

CHAPTER

# 36

## Lipid Mediators: Eicosanoids, Docosanoids and Platelet-Activating Factor

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## STORAGE OF LIPID MESSENGERS IN NEURAL MEMBRANE PHOSPHOLIPIDS

**Excitable membranes maintain and rapidly modulate substantial transmembrane ion gradients in response to stimuli**

This function requires the presence of ion pumps, neurotransmitter receptors and other associated membrane proteins (see also Chs. 2–5). Excitable membranes have a phospholipid composition that differs from that of other membranes, a property assumed to be related to their highly specialized functions. The understanding of excitable membrane organization has conceptually evolved from the lipid bilayer with embedded proteins to a highly dynamic, heterogeneous patchwork of microdomains that contain ion channels, receptors, transporters and other proteins. Cellular membranes in the nervous system were divided in the past into relatively more fluid membranes (e.g., those of cells of gray matter) and relatively more rigid membranes (e.g., oligodendrocyte plasma membrane that spirals around the axon to

form the myelin), according to the higher or lower content of polyunsaturated fatty acids (PUFA) in phospholipids. Several phospholipid pools in neurons, glia and endothelial cells of the cerebrovasculature are now recognized as reservoirs of lipid messengers. In addition, there are lipid rafts, which are cholesterol- and sphingolipid-rich microdomains in specific cellular compartments of the nervous system, including dendrites, where they are associated with specific postsynaptic proteins. Interestingly, there are subtypes of lipid rafts, which vary by their resistance to detergent extraction, their density and their raft marker proteins, Thy-1 and caveolin. Several proteins have been found segregated in these microdomains, including glycoprophosphatidyl (GPI) anchors, signaling proteins, proteins interacting with the actin cytoskeleton and proteins involved in cell trafficking and in endocytosis. PSD-95, GRIP and glutamate AMPA receptors have been found in lipid rafts isolated from rat brain. Moreover, the normal density of synapses and dendritic spines seems to depend on lipid rafts, since changes in cholesterol availability modify rafts and in turn the properties of synapses and spines. AMPA receptor internalization is affected by compositional changes in the rafts.

## Specific lipid messengers are cleaved from reservoir phospholipids by phospholipases upon activation by various stimuli

These stimuli include neurotransmitters, neurotrophic factors, cytokines, membrane depolarization, ion channel activation and others. Several lipid mediators generated in response to such stimuli regulate and interact with many other signaling cascades, contributing to the development, differentiation, function, protection and repair of the cells of the nervous system (Shimizu, 2009). Under physiologic conditions, the balance of membrane lipid metabolism, particularly that of arachidonoyl and docosahexaenoyl chains, favors a very small and tightly controlled cellular pool of free arachidonic acid (AA, 20:4n-3) and docosahexaenoic acid (DHA, 22:6n-3), but levels increase very rapidly upon cell activation, cerebral ischemia, seizures and other types of brain trauma (Sun et al., 2004). Other free fatty acids (FFAs) released during cell activation and the initial stages of focal and global cerebral ischemia are stearic acid (18:0), palmitic acid (16:0) and oleic acid (18:1).

## Phospholipids in synaptic membranes are an important target in seizures, traumatic brain injury, neurodegenerative diseases and cerebral ischemia

Synaptic membranes are excitable membranes enriched in phospholipids esterified with the polyunsaturated fatty acids AA and DHA, which form a significant proportion of the FFAs rapidly released during ischemia, seizure activity and other brain trauma.

## Some molecular species of phospholipids in excitable membranes are reservoirs of bioactive lipid mediators that act as messengers

Signals, such as those resulting from neurotransmitter receptor occupancy, trigger the release of phospholipid moieties via the activation of phospholipases. Some of the breakdown products are bioactive, such as inositol 1,4,5-trisphosphate (IP<sub>3</sub>), diacylglycerol (DAG) and AA. Another product, lysophatelet-activating factor (lyso-PAF), upon further metabolism, gives rise to the bioactive lipid PAF (1-octadecyl-2-acetyl-sn-glycero-3-phosphocholine). Eicosanoids are derived from enzyme-mediated oxygenation of AA. AA and its metabolites function as intra- and intercellular messengers. In contrast, the full significance of DHA still remains an enigma. DHA-containing phospholipids may provide a specific milieu in which the membrane proteins of excitable membranes function. For example, the high concentrations of DHA phospholipids of outer segments of retinal rod photoreceptors provide a highly specialized membrane environment for rhodopsin. In fact, a specific requirement for DHA phospholipids in rhodopsin function has been documented (Niu et al., 2004). The DHA-derived lipid mediator, neuroprotectin D1 (NPD1), was isolated from ischemic brain (Marcheselli et al., 2003) and the retinal pigment epithelium. This lipid is called neuroprotectin D1

because of (1) its *neuroprotective* properties in brain ischemia-reperfusion and in oxidative stress-challenged retinal pigment epithelial cells, (2) its potent ability to inactivate proapoptotic signaling, and (3) its status as the first identified neuroprotective mediator derived from DHA.

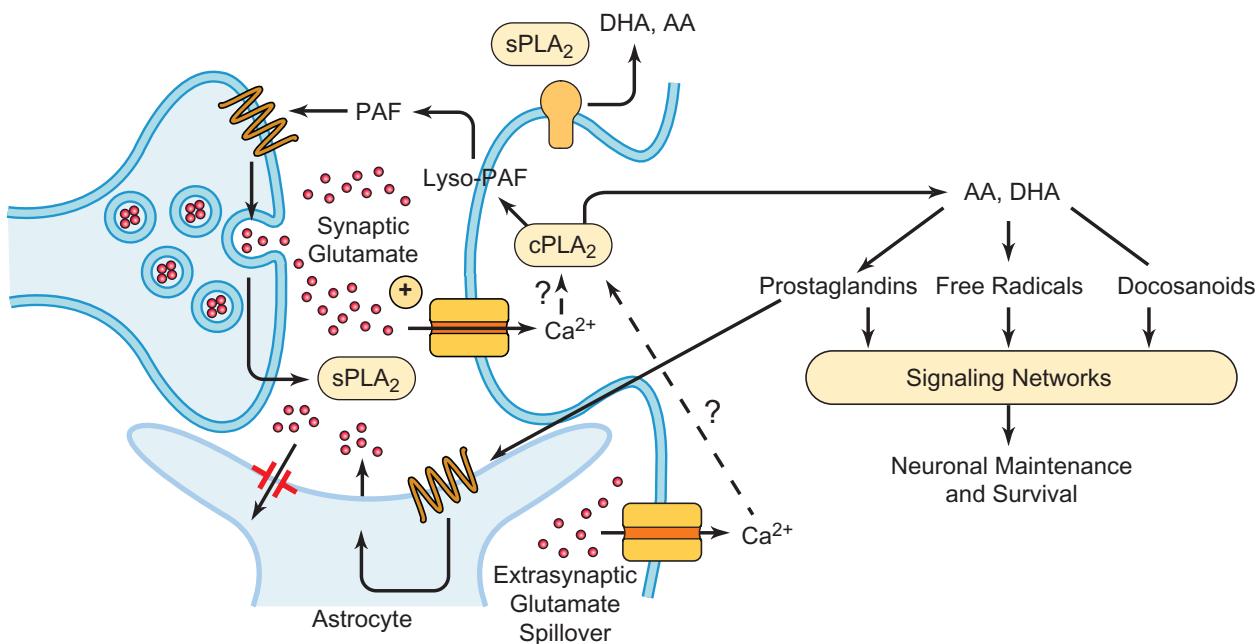
## Mammalian phospholipids generally contain polyunsaturated fatty acyl chains almost exclusively esterified to the second carbon of glycerol

Phospholipase (PL)A<sub>2</sub> activation cleaves the acyl chains at the *sn*-2 position of the glycerol backbone, thus releasing free polyunsaturated fatty acids AA and DHA. There are a number of potential fates for the phospholipase products. They may be reincorporated into membrane lipids or further metabolized to biologically active derivatives. The remaining lysophospholipid can be either re-esterified and reincorporated into a membrane phospholipid or further metabolized. The discovery of neuroprotectin D1 from DHA suggests that docosanoids may arise as well.

This chapter surveys the neurochemistry of lipid messengers, as well as the mechanisms by which bioactive lipids accumulate upon stimulation in response to injury, cerebral ischemia, seizures, neurotrauma or neurodegenerative diseases, and their significance in pathophysiology. Emphasis is placed on three groups of bioactive lipids: AA and its metabolites, known collectively as eicosanoids; PAF, a highly potent ether phospholipid; and the DHA-derived mediator, neuroprotectin D1.

AA metabolites and PAF have initially been studied in terms of their roles in the acute inflammatory response, such as increased vascular permeability and the activation of and infiltration by inflammatory cells. However, these bioactive lipids have additional neurobiological actions in ion channel functions, receptors, neurotransmitter release, synaptic plasticity and neuronal gene expression.

Bioactive lipids may be considered dual messengers: they modulate cell functions as messengers and they become part of the response of the nervous tissue to injury, broadly referred to as the inflammatory response. This response occurs in ischemia-reperfusion damage associated with stroke, various forms of neurotrauma, infectious diseases and neurodegenerative diseases such as Alzheimer's disease (see Ch. 34). Inflammation in the nervous system differs from that in other tissues. If the blood-brain barrier is broken, blood-borne inflammatory cells (e.g., polymorphonuclear leukocytes, monocytes, macrophages) invade the intercellular space and glial cells, particularly microglia, are activated, and play a prominent role in the inflammatory response. These responses may lead to neuronal cell injury and death. In addition, ischemia, seizures and other forms of injury upregulate lipid signaling in neurons, mainly through N-methyl-D-aspartate (NMDA)-type glutamate (Fig. 36-1) receptors. As a consequence, PLA<sub>2</sub> is activated, AA is released, eicosanoids and PAF are synthesized and cyclooxygenase (COX)-2 is induced in neurons. The location of NMDA receptors (i.e., synaptic vs extrasynaptic) has a critical effect on downstream signaling pathways; synaptic NMDA receptors couple to survival



