thematic review

Synaptic lipid signaling: significance of polyunsaturated fatty acids and platelet-activating factor

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Abstract **Neuronal cellular and intracellular membranes are rich in specialized phospholipids that are reservoirs of lipid messengers released by specific phospholipases and stimulated by neurotransmitters, neurotrophic factors, cytokines, membrane depolarization, ion channel activation, etc. Secretory phospholipases A_2 may be both intercellular messengers and generators of lipid messengers. The highly networked nervous system includes cells (e.g., astrocytes, oligodendrocytes, microglial cells, endothelial microvascular cells) that extensively interact with neurons; several lipid messengers participate in these interactions. This review highlights modulation of postsynaptic membrane excitability and long-term synaptic plasticity by cyclooxygenase-2-generated prostaglandin E2, arachidonoyldiacylcylglycerol, and arachidonic acid-containing endocannabinoids. The peroxidation of docosahexaenoic acid (DHA), a critical component of excitable membranes in brain and retina, is promoted by oxidative stress. DHA is also the precursor of enzyme-derived, neuroprotective docosanoids. The phospholipid platelet-activating factor is a retrograde messenger of long-term potentiation, a modulator of glutamate release, and an upregulator of memory formation. Lipid messengers modulate signaling cascades and contribute to cellular differentiation, function, protection, and repair in the nervous system. Lipidomic neurobiology will advance our knowledge of the brain, spinal cord, retina, and peripheral nerve function and diseases that affect them, and new discoveries on networks of signaling in health and disease will likely lead to novel therapeutic interventions.**—Bazan, N. G. **Synaptic lipid signaling: significance of polyunsaturated fatty acids and platelet-activating factor. *J. Lipid Res.* 2003.** 44: **2221–2233.**

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The highly networked organization of the nervous system encompasses a variety of cells that display, as a distinctive feature, one of the largest membrane surface areas of all cells. This would be very clearly illustrated if the plasma membrane from a neuron (e.g., hippocampal CA1 or Purkinje cell of the cerebellum) could be spread out on a flat surface: the branching and complexities of dendrites that form the dendritic spines are very extensive, largely comprising postsynaptic elements. An important additional feature of neuronal dendrites is that they undergo dramatic changes in shape and length during development and learning; they are also affected in aging and in several pathologic conditions. Astrocytes, oligodendrocytes, and microglial cells have very large plasma membrane surface areas as well. To add a further level of complexity, all of these cells extensively interact with neurons. How membranes are organized 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 PUFAs in phospholipids. Currently, in neurons, glia, and endothelial cells of the cerebrovasculature, several phospholipid pools are increasingly being recognized as reservoirs of lipid messengers. Specific lipid messengers are cleaved from these reservoir phospholipids by phospholipases upon stimulation by neurotransmitters, neurotrophic factors, cytokines, membrane depolarization, ion channel activation, etc. 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

To highlight the significance of PUFA and of a bioactive phospholipid, platelet-activating factor (PAF), we provide here examples of lipid signaling in synaptic plasticity and

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memory, as well as in pathologic conditions relevant to retinal degeneration, stroke, epilepsy, and Alzheimer's disease. This article describes certain aspects of a field that is rapidly growing and does not represent a complete account. Only some of the several contributions made to this theme are discussed: omission of papers was unavoidable due to the relative brevity of this article and the large scope of this field.

CYCLOOXYGENASE-2-GENERATED PROSTAGLANDIN E2 MODULATES POSTSYNAPTIC MEMBRANE EXCITABILITY AND LONG-TERM SYNAPTIC PLASTICITY IN HIPPOCAMPAL NEURONS

Brain contains various cyclooxygenases (COXs) for the enzymatic conversion of free arachidonic acid (AA) into PGH2, the precursor of prostaglandins, thromboxanes, and prostacyclin. COX-1 is thought to be constitutively expressed, whereas COX-2 is both constitutive as well as inducible. COX-3 is a third isoenzyme that is highly expressed in nervous tissue. This enzyme seems to be generated by COX-1 intron-1 retention. Partial COX-1a and b proteins were also reported as additional COXs (1, 2).

Basal, "constitutive" COX-2 expression is regulated by synaptic activity and is up-regulated by high-frequency stimulation associated with long-term synaptic plasticity (3). Furthermore, COX-2 is enriched in dendritic spines in excitatory synapses (4). These findings implicate both constitutive and inducible COX-2 in synaptic plasticity.

More direct evidence on the significance of COX-2 in synaptic plasticity was obtained using COX-2 inhibitors and prostaglandins in hippocampal dentate granule neurons (5). These studies demonstrated that selective COX-2 inhibitors, but not COX-1 inhibitors, decrease induction of long-term potentiation (LTP) in hippocampal dentate granule neurons. Prostaglandin E2 (PGE2), but not PGD2 or PGF2α, reverses COX-2 inhibitor-induced suppression of LTP. This is in agreement with the observation that COX-2 preferentially channels the conversion of AA toward PGE2 and prostacyclin (6). PGE2 receptors are expressed in hippocampus, implying that the action of COX-2 inhibition on LTP induction in dentate neurons results from blocking the synthesis of PGE2. Therefore, it is very likely that PGE2 is the effector of COX-2-mediated activity-dependent synaptic plasticity (5).

