NS/202: Unit 4
Lipid mediators: Eicosanoids, Docosanoids and Platelet-activating factor

Storage of Lipid Messengers in Neural Membrane Phospholipids

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

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

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

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

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

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

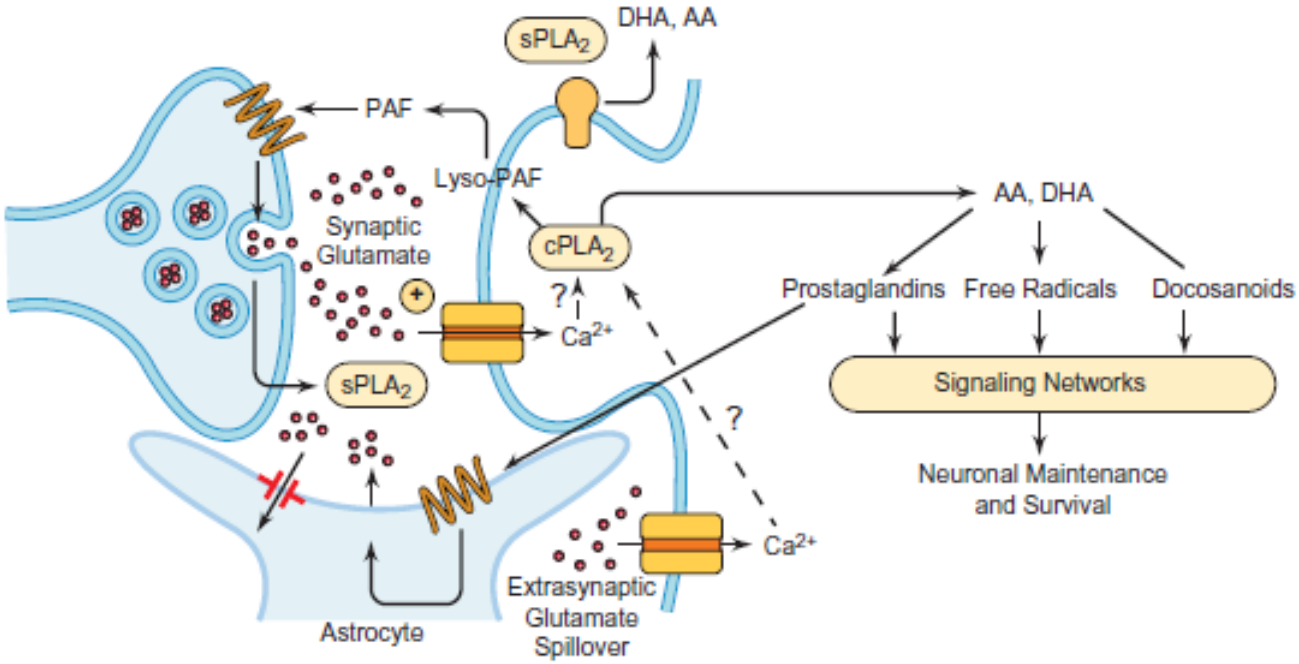
1. This function requires the **presence of ion pumps, neurotransmitter receptors and other associated membrane proteins**. Excitable membranes have a phospholipid composition that differs from other membranes, a property assumed to be related to their highly specialized functions.
2. Cellular membranes 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. neurons, glia and endothelial cells of the cerebrovasculature, several phospholipid pools are recognized as reservoirs of lipid messengers.
3. ***lipid rafts*** *have been* described, isolated and their chemical composition studied in specific cellular compartments of the nervous system, including dendrites, where they are associated with specific postsynaptic proteins. **Lipid rafts are microdomains enriched in cholesterol and sphingolipids.**
   Interestingly, there are subtypes of lipid raft, which vary by their resistance to detergent extraction, density and their raft marker proteins, Thy-1 and caveolin

4. Several proteins have been found segregated in these microdomains, including **glycophosphatidyl (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.
5. 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.

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

1. These stimuli include **neurotransmitters, neurotrophic factors, cytokines, membrane depolarization, ion channel activation and others**. Lipid messengers regulate and interact with multiple other signaling cascades, contributing to the **development, differentiation, function, protection and repair of the cells of the nervous system.**
2. 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) and docosahexaenoic acid (DHA),** but levels increase very rapidly upon **cell activation, cerebral ischemia, seizures and other types of brain trauma.**

- ✓**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.**
- ✓**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 are activated, particularly microglia**, which 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-daspartate*** **(NMDA)-type glutamate receptors.**
- ✓As a consequence, **PLA2 is activated, AA is released, eicosanoids and PAF are synthesized and cyclooxygenase (COX)-2 is induced in neurons.**
- ✓**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.**



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. Calcium-mediated activation of the cytoplasmic PLA2 (*cPLA2)* 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 [7]. PAF binds to its presynaptic 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 PLA2 (*sPLA2) may be released from the presynaptic terminal* [8]: *sPLA2 binding sites are present in neurons [9*– 12], and sPLA2 promotes active AA remodeling in neurons in culture [13] and may also promote DHA release. DHA may also be released by cPLA2. Free DHA may subsequently follow enzyme-mediated oxygenation pathways and lead to the synthesis of docosanoids, messengers made in the retina [14] and brain** [4]. 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 Ca2+-dependent mechanism [15].**

Phospholipases A2

- *Phospholipases A2 catalyze the cleavage of the fatty acyl chain from the sn-2 carbon of the glycerol backbone of phospholipids*

- *Cytosolic phospholipases A2 are involved in bioactive lipid formation*

- *Ischemia and seizures activate phospholipases A2, releasing arachidonic and docosahexaenoic acids*