**FIGURE 36-1** A depolarizing stimulus at the presynaptic terminal triggers glutamate release. Glutamate binds to the NMDA receptor and, as a consequence, an influx of calcium ions occurs in the postsynaptic neuron. During certain pathological scenarios such as stroke, extrasynaptic NMDA receptors are also activated (Hardingham & Bading, 2010). Although the unique contributions of synaptic and extrasynaptic NMDA receptors to cPLA<sub>2</sub> activation remain unclear, calcium-mediated translocation/activation of the cytoplasmic PLA<sub>2</sub> (cPLA<sub>2</sub>) results in the release of arachidonic acid (AA), docosahexaenoic acid (DHA) and lyso-PAF, the PAF precursor. Although PAF has a very short biological half-life, on repeated stimulus sufficient PAF accumulates to diffuse back across the synaptic cleft. Experimental evidence for this was provided by injecting PAF into the postsynaptic neuron and monitoring neurotransmitter release. PAF binds to its pre-synaptic receptor and enhances glutamate exocytosis by an as-yet-undefined mechanism. During synaptic plasticity events, PAF may also activate gene expression that in turn is probably involved in long-term alterations of synaptic function (not shown here). Cell-surface PAF-receptor antagonists confer neuroprotection during ischemia–reperfusion and inhibit PAF-induced glutamate release from hippocampal neurons and CA1 LTP formation, presumably through the same mechanism. The inhibitory effects of this antagonist on glutamate release could account in part for its neuroprotection in ischemia–reperfusion. Secretory PLA<sub>2</sub> (sPLA<sub>2</sub>) may be released from the presynaptic terminal: sPLA<sub>2</sub> binding sites are present in neurons, and sPLA<sub>2</sub> promotes active AA remodeling in neurons in culture (Kolko et al., 1996) and may also promote DHA release. DHA may also be released by cPLA<sub>2</sub>. Free DHA may subsequently follow enzyme-mediated oxygenation pathways and lead to the synthesis of docosanoids, messengers made in the retina (Bazan et al., 1984) and brain (Marcheselli et al., 2003). Free radicals would accumulate during oxidative stress. Downstream lipid signaling modulates neuronal function and survival. Synapses are intimately surrounded by astrocytes, which express glutamate transporters that remove the excitatory neurotransmitter from the vicinity of the synaptic cleft. Astrocytes also respond to prostaglandins by releasing glutamate through a Ca<sup>2+</sup>-dependent mechanism, although the quantitative importance and therefore functional relevance of astrocyte exocytosis of glutamate and other astrocyte derived “gliotransmitters” has come into question (Hamilton & Attwell, 2010). Neurons also express prostaglandin receptors (Sang et al., 2005) that modulate synaptic plasticity and excitotoxicity.

signaling, while extrasynaptic NMDA receptors couple to death signaling (Hardingham & Bading, 2010). How these spatially distinct NMDA receptors uniquely contribute to PLA<sub>2</sub> activation and downstream lipid signaling is unknown and a subject of intense interest.

Other neuronal correlates of the inflammatory response include signaling by cytokines, nitric oxide and various growth factors. Activation of arriving inflammatory cells also plays a role in initial defenses against injury, removal of cellular debris and the longer-term repair/wound healing of the nervous system. Several lipid messengers are released from these cells and may participate in beneficial actions. Much remains to be learned in this area of integration of our knowledge of the inflammatory response and neuroimmune/repair signaling.

Identification of lipids with biological activity has progressed remarkably over the last decade. While the inositol phosphates have been known for some time to play fundamental roles in cell biology (see Ch. 23), others—such as lysophosphatidic acids, previously considered only as intermediates in phospholipid metabolism—have more recently been found to possess important functions, and receptors for lysophosphatidic acid and sphingosine have been cloned. This review should give the reader a broad appreciation of the diversity of bioactive lipids—particularly eicosanoids, docosanoids and PAF, which are produced in brain during injury (e.g., trauma or ischemia), during seizures, and under inflammatory conditions—and point to gaps in our knowledge still to be explored.

## PHOSPHOLIPASES A<sub>2</sub>

**Phospholipases A<sub>2</sub> catalyze the cleavage of the fatty acyl chain from the sn-2 carbon of the glycerol backbone of phospholipids**

The phospholipase A<sub>2</sub> (PLA<sub>2</sub>) superfamily of enzymes is composed of a total of 15 groups (designated with Roman numerals I to XV) to which particular enzymes are assigned based on the catalytic mechanism utilized, along with functional and structural characteristics (Schaloske & Dennis, 2006). Each member of the 15 groups is also assigned to one of five more general categories called “types.” The five types of PLA<sub>2</sub> include small (14–18 kDa), secreted sPLA<sub>2</sub>s; cytosolic cPLA<sub>2</sub>s; Ca<sup>2+</sup>-independent iPLA<sub>2</sub>s; PAF acetylhydrolases (PAF-AHs); and lysosomal PLA<sub>2</sub>. In brain, the most studied PLA<sub>2</sub>s are Group IVA cPLA<sub>2</sub>, Group VIA iPLA<sub>2</sub> and Group IIA sPLA<sub>2</sub>. Catalysis by sPLA<sub>2</sub>s requires Ca<sup>2+</sup>, whereas Ca<sup>2+</sup> is required for cytosol-to-membrane translocation of cPLA<sub>2</sub> but not cPLA<sub>2</sub>-mediated catalysis. Instead of cleaving a fatty acyl chain from the sn-2 carbon of glycerophospholipids, PAF-AH hydrolyzes the acetyl group from the sn-2 position of PAF, resulting in formation of inactive lyso-PAF.

The sPLA<sub>2</sub> is synergistic with glutamate-induced neuronal damage (Kolko et al., 1996). Whereas pathways leading to PLA<sub>2</sub> activation are part of normal neuronal function, ischemia–reperfusion enhances these events, overproducing PLA<sub>2</sub>-derived lipid messengers—such as enzymatically produced AA- and DHA-oxygenation derivatives, non-enzymatically generated lipid-peroxidation products, and other reactive oxygen species (ROS)—all of which may be involved in neuronal damage. Among the consequences of PLA<sub>2</sub> activation by ischemia are alterations in mitochondrial function by the rapid increase in the brain FFA pool size, for example, the un-coupling of oxidative phosphorylation from electron transport in the mitochondrial respiratory chain and the generation of ROS. Intracellular PLA<sub>2</sub>s (*i.e.* cPLA<sub>2</sub>s and iPLA<sub>2</sub>s) are located either in the cytosol or in noncovalent association with membranes.

### Cytosolic phospholipases A<sub>2</sub> are involved in bioactive lipid formation

Cytosolic phospholipases A<sub>2</sub> (cPLA<sub>2</sub>s) are regulated by transcription, post-translational modulation of enzyme activity and membrane translocation. Membrane translocation of cPLA<sub>2</sub> to endoplasmic reticulum and nuclear membranes is mediated via a specific Ca<sup>2+</sup>-dependent C2 domain similar to those seen in protein kinase C, phospholipase C and GTPase-activating protein. This domain is consistent with that of other enzymes of AA metabolism, such as prostaglandin G/H synthases (PGS), also termed cyclooxygenases (COX-1 or -2), and 5-lipoxygenase (5-LO); however, unlike 5-LO, cyclooxygenase association with membranes through its membrane binding domain (MBD) does not appear to require Ca<sup>2+</sup>.

The catalytic activity of cPLA<sub>2</sub> is stimulated by phosphorylation catalyzed by the mitogen-activated protein kinase (MAPK) at Ser505. This modification stimulates enzyme activity only, indicating that translocation and phosphorylation are independent mechanisms of cPLA<sub>2</sub> regulation.

### Ischemia and seizures activate phospholipases A<sub>2</sub>, releasing arachidonic and docosahexaenoic acids

Ischemia or seizure triggers accumulation of free AA, DHA and other FFA in the brain. This reflects PLA<sub>2</sub> activation in excitable membranes. While little is known about the mechanisms that control its activity, the importance of cPLA<sub>2</sub> in ischemic brain injury is strongly supported by the finding that cPLA<sub>2</sub>-knockout mice have substantially reduced infarcts and neurologic deficits in a model of stroke (Bonventre et al., 1997). The cPLA<sub>2</sub> is also thought to be involved in the pathogenesis of other neurological disorders, *e.g.*, Alzheimer’s disease (Sanchez-Mejia et al., 2008).

Shifts in intracellular pH may be another mechanism by which intracellular PLA<sub>2</sub> activity can be regulated. Glutamate-induced AA release in mouse cortical neuronal cultures is mediated in part by a membrane-associated PLA<sub>2</sub> activity, which is upregulated in alkaline pH and is therefore sensitive to the shifts in pH induced by excitatory neurotransmission.

There are also mechanisms in the brain for the downregulation of intracellular PLA<sub>2</sub> activity by lipocortins (also known as annexins), a family of Ca<sup>2+</sup>- and phospholipid-binding proteins that act as endogenous inhibitors of PLA<sub>2</sub>. The steroid-inducible lipocortin-1 is present in neuronal and glial cells, especially in the hippocampus, where it may act as an endogenous neuroprotective agent. Exogenous lipocortin-1 administered intracerebroventricularly to rats significantly reduces the infarct size and edema induced by cerebral ischemia, and attenuates excitotoxic damage mediated by NMDA receptors.

### Secretory phospholipases A<sub>2</sub> are of relatively low molecular weight and have a high number of disulfide bridges, making them relatively more resistant to denaturation

The mammalian sPLA<sub>2</sub> groups can be assigned on the basis of amino acid sequence. These include pancreatic or Group I sPLA<sub>2</sub>s, the members of which function in pancreatic secretions, smooth muscle contraction, cell proliferation and fertilization, and synovial or Group II sPLA<sub>2</sub>s, the members of which function in inflammatory responses. Both have been found also to be expressed in the nervous system.

### There are high-affinity receptors that bind secretory phospholipases A<sub>2</sub>

The muscle (M-type) and neuronal (N-type) receptors are structurally and pharmacologically distinct. The M-type consists of a single 180 kDa subunit and binds both OS1 and OS2 sPLA<sub>2</sub> (purified from the venom of the Australian taipan snake *Oxyuranus scutellatus scutellatus*). The N-type is composed of three major polypeptides of 34, 48 and 82 kDa, and binds OS2 and bee venom sPLA<sub>2</sub> but not OS1. Expression of the M- and N-type receptors is not limited to their nominal sites. In fact, both types are widely distributed in different cells and tissues. The M-type receptor has been cloned from rabbit and human tissues and, independently, from mouse and bovine tissues; it mediates Group I sPLA<sub>2</sub> cellular

actions. The receptor consists of a single 180–200 kDa glycoprotein subunit that bears significant sequence homology to the macrophage mannose receptor and to other members of the C-type lectin superfamily. The recombinant receptor binds both mammalian Group I and Group II sPLA<sub>2</sub> with high affinity. Studies using enzymatically inactive mutants of Group I sPLA<sub>2</sub> to stimulate prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) synthesis in rat mesangial cells suggest that at least some of the effects mediated through the receptor are independent of sPLA<sub>2</sub> enzymatic activity. Potential ligands for the N-type receptor may be the Group II sPLA<sub>2</sub> induced in rat brain during ischemia.

prostaglandins (PGs), the thromboxanes (TXs), the leukotrienes (LTs) and the lipoxins (LXs), which modulate cell function and are also involved in pathophysiology. Several nervous system functions engage eicosanoids. In addition, these messengers participate in inflammatory responses and other pathologic processes in the nervous system. Cyclooxygenases (COXs) are inhibited by nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin or ibuprofen. Mainly as a consequence of studies on NSAIDs, the significance of prostaglandins as critical modulators of immune responses, pain, fever, inflammation, mitogenesis and apoptosis has been established.