The phospholipid messenger PAF has been proposed as a retrograde messenger of LTP (7) and a presynaptic site of action has been evidenced (see below). The action of PGE2 on synaptic modification, however, is not on presynaptic endings, but rather on postsynaptic sites. PGE2 regulates membrane excitability by modulating K^+, hyperpolarization-activated current, and tetrodotoxin-resistant Na^+-channel currents in sensory neurons (8–10). Moreover, spatiotemporal correlation of coincidence of postsynaptic firing and excitatory postsynaptic potentials are key in synaptic efficacy (11–14). In fact, decreasing postsynaptic neuron membrane excitability, which leads to a decrease in the number of postsynaptic action potentials during high-frequency stimulation of the presynaptic ending, decreases the probability of LTP induction. It was found that COX-2 inhibition attenuates postsynaptic membrane excitability. Thus, PGE2 derived from the tonic activity of constitutive COX-2 participates in neuronal membrane excitability. One other important factor in LTP is the active propagation of axonally generated action potentials back into the dendrites, where associated dendritic influx plays a significant role (13). Pharmacologic COX-2 blocking decreases back-propagating dendritic action potential-associated Ca^{2+} influx (5). Overall, constitutively inducible COX-2 regulates membrane excitability and activity-dependent LTP induction in hippocampus.

Depolarizing concentrations of K^+ promote active release of AA in cortical neurons (15), retina (16), and synaptosomes (17). One interpretation of these results is that high K^+ promotes a depolarization-dependent Ca^{2+} influx through voltage-gated Ca^{2+} channels. This in turn activates presynaptic glutamate release that modulates excitatory glutamate receptors (15). Thus Ca^{2+} influx through the *N*-methyl-D-aspartate (NMDA) receptor, and direct Ca^{2+} entrance through voltage-dependent calcium channels, activate phospholipase A_2 (PLA_2) to result in AA release via phospholipid hydrolysis. In addition, glutamate may activate metabotropic receptors that, through phospholipase C (PLC) activation, generate diacylglycerol.

Neuronal COX-2 expression is also up-regulated in experimental epileptogenesis, when aberrant synaptic plasticity is thought to be activated. The experimental kindling model resembles aspects of mesial temporal lobe epilepsy (18). Kindling epileptogenesis is triggered by repeated subconvulsive stimulation, which gradually results in intensified seizures. Both COX-2 and $cPLA_2$ expression are activated, indicating that the free AA released is converted into prostaglandins during epileptogenesis (19). 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(s) and/or PAF synthesis, lipid messengers that are both involved in synaptic facilitation (20). 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 (19). Kindling is also a model of bipolar disorders, and valproate, a mood stabilizer, when chronically administered to rats, down-regulates AA conversion into prosta-

glandins by decreasing COX brain expression (21). Lithium, also used to treat bipolar disorders, potentiates brain AA signaling (22).

Astrocytes are also active in the synthesis of prostaglandins, particularly in response to interleukin-1β (IL-1β) (23). Astrocytes form an intimate sheath around synapses and increase their intracellular Ca^{2+} content when excitatory neurotransmission is activated (24). As a consequence, there is a calcium-dependent glutamate release from astrocytes (25), an event that is activated by prostaglandins (**Fig. 1**). Thus, these lipid messengers may be an intercellular signal of an integrated glutamatergic cross-talk between neurons and astrocytes (25). At present there are several evolving concepts on the nature of the signaling that allows neurons and astrocytes to communicate. In fact, this communication is bidirectional, and also has consequences on activity-dependent modulation of cerebral blood flow (26, 27) through multiple astrocyte signaling, including possible cytochrome P450 epoxygenase, which synthesizes epoxyeicosatrienoic acids that in turn elicit vasodilatory properties on the cerebral microcirculation (28). Astrocytes also release AA in response to oxidative stress by activation of $cPLA_2$ and Ca^{2+}-independent PLA_2 ($iPLA_2$) (29). These cells have complex regulatory mechanisms for $cPLA_2$ activation involving protein kinase C (PKC) and MAP kinases (30). Moreover, the astrocytes' response to proinflammatory cytokines includes induction of secretory PLA_2 ($sPLA_2$) expression (31).

20:4-DAG IN SYNAPTIC PLASTICITY

Another example of lipid signaling in synaptic function involves the messengers released from inositol lipids. The excitatory neurotransmitter glutamate, in addition to activating PLA_2, activates PLC through G-protein-linked glutamate metabotropic receptors. As a consequence, the pool size of DAG increases as does that of inositol-1,4,5-trisphosphate (IP3). The DAG are complex in terms of their fatty acid composition because their pool size is also modulated by another phospholipase (PLD)-mediated degradation of phosphatidylcholine (PC) to phosphatidic acid (PA), followed by lipid phosphate phosphatase-mediated generation of DAG. Now we know that cellular compartments are made up also by scaffold/anchoring proteins that contain anchoring sites for multiple enzymes of convergent as well as divergent signal-transduction pathways. Thus, the fatty acid composition analyzed in a tissue or cell extract does not represent the specificities of subcellular signaling. Inositol lipid-derived DAG display, as