- *Secretory phospholipases A2 are of relatively low molecular weight and have a high number of disulfide bridges, making them relatively more resistant to denaturation*

- *There are high-affinity receptors that bind secretory phospholipases A2*

PHOSPHOLIPASE A2

Phospholipase A2 catalyzes the cleavage of the fatty acyl chain from the second carbon of the glycerol backbone of phospholipids

1. There are a wide variety of PLA2 types that include, in addition to intracellular PLA2, a low-molecular-weight **secretory PLA2 (sPLA2)** that **synergizes glutamate-induced neuronal damage.**
2. Whereas **pathways leading to PLA2 activation are part of normal neuronal function, ischemia–reperfusion enhances these events, overproducing PLA2-derived lipid messengers, such as enzymatically produced AA- and DHA-oxygenation derivatives, and nonenzymatically generated lipidperoxidation products and other reactive oxygen species (ROS), all of which may be involved in neuronal damage.**
3. **Intracellular PLA2 types** are located either in the cytosol or in noncovalent association with membranes and consist of **Ca2+-dependent types (iPLA2).**

Calcium-ion-dependent phospholipase A2 with a preference for polyunsaturated fatty acyl chains is involved in bioactive lipid formation

1. Membrane translocation of cPLA2 to endoplasmic reticulum and nuclear membranes is mediated via a specific Ca2+-dependent domain similar to those seen in **protein kinase C, phospholipase C and GTPase-activating protein.**
2. This site is consistent with that of other enzymes of AA metabolism, such **as prostaglandin synthases (PGS), also termed cyclooxygenases (COX-1 or -2), as well as 5-lipoxygenase and its activator protein.** The catalytic activity of cPLA2 is stimulated by phosphorylation catalyzed by the mitogen-activated protein kinase (MAPK).

Secretory phospholipases A2 are of relatively low molecular weight and have a high number of disulfide bridges, making them relatively more resistant to denaturation.

The mammalian sPLA2 types can be subdivided on the basis of amino acid sequence into several groups that include pancreatic or **group I sPLA2**, the members of which function in pancreatic secretions, smooth muscle contraction, cell proliferation and fertilization; and synovial or **group II PLA2**, 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 A2

1. 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 sPLA2** (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 sPLA2 but not OS1.***
2. The **M-type receptor** has been cloned from rabbit and human tissues and, independently, from mouse and bovine tissues; it **mediates group I sPLA2 cellular actions.**
3. Potential ligands for the **N-type receptor may be the group II sPLA2** induced in rat brain during ischemia.

Eicosanoids

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

- *Prostaglandins are very rapidly released from neurons and glial cells*

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

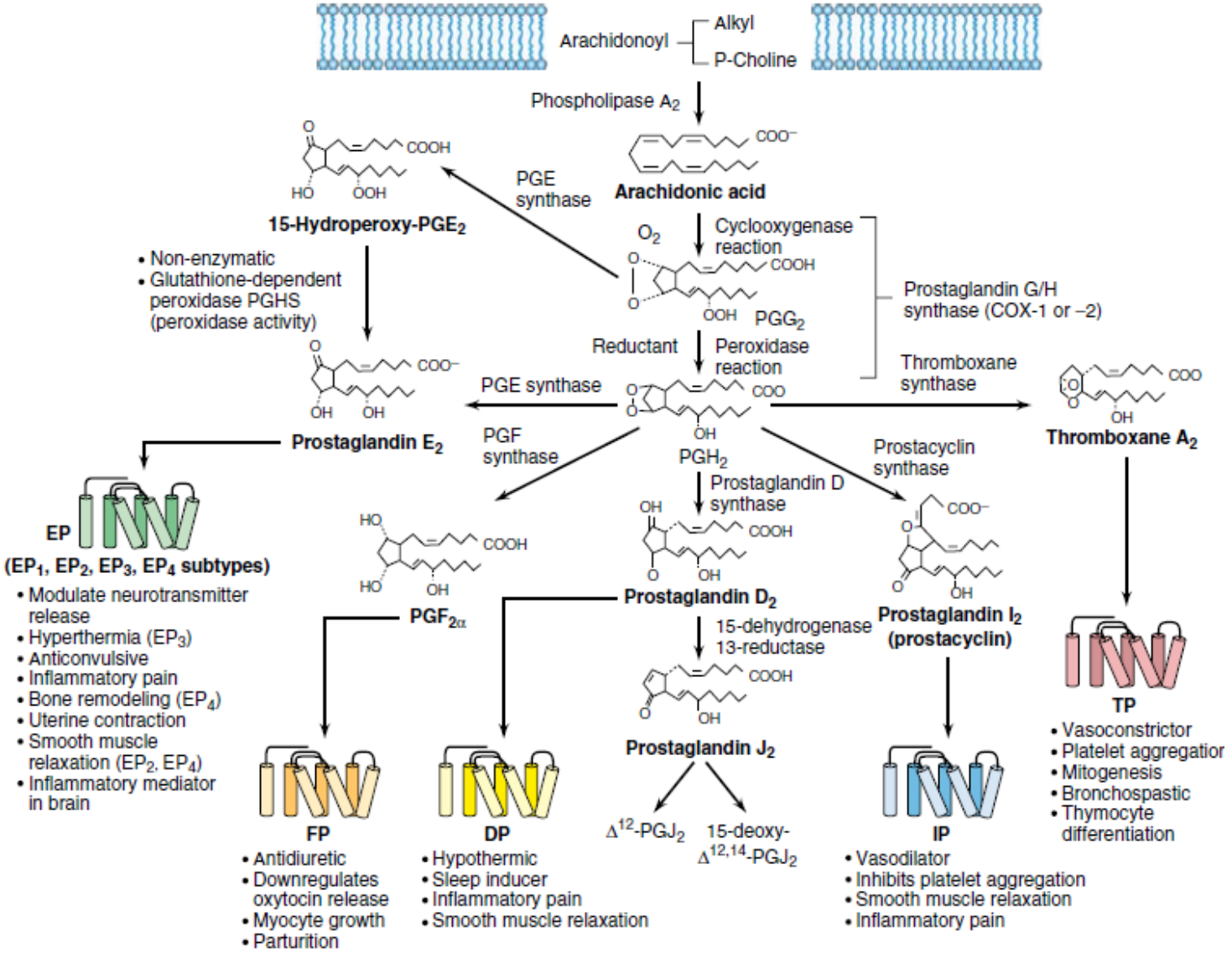
Arachidonic acid is converted to biologically active derivatives by cyclooxygenases and lipoxygenases