## EICOSANOIDS

### Arachidonic acid is converted to biologically active derivatives by cyclooxygenases and lipoxygenases

These metabolites, referred to collectively as eicosanoids (Figs. 36-2, 36-3), include potent lipid mediators such as the

### Prostaglandins are very rapidly released from neurons and glial cells

Synaptic activation and certain forms of injury, such as ischemia–reperfusion or seizures, trigger prostaglandin synthesis and rapid efflux into the intercellular space. These lipid mediators, in turn, elicit their signaling through autocrine and paracrine routes. Several prostaglandin receptors have been cloned. Figure 36-4 depicts the PGE<sub>2</sub> receptor (EP receptor),

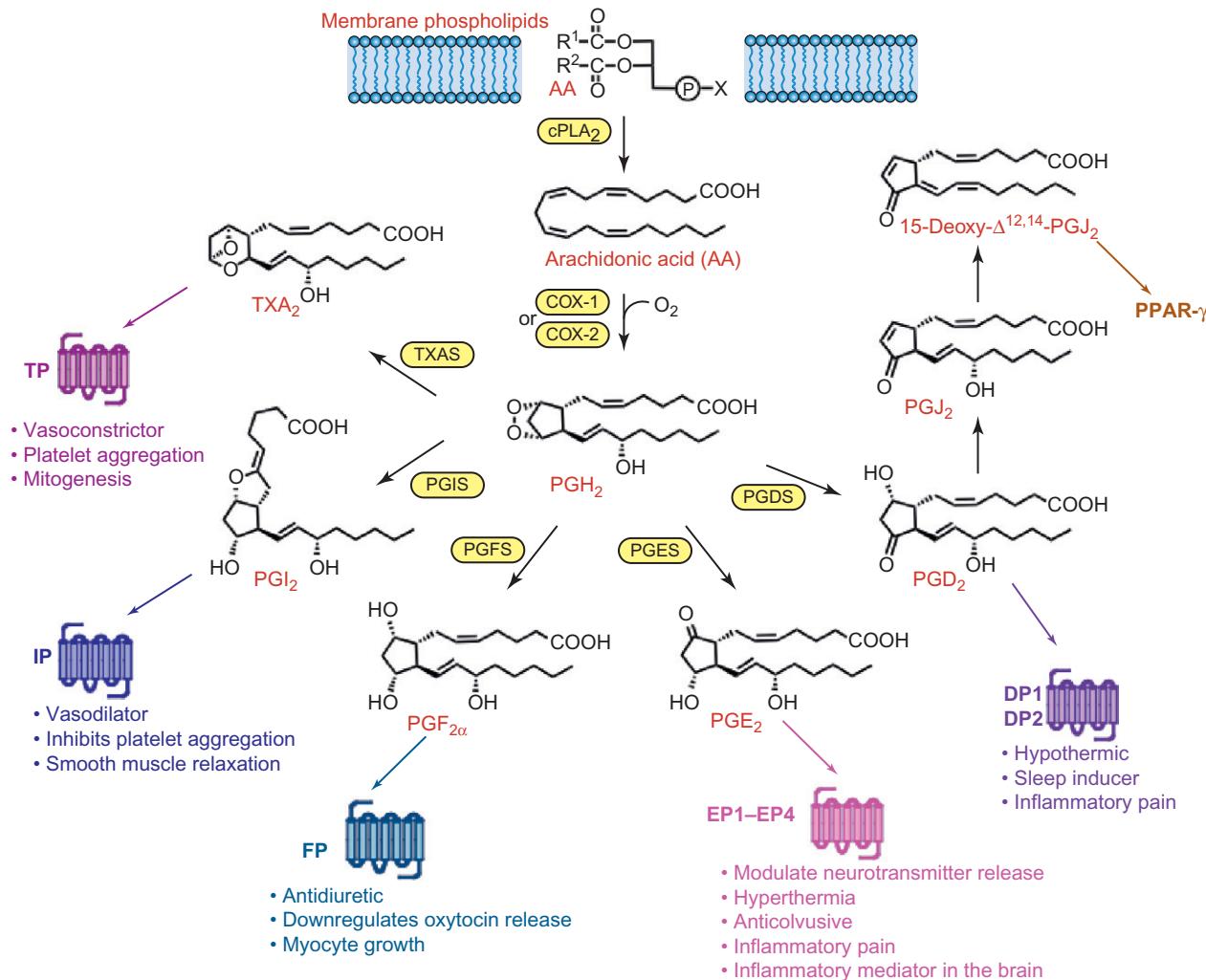


FIGURE 36-2 The arachidonic acid cyclooxygenase cascade.

which in turn generates cAMP by activating adenylyl cyclase. The PGE<sub>2</sub> receptor exists in four isoforms (EP1–EP4). It belongs to the seven-transmembrane-domain G protein-coupled receptor (GPCR) family and is coupled to cAMP signaling. This depends on the activity of protein kinase A (PKA), a heteromeric enzyme that, upon binding cAMP to its regulatory subunit, releases the catalytic subunit. The catalytic subunit, in turn, activates gene transcription by phosphorylation of a DNA-binding protein, namely, the cAMP response element-binding (CREB) protein. Several genes contain consensus sequences for CREB. Expression of these genes in turn is modulated by this lipid-signaling pathway, as well as by other signaling pathways. CREB has been implicated in plasticity changes of synaptic circuits, memory formation and behavior.

### Arachidonic acid is also the substrate for lipoxygenases and, as in the case of cyclooxygenases, molecular oxygen is required

The exact mechanisms controlling the channeling of AA through COXs or lipoxygenases (LOs) are not clearly understood.

However, there is growing evidence that subcellular compartmentalization is a major factor in the channeling of AA through either pathway.

### PLATELET-ACTIVATING FACTOR

Phospholipid molecules of membranes from neurons and glial cells store a wide variety of lipid messengers. Receptor-mediated events and changes in intracellular Ca<sup>2+</sup>, such as occur during excitatory neurotransmission and activity-dependent synaptic plasticity, activate phospholipases that catalyze the release of bioactive moieties from phospholipids, which then participate in intra- and/or intercellular signaling pathways. PLA<sub>2</sub>-mediated cleavage of AA from the 2-position of ether phospholipids, followed by enzymatic acetylation, forms a lipid mediator known as platelet-activating factor (PAF) (Fig. 36-5). Deacetylation of PAF regenerates its inactive precursor lyso-PAF, which can be re-esterified into membrane phospholipids (Shimizu, 2009).

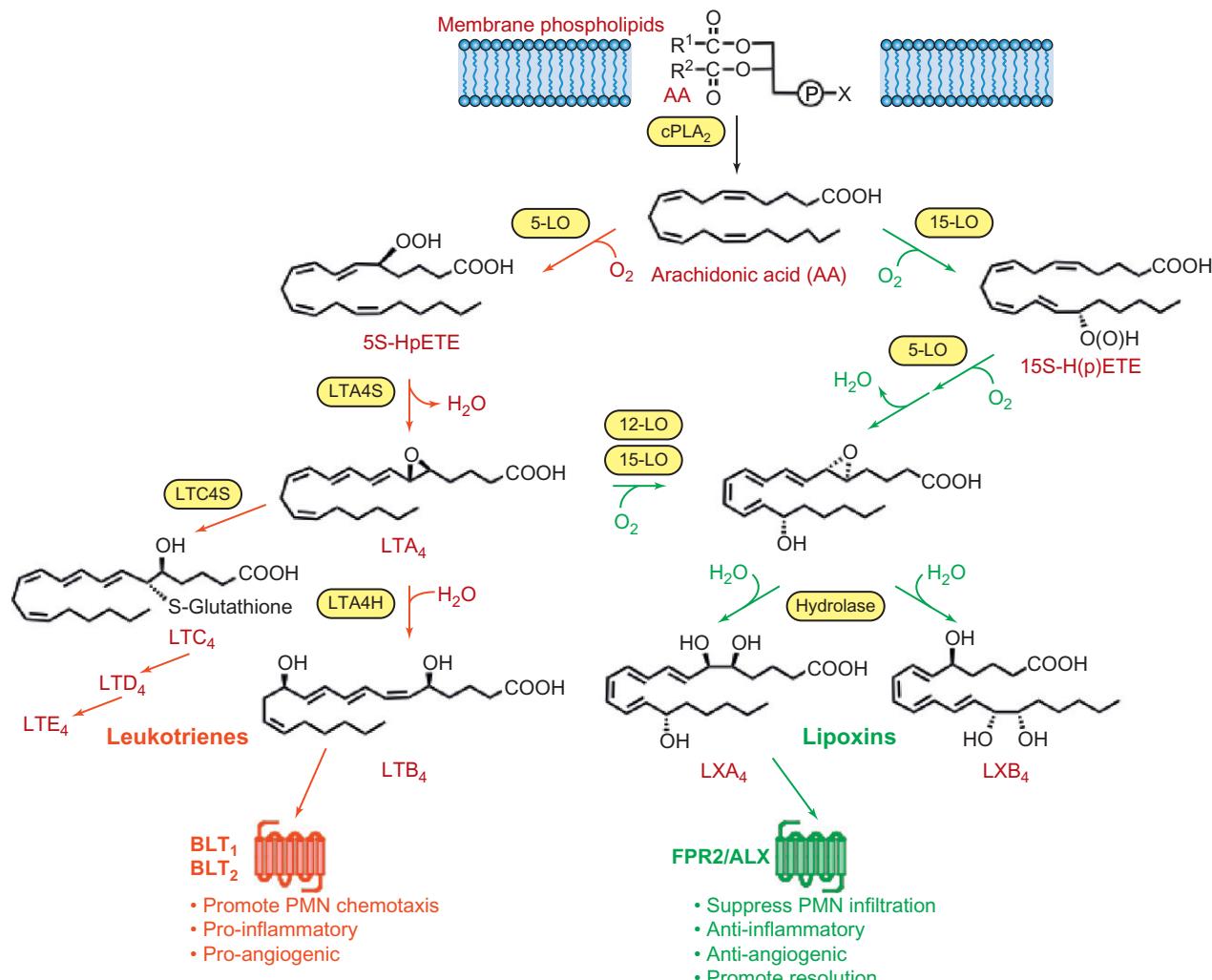
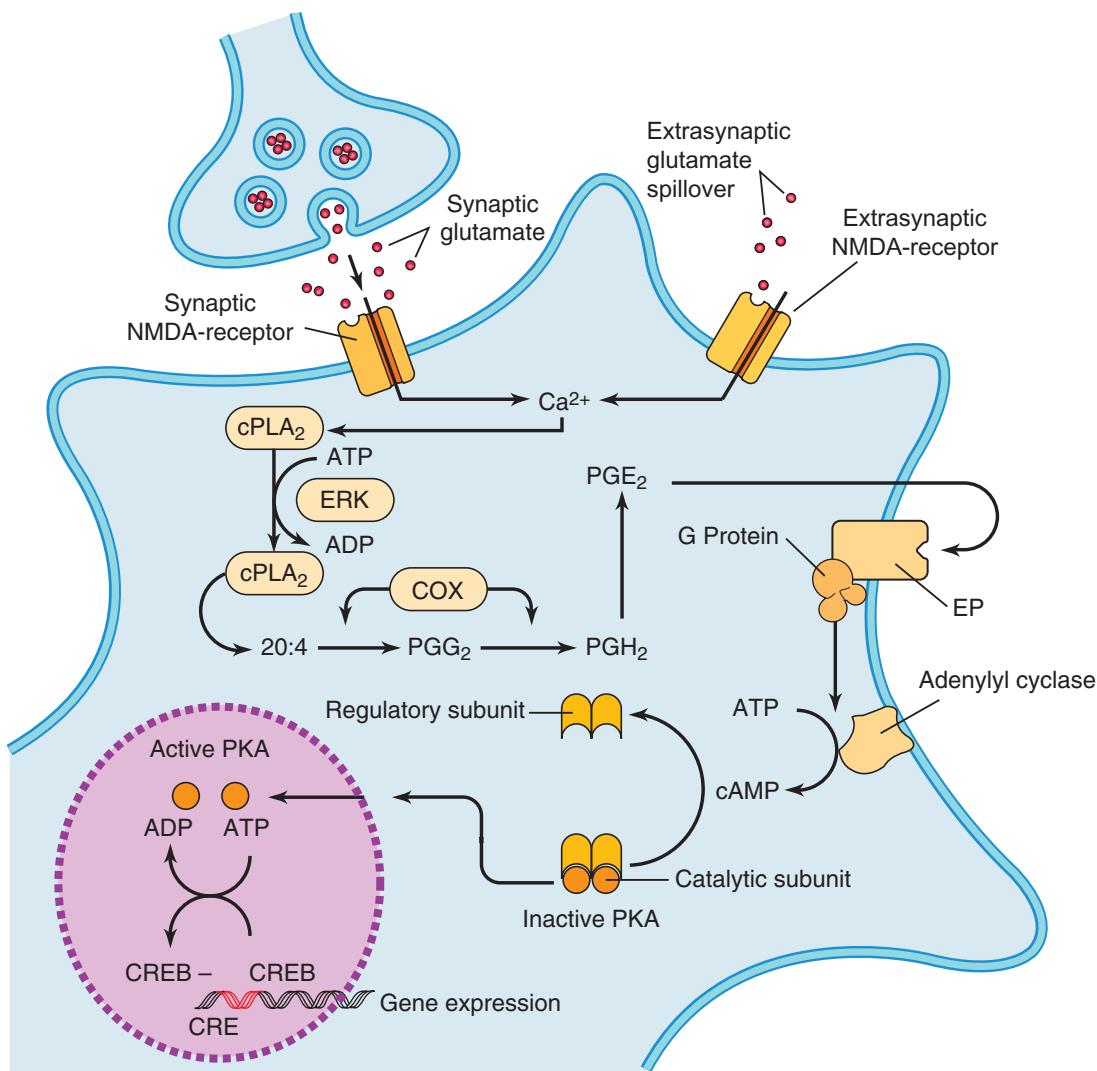