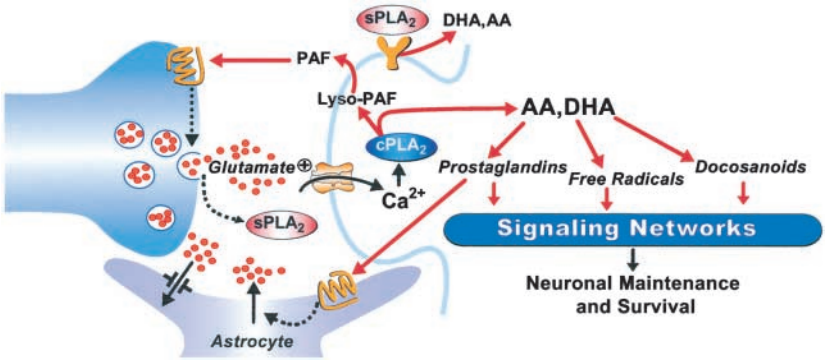

Fig. 1. Simplified cartoon of phospholipases A_2 at the synapse. A depolarizing stimulus at the presynaptic terminal triggers glutamate release. Glutamate binds to the *N*-methyl-D-aspartate receptor and as a consequence an influx of calcium in the postsynaptic neuron occurs. Calcium-mediated activation of the cytoplasmic PLA_2 results in the release of arachidonic acid (AA), docosahexaenoic acid (DHA), and lyso-platelet-activating factor (PAF), the PAF precursor. Although PAF has a very short biologic 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 in this diagram). Cell surface PAF-receptor antagonists confer neuroprotection during ischemia-reperfusion and inhibit PAF-induced glutamate release from hippocampal neurons and CA1 long-term potentiation 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_2 ($sPLA_2$) may be released from the presynaptic terminal (63): $sPLA_2$ binding sites are present in neurons (55–58), and $sPLA_2$ promotes active AA remodeling in neurons in culture (59) and may also promote DHA release. DHA may also be released by $cPLA_2$. Free DHA may subsequently follow enzyme-mediated oxygenation pathways and lead to the synthesis of docosanoids, messengers made in the retina (94) and brain (95). 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^{2+}-dependent mechanism (25). For the sake of simplicity, endocannabinoids and the CB_1 receptor are not included.

the prevalent molecular species, arachidonoyl-stearoyl-glycerol. This composition also corresponds to the major molecular species of the membrane reservoir, phosphatidylinositol-4,5-bisphosphate (PIP2), critical in inositol lipid signaling.

Inositol lipid synthesis in neurons is maintained by a de novo route through PA, and the DAG kinase step contributes to their replenishment (by its product PA) after synaptic activity-induced PIP2 hydrolysis. Thus, the DAGε (specific for 20:4-DAG, see below) becomes critical for the synthesis of PIP2 containing 20:4.

Diacylglycerols are messengers that modulate certain PKC isoforms, whereas IP3 releases Ca^{2+} from intracellular stores. DAG targets may discern DAG fatty acid composition (32). Thus, DAG generated from PC through PLD has different fatty acid composition from that generated from PIP2. PC at *sn*-2 is mainly oleate, 18:1,n-3 (33), as compared with arachidonate, which is the major *sn*-2 acyl chain in PIP2. During seizures, the main DAG that transiently accumulates in brain is 20:4-DAG (34–36); however, early and late DAG peaks display different fatty acid composition, strongly implying sources other than PIP2. Thus, it is clear that the fatty acid composition of inositol lipids is important in the generation of 20:4-DAG. To achieve enrichment in 20:4 in PIP2, enzymes for inositol lipid synthesis selectively employ 20:4-containing lipids as substrates (37). A critical enzyme is DAG kinase, which catalyzes the phosphorylation of DAG to PA. There are nine genes encoding these enzymes. DGKε, which selectively phosphorylates 20:4-DAG, when deleted has helped to dissect several of these biochemical and neurobiological issues (38).

DGKε brain cellular distribution as mGluR1 is localized in Purkinje cerebellar neurons, mitral cells of the olfactory bulb, hippocampal interneurons, and neurons of the thalamus and substantia nigra (39, 40). The neurotransmitter glutamate activates G-protein-coupled mGluR1, promoting PIP2 degradation by PLC and the subsequent activation of PKCβ. DGKε-deficient mice displayed a marked down-regulation of 20:4-inositol lipid signaling when exposed to electroconvulsive shock (ECS). This was reflected by lower PIP2 degradation and lower accumulation of DAG, free arachidonic, and stearic acids after ECS. These studies on DGKε suggest that this signaling pathway participates in synaptic function, long-term potentiation, seizures, and neuroprotection.

ARACHIDONIC ACID-CONTAINING MESSENGERS IN THE PSYCHOACTIVE ACTIONS OF ENDOCANNABINOIDS

N-arachidonoyl ethanolamine and 2-arachidonoyl glycerol are endogenous ligands (endocannabinoids) of the G-protein-coupled CB_1 receptor, to which the main psychoactive component of marijuana, tetrahydrocannabinol, binds. Interestingly, the cellular distribution in brain of the membrane-associated enzyme fatty acid amide hydrolase is complementary to that of the CB_1 receptor (41, 42). This accounts for the fact that the half-life of *N*-arachidonyl ethanolamine (anandamide) is only a few minutes (43). Thus, this hydrolytic enzyme degrades a potent lipid messenger that modulates neuronal function, as is also the case with PAF acetylhydrolase (discussed below).

