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Lipid Signaling in Neural Plasticity, Brain Repair, and Neuroprotection

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Abstract

The extensive networking of the cells of the nervous system results in large cell membrane surface areas. We now know that neuronal membranes contain phospholipid pools that are the reservoirs for the synthesis of specific lipid messengers on neuronal stimulation or injury. These messengers in turn participate in signaling cascades that can either promote neuronal injury or neuroprotection. Prostaglandins are synthesized as a result of cyclooxygenase activity. In the first step of the arachidonic acid cascade, the short-lived precursor, prostaglandin H2, is synthesized. Additional steps in the cascade result in the synthesis of an array of prostaglandins, which participate in numerous physiological and neurological processes. Our laboratory recently reported that the membrane polyunsaturated fatty acid, docosahexaenoic acid, is the precursor of oxygenation products now known as the docosanoids, some of which are powerful counter-proinflammatory mediators. The mediator 10,17S-docosatriene (neuroprotectin D1, NPD1) counteracts leukocyte infiltration, NF-κ activation, and proinflammatory gene expression in brain ischemia-reperfusion and is an apoptostatic mediator, potently counteracting oxidative stress-triggered apoptotic DNA damage in retinal pigment epithelial cells. NPD1 also upregulates the anti-apoptotic proteins Bcl-2 and Bcl-xL and decreases pro-apoptotic Bax and Bad expression. Another biologically active messenger derived from membrane phospholipids in response to synaptic activity is platelet-activating factor (PAF). The tight regulation of the balance between synthesis (via phospholipases) and degradation (via acetylhydrolases) of PAF modulates the functions of this lipid messenger. Under pathological conditions, this balance is tipped, and PAF becomes a proinflammatory mediator and neurotoxic agent. The newly discovered docosahexaenoic acid signaling pathways, as well as other lipid messengers related to synaptic activation, may lead to the clarification of clinical issues relevant to stroke, age-related macular degeneration, spinal cord injury, Alzheimer's disease, and other diseases that include neuroinflammatory components.

Index entries: Cyclooxygenases; diacylglycerol kinase ε; docosahexaenoic acid; docosanoids; neuroprotection; platelet-activating factor; prostaglandins.

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Introduction

I was honored and delighted to present the First Leon Wolfe Killam Lecture because, from early in my career, although I never had the pleasure of working with him, I enjoyed a great friendship and many interactions with Dr. Wolfe. In fact, Dr. Wolfe's support and encouragement were the primary motivation for me to move to Toronto, where I set up my first laboratory at the Clarke Institute of Psychiatry. Thereafter, he and I frequently met at conferences. This article is a "progress report" on several lipid signaling studies that Dr. Wolfe and I discussed over the years *(1)*.

A distinctive feature of the nervous system is its highly networked organization caused by the variety of intervening cells. The result is one of the largest membrane surface areas of all cells. This can be illustrated by imagining the plasma membrane from a neuron (e.g., hippocampal CA1 or Purkinje cell of the cerebellum) spread out on a flat surface: the extensive branching and complexities of dendrites comprising the dendritic spines are largely composed of postsynaptic elements. Neuronal dendrites undergo profound changes in shape and length during development and learning, and they are also affected in aging and in pathological conditions such as Alzheimer's disease, in which neurodegeneration promotes selective loss of synapses. Astrocytes, oligodendrocytes, and microglia also have very large plasma membrane surface areas, and all of these cells extensively interact with neurons, enhancing intercellular interactions and complexity.

Membrane organization has conceptually evolved from the notion of a lipid bilayer with embedded proteins to that of a highly dynamic, heterogeneous patchwork of microdomains containing ion channels, receptors, transporters, and other proteins. In the past, cellular membranes in the nervous system were divided into relatively more fluid membranes (e.g., those of cells of gray matter) and relatively more rigid membranes (e.g., the oligodendrocyte plasma membrane that spirals around the axon to form myelin) based on a higher or lower content of polyunsaturated fatty acids (PUFAs) in phospholipids. We now know that neurons, glia, and endothelial cells of the cerebrovasculature are endowed with phospholipid pools that are increasingly recognized as reservoirs of lipid messengers. Specific lipid messengers are cleaved from these reservoir phospholipids by phospholipases after stimulation by neurotransmitters, neurotrophic factors, cytokines, membrane depolarization, ion channel activation, and so forth. In turn, these lipid messengers regulate and interact with other signaling cascades and contribute to the development, differentiation, function, protection, and repair of the cells of the nervous system *(2)*.