FIGURE 36-3 Arachidonic acid lipoxygenase pathways.



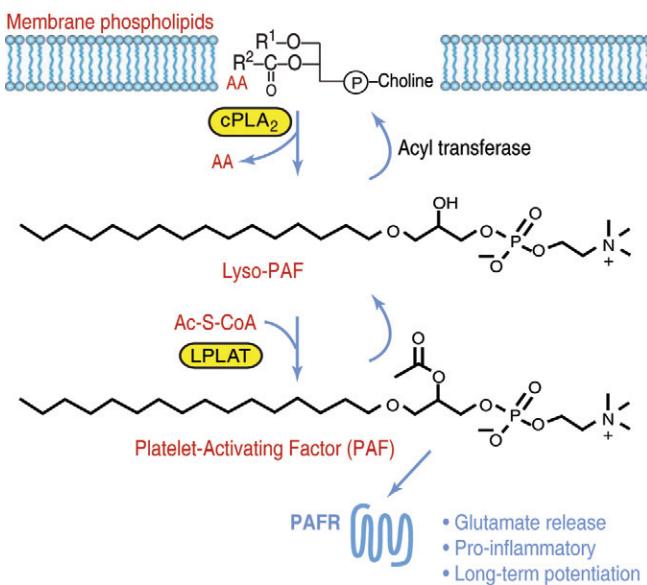
**FIGURE 36-4 Prostaglandin signaling pathway triggered by the excitatory amino acid neurotransmitter glutamate.** NMDA receptors initiate changes in intracellular  $\text{Ca}^{2+}$  concentration that modulate prostaglandin signaling ( $\text{PGE}_2$  in this example) via regulation of cPLA<sub>2</sub> and COX-2. The respective contributions to these pathways by the spatially distinct synaptic and extrasynaptic NMDA receptors have not been defined. For illustration, a generic EP receptor is shown as a positive regulator of CRE-dependent transcription via increased adenylyl cyclase activity, but different prostaglandin receptor types or isoforms may have opposing effects on cAMP. COX, cyclooxygenase; cPLA<sub>2</sub>, cytosolic phospholipases A<sub>2</sub>; CRE, cAMP response element; CREB, cAMP-response element binding protein; EP receptor, prostaglandin E2 receptor; ERK, extracellular signal-regulated kinase; NMDA, N-methyl-D-aspartate; PGG<sub>2</sub> and PGH<sub>2</sub> are short-lived intermediates in the synthesis of prostaglandin E<sub>2</sub> ( $\text{PGE}_2$ ); PKA, protein kinase A.

### Platelet-activating factor is a very potent and short-lived lipid messenger

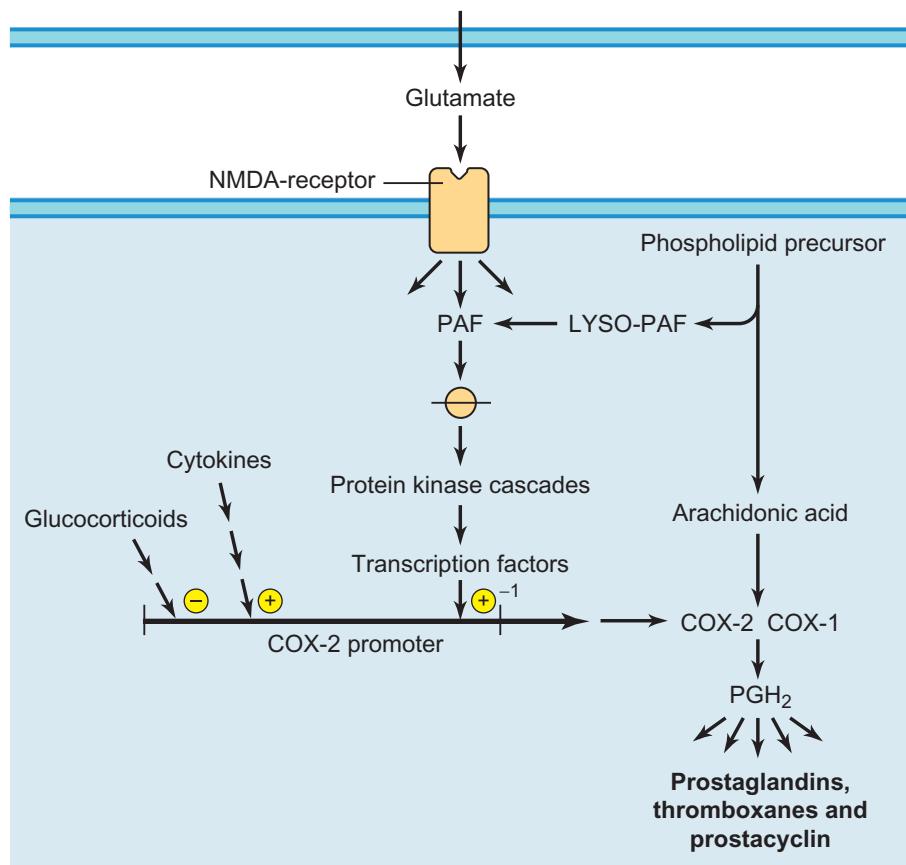
Platelet-activating factor (PAF) is known to have a wide range of actions, operating as a mediator of inflammatory and immune responses, a second messenger, and a potent inducer of gene expression in neural systems (Fig. 36-6). Thus, in addition to its role in acute inflammation, PAF can potentially mediate longer-term effects on cellular physiology and brain functions.

PAF enhances glutamate release in synaptically paired rat hippocampal neurons in culture (Clark et al., 1992). The PAF analog methylcarbamoyl (mc-PAF), but not the biologically

inactive lyso-PAF, increases excitatory synaptic responses. Action of the inhibitory neurotransmitter GABA is unaffected by mc-PAF under these conditions. The presynaptic PAF-receptor antagonist BN 52021 blocks the mc-PAF-enhanced glutamate release. In addition, mc-PAF increases presynaptic glutamate release, since it does not augment the effects of exogenously added glutamate, and evokes spontaneous synaptic responses characteristic of enhanced neurotransmitter release. Therefore, as a modulator of glutamate release, PAF participates in long-term potentiation (LTP) (Kato et al., 1994; Wieraszko et al., 1993), synaptic plasticity and memory formation (Izquierdo et al., 1995).



**FIGURE 36-5** The platelet-activating factor (PAF) pathway. Activated cytosolic phospholipase A<sub>2</sub> (cPLA<sub>2</sub>) catalyzes the cleavage of the arachidonoyl (AA) ester group at the 2-position of ether glycerophospholipids to form free arachidonic acid (AA) and lyso-PAF, which is rapidly re-incorporated into the phospholipid via an acyl transferase enzyme, or is acetylated by a lysophospholipid acyltransferase enzyme (LPLAT) to form PAF. Deactivation of PAF takes place via the acetyl group hydrolysis and re-acylation, followed by reuptake into the membrane phospholipids. PAF is short lived and exerts potent actions via its GPCR presynaptic receptor (PAFR).



**FIGURE 36-6** Seizure- or ischemia-triggered signaling events linking synapse activation and cyclooxygenase-2 (COX-2) gene expression in neurons. *N*-methyl-D-aspartate (NMDA) receptor activation by glutamate leads to phospholipase A<sub>2</sub> (PLA<sub>2</sub>) activation and the generation of platelet-activating factor (PAF) and of arachidonic acid. PAF is synthesized through other metabolic routes as well [119], and elicits its actions through a PAF receptor. PAF activates COX-2 gene expression through protein kinase cascades and transcription factors. The COX-2 promoter is also a target for cytokines (activation) and glucocorticoids (inhibition). COX-2 protein then catalyzes the conversion of arachidonic acid into prostaglandin H<sub>2</sub> (PGH<sub>2</sub>), the precursor of eicosanoids. Constitutive activity of COX-1 also catalyzes this metabolic step.

## Ischemia and seizures increase platelet-activating factor content in the brain

Furthermore, the brain is endowed with a variety of degradative enzymes that rapidly convert PAF to biologically inactive lyso-PAF. Presynaptic membranes display PAF binding that can be displaced by BN 52021, a terpenoid extracted from the leaf of the *Ginkgo biloba* tree. It is likely that this PAF-binding site is identical to a seven-transmembrane PAF receptor. BN 52021 inhibits both PAF-induced glutamate release (Clark et al., 1992) and LTP (Kato et al., 1994). Moreover, this antagonist is neuroprotective against ischemia–reperfusion damage in the gerbil brain (Panetta et al., 1987). These findings together indicate that PAF, when overproduced during ischemia, promotes enhanced glutamate release, which in turn contributes to excitotoxicity.