Endocannabinoids are postulated to signal another mode of transynaptic interaction, the "depolarization-induced suppression of inhibition" (44–46). This is a retrograde mode of communication, where endocannabinoids disinhibit neuronal activity by blocking neurotransmitter release from gamma aminobutyric acid (GABA) ergic neurons (47). Consequently, the outcome is stimulation of neurotransmission. In different neuronal circuits, endocannabinoids may exert excitatory or inhibitory modulatory actions (46, 48).

$sPLA_2$: AN INTERCELLULAR MESSENGER

We refer to PLA_2 in several parts of this article. These enzymes represent a growing family of enzymes that overall include the higher molecular weight enzymes, the $cPLA_2$s, the lower molecular weight secretory $sPLA_2$, and the Ca^{2+}-$iPLA_2$ (49–52). At present, there is still an incomplete understanding of the properties and significance of these enzymes in the nervous system. When the $cPLA_2$ was deleted, there was an increased resistance to cerebral ischemia, indicating an important function of this enzyme in ischemic neuronal damage (53). Newer, noninvasive imaging approaches to study brain PLA_2 have recently linked the activation of this enzyme with 5-HT(2a) receptors (54).

$sPLA_2$s binding to N (neural)- and M (muscle)-type receptors were discovered by using various purified snake and bee venom $sPLA_2$s as ligands (55–58). The activation of $sPLA_2$ receptors in primary neurons in culture, using purified $sPLA_2$ as a ligand, promotes a synergy with glutamate, inducing neuronal cell death (59). Moreover, AA metabolism (59), COX-2 activation (60), ischemic neuronal survival (61), and calcium influx (62) are clearly engaged in this response. Figure 1 outlines the potential involvement of $sPLA_2$ at the synapse. It has been suggested that $sPLA_2$ is coreleased along with neurotransmitters (63). This issue needs to be further explored in relation to specific synapses. At any rate $sPLA_2$ in the intercellular space is able to generate lipid mediators, and the enzyme protein itself, when secreted, becomes a messenger. It is of interest that the consequences of $sPLA_2$ acting on cells is the release of lipid messengers, either on the cell surface or intercellularly. It is not clear yet how $sPLA_2$s may act on astrocytes, microglia, and neurons, or whether they are restricted to some cells.

Another remarkable case of a lipid-metabolizing enzyme that is present in the extracellular space is the major component of the cerebrospinal fluid, prostaglandin D synthase (64–66).

$sPLA_2$ receptor activation in neurons induces sustained modifications in neuronal metabolism of arachidonoyl-phospholipids, although the specific messengers formed are not yet defined. $sPLA_2$ receptor activation and

glutamate synergize AA mobilization and neurotoxicity. Therefore, excitotoxicity may not only involve glutamate, as often assumed, but also $sPLA_2$ (59). It is also possible that $sPLA_2$ may have modulatory physiological actions at the synapse and other sites of the nervous system.

DOCOSAHEXAENOIC ACID IN NEURONS AND PHOTORECEPTORS

Docosahexaenoic acid (DHA) is a major PUFA in the central nervous system. Brain and retina contain the highest content of DHA in any tissues. There is still a void in knowledge about the significance of DHA. Here we highlight some aspects of DHA in brain and retina that may clarify its participation in cell function and in neurodegenerations. DHA is used continuously for the biogenesis and maintenance of neuronal and photoreceptor membranes. This system is supported by the liver, which supplies DHA incorporated into plasma lipoproteins, and is directed to the retina through choriocapillaries (67–71). The uptake of DHA by the retina involves the retinal pigment epithelium (RPE) and its delivery to photoreceptors (68–70, 72–74). During the daily photoreceptor-renewal process, an active recycling of phagosome-derived DHA by RPE via the interphotoreceptor matrix retains DHA within photoreceptors (73, 75, 76). Endothelial cells and RPE (69, 70) are able to synthesize DHA from dietary precursors, and levels of n-3 fatty acids, such as 18:3n-3 and 20:5n-3, are very low in plasma; therefore, the retina must rely largely on DHA supplied by the liver, either derived from dietary sources or synthesized in the liver from 18:3n-3. A signal generated in brain or retina to control DHA delivery from the liver has been postulated (67); 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 neurodegenerations result in loss of DHA from excitable membranes and replenishment of DHA is needed (67, 71).

Rhodopsin in photoreceptors is immersed in a lipid environment highly enriched in phospholipids containing DHA. Some DHA is found in close association with rhodopsin (74, 77) and DHA is essential for rhodopsin function (78–80). Moreover, retinal DHA, like brain DHA, is very resistant to n-3 fatty acid dietary deprivation.

Because of this constant systemic flow of DHA via a "long loop," it is highly relevant that retinitis pigmentosa (RP) is associated with alterations in lipoprotein metabolism (73). The most frequently reported phenotype is low plasma and red blood cell levels of DHA (81–84). In Usher's syndrome, an autosomal recessive RP that is associated with hearing loss, lower plasma levels of 22:6- and 20:4-phospholipids are also found (85). Interestingly, changes show a direct correlation to 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 (86).

Low plasma levels of 22:6n-3 in RP patients have also been interpreted as a mechanism to protect the retina from oxidative damage resulting from photochemical function of rhodopsin (87). Stress signals originating from retinas undergoing degeneration (e.g., RP) may shut off the communication between the retina and the liver (67), reducing the systemic liver supply of 22:6n-3. In dogs with progressive rod-cone degeneration (*prcd*) (88) as well as in other retinal-degeneration models (87), 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 (89), and dietary supplementation with DHA failed to prevent the loss of photoreceptor DHA and the *prcd* phenotype (88). Moreover, cultured hepatocytes from *prcd* dogs display higher accumulation of 22:6-phospholipids in the liver and impaired hepatocyte secretion of DHA-containing VLDLs (85), 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 RP patients is not known.

