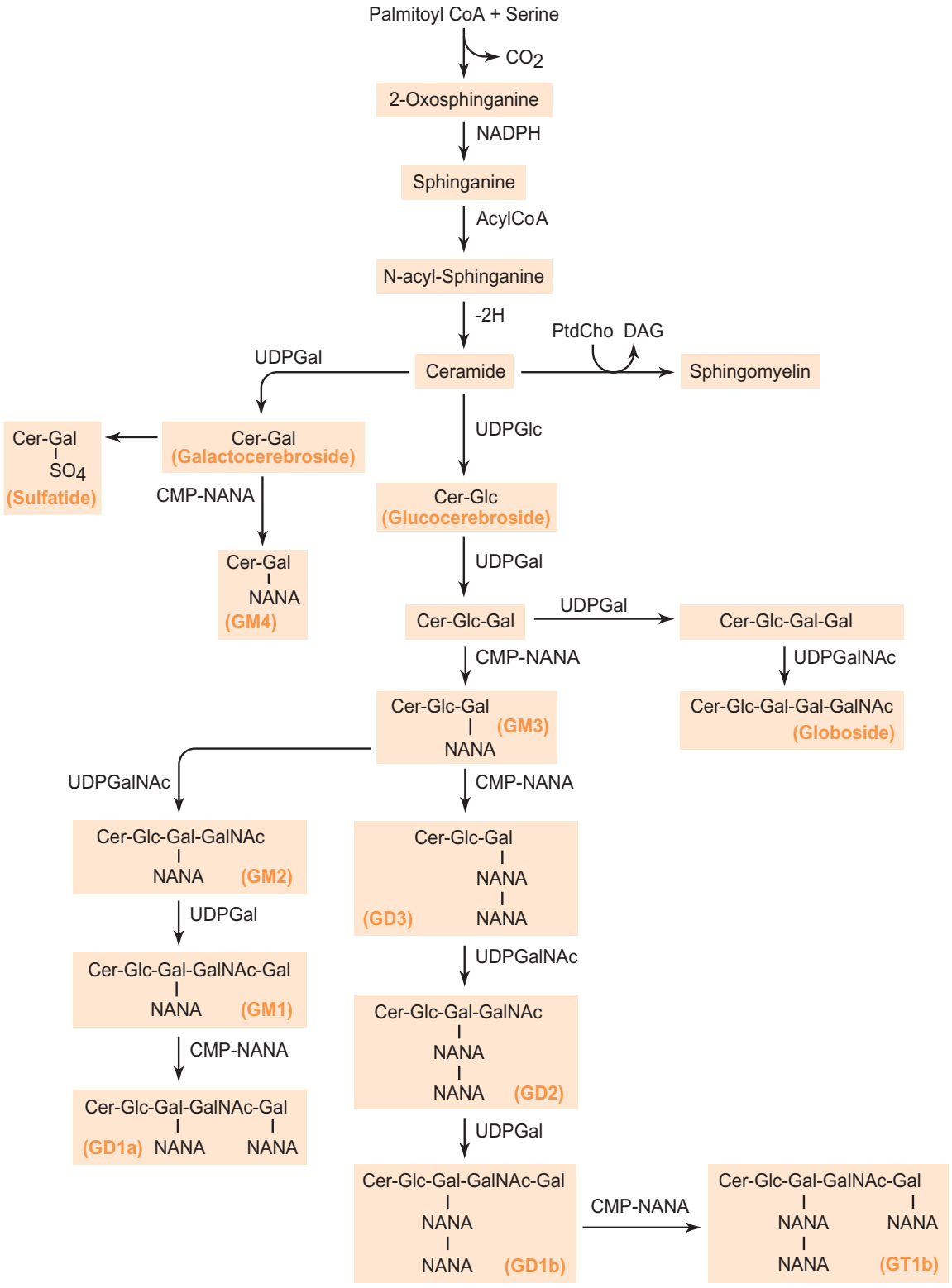



FIGURE 5-9 **Pathways for biosynthesis of sphingolipids**. Ceramide (Cer) is the precursor of all sphingolipids. Ceramide is converted to cerebroside (Cer-Gal), the main brain glycolipid, which is further converted to cerebroside sulfate (sulfatide) as shown. Cer-Gal is also converted to ganglioside (GM4), which is present in brain myelin. Most other gangliosides originate from Cer-Glc, and the main pathways for formation of these lipids are shown. The abbreviations using Svennerholm's nomenclature are shown in parentheses. (See Figs. 5-4 and 5-5.) The first letter, G, is for ganglioside. The second letter, M, D, T or Q, represents the number of sialic acid residues. Isomeric configurations of NANAs are distinguished by a and b. The main gangliosides of adult human brain are GM1, GD1a, and GD1b.

that sulfatide plays a critical role in the proper localization and maintenance of $Na^+$ channels at the paranode (Ishibashi et al., 2002) (see Ch. 10). Negative phenotypes can be informative as well. The hypothesis that complex gangliosides play a role in synaptic transmission was examined by deleting GM2/GD2 synthase, an enzyme that catalyzes an early step in ganglioside biosynthesis, then testing neurotransmitter release at the neuromuscular junction (Bullens et al., 2002). Transmitter release was not altered under normal conditions, suggesting a redundancy or compensatory action of complex ganglioside function at the synapse.