## CYCLOOXYGENASES

### The cyclooxygenases are heme-containing enzymes that convert arachidonic acid to prostaglandin H<sub>2</sub>

Cyclooxygenase-1 (COX-1) is a constitutive enzyme, while cyclooxygenase-2 (COX-2) is inducible by cytokines, glutamate, growth factors, PAF and other mediators, and is inhibited by glucocorticoids. In the brain, COX-2 has both constitutive and inducible functions (Phillis et al., 2006). This enzyme is encoded by an early-response gene, and its mRNA has a short half-life. In the human neocortex, the half-life is about 3 hours, as compared to 12 hours for COX-1 mRNA. In most tissues, stimulation, injury, inflammatory stimuli and other forms of cellular stress trigger expression of the COX-2 gene. However, brain, macula densa of kidney, testes and the female reproductive system also display constitutive levels of COX-2. In brain, the relatively high constitutive COX-2 expression appears to be almost exclusively neuronal. Dendrites and the perinuclear region are enriched in COX-2. Moreover, COX-2 expression seems to be regulated by synaptic activity (Yamagata et al., 1993).

COXs thus catalyze the same first committed step of the AA cascade (Fig. 36-2). COX-2, however, is expressed in response to mitogenic, inflammatory, and synaptic stimuli and encoded by an early-response gene. In contrast, COX-1 expression is not subject to short-term regulation. Neurons in the hippocampus, as well as in a few other brain regions, are unlike other cells in that they display basal COX-2 expression (Kaufmann et al., 1996). This expression is modulated by synaptic activity, and involves the NMDA glutamate receptors (Yamagata et al., 1993; Kaufmann et al., 1996).

### Platelet-activating factor is a transcriptional activator of cyclooxygenase-2

PAF induces mouse COX-2-promoter-driven luciferase activity transfected into neuroblastoma cells, such as NG108-15 or SH-SY5Y, and in NIH 3T3 cells (Fig. 36-6). The intracellular PAF antagonist BN 50730 inhibits PAF activation of this construct (Bazan et al., 1994). The abundance in brain of several

early-response gene transcripts shows rapid and transient increases during cerebral ischemia and after seizures. Several early-response genes encode transcription factors, which in turn modulate the expression of other genes, whereas others encode inducible enzymes (Fig. 36-6). The glutamate analog kainic acid promotes extensive neuronal damage, particularly in the hippocampus, and induces early-response genes such as the transcription factor *zif-268*. COX-2 is also induced under these conditions, but there are striking differences in the magnitude and duration of the induction of COX-2 as compared with *zif-268*. COX-2 mRNA, two hours after kainic acid injection, showed a 35-fold increase in hippocampus, as compared to only a 5.5-fold increase for *zif-268* (Marcheselli & Bazan, 1996). Also, a peak in COX-2 mRNA abundance was evident at three hours, a 71-fold increase, as compared with one hour for *zif-268*, a 10-fold increase. The *zif-268* mRNA time course of changes in the hippocampus corresponds to the expected profile of early-response genes, that is, a rapid decrease in abundance after the peak. COX-2, however, displayed sustained upregulation for several hours after kainic acid injection, a 5.2-fold increase remaining at 12 hours (Marcheselli & Bazan, 1996).

### COX-derived AA metabolites play multiple important roles in CNS

Under normal conditions Cox-derived AA metabolites are involved in synaptic function, cerebral blood flow regulation and angiogenesis (Phillis et al., 2006). Excessive COX activity is linked to neuronal injury and neuroinflammation associated with a variety of disease states, including ischemia, epilepsy, Alzheimer's disease, and Parkinson's disease (see Ch. 34).

### Cyclooxygenase-2 participates in aberrant synaptic plasticity during epileptogenesis

Neuronal COX-2 expression is also upregulated in experimental epileptogenesis, when aberrant synaptic plasticity is thought to be activated. The experimental kindling model resembles aspects of mesial temporal lobe epilepsy. Kindling epileptogenesis is triggered by repeated subconvulsive stimulation, which gradually results in intensified seizures. Both COX-2 and cPLA<sub>2</sub> expression are activated, indicating that the free AA released is converted into prostaglandins during epileptogenesis (Tu & Bazan, 2003). Nimesulide, a COX-2 blocker, decreases kindling epileptogenesis. The inability of nimesulide to completely inhibit kindling development suggests either a limited bioavailability of the drug to neuronal COX-2 to attain full blockade and/or a redundancy of the signaling involved. For example, COX-1, which is not inhibited by nimesulide, may catalyze the synthesis of prostaglandins, minimizing the action of nimesulide.

The exact mechanism by which COX-2 inhibition attenuates kindling epileptogenesis is not understood. Notwithstanding, COX-2 inhibition may diminish prostaglandin and/or PAF synthesis, lipid messengers that are both involved in synaptic facilitation. Moreover, kindling epileptogenesis promotes selective neuronal COX-2 expression, initially in the hippocampus and subsequently in the neocortex. The stimulating

electrode in this particular experiment was placed in the ventral hippocampus. Taken together, these studies suggest that the spreading of kindling-induced COX-2 expression from hippocampal neurons to neocortical neurons is a major event in the permanent facilitation of aberrant functional connectivity, and that COX-2 is a mediator (Tu & Bazan, 2003).

Several tissue-specific, eicosanoid-selective isomerases or synthases (PGES, PGFS, PGDS, PGIS, TXAS) catalyze the conversion of PGH<sub>2</sub> into the five principal bioactive prostaglandins: PGE<sub>2</sub>, PGF<sub>2α</sub>, PGD<sub>2</sub>, PGI<sub>2</sub>, and thromboxane (TXA<sub>2</sub>), respectively (Fig. 36-2). An alternative pathway is the direct conversion of PGG<sub>2</sub> into PGE<sub>2</sub> through 15-hydroperoxy-PGE<sub>2</sub>. This route is not well understood, although it may have modulatory implications. The synthesis of PGE<sub>2</sub> is catalyzed by three different enzymes. Two of them are membrane-bound (mainly to the endoplasmic reticulum) and one is soluble. Interestingly, these enzymes are differentially coupled to the COXs, thus channeling released AA through constitutive COX-1 or through inducible COX-2, which has important regulatory consequences on the availability of PGE<sub>2</sub>. Membrane-bound prostaglandin E synthase-1 (mPGES-1) is a glutathione (GSH)-requiring perinuclear-enriched protein that belongs to the MAPEG (membrane-associated proteins involved in eicosanoid and glutathione metabolism) family. This enzyme is functionally coupled to COX-2; it is induced by proinflammatory stimuli, cytokines, growth factors or lipopolysaccharide, and its induction is downregulated by anti-inflammatory glucocorticoids. Kainic acid microinjection into rodent hippocampus induces mPGES-1 expression in brain endothelial cells, but not neurons or astrocytes, and mPGES-1 deficient mice are resistant to kainic acid-induced neuronal loss (Takemiya et al., 2010). The second membrane-bound PGE synthase is mPGES-2, which, unlike the former, is constitutively expressed and may be coupled to COX-1 or COX-2. This enzyme has glutaredoxin-thioredoxin-like domains and is activated by thiol reagents. The cytosolic enzyme (cPGES) is coupled to COX-1 and allows rapid PGE<sub>2</sub> synthesis upon free AA release. It is a 23 kDa glutathione-requiring enzyme regulated by phosphorylation through casein kinase II and requiring the molecular chaperone HSP90 (Kobayashi et al., 2004).

Each of the five principal prostaglandins acts on specific membrane bound GPCR-type receptors. These prostaglandin receptors are classified according to the prostaglandin ligand that each binds with highest affinity (e.g., EP, FP, DP, IP, TP), resulting in specialized intra- and intercellular signaling cascades that lead to mediator-specific actions (Fig. 36-2).

## LIPOXYGENASES

**The lipoxygenases are involved in the rate-determining step in the biosynthesis of leukotrienes, lipoxins, resolvins, and protectins**

These membrane-bound non-heme iron-containing enzymes catalyze the dioxygenation of AA and DHA stereoselectively and with positional specificity. Thus they are named the 5-, 8-, 11-, 12-, and 15-lipoxygenases (LOs) and catalyze reactions that

add dioxygen at the respective AA carbon atom. The most common LOs in the brain are 5-LO, 12-LO and 15-LO (Phillis et al., 2006).

Individual LOs convert AA and DHA to the corresponding hydroperoxides (HpETEs), which are reduced by peroxidase enzymes to the hydroxy derivatives (HETEs). When acting sequentially or in a transcellular manner, LOs are involved in the conversion of AA, EPA, and DHA to di- and tri-hydroxy derivatives, including the lipoxins, the resolvins and the protectins (Serhan, 2010; Serhan et al., 2008; Serhan et al., 2008).

### 5-Lipoxygenase catalyzes the oxygenation of arachidonic acid at the 5-position to form 5-HpETE

5-LO plays a key role in the conversion of AA to several potent lipid mediators involved in inflammation, including the pro-inflammatory leukotrienes (LTs) and the anti-inflammatory lipoxins (LXs) (Fig. 36-3).

In a two-step transformation, 5-LO is able to convert AA to leukotriene A4 (LTA4) via a calcium-dependent translocation to the nuclear membrane and co-localization with the helper protein FLAP. This protein system serves as an efficient LTA4 synthase (LTA4S), forming the labile epoxide LTA4, which is enzymatically hydrolyzed to leukotriene B4 (LTB4) or is converted to the cysteinyl leukotrienes LTC4, LTD4 and LTE4.

### 15-Lipoxygenase catalyzes the oxygenation of arachidonic acid at the 15-position to Form 15-HpETE

15-LO exists in different mammalian isoenzymes designated together as 12/15-LO since they have variable positional specificity for both the 15-position and the 12-position of AA (Dobrian et al., 2011). Unlike other LOs, 15-LO oxidizes both the free AA as well as AA bound in phospholipids and lipoproteins.

15-LO is a key enzyme involved in the biosynthesis of anti-inflammatory lipid mediators, including lipoxins, resolvins and protectins (Serhan, 2010; Serhan et al., 2008; Serhan et al., 2008). Two different 12/15-LO pathways convert AA to lipoxins (Fig. 36-3). The first one involves oxygenation of LTA4 mediated by 12/15-LO to generate an epoxy-tetraene intermediate, which undergoes enzymatic hydrolysis to form lipoxin A4 (LXA4) and lipoxin B4 (LXB4). An alternative pathway begins with the oxygenation of AA by 15-LO to form 15S-HpETE or its reduced product 15S-HETE, followed by the action of 5-LO to generate the same epoxide precursor to LXA4 and LXB4. An analogous pathway (not shown in Fig. 36-3), initiated by acetylated COX-2 (formed in the presence of COX-2 and aspirin) or via a cytochrome P-450 enzyme, converts AA to the epimeric oxygenation product 15R-H(p)ETE, which can participate in the same enzymatic transformation to produce the epimeric lipoxins 15-epi-LXA4 (or aspirin-triggered LXA4, AT-LXA4) and 15-epi-LXB4 (or aspirin-triggered LXB4, AT-LXB4).