DHA: TARGET OF FREE RADICAL-CATALYZED PEROXIDATION AND PRECURSOR OF DOCOSANOIDS

DHA-containing phospholipids are a target for lipid peroxidation, and as a result of free radical-catalyzed peroxidation, F4-isoprostanes are formed (90, 91). F2-isoprostanes are also derived from free-radical-catalyzed peroxidation, although from AA instead (92). F4-isoprostanes are found esterified in phospholipids, and it has been reported that their content is increased in the brains of patients with Alzheimer's disease (93).

Docosanoids, enzyme-derived DHA metabolites, were identified initially in the retina (94), and have been proposed to elicit neuroprotective actions (78). Very recently it was ascertained by tandem liquid chromatography-photodiode array-electrospray ionization-mass spectrometric-mass spectrometry (LC-PDA-ESI-MS-MS)-based lipidomic analysis that the synthesis of DHA-oxygenation messengers occurs during brain ischemia-reperfusion (95). Two DHA-oxygenation pathways operate: one that gives rise to 10,17*S*-docosatriene, and the second that results in the synthesis of resolvin-type messengers (17*R*-DHA). Resolvins were elegantly identified outside the nervous system as a response to aspirin treatment (96). The brain resolvins are also made upon treatment with aspirin (95). Both DHA-oxygenation pathways generate messengers that are counter-proinflammatory signals (96–98). The docosanoid 10,17*S*-docosatriene is a potent inhibitor of brain ischemia-reperfusion-induced polymorphonuclear leukocyte (PMN) infiltration, as well as of nuclear factor-κB (NF-κB) and COX-2 expression (95). Moreover, marked attenuation of the stroke volume was observed when 10,17*S*-docosatriene was infused into the third ventricle during ischemia-reperfusion (95). Discovery of these 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. In particular, the very high biological activity of the docosanoid 10,17*S*-docosatriene marks it as potential effector of neuroprotection.

PAF-MEDIATED CELL SIGNAL TRANSDUCTION IN THE NERVOUS SYSTEM

Excitatory synaptic neurotransmission promotes activation of phospholipases that in turn cleave synaptic membrane phospholipid reservoirs, releasing biologically active moieties: the lipid messengers. One such reservoir is the phospholipids that typically contain an alkyl-acyl chain in the C1 position and either arachidonate (20:4) or docosahexaenoate (22:6) in the C2 position of the glycerol backbone, and that have a phosphorylcholine ester. These membrane phospholipids are the PCs, and are the target of phospholipases A_2, which cleave the fatty acid linked to C2. The products of this reaction are lyso-PAF and a free PUFA, either arachidonic or DHA. Subsequently, lyso-PAF is acetylated and its product, PAF, is a potent phospholipid messenger (remodeling pathway; **Fig. 2**). Therefore, PAF, like prostaglandins and other lipid messengers, is not maintained in membranes as a preformed structure; rather, it is rapidly synthesized upon specific stimulation.

PAF is a physiologic mediator that modulates glutamate release and acts as a retrograde messenger of LTP (7, 99–101). PAF modulates neuronal calcium ionization through the PAF receptor (102) and is also generated by NMDA receptor activation. PAF functions are thought to be modulated by synthesis and degradation of the lipid messenger by tightly regulated enzymes. In fact, brain contains several PAF acetylhydrolases (PAF-AH) (103), which rapidly remove the acetate moiety from C2 (see below), thus rendering PAF biologically inactive. The existence of these "off-signal" enzymes highlights the fact that PAF is a potent messenger (104).

PAF and the free PUFAs released during PAF synthesis accumulate in brain upon stimulation (105). In the retina and in neurons in culture, neurotransmitters trigger Ca^{2+}-independent de novo PAF synthesis through cytidine 5′-diphosphocholine (106, 107). Since PAF stimulates ATP release from PC12 cells, it has been implicated in vesicular release of neurotransmitters (108), perhaps through a mech-