# LIPIDS IN THE CELLULAR MILIEU

## Lipids are transported between membranes

As indicated above, lipids are often biosynthesized in one intracellular membrane and must be transported to other intracellular compartments for membrane biogenesis. Because lipids are insoluble in water, special mechanisms must exist for the inter- and intracellular transport of membrane lipids. Vesicular trafficking, cytoplasmic transfer-exchange proteins and direct transfer across membrane contacts can transport lipids from one membrane to another. The best understood of such mechanisms is vesicular transport, wherein the lipid molecules are sorted into membrane vesicles that bud out from the donor membrane and travel to and then fuse with the recipient membrane. The well-characterized transport of plasma cholesterol into cells via receptor-mediated endocytosis is a useful model of this type of lipid transport (Brown & Goldstein, 1986; Maxfield & Wustner, 2002). A brain-specific transporter for cholesterol has been identified (see Chapter 2). It is believed that transport of cholesterol from the endoplasmic reticulum to other membranes and of glycolipids from the Golgi bodies to the plasma membrane are mediated by similar mechanisms. The transport of phosphoglycerides is less clearly understood. Recent evidence suggests that net phospholipid movement between subcellular membranes may occur via specialized zones of apposition, as characterized for transfer of PtdSer between mitochondria and the ER (Voelker, 2003).

## Membrane lipids may be asymmetrically oriented

In the 'fluid-mosaic' model of biomembranes, the lipids form a bimolecular leaflet in which proteins are embedded (Ch. 2). This model, with some modifications, is useful in explaining a number of membrane phenomena, but it does not take into account the complex arrangement and function of various polar head groups and different fatty acids present in biomembrane lipids, nor the enrichment of specific proteins and lipids in lateral domains, or rafts, within the bilayer. Lipids can move freely within the same plane of the bilayer, but their movement from one leaflet of the bilayer to another is thermodynamically restricted. It is not clear how this asymmetric distribution of lipids in biomembranes originates. It is postulated that membranes contain ATP-dependent transporters that catalyze a 'flip-flop' transbilayer movement of lipids (Contreras et al., 2010). Specificity of such 'flippase', 'floppase' and 'scramblase' activities may be responsible for the asymmetric distribution of lipids between the inner and outer leaflets of the membrane bilayer. Transfer of lipids from one side of the bilayer to the other may also be the result of the enzymatic generation of lipids, such as diacylglycerol or ceramide at one side of the membrane, and may play a role in the formation of lipid rafts (Ch. 2).

In some biomembranes, such as those of red blood cells, the choline-containing phospholipids PtdCho and sphingomyelin are known to be enriched in the outer leaflet, while the amino lipids PtdEtn and PtdSer are concentrated in the inner leaflet of the plasma membrane. This arrangement probably also exists in the plasma membrane of most other cells. Studies with the serine-binding protein annexin V indicate that PtdSer appears in the plasma membrane outer leaflet in apoptosis (Williamson & Schlegel, 2002). The glycolipids, especially the gangliosides, are enriched in the extracellular side of the plasma membrane, where they may function in intercellular communication and act as receptors for certain ligands; for example, GM1 acts as a receptor for cholera toxin and GD1b for tetanus toxin. In support of this hypothesis, it has been shown that plasma membrane contains an ATP-dependent phospholipid translocase that selectively catalyzes the transport of the aminophospholipids PtdSer and PtdEtn but not of PtdCho from the outer to the inner lipid layer of the membrane (Daleke & Lyles, 2000). A glucosyl ceramide translocase, required for transport of glucosyl ceramide from the cytoplasmic surface to the luminal surface of the Golgi membrane for synthesis of neutral glycosphingolipids, has been identified as ABCA1 or multiple drug resistance protein 1 (De Rosa et al., 2003) (Ch. 3).