## LOs and LO-derived products play important roles in a variety of inflammatory disorders

The leukotrienes and lipoxins have potent actions mediated by binding to their perspective GPCR-type receptors, such as: BLT1 and BLT2 for LTB4, CysLT1 and CysLT2 for LTC4 and LTD4, and the FPR2/ALX receptor for LXA4 and AT-LXA4. While the leukotrienes promote leukocyte chemotaxis and are pro-inflammatory, the lipoxins and resolvins have opposite properties: suppressing PMN infiltration, reducing inflammation, and promoting resolution (Serhan, 2010; Serhan et al., 2008a; Serhan et al., 2008b). In the brain, these lipid mediators are key players in inflammation resulting from brain injury and in neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease (Phillis et al., 2006) (also see Ch. 34).

## DIACYLGLYCEROL KINASES

### The slow glutamate responses are mediated through metabotropic receptors coupled to G proteins

For review of metabotropic receptors (mGluRs) and G proteins, see Chapters 17 and 21. One of the mGluR family, mGluR1, is linked to the inositol lipid-PLC pathway. Activation of neuronal inositol lipid signaling through mGluR1 and PKC $\gamma$  is involved in synaptic plasticity such as in learning, memory, LTP and long-term depression (Chs. 23, 56) and has been implicated in neurological and psychiatric diseases such as epilepsy, Alzheimer's disease and depression. Activation of mGluR1 triggers a short-lived signal with potent and sustained consequences in other signaling pathways. DAG-activated PKC contributes to feedback inhibition of the PLC pathway and, at the same time, may lead to PLD and PLA<sub>2</sub> activation. The termination of the DAG signal is mainly accomplished by DAG kinase (DGK)<sub>e</sub>, which selectively phosphorylates AA-DAG to generate AA-PA. Eight other mammalian DGKs have been identified, but only DGK<sub>e</sub> uses AA-DAG. The central role played by DGK<sub>e</sub> in the inositol lipid-PLC pathway was revealed using mice with targeted disruption of the DGK<sub>e</sub> gene (Rodriguez de Turco et al., 2001). DGK<sub>e</sub><sup>-/-</sup> mice display higher resistance to seizures and attenuation of LTP in the hippocampus. Interestingly, not only the inositol lipid-PLC pathway was greatly affected by the mutation, but also the cPLA<sub>2</sub>-AA and PLD-DAG pathways, reflecting the impact of the former in modulating multiple pathways that participate in synaptic activity and neuronal plasticity.

The DAG signal itself can also be terminated by DAG lipases with the sequential release of 2-AA glycerol (2-AG) and then free AA and glycerol. As the primary second messengers are removed to terminate their functions, other bioactive lipids are generated; 2-AG is an endogenous ligand for the CA1 cannabinoid receptor with neuromodulatory functions (Piomelli, 2003), while PA and its PLA<sub>2</sub> product lyso-PA are potent intra- and intercellular messengers. Lyso-PA is released from the cells and interacts with cell surface G-protein-coupled receptors. Lyso-PA inhibits neurite

growth and neuroblastoma differentiation, stimulates neurotransmitter release in PC12 cells and has been implicated in Alzheimer's disease.

## LIPID SIGNALING IN NEUROINFLAMMATION

### A platelet-activating-factor-stimulated signal-transduction pathway is a major component of the kainic-acid-induced cyclooxygenase-2 expression in hippocampus

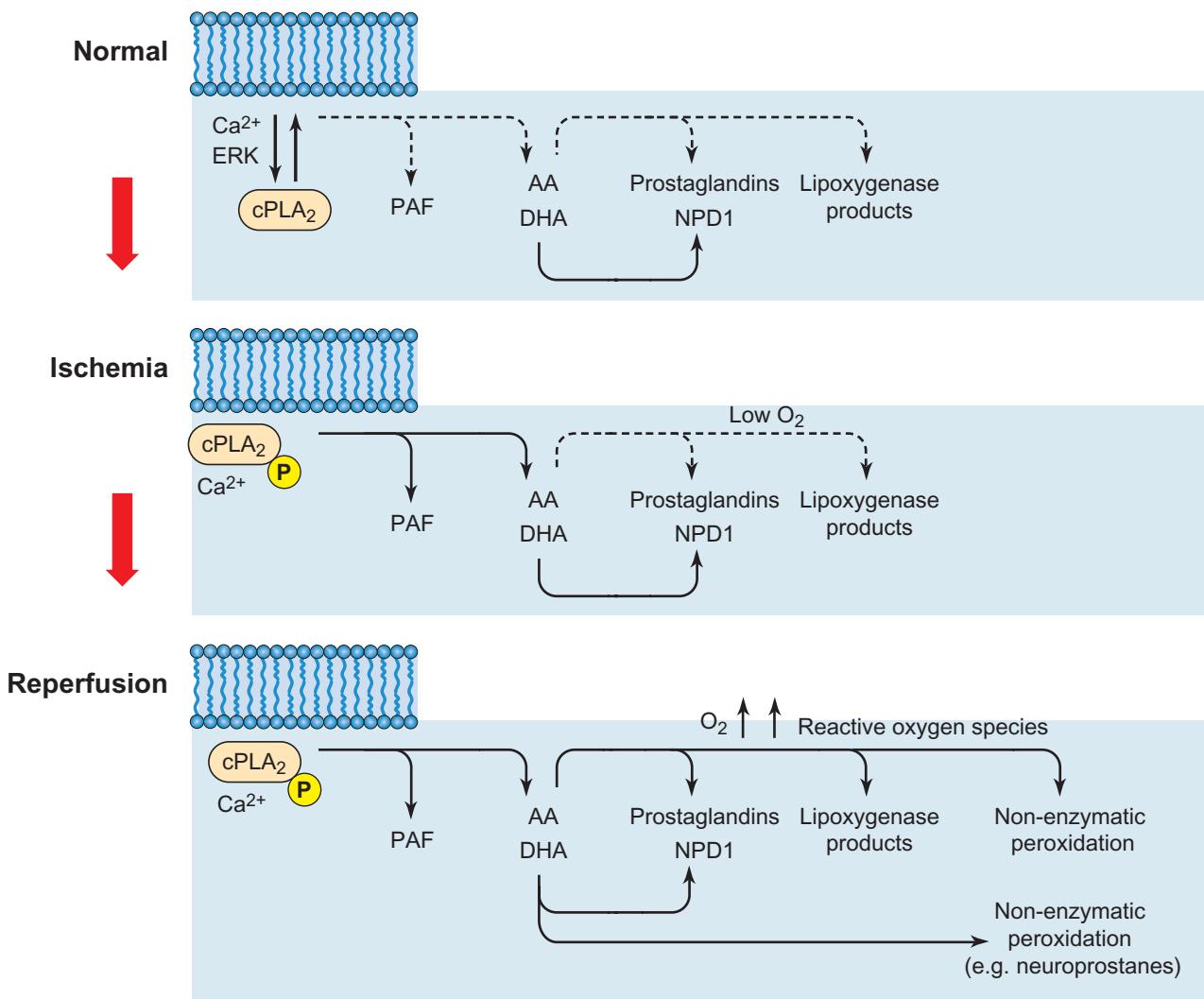
Both PAF and COX-2 are potent mediators of the injury/inflammatory response (Fig. 36). PAF and COX-2 are also interrelated in neuronal plasticity. The PAF transcriptional activation of COX-2 may provide clues about novel neuronal cell-death pathways. In fact, the delayed induction of COX-2 by kainic acid precedes selective neuronal apoptosis by this agonist in the hippocampus (Marcheselli & Bazan, 1996), although some evidence suggests that non-neuronal COX-2 may be an important mediator of kainic acid-induced neuronal cell death (Takemiya et al., 2010; Takemiya et al., 2006).

### In cerebrovascular diseases, the phospholipase-A<sub>2</sub>-related signaling triggered by ischemia–reperfusion may be part of a delicate balance between neuroprotection and neuronal cell death

Events that would tilt the balance toward neuroprotection are possible therapeutic targets. It is interesting to note that PAF, being short-lived and rapidly degraded by PAF acetylhydrolase, is a long-term signal with consequences for neurons through sustained expression of COX-2 (Fig. 36-7). Current investigations aim at determining whether other messengers, such as nitric oxide, cooperate to enhance neuronal damage and to what extent astrocytes and microglial cells are involved. On the other hand, a number of studies have implicated particular prostaglandin receptor subtypes as neuroprotective in a variety of brain injury models (Jiang et al., 2010; McCullough et al., 2004; Wu et al., 2007). Further understanding of these potentially neurotoxic events involving lipid messengers and COX-2 will contribute to the identification of new therapeutic strategies for the management of cerebrovascular diseases, neurodegenerative diseases and other pathologic conditions involving neuroinflammation.

### Free arachidonic acid, along with diacylglycerols and free docosahexaenoic acid, are products of membrane lipid breakdown at the onset of cerebral ischemia, seizures and other forms of brain trauma

Because polyunsaturated fatty acids are the predominant FFA pool components that accumulate under these conditions, this further supports the notion that fatty acids released



**FIGURE 36-7** Phospholipases A<sub>2</sub> (cPLA<sub>2</sub>) in the generation of eicosanoids, docosanoids and lipid peroxidation products during brain, retina, or spinal cord ischemia and reperfusion. During the ischemic phase, phospholipase overactivation and the downregulation of oxidative and energy metabolism, and of fatty acid reacylation, promote the accumulation of free arachidonic acid (AA), free docosahexaenoic acid (DHA) and lysophospholipids such as lyso-PAF. The reperfusion phase facilitates eicosanoid and docosanoid synthesis. Reactive oxygen species are generated at rates that can overload the antioxidant and free radical-scavenger systems of the brain, thus promoting peroxidation of polyunsaturated fatty acids. ERK, extracellular signal-regulated kinase; NPD1, neuroprotectin D1.

from the sn-2 position of membrane phospholipids are major contributors to the FFA pool, implicating PLA<sub>2</sub> activation as the critical step in FFA release (Fig. 36-7).

### Free fatty acid release during cerebral ischemia is a complex process that includes the activation of signaling cascades

In addition to cPLA<sub>2</sub>, these cascades probably involve the simultaneous and/or sequential activation of other phospholipid degradation pathways and the successive recruitment of different phospholipid pools (see also Ch. 35). This is alluded to by the fact that, in focal and global cerebral ischemia, FFA accumulation is preceded by a lag of a few seconds after the start of ischemia (Fig. 36-7). Moreover, the initial release of

FFA is, at least partially, derived from the activity of phospholipase C on inositol lipids (mainly on phosphatidylinositol 4,5-bisphosphate), with FFAs being subsequently released from DAG by DAG lipase and monoacylglycerol lipase. FFAs and DAGs enriched in stearic and AAs accumulate during cerebral ischemia and seizures, concomitant with a rapid decrease in the inositol lipid pool. A sequential activation of PLC and is suggested as a mechanism for the time-dependent metabolism of different phospholipid pools during brain ischemia. The inositol lipid pool is initially a contributor to release of FFAs that are further metabolized via the PLC-DAG lipase pathway. However, as the ischemia progresses or after seizures, the cPLA<sub>2</sub> pathway prevails, with the FFA now derived from other phospholipids. A mechanism could be envisaged to explain these observations in which PI-PLC activation leads to the release of DAG and inositol trisphosphate (IP<sub>3</sub>).