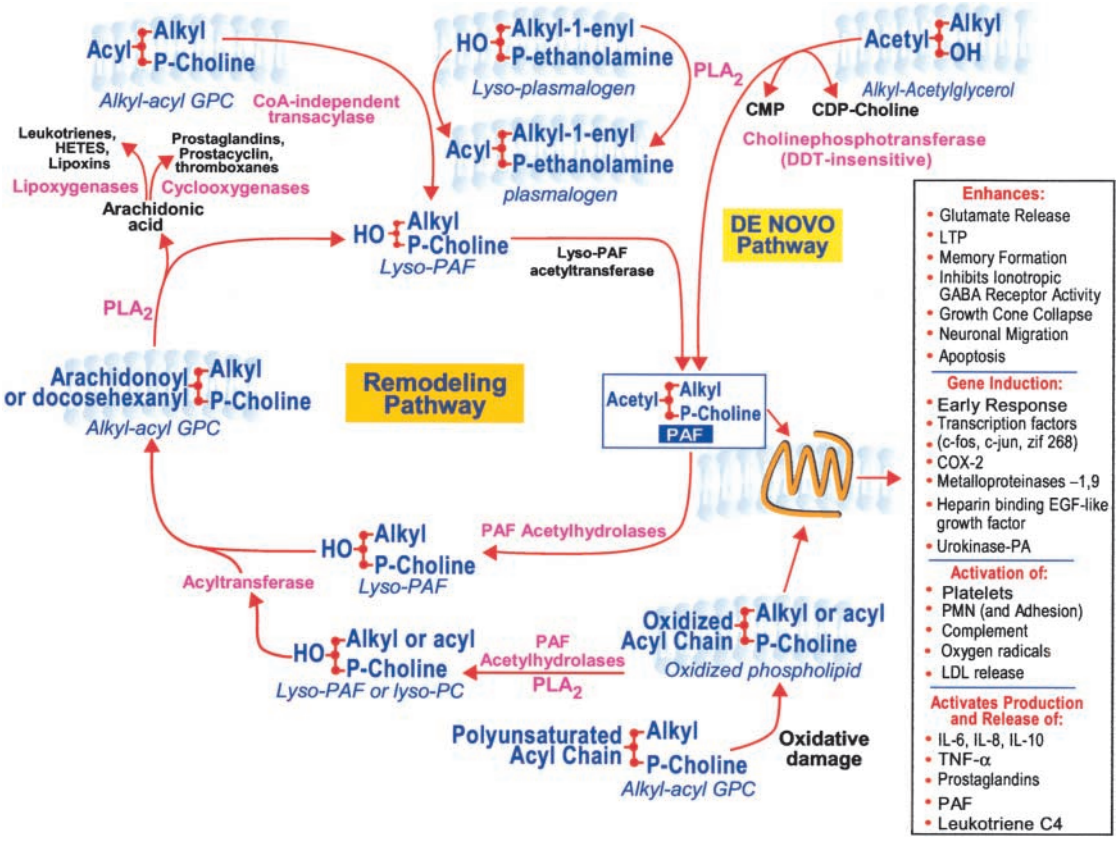

Fig. 2. Pathways of PAF synthesis and degradation. In the center of this figure is the remodeling pathway or "PAF cycle" from the membrane phospholipid PAF precursor, alkyl-acyl-glycerophosphorylcholine (GPC, on the left) to the the biologically active PAF (at right). The remodeling route includes the production of lyso-PAF, which is generated from the PAF precursor alkyl-acyl-GPC either directly by the action of phospholipase A_2 or by the transfer of the *sn*-2 acyl moiety to a "donor" lyso-plasmalogen (top), which is itself mobilized from membrane plasmalogen by phospholipase action. The de novo route of PAF synthesis (upper right) involves the direct transfer of a choline moiety to alkyl-acetylglycerol. Note that PAF acetylhydrolase (bottom) inactivates all PAF molecules, regardless of their biosynthetic route, and additionally inactivates oxidatively damaged phospholipids (shorter peroxidated acyl group at C2) that possess biological activity at the PAF receptor.

anism analogous to that engaged in vesicular release of vasoactive substances from platelets and eosinophils (109).

PAF is rapidly degraded by deacetylation to the biologically inactive lipid, lyso-PAF. Several PAF-AH are expressed in the central nervous system (99). Use of the stable PAF analog, methyl carbamyl-PAF (mc-PAF), allows the study of PAF action while avoiding enzyme-mediated inactivation. Bath-applied mc-PAF specifically augments hippocampal excitatory synaptic transmission using synaptic pairs of neurons in culture (100). This study also demonstrated that PAF action is receptor mediated and presynaptically localized, and supported the notion that PAF is a second messenger in the central nervous system. Additional mc-PAF enhanced the amplitude of evoked postsynaptic currents, while diminishing the size of presynaptic action potentials. Interestingly, the mc-PAF effect on excitatory synaptic transmission is very selective, since it does not increase general presynaptic function (100). Also, c-PAF elicts these actions independently of postsynaptic glutamate receptors. Moreover, mc-PAF augmented the frequency of spontaneous miniature excitatory synaptic events, sparing their amplitude and their time course.

Retrograde messengers of LTP are candidates to up-regulate memory. Retrograde messengers released from the postsynaptic neuron are thought to enhance excitatory neurotransmitter release from the presynaptic neuron; this is believed to increase synaptic efficiency of LTP. The signaling that links the postsynaptic neuron to presynaptic modulation of neurotransmitter release is in accord with the Hebbian concept of synaptic potentiation. Several mediators have been postulated to be retrograde messengers, including arachidonic acid, nitric oxide, carbon monoxide, and PAF. In the case of arachidonic acid, even though this fatty acid is released and increases excitatory synaptic transmission when coupled with presynaptic stimulation, it requires relatively high concentrations (110), and in CA1 neurons, AA effects are blocked by DL-2-amino-phosphopentanoic acid (APV) (111, 112), consequently limiting arachidonic acid's potential as a retrograde messenger.

PAF, on the other hand, fulfills several aspects of a retrograde messenger in LTP: it is synthesized in brain during stimulation as well as in neurons in culture, it activates hippocampal excitatory synapses by increasing presynaptic glutamate release, and it augments hippocampal CA1 LTP.