These metabolites, referred to collectively as **eicosanoids are potent messengers** that 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. 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.

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.

The **PGE2 receptor** belongs to the seven-transmembrane-domain receptor family and is coupled **via G proteins 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.**
CREB has been implicated in plasticity changes of synaptic circuits, memory formation, and in behavior.



The arachidonic acid cascade. Free AA released by phospholipase A2 (or PLC-diacylglycolipase-monoacylglycerollipase) undergoes cyclooxygenation and peroxidation mediated by prostaglandin G/H synthase. Specific tissue and product-specific isomerases mainly utilize PGH2 to generate prostaglandins, prostacyclin and thromboxane. Receptors as well as some of the bioactivities of specific eicosanoids are illustrated.

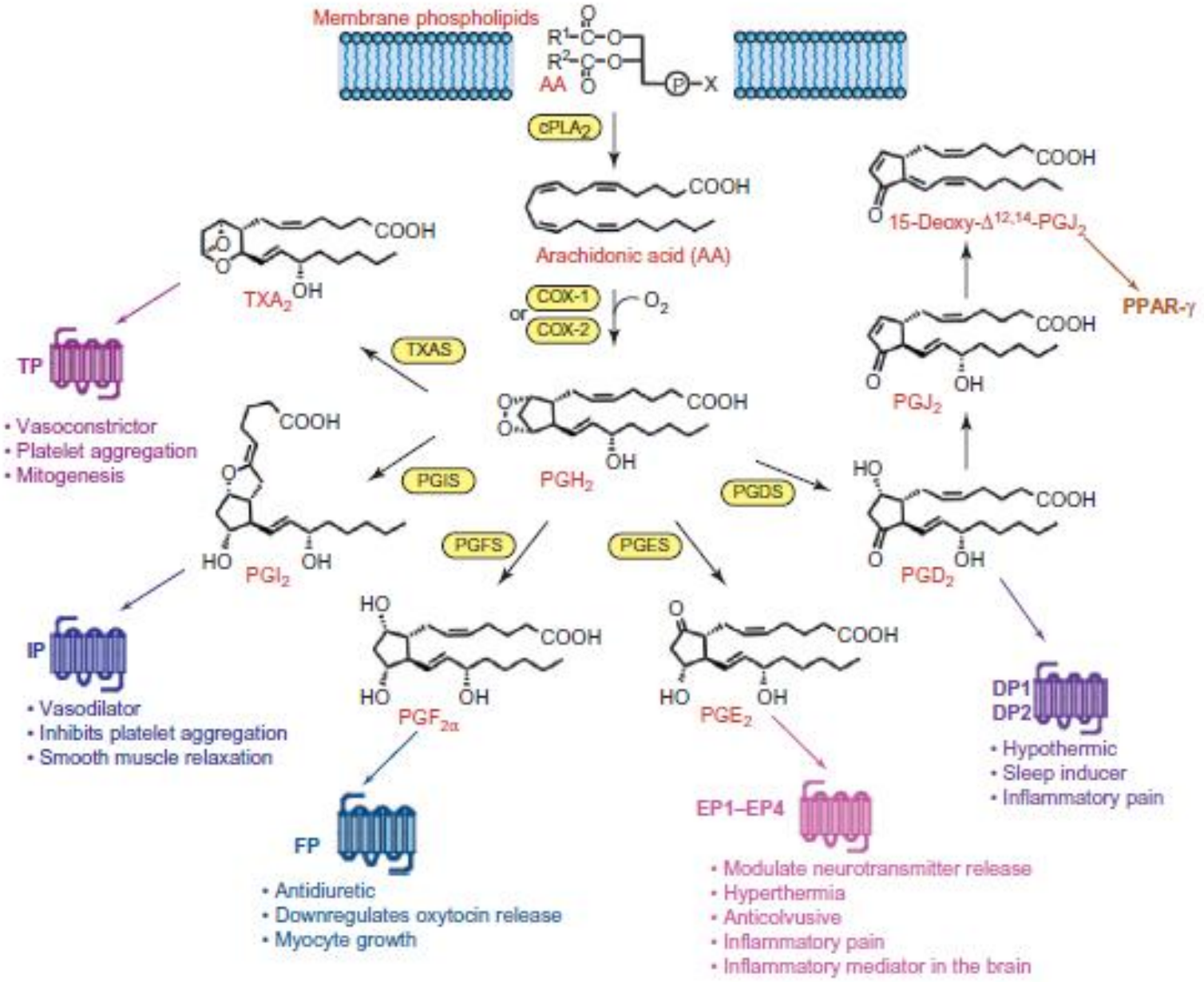


FIGURE 36-2 **The arachidonic acid cyclooxygenase cascade.**

Arachidonic acid is also the substrate for lipoxygenase and, as in the case of cyclooxygenase, molecular oxygen is required

The exact mechanisms controlling the channeling of AA through COXs or lipoxygenases are not clearly understood. However, there is growing evidence that subcellular compartmentalization is a major factor in the channeling of AA through either pathway.