DAG activates protein kinase C, which in turn may modulate PI-PLC. At the same time, IP<sub>3</sub> mobilizes intracellular calcium and thus stimulates cPLA<sub>2</sub> translocation to the membrane.

### The rate of free fatty acid production in the mammalian brain correlates with the extent of resistance to ischemia

FFA production rate is much lower in the brains of neonatal mammals and poikilothermic animals, organisms that display a greater resistance to cerebral ischemic insults than mature mammals. In addition, within the mammalian brain, FFA release is higher in the gray matter compared with white matter, and there is a greater accumulation of AA in areas of the brain, such as the hippocampus, selectively vulnerable to cerebral ischemic damage.

### Activation of the arachidonic acid cascade during ischemia–reperfusion is a multistage process

During the ischemic phase, activation of phospholipases, inhibition of energy-dependent reacylation of membrane lipids and the lowering of oxygen tension in the brain promote the accumulation of free AA. When, or if, blood flow is restored, the rise in oxygen tension, coupled with a large pool of free AA, stimulates oxygenation-dependent reactions, some of them enzyme-mediated (e.g., for the synthesis of eicosanoids; Figs. 36-2, 36-3) and others non-enzyme-mediated, which promote the formation of lipid hydroperoxides. Platelet-activating factor (PAF) synthesis is also enhanced under these conditions. Accordingly, eicosanoids accumulate during reperfusion.

### Cyclooxygenase and lipoxygenase products accumulate during reperfusion following cerebral ischemia

The levels of lipoxygenase products (Fig. 36-3) increase more slowly than COX products but remain raised for longer periods of time. The cellular sites of synthesis of ischemia–reperfusion-induced eicosanoids within the brain have not yet been fully assessed. Glia and microglia comprise major inflammatory cells of the brain, as they are leukocytes that infiltrate the brain during reperfusion or traumatic injury (Marcheselli et al., 2003). Induction of COX-2 transcription is rapid in ischemia and in seizures. Given the involvement of glutamatergic neurotransmission in the induction of neuronal COX-2, it may be predicted that the release of glutamate triggered by cerebral ischemia would provide a trigger for COX-2 induction, although the contributions of synaptic and extrasynaptic NMDARs to this process remain unclear (Hardingham & Bading, 2010). There are several early-response genes that encode transcription factors that are rapidly induced under these conditions, such as hypoxia-inducible factor (HIF)-1 $\alpha$  and NF- $\kappa$ B. The COX-2 promoter contains consensus sequences for these transcription factors. Nonetheless, brain displays ‘constitutive’ inducible COX-2 under physiological conditions. This may be related to the

involvement of COX-2 in synaptic plasticity and long-term potentiation (Chen et al., 2002).

### The cerebrovasculature is also an abundant source of eicosanoids

Platelets, leukocytes and vascular endothelium are all capable of synthesizing eicosanoids (see above). Brain microvessels isolated from ischemic rat brain demonstrate enhanced synthesis of eicosanoids, and leukocytes and platelets may account for much of the LTD4 produced during ischemia–reperfusion in the gerbil.

## DOCOSAHEXAENOIC ACID

### Brain and retina are the tissues containing the highest contents of docosahexaenoic acid

DHA is used continuously for the biogenesis and maintenance of neuronal and photoreceptor membranes (see Vision in Ch. 51). This system is supported by the liver, which supplies DHA incorporated into plasma lipoproteins (Scott & Bazan, 1989). The uptake of DHA by the retina involves the retinal pigment epithelium (RPE) and its subsequent delivery to photoreceptors. During the daily photoreceptor renewal, an active recycling of phagosome-derived DHA by RPE via the interphotoreceptor matrix retains DHA within photoreceptors. A signal generated in brain or retina to control DHA delivery from the liver has been postulated (Scott & Bazan, 1989); this would allow activation of DHA export during development, when DHA is required for active synapse and photoreceptor outer segment formation. This activation may also occur when injury or neurodegeneration results in loss of DHA from excitable membranes and replenishment of DHA is needed.

### Rhodopsin in photoreceptors is immersed in a lipid environment highly enriched in phospholipids containing docosahexaenoic acid, which is essential for rhodopsin function

Moreover, retinal DHA, like brain DHA, is very resistant to n-3 fatty acid dietary deprivation (Scott & Bazan, 1989). Because of this constant systemic flow of DHA via a ‘long loop’, it is highly relevant that retinitis pigmentosa is associated with alterations in lipoprotein metabolism. The most frequently reported phenotype is low plasma and red blood cell levels of DHA. In Usher’s syndrome, an autosomal recessive retinitis pigmentosa that is associated with hearing loss, lower plasma levels of 22:6- and 20:4-phospholipids are also found (Bazan et al., 1986). Interestingly, changes show a direct correlation with the severity of disease and are more accentuated in patients with Usher’s type I than in those with the less severe type II form.

Low plasma levels of 22:6n-3 in retinitis pigmentosa patients have also been interpreted as a mechanism to protect the retina from oxidative damage resulting from photochemical function of rhodopsin (Anderson et al., 1999). Stress signals originating from retinas undergoing degeneration

(e.g., retinitis pigmentosa) may shut off the communication between the retina and the liver, reducing the systemic liver supply of 22:6n-3. In dogs with progressive rod-cone degeneration (PRCD) as well as in other retinal degeneration models (Anderson et al., 1999), low levels of DHA in photoreceptor phospholipids occur. Moreover, the synthesis of DHA from 20:5n-3 in RPE cultures from PRCD is not affected by the retinal degeneration, and dietary supplementation with DHA failed to prevent the loss of photoreceptor DHA and the PRCD phenotype. Moreover, cultured hepatocytes from PRCD dogs display higher accumulation of 22:6-phospholipids in the liver and impaired hepatocyte secretion of DHA-containing very low-density lipoproteins, supporting the hypothesis that in retinal degenerations, a systemic DHA metabolic defect occurs that leads to reduced liver DHA supply to the retina. Whether DHA dietary supplementation protects the retina of retinitis pigmentosa patients is not known.

## LIPID PEROXIDATION AND OXIDATIVE STRESS

### Docosahexaenoic-acid-containing phospholipids are targets for lipid peroxidation

As a result of non-enzymatic free radical-catalyzed peroxidation, F4-isoprostananes are formed (Roberts et al., 1998). F2-isoprostananes are also derived from free radical-catalyzed peroxidation, although from AA instead. F4-isoprostananes are found esterified in phospholipids and it has been reported that their content is increased in the brains of patients with Alzheimer's disease (Reich et al., 2001).

## DOCOSANOIDS

### Sequential oxygenation of DHA leads to several types of potent bioactive lipid mediators, including resolvins and protectins

The D-series resolvins, including resolvins generated in the brain, are DHA-derived mediators that were identified by incubations upon treatment with aspirin (Marcheselli et al., 2003; Serhan et al., 2002). These DHA-oxygenation pathways generate messengers that are counter-proinflammatory signals and promote resolution (Serhan, 2010; Serhan et al., 2008a; Serhan et al., 2008b; Serhan et al., 2002; Serhan et al., 2000; Hong et al., 2003).

### NEUROPROTECTIN D1: A DOCOSAHEXAENOIC-ACID-DERIVED MEDIATOR

### Docosanoids, enzyme-derived docosahexaenoic acid metabolites, were identified initially in the retina

These have been proposed to elicit neuroprotective actions (Bazan et al., 1984). It has been ascertained by tandem

LC-PDA-ESI-MS-MS-based lipidomic analysis that the synthesis of DHA-oxygenation messengers occurs during brain ischemia-reperfusion (Fig. 36-7), leading to the isolation of neuroprotectin D1 (NPD1), a DHA-derived lipid mediator (Fig. 36-8). Structural characterization (Marcheselli et al., 2003; Mukherjee et al., 2004; Hong et al., 2003) and stereochemical assignment (Serhan et al., 2006) defined the complete structure of NPD1 (10R,17S-dihydroxy-docosa-4Z,7Z,11E,13E,15Z,19Z-hexaenoic acid), and further investigations established its potent anti-inflammatory and neuroprotective actions (Mukherjee et al., 2007; Mukherjee et al., 2007; Calandria et al., 2009; Marcheselli et al., 2010).

The biosynthesis of NPD1 (Fig. 36-8) begins with the cPLA<sub>2</sub>-mediated release of free DHA and the specific dioxygenation at carbon-17 catalyzed by 15-LO to form 17S-HpDHA. Enzymatic dehydration to the 16S,17S-epoxide intermediate, followed by enzymatic hydrolysis, generates NPD1.

### Neuroprotectin D1 is a potent inhibitor of brain ischemia-reperfusion-induced PMN infiltration, as well as of NF-κB and COX-2 expression

Marked attenuation of stroke volume was observed when neuroprotectin D1 was infused into the third ventricle during ischemia-reperfusion (Marcheselli et al., 2003). Discovery of this and other DHA oxygenation messengers sheds new light on how the brain modulates its response to inflammatory injury and raises the exciting potential for designing new drugs to treat neurologic disorders that have a neuroinflammatory component, such as stroke, traumatic brain injury or spinal cord injury.