## Some proteins are bound to membranes by covalently linked lipids

A number of membrane-bound proteins are covalently linked with various lipids, which promotes association of the protein with the lipid bilayer. These modifications include glycerophosphatidyl inositol (GPI) anchors, cysteine acylation, N-terminal myristoylation, isoprenylation, and C-terminal sterol addition (Levental et al., 2010). Addition of GPI with saturated fatty acids tethers a major family of extracellular proteins to the membrane, predominantly in lipid rafts. Transmembrane and intracellular proteins can undergo S-acylation with saturated fatty acids, again leading to association with lipid rafts. For example, in myelin proteolipid protein, fatty acids (16:0, 18:0, 18:1) are attached to the cysteine moieties in the protein as thioesters [Chap. 10]. Analysis of the neuronal palmitoyl-proteome has identified palmitoylaton of neurotransmitter receptors, transporters, adhesion molecules, SNAREs and other vesicular trafficking proteins (Kang et al., 2008). Drug-induced activity paradigms suggest that palmitoylation contributes to activity-dependent changes in synaptic morphology and function. A number of cellular proteins are also acylated with myristic acid (14:0) on the free amino group of *N*-terminal amino acids. A class of proteins, including Ras, a proto-oncogene product, has been shown to form covalent links with farnesyl ($C_{15}$) or $C_{20}$ isoprenes via a

thioether linkage to cysteine (Glomset et al., 1990). The lipid anchor in Ras, which occupies a central position in intracellular signal transduction, is essential for its activity. The Hedgehog family of proteins contains palmitic acid linked to the amino terminal domain and cholesterol covalently linked to the carboxy terminal signaling domain (Bijlsma et al., 2004), making them the only sterolated proteins identified to date. These proteins are of critical importance in patterning during development and in tumorigenesis. Lipidated Hedgehog proteins are secreted from cells via the membrane transporter Dispatched, and then transduce signals through two membrane-bound receptors, Patched and Smoothened.

## Lipids have multiple roles in cells

Many lipids have both structural and regulatory roles in the cell. For example, while arachidonic acid (20:4n-6) is a major constituent of brain inositides and PtdEtn, the free acid is also a precursor of a number of important biomessengers, the eicosanoids, such as prostaglandins, prostacyclins, leukotrienes and thromboxanes (see Ch. 36). Arachidonic acid itself acts as a biomessenger by activating certain isoforms of PKC. It may also be found in a derivatized form. It has been identified in amide linkage with ethanolamine as anandamide or esterified to the 2-position of glycerol, and both have been proposed as possible endogenous ligands for brain cannabinoid receptors (Sugira & Waku, 2002). Oleic acid amide has been reported to be an endogenous sleep-promoting factor (Mendelson & Basile, 2001), as has the prostanoid prostaglandin $D_2$ (Satoh et al., 1996). DAG is an important precursor for lipid biosynthesis in the endoplasmic reticulum, but in the plasma membrane it acts as a second messenger, activating PKC. Major structural lipids, such as PtdIns and PtdCho, are also intimately involved in the signal-transduction process (see Ch. 23). The ether lipid 1-*O*-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine, termed platelet activating factor and commonly referred to as PAF, has potent biomessenger activity in aggregating platelets, releasing eicosanoids and modulating glutamate release and plasticity at the synapse (Bazan, 2003) (see Ch. 36). Lysophosphatidic acids have been identified as extracellular ligands acting via several G-protein-coupled receptors. Sphingolipids, including ceramide, sphingosine and sphingosine-1-phosphate, have been implicated in neurodegeneration and in numerous cell regulatory processes (Soliven et al., 2011). For example, tumor necrosis factor-$\alpha$, the cytokine interleukin-1$\beta$ and nerve growth factor act through their receptors to induce sphingomyelin hydrolysis to ceramide, which then activates a number of downstream activities, including protein kinases and phosphatases, triggering cell-cycle arrest, proliferation, differentiation or cell death (Buccliero & Futerman, 2003; Gulbins, 2003; Jana et al., 2009). At the neuronal presynaptic terminal, ceramide, sphingosine and sphingosine 1-phosphate appear to be involved in priming and docking of synaptic vesicles, vesicle fusion and regulation of neurotransmitter release (Haughey, 2010).

## SUMMARY

Lipids have critical roles in nervous system structure and function. They contribute to the unique composition of synaptic complexes and myelin, and participate in multiple signaling pathways involving lipid intermediates. Lipid modification of proteins is a key mechanism for modulating the activity of trophic factors and receptors. The structure and synthesis of cholesterol and phospholipids, as well as complex lipids including gangliosides, cerebrosides, sulfatides and phosphoinositides, have been characterized in detail. Chromatography and mass spectrometry can directly analyze the molecular profile or 'lipidome' of different lipid classes in very small samples. Lipids are transported within the cell and transferred enzmatically from one side of the membrane bilayer. Signaling across cell membranes occurs primarily in lateral domains, or rafts, enriched in specific proteins and lipids, especially cholesterol and glycolipids.

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