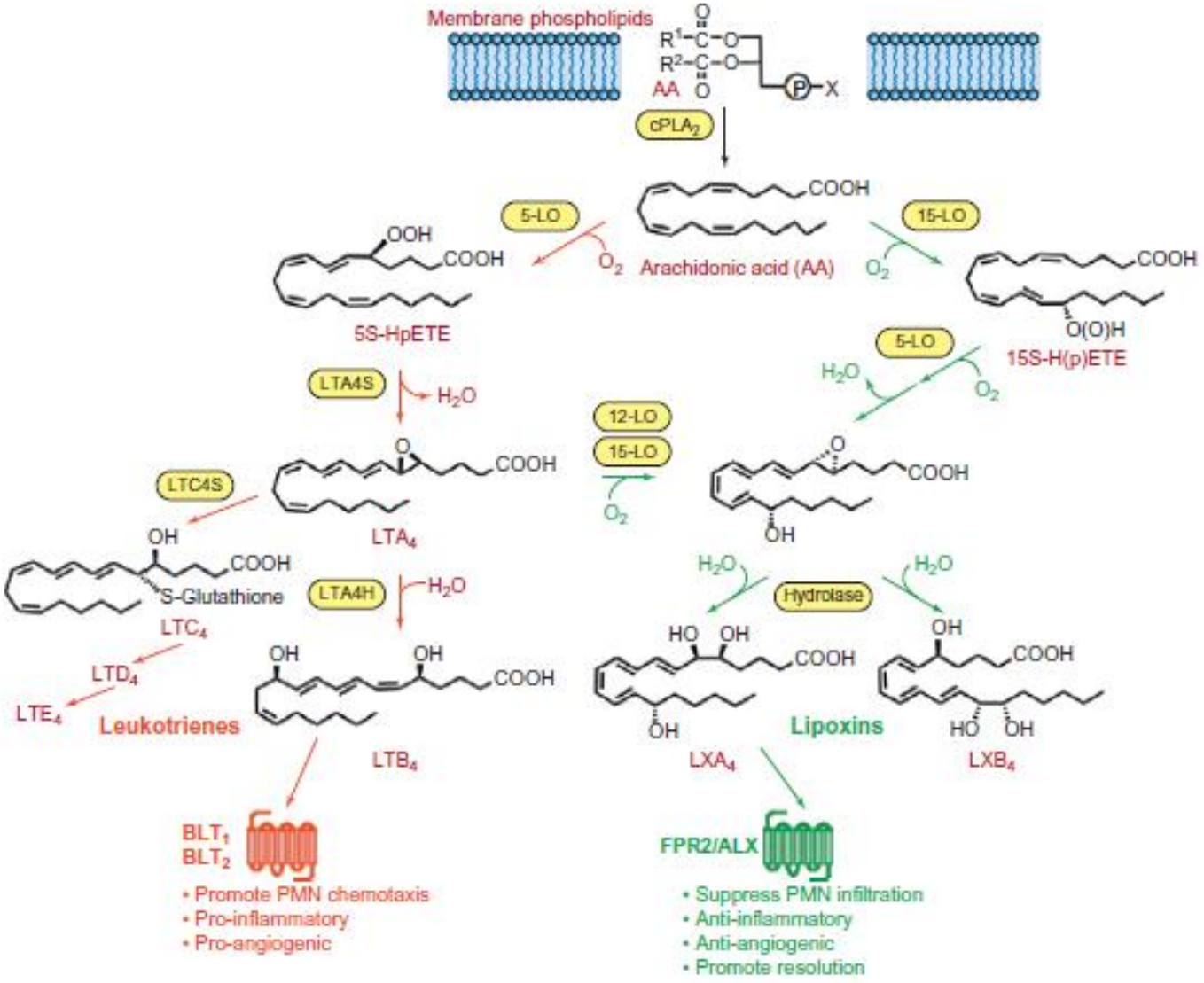


FIGURE 36-3 **Arachidonic acid lipoxygenase pathways.**

PLATELET-ACTIVATING FACTOR

Platelet-activating factor is a very potent and shortlived lipid messenger

1. It is known to have a wide range of actions: as a **mediator of inflammatory and immune responses, as a second messenger, and as a potent inducer of gene expression** in neural systems. PAF can potentially mediate longer-term effects on **cellular physiology and brain functions.**
2. PAF **enhances glutamate release in synaptically paired** rat hippocampal neurons in culture. 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.
3. The **presynaptic PAF-receptor antagonist BN 52021 (**a terpenoid extracted from the leaf of the *Ginkgo biloba tree)* 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 it evokes spontaneous synaptic responses characteristic of enhanced neurotransmitter release.
4. Therefore, as a modulator of glutamate release, **PAF participates in long-term potentiation (LTP), synaptic plasticity and memory formation.**

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

1. The brain is endowed with a variety of degradative enzymes that rapidly convert PAF to biologically inactive lyso-PAF .
2. **BN 52021** (terpenoid extracted from the leaf of the *Ginkgo biloba tree)* inhibits both PAF-induced glutamate release and long-term Potentiation. Moreover, this antagonist is neuroprotective in ischemia–reperfusion damage in the gerbil brain.
3. PAF, when overproduced at the synapse during ischemia, promotes enhanced glutamate release, which in turn contributes to **excitotoxicity through the activation of postsynaptic receptors.**