## THE FUTURE OF NEUROLIPIDOMIC SIGNALING

### Knowledge of the significance of lipid signaling in the nervous system is being expanded by advances in experimental approaches

Following the genomics era, we are now in 'proteomics times,' when not only is the proteome being defined but metabolomics are emerging. Among the powerful new tools available are lipidomic analyses. Lipidomics is beginning to allow us to precisely define lipid organization, metabolism, synthesis of stereospecific mediators (e.g., neuroprotectin D1) and signal transduction in a given cell or part of a cell (e.g., dendrites). This is mainly due to the power, ease and versatility of what has evolved from the development of electrospray ionization mass spectrometry (ESI/MS) (Piomelli et al., 2007). Recent developments in Matrix Assisted Laser Desorption Ionization (MALDI) mass spectrometry now allow visualization of specific membrane phospholipid and other lipid species *in situ* (Murphy et al., 2009). Thus, the detailed composition of lipid classes and molecular species can now be approached more accurately. Moreover, the detailed identification of changes in the lipidome during

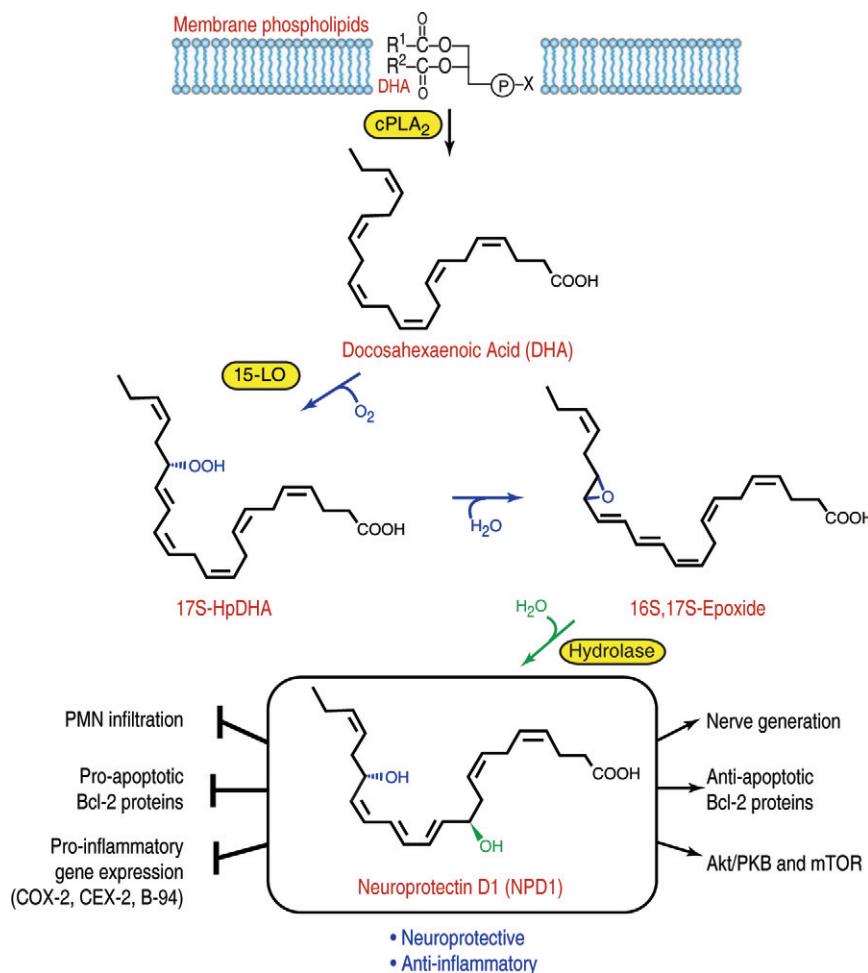


FIGURE 36-8 Biosynthesis and actions of neuroprotectin D1.

the development, function, aging and dysfunctions of the nervous system will be tackled. These studies will be integrated with nutritional approaches and clinical neurosciences (Box 36-1).

### Understanding of the fundamental workings of the dendrites, which contain complex intracellular membranes rich in polyunsaturated phospholipids, continues to evolve

Dendrites undergo profound changes during neuronal function, including the membrane vesicular transport of neurotransmitter receptors, ion channels and other proteins destined to the dendritic spine, where critical postsynaptic elements of neurotransmission are located. Definition of the dendritic lipidome will also define the participation of lipid signaling in dendritic development and in the establishment of synaptic contacts as well as overall dendritic plasticity.

**Arachidonic acid is widely implicated in signaling in brain, and research continues toward understanding the release of this fatty acid from membrane reservoirs**

Modulation of PLA<sub>2</sub> by Ca<sup>2+</sup> and protein kinase needs to be better defined. It is clear that NMDA receptor activation promotes the release of AA (Taylor et al., 2008; Dumuis et al., 1988), and that a variety of eicosanoids are then generated (Fig 36-2, 36-3). The modulatory events that channel AA towards specific eicosanoids are not understood. The endocannabinoid family of lipid messengers will remain an active focus of interest because of the growing evidence of their actions in synaptic function, learning, memory, and other forms of behavior (Piomelli, 2003).

The essential fatty acid of the linolenic acid family, DHA, is most highly concentrated in brain and retina. The significance of this polyunsaturated fatty acid, in addition to being a target for nonenzymatic peroxidation under various pathologic

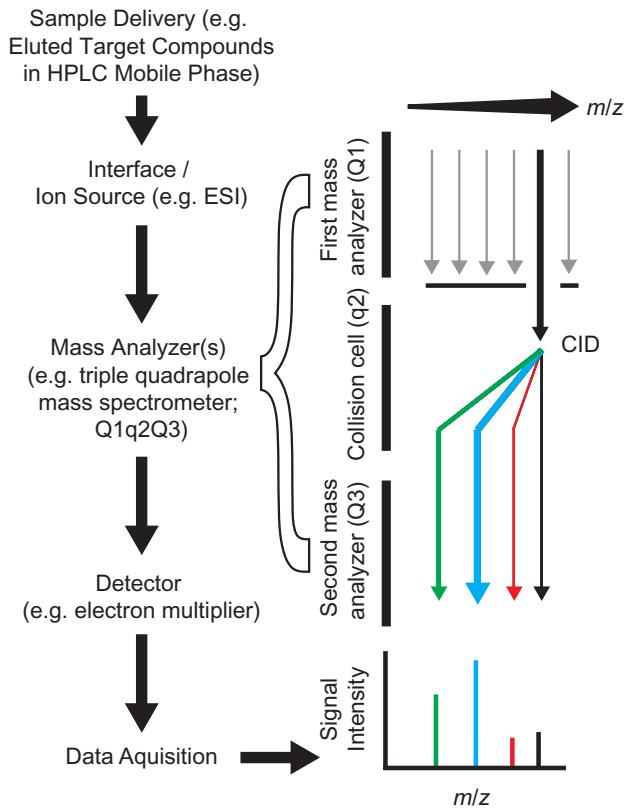
## MEDIATOR LIPIDOMICS IN TRANSLATIONAL NEUROSCIENCES

David T. Stark, Nicolas G. Bazan

The application of mass spectrometry (MS) in biology has increased dramatically since the advent of “soft” ionization techniques such as electrospray ionization (ESI) and matrix assisted laser desorption/ionization (MALDI). The field of neurolipidomics has advanced radically as a result of these developments. These techniques allow delivery of target compounds to a mass spectrometer without the need for prior fragmentation or derivatization. Thus, a huge diversity of target lipid molecules in complex matrices (e.g., serum, cerebrospinal fluid, brain interstitial fluid obtained by microdialysis; brain tissue itself or neural cells) can be analyzed with only minimal sample preparation. Typical components of an HPLC-ESI-MS/MS-based system, operated in “product ion” mode, are illustrated in the schematic diagram. A sample extract is loaded onto a high performance liquid chromatography (HPLC) analytical column and eluted by flowing mobile phase solvent, which is interfaced with the mass spectrometer via an ESI source. The ESI source desolvates the target compounds as they elute from the column, and the target compounds enter the mass spectrometer in the form of gaseous ions. A popular format for mass spectrometers is the triple quadrupole design. Tandem quadrupolar electromagnetic fields are used to separate target compounds from matrix on the basis of mass-to-charge ratio ( $m/z$ ). In this example, the first quadrupole (Q1) selects compounds with a specific  $m/z$  value and these compounds enter the collision cell (q2), where they undergo collision-induced dissociation (CID) as a result of interaction with an inert gas. The resulting product ions produce a characteristic mass spectrum, which can be used in combination HPLC retention time matching and auxiliary on-line tools such as UV spectroscopy to confirm the identity of a target compound and to quantify its abundance (Figure 1). HPLC-ESI-MS/MS-based lipidomic analysis in combination with other technologies is used to characterize novel lipids in the nervous system and other tissues. Analysis of lipid oxidation products holds particular promise for uncovering early markers of the initiation and progression of neurodegenerative diseases as well as in mechanistic studies designed to reveal new therapeutic targets in brain disease. For instance, DHA autoperoxidation products (neuroprostanes) are increased in the brains of Alzheimer’s disease patients (Reich et al., 2001), whereas the stereospecific DHA-derived mediator neuroprotectin D1 (NPD1) is decreased 2 (Lukiw et al., 2005). Moreover, recently mediator lipidomic-identified NPD1 and its precursor enhanced in the penumbra of a middle cerebral artery occlusion (MCAo) model of ischemic stroke upon intravenous DHA injection (Belayev et al., 2011). The selective oxidation of a mitochondria-specific phospholipid, cardiolipin, has been analyzed in clinical traumatic brain injury and is thought to be a marker of neuronal injury as well as a target for prevention of neuronal apoptosis (Sparvero et al., 2010).

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**BOX FIG. 36-1** Schematics of the typical components of an HPLC-ESI-MS/MS-based system.

conditions, will be a focus of continued research. Clearly, DHA-containing molecular species of phospholipids confer a unique environment for ion channels, receptors, transporters and protein–protein interactions critical in signaling. How these events are regulated, including the synthesis and remodeling of these highly unsaturated phospholipids, is not clearly understood. The enzyme-mediated synthesis of docosanoids raises the possibility of exploring the specific generation of novel DHA-oxygenation messengers that are neuroprotective. How these docosanoid messengers are synthesized, through which receptors they elicit their actions, how they are affected by pharmacologic agents and how the docosanoids themselves may become a template for drug design are questions for the near future. The use of tandem LC-PDA-ESI-MS-MS-based lipidomic analysis in combination with other experimental approaches will greatly contribute to our understanding of the significance of DHA in health and in pathologic conditions.

### The knowledge evolving from lipidomic neurobiology will be potentiated by multidisciplinary approaches such as multiphoton confocal analysis

Structural neurobiology will also come into play, because the lipidome will provide new insights into the precise stereochemical structure of lipids of excitable membranes, as well as of intracellular membranes. There is also growing evidence of the exquisite signaling interplay among neurons, astrocytes, oligodendrocytes and microglia. Prostaglandins are among the lipid messengers explored to date as modulators of astrocyte release of glutamate (Hamilton & Attwell, 2010). Microglia also actively make prostaglandins in response to injury (Block et al., 2007), although prostaglandin E2 may be engaged in neuroprotection (Jiang et al., 2010; McCullough et al., 2004; Wu et al., 2007). Moreover, the renewed interest in defining the significance of non-neuronal cells in the nervous system will be greatly enhanced by lipidomic approaches. The fact that docosahexaenoic acid (DHA) is avidly retained and uniquely concentrated in the nervous system, particularly in photoreceptors and synaptic membranes, will be further studied in terms of its significance in vision, neuroprotection, successful aging, memory and other functions. In addition, the anti-inflammatory properties of DHA and docosanoids in contrast to pro-inflammatory actions of several eicosanoids, as well as their synaptic bioactivities, will be investigated. DHA signalolipidomics will evolve as a field within the broader field of lipidomics. DHA signalolipidomics includes the cellular organization of DHA uptake, its distribution among cellular compartments, the organization and function of membrane domains rich in DHA-containing phospholipids, specific roles in synaptic function, and the cells and molecules regulated by DHA and neuroprotectin D1 (NPD1) as well as by other docosanoids. The detailed mechanisms of the NPD1 synthesis agonists neurotrophins and oxidative stress will be defined. Moreover the significance of eicosanoids, docosanoids and PAF in Alzheimer's disease, macular degeneration, Parkinson's disease and other brain disorders will be determined. Signalolipidomics in the CNS will uncover novel targets for clinical translation and pharmaceutical intervention.

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