Infusion of PAF into specific brain regions promotes memory facilitation in rats. In experiments using the step-down inhibitory avoidance or spatial-habitation task, PAF infused 10 min before training or immediately after training enhanced retention scores. However, PAF infused 60 min post-training had no effect on retention (113). In PAF-receptor-deficient mice, marked attenuation of LTP was observed. Infusion of PAF into brain regions enhanced memory formation in a time-dependent, neuroanatomically specific fashion. A presynaptic PAF-receptor antagonist had a clear amnesic effect in several memory-task tests.

Post-training intrahippocampal infusion of intracellular forms of the PAF receptor did not modify memory for an inhibitory avoidance task (113, 114). However, post-training injection of PAF into either amygdala, hippocampus, or entorhinal cortex enhances memory in an inhibitory avoidance task (113), whereas injection of a PAF-receptor antagonist that selectively targets the presynaptic PAF receptor impairs memory in this task. Moreover, post-training intrahippocampal and intradorsal striatal injections of either PAF or PAF-receptor antagonists modulate memory in hidden and visible platform water-maze tasks, respectively (115–117).

The NMDA-receptor antagonist MK-801 markedly attenuated the memory-enhancing effects of intradorsal striatal infusion of PAF on the visible platform water-maze task. On the other hand, in a hidden platform water-maze task, intrahippocampal infusion of PAF blocks the memory-perturbing actions of MK-801. The mechanism allowing interactions between the phospholipid messenger and the NMDA receptor in the mnemonic functions of hippocampus and striatum may underlie the participation of the intracellular form of the PAF receptor or of signaling cascades evolving from the cell-surface PAF receptor to transcription factors and genes (118).

PAF-receptor antagonists block LTP in rat hippocampal CA1 neurons, dentate gyrus, and medial vestibular nuclei. PAF itself increases excitatory postsynaptic responses and the frequency of spontaneous excitatory postsynaptic currents, and enhances the receptor-mediated release of the excitotoxic amino acid, glutamate, from presynaptic terminals. Excitatory synaptic transmission is modulated by a variety of signaling systems, including the inhibitory neurotransmitter GABA. In a study of the effects of PAF on GABA neurotransmission in hippocampal neurons in primary culture, extracellular PAF reduced GABA-gated chloride ion current in more than 65% of cells while enhancing it in ~23% of cells. This heterogeneous modulation of inhibitory neurotransmission may be the result of GABA receptor subtypes differing in their subunit composition or in phosphorylation sites responding to intracellular signaling molecules that transduce the extracellular PAF signal to the GABA receptor, or it may be the result of allosteric effects on the receptor regulatory sites (119).

Because PAF predominantly inhibits ionic GABA-receptor activity in hippocampal neurons, it is not surprising that PAF enhances excitatory presynaptic glutamatergic neurotransmission as well as decreases inhibitory GABAergic postsynaptic activity (119).

The observation that PAF-receptor antagonists elicit neuroprotection in the gerbil brain ischemia-reperfusion model (120) suggested that PAF may work at the synapse. In this model, the ischemia-induced accumulation of free PUFA was decreased by the PAF antagonists that were also neuroprotective by restoring cerebral blood flow (120). The ischemia-induced PUFA release is believed to result from synaptic phospholipase(s) A_2 activity (116). In addition, the PAF antagonist shown to be neuroprotective in the gerbil ischemia-reperfusion model also selectively displaced radiolabeled PAF binding from synaptic membranes (121). This synaptic membrane binding site was therefore suggested to be the modulator of glutamate neurotransmitter release (100). Moreover, intracellular PAF-binding sites identified in a microsomal fraction were distinct from those at the synaptic membrane fraction in-

sofar as responses to several antagonists (121). At that time it was known that PAF activates early-response genes (122–124), therefore the intracellular receptor was thought to be the signaling linkage to the nucleus. Cloning of the seven-transmembrane domain PAF receptor (125–127) and the discovery of specific PAF receptor-mediated Ca^{2+} influx into neurons (102) further illuminated neural PAF signaling. In studies of LTP in hippocampal neurons from PAF-receptor-deficient mice, both incidence and size of LTP, defined as increased excitatory postsynaptic potentials, were attenuated in PAF-receptor-knockout mice, as compared with LTP in wild-type mice. Moreover, PAF receptor-deficient mice display a marked attenuation of LTP upon stimulation of the lateral, but not the medial, perforant path (128). In another study, PAF-deficient mice had unaltered LTP; however, there were substantial differences between the experimental conditions of the two studies (129). Furthermore, PAF-receptor antagonists reduced LTP in wild-type mice, but not in PAF-receptor knockout mice. These results further support the hypothesis that PAF is involved in hippocampal synaptic plasticity (128). Most recently, the intracellular PAF receptors were further characterized. One form is confined to the endosomes (130) and the other to the nuclear membrane (131). Both intracellular forms of the receptor may be part of the intracellular microsomal form described earlier (121). At any rate, we know that neurons, astrocytes, and microglia, as well as endothelial cells, express the PAF seven-transmembrane domain receptor. It remains to be defined whether the intracellular PAF receptor(s) have a different molecular structure or whether they are in fact an intracellular state of the cell-surface PAF seven transmembrane domain receptor. Does the cell-surface PAF receptor internalize? Is the cell-surface PAF receptor destined to insertion in the membrane already active, and if so, can PAF itself internalize to access either of these PAF-receptor forms located inside the cell? Finally, regarding PAF's transcriptional actions, at least two mechanisms are possible: the cell surface receptor triggers the signaling cascade, or the intracellular form establishes interactions with specific kinases/phosphatases or transcription factors with or without specific scaffolding proteins.