CYCLOOXYGENASES

1. Cyclooxygenase-1 is a constitutive enzyme that converts arachidonic acid to prostaglandin H2
2. COX-2 is inducible by cytokines, glutamate, growth factors, PAF and other mediators and is inhibited by glucocorticoids.
3. In most tissues, stimulation, injury, inflammatory stimuli and other forms of cellular stress trigger expression of the COX-2 gene.
4. 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. **COX-3** is expressed in brain, brain microvasculature and has been proposed to be a target of the **analgesic/antipyretic acetaminophen.**

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

Cyclooxygenase-2 participates in aberrant synaptic plasticity during epileptogenesis.

1. Both COX-2 and cPLA2 expression are activated, indicating that the free AA released is converted into prostaglandins during epileptogenesis.
2. **Nimesulid**e, 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.

3. COX-1, which is not inhibited by nimesulide, may catalyze the synthesis of prostaglandins, minimizing the action of nimesulide.
4. COX-2 inhibition may diminish prostaglandin and/or PAF synthesis, lipid messengers that are both involved in synaptic facilitation.

DOCOSAHEXAENOIC ACID

Docosahexaenoic acid, **enriched in the brain and retina**, generates docosanoids in response to disruptions of cellular homeostasis. Docosanoids include neuroprotectin D1 (NPD1), which is decreased in the CA1 hippocampal area of patients with early-stage Alzheimer's disease (AD)

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

Docosahexaenoic Acid (DHA)

Vital Part of Brain and Retina Structure

- The total dry weight of the adult brain is 50-60 percent lipid (fat), and more than 17 percent is in the form of DHA. In the retina, DHA makes up more than 33 percent of the total fatty acids.

Necessary for Infant Brain and Eye Development, Adult Cognitive Function

- Because of its structure, DHA may help keep the membranes of the your brain cells fluid and permeable, allowing for better signaling and better stress response.
- DHA is so important for development the placenta selectively takes up DHA for the developing baby.
- Higher DHA status from diets rich in long-chain fatty acids may be associated with decreased risk for age-related cognitive challenges.