During ischemia, seizures, and in other pathologic conditions involving oxidative stress, the rates of PAF synthesis and degradation no longer maintain a modulated PAF pool size; consequently, PAF concentration increases and it becomes a pro inflammatory messenger and a mediator of neurotoxicity. As such, PAF activates COX-2 expression (132) as well as that of several early-response genes that encode transcription factors (122, 123). PAF activates apoptosis and PMN and their adhesion to the microvasculature (109). This event has critical consequences for cell survival. Leukocyte infiltration mediates neural injury in head trauma, stroke, spinal cord injury, and other diseases. PAF also enhances the synthesis and release of IL-6, IL-8, IL-10, tumor necrosis factor-α, and of other mediators of the inflammatory response. PAF activates phospholipases through its receptor, resulting in additional PAF synthesis, along with that of prostaglandins and leukotrienes. Overall, PAF is a potent neuronal injury messenger. PAF also plays a prominent role in astrocytes and in microglial cells. Many of these actions have been studied in nonneural cells and are assumed to occur in the nervous system as well. Therefore, excessive PAF promotes neuronal damage, and PAF-receptor antagonists elicit neuroprotection in various models of neural injury (120, 133–137).

PAF DEGRADATION IN THE CENTRAL NERVOUS SYSTEM

Multiple PAF-degrading enzymes in the central nervous system further highlight the importance of maintaining a well-controlled PAF pool size. These include tissue PAF-AH (103) as well as plasma-type PAF-AHs (109). One plasma-type PAF-AH of molecular mass 45 kDa is a form of $sPLA_2$. There is also an intracellular form, PAF-AHIb, isolated from brain. PAF-AHIb is a heterotrimer with two catalytic subunits (α1, 29 kDa and α2, 30 kDa) and one regulatory β subunit (45 kDa); the latter gene is analogous to the LIS1 gene, which causes lissencephaly in Miller-Dieker syndrome (104). Because lissencephaly results from defects in neuronal cell migration, PAF-AHIb may modulate neuronal migration during nervous system development. Moreover, PAF has been found to promote growth-cone collapse, implicating further the phospholipid messenger in developmental neurobiology (100).

PAF-AH cleaves the acetate moiety of PAF and comprises an "off" signal when PAF is no longer needed to perform a function or when the concentrations of PAF become neurotoxic. We tested this hypothesis using recombinant plasma-type PAF-AH (rPAF-AH) in primary neuronal cultures, and found that rPAF-AH in fact elicits neuroprotection (138). The human form of PAF-AH is effective in inhibiting inflammation in nonneural cells (139). rPAF-AH elicits concentration-dependent neuroprotection against NMDA-induced apoptosis in hippocampal neurons in primary culture (138). The criteria used in this study included electron microscopy to monitor nuclear condensation, histone release, TUNEL staining, and DNA laddering. Therefore, rPAF-AH may be an alternative to PAF antagonists in regulating pathologic accumulation of PAF in several conditions involving excitotoxicity, including epileptic brain damage, head injury, stroke, glaucoma, and neurodegenerative diseases. The development of these concepts for therapeutic interventions may be helpful because rPAF-AH is relatively resistant to proteolytic degradation, contributing to sustained bioavailability after administration. Also rPAF-AH is able to hydrolyze oxidized phospholipids, which are also recognized by the PAF receptor (109). PAF-like oxidized phospholipids accumulate in several pathologic conditions that involve oxidative stress.

LIPIDOMIC NEUROBIOLOGY ON THE HORIZON

The significance of PUFAs and phospholipids in the nervous system, particularly their specific involvement in

cell signaling, will be greatly expanded by newer experimental approaches. Some of the new avenues of exploration in the nervous system are also applicable to other tissues. Furthermore, newer experimental approaches are already here, and will soon be expanded. 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, 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 (140). The detailed composition of lipid classes and molecular species can now be approached in a more accurate and efficient manner. Moreover, the detailed identification of changes in the lipidome during the development, function, aging, and dysfunctions of the nervous system will be tackled.

An example of an evolving area of the neurosciences is the understanding of the fundamental inner workings of the dendrites, which contain complex intracellular membranes rich in polyunsaturated phospholipids. 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.

Although AA is widely implicated in signaling in brain, there are several gaps in our understanding of the release of this fatty acid from membrane reservoirs. Reports that AA is released primarily by G-protein-mediated PLA_2 activation remain to be confirmed (141, 142). In addition, modulation of PLA_2 by Ca^{2+} and protein kinase needs to be better defined. It is clear that NMDA-receptor activation promotes the release of AA (143), and that a variety of eicosanoids are then generated. The modulatory events that channel AA toward 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 (144, 145).

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, above and beyond its being a target for nonenzymatic peroxidation under various pathologic 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 (95). 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 might become a base for new 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 also be potentiated by multidisciplinary approaches (e.g., 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 (25). Microglia also actively make prostaglandins in response to injury (146–148), although prostaglandin E2 may be engaged in neuroprotective mechanisms (149). Moreover, the renewed interest in defining the significance of nonneuronal cells in the nervous system (150–152) will be greatly enhanced by lipidomic approaches. Overall, the near future will witness breakthroughs using lipidomic neurobiology to more precisely define synaptic lipid signaling that will contribute to a renewed vista of the function of the nervous system as well as of the neurobiology of diseases.

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