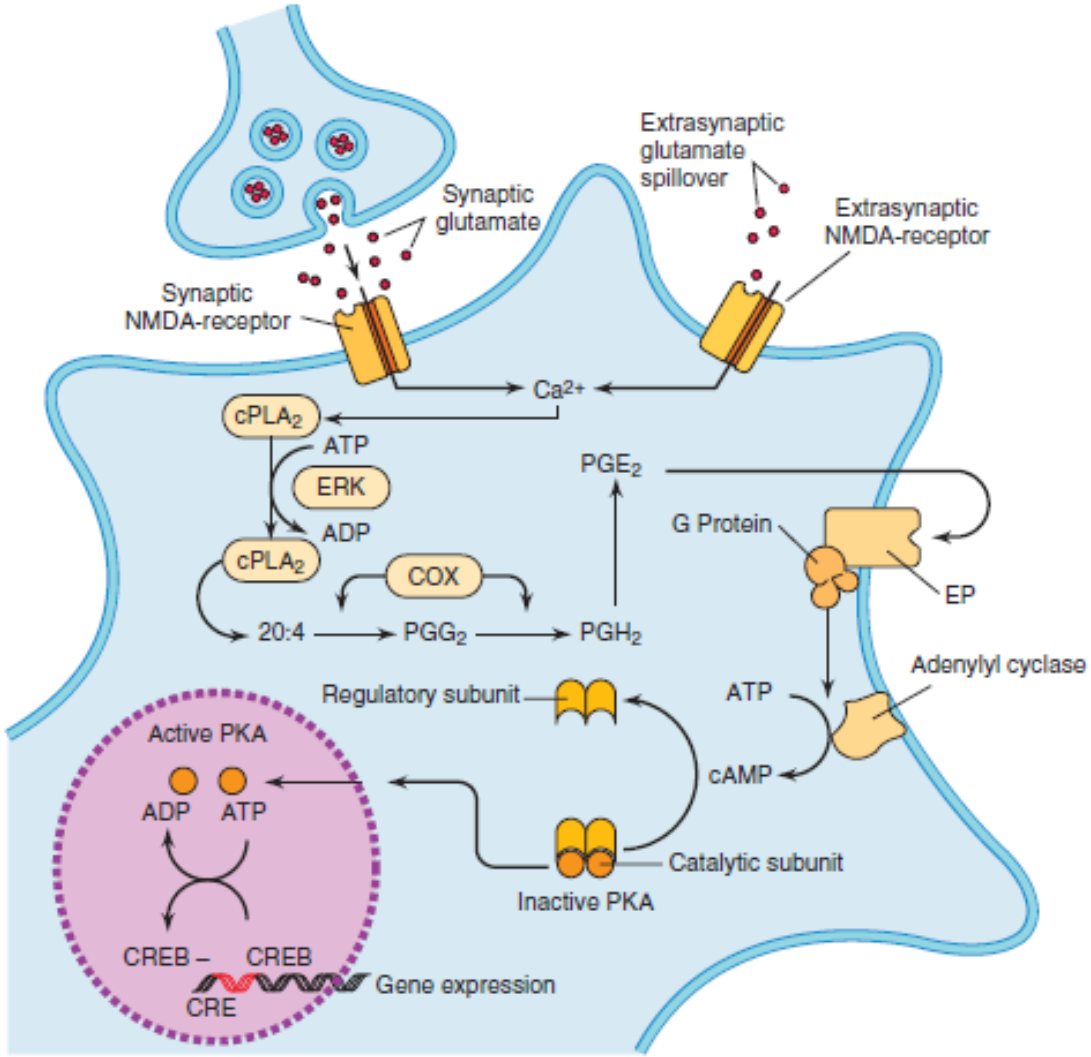


FIGURE 36-4 **Prostaglandin signaling pathway triggered by the excitatory amino acid neurotransmitter glutamate.** NMDA receptors initiate changes in intracellular Ca^{2+} concentration that modulate prostaglandin signaling (PGE_2 in this example) via regulation of $cPLA_2$ and COX-2. The respective 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*$_2$, cytosolic phospholipases A_2; *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*$_2$ and *PGH*$_2$ are short-lived intermediates in the synthesis of prostaglandin E_2 (*PGE*$_2$); *PKA*, protein kinase A.

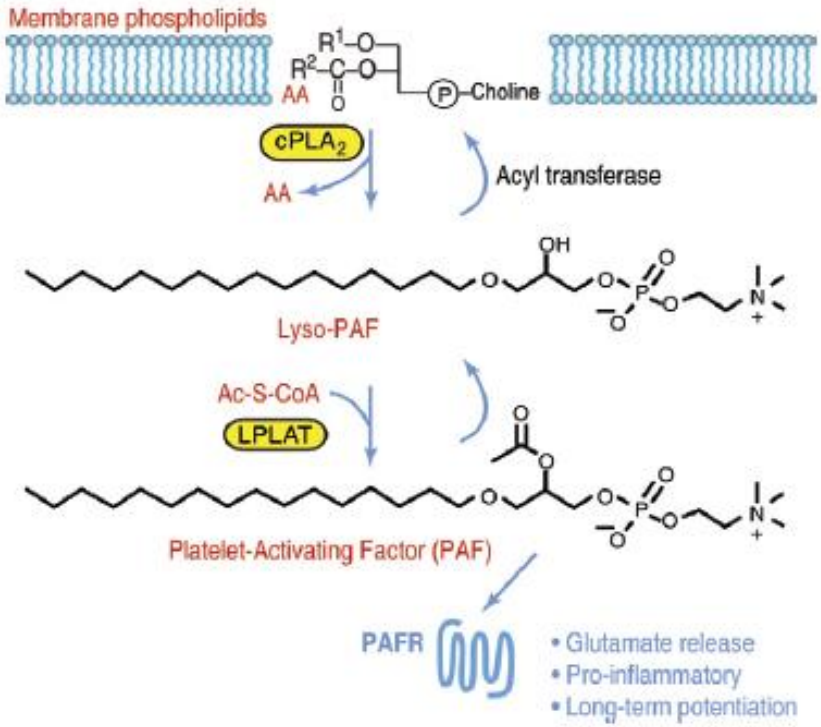


FIGURE 36-5 **The platelet-activating factor (PAF) pathway.** Activated cytosolic phospholipase A2 (cPLA2) catalyzes the cleavage of the arachidonoyl (AA) ester group at the 2-position of ether glycerophospholipids to form free arachidonic acid (AA) and lyso-PAF, which is rapidly re-incorporated into 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).

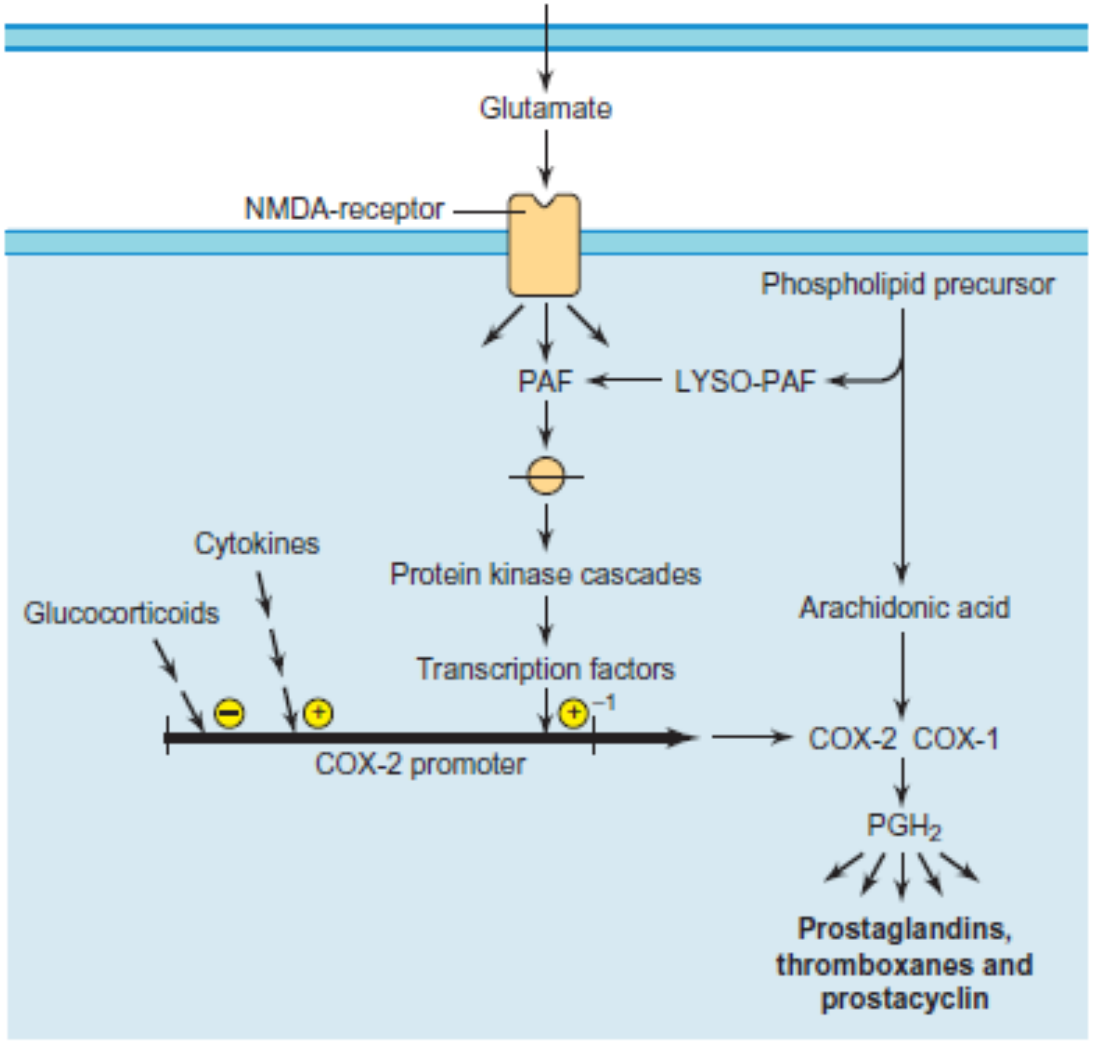


FIGURE 36-6 **Seizure- or ischemia-triggered signaling events linking synapse activation and cyclooxygenase-2 (COX-2) gene expression in neurons.** *N*-methyl-D-aspartate (NMDA) receptor activation by glutamate leads to phospholipase A_2 (PLA_2) activation and the generation of platelet-activating factor (*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 H2 (PGH2), the precursor of eicosanoids. Constitutive activity of COX-1 also catalyzes this metabolic step.

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**

- **In cerebrovascular diseases, the phospholipase- A2-related signaling triggered by ischemia–reperfusion may be part of a delicate balance between neuroprotection and neuronal cell death**

a) Overexpression of hippocampal COX-2 during cerebral ischemia and seizures may in turn lead to the formation **of neurotoxic metabolites, such as ROS.**

b) The regulatory subunit of PAF acetylhydrolase (intracellular) is the *lis-1 gene,* mutated in Miller–Diecker syndrome, a neuronal disease characterized by the absence of gyri and sulci in the cerebral cortex (lissencephaly). A defect in neuronal migration during brain development may underlie the smooth cerebrum of people afflicted with the syndrome, and PAF and PAF-acetylhydrolase may be involved in neuronal migration.

c) 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

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

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

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

• Cyclooxygenase and lipoxygenase products accumulate during reperfusion following cerebral ischemia

• The cerebrovasculature is also an abundant source of eicosanoids

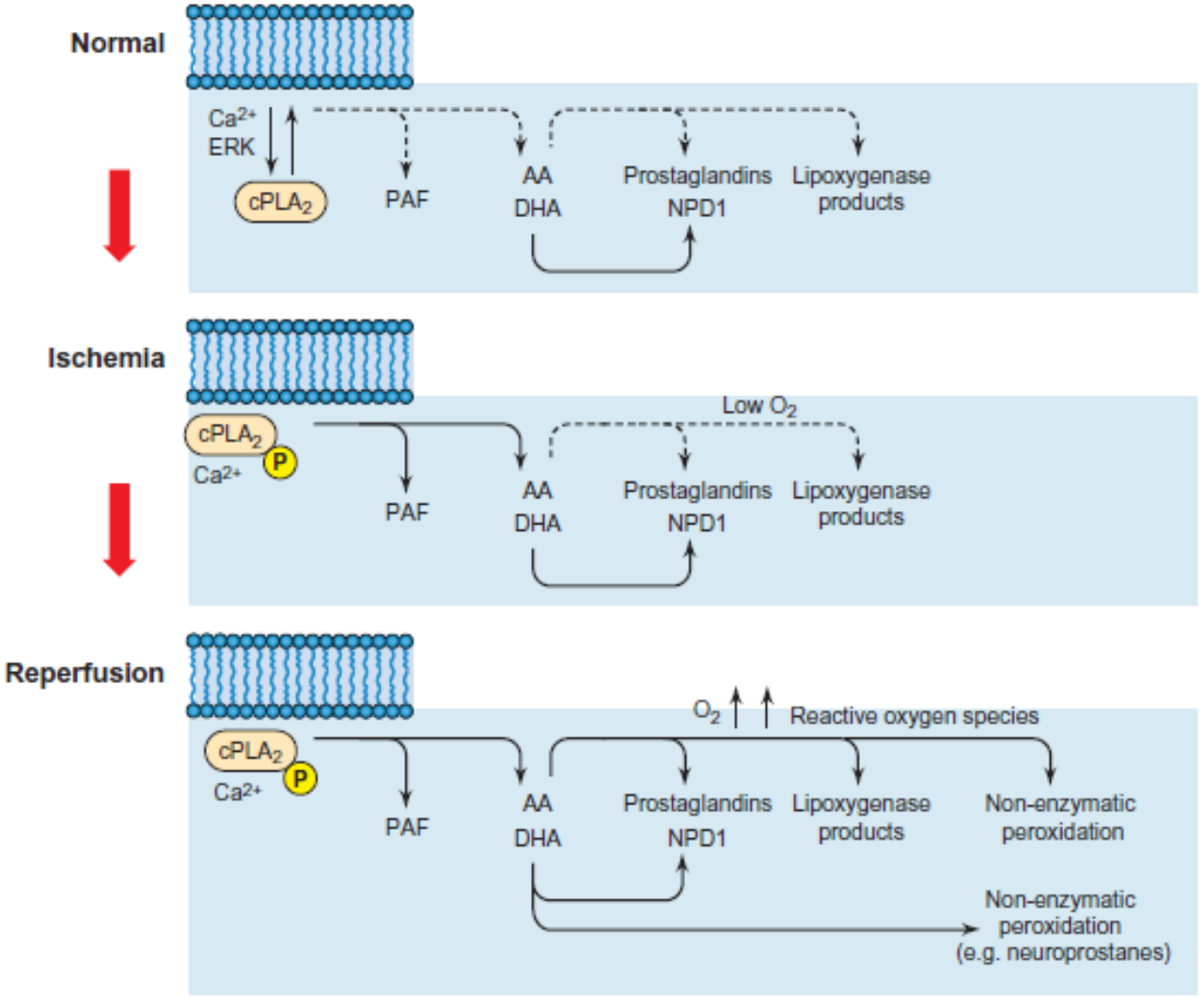


FIGURE 36-7 **Phospholipases A_2 ($cPLA_2$) in the generation of eicosanoids, docosanoids and lipid peroxidation products during brain, retina, or spinal cord ischemia and reperfusion.** During the ischemic phase, phospholipase overactivation and the downregulation of oxidative and energy metabolism, 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.

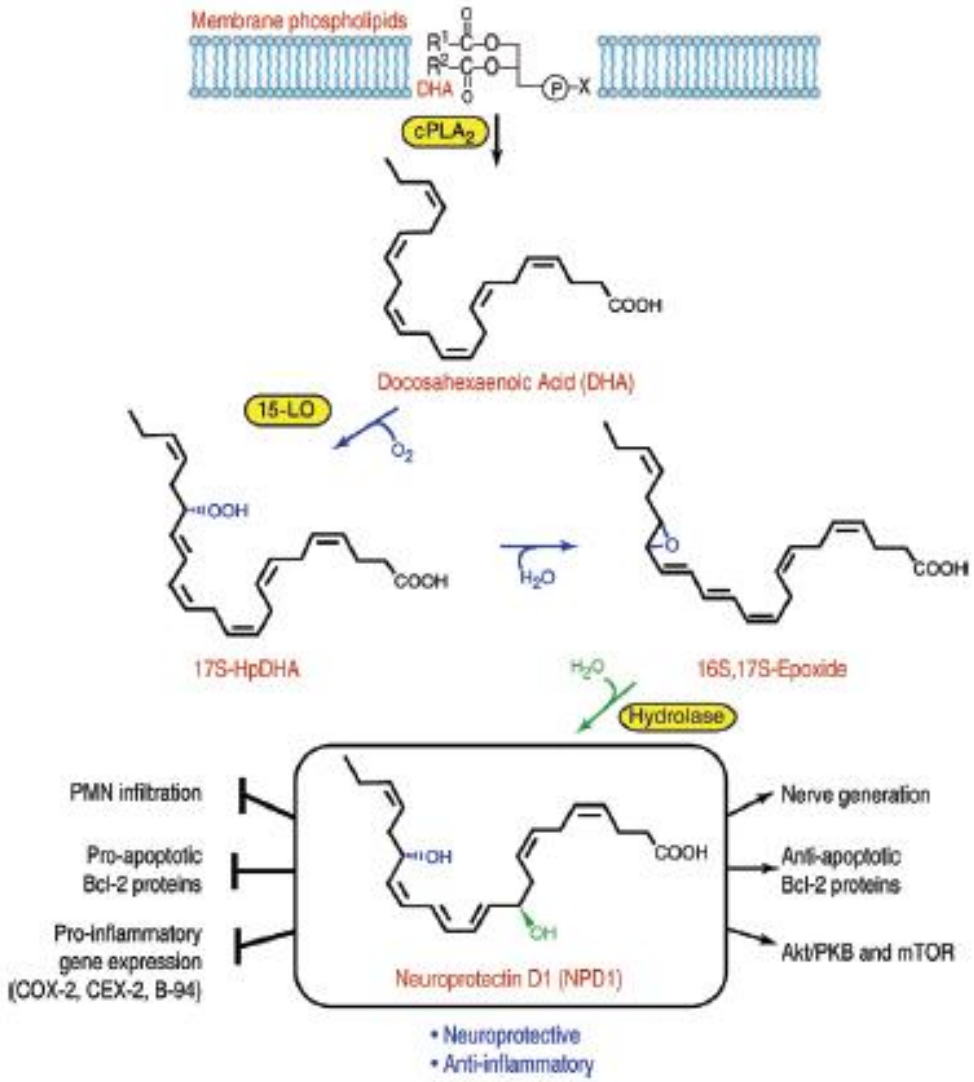


FIGURE 36-8 **Biosynthesis and actions of neuroprotectin D1.**

THE FUTURE OF LIPID SIGNALING IN THE NERVOUS SYSTEM

A. The significance of lipid signaling in the nervous system will be greatly expanded by newer experimental approaches
B. An evolving area is the understanding of the fundamental inner workings of the dendrites, which contain complex intracellular membranes rich in polyunsaturated Phospholipids
C. Although arachidonic acid is widely implicated in signaling in brain, there are several gaps in our understanding of the release of this fatty acid from membrane reservoirs.
D. The knowledge evolving from lipidomic neurobiology will also be potentiated by multidisciplinary approaches such as multiphoton confocal analysis