In this article, the roles of PUFA and of the bioactive phospholipid platelet-activating factor (PAF) provide examples of lipid signaling in synaptic plasticity and memory as well as in pathological conditions that prevail in retinal degenerations, stroke, epilepsy, and Alzheimer's disease. Not all of the contributions made to this rapidly expanding field can be discussed: omission of papers was unavoidable because of the relative brevity of this article and the broad scope of lipid signaling in the nervous system.

Prostaglandin E_2 Modulates Neuronal Function

Cyclooxygenase (COX)-2, an enzyme encoded by an early response gene, is inducible by cytokines, glutamate, growth factors, PAF, and other mediators and is inhibited by glucocorticoids. COX-2 messenger RNA (mRNA) has a short half-life: about 3 h in human neocortex, compared with 12 h for the constitutive COX-1 mRNA. 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 exclusively neuronal. Dendrites and the perinuclear region are enriched in COX-2. Moreover, COX-2 expression appears to be regulated by synaptic activity *(3)*.

Recently, COX-3 was characterized as generated from COX-1 intron-1 retention *(4)*. COX-3 is expressed in brain *(4)* and brain microvasculature *(5)* and has been proposed to be a target of the analgesic/antipyretic acetaminophen *(4,6)*. Neurons in the hippocampus display basal COX-2 expression *(3)* modulated by synaptic activity (such as long-term potentiation [LTP]) and involving the *N*-methyl-D-aspartate (NMDA) glutamate receptors *(3,7)*. The COXs catalyze the same first committed step of the arachidonic acid (AA) cascade: the generation of a very short-lived intermediate, prostaglandin H_2, which is the precursor to various lipid derivatives.

COX-2 participates in aberrant synaptic plasticity, because neuronal COX-2 expression is upregulated in experimental kindling epileptogenesis. The experimental kindling model resembles aspects of mesial temporal lobe epilepsy *(8)*, whereby repeated subconvulsive stimulation gradually results in intensified seizures. Under these conditions, both COX-2 and cytosolic phospholipase A_2 (PLA_2) expressions are enhanced, indicating that the free AA released is converted into prostaglandins during epileptogenesis *(9)*. The inability of nimesulide (a COX-2 inhibitor) 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 *(10)*. For example, COX-1, which is not inhibited by nimesulide, may catalyze the synthesis of prostaglandins, thus minimizing the action of nimesulide. The exact mechanism by which COX-2 inhibition attenuates kindling epileptogenesis is not understood, but this inhibition may diminish synthesis of the lipid messengers prostaglandin and/or PAF, both of which are involved in synaptic facilitation *(10)*. Moreover, kindling epileptogenesis promotes selective neuronal COX-2 expression, initially in the hippocampus and subsequently in the neocortex. Together, these studies suggest that the spread 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 *(10)*.

In a recent study of lipid messenger regulation of membrane excitability and synaptic transmission, we found that endogenous prostaglandin E_2 (PGE_2) selectively regulates certain fundamental synaptic properties. Somatic and dendritic membrane excitability was reduced when endogenous PGE_2 was eliminated by the selective COX-2 inhibitor NS398 in rat hippocampal CA1 pyramidal neurons in a slice preparation *(11)*. Exogenous application of PGE_2 produced increases in frequency of firing, excitatory postsynaptic potential (EPSP) amplitude, and temporal summation in hippocampal slices treated with the COX-2 inhibitor. Application of the EP_2 receptor agonist induced a long-lasting enhancement of EPSPs, whereas EP_1 and EP_3 receptor agonists did not. The PGE_2-induced enhancement of EPSPs was blocked by both protein kinase (PK)-A and -C inhibition.

These findings indicate that PGE_2 regulates membrane excitability and synaptic transmission via $EP_{2/4}$-cyclic adenosine monophosphate (cAMP)–PKA and –PKC pathways in hippocampal CA1 pyramidal neurons *(11)*. However, the precise mechanisms by which PGE_2 modulates COX-2-mediated synaptic function are still not clear. In this connection, we also demonstrated that PGE_2 increased the frequency of miniature excitatory postsynaptic currents (mEPSCs) in hippocampal neurons in culture. These actions were also mimicked by an EP_2 agonist and were attenuated by PKA inhibitors. Additionally, the frequency of mEPSCs was enhanced in neurons pretreated with interleukin (IL)-1β or lipopolysaccharide, and this enhancement was inhibited by a COX-2 inhibitor. We further identified that the EP_2 receptor is predominantly expressed in presynaptic terminals, whereas COX-2 and microsomal PGE synthases-1 and -2 are localized in postsynaptic dendritic spines. Decreased EP_2 expression through EP_2 gene silencing eliminated PGE_2-induced increase of the frequency of mEPSCs. Our results demonstrate that PGE_2 synthesized by postsynaptically localized COX-2 functions as a retrograde messenger in hippocampal synaptic signaling via a presynaptic EP_2 receptor *(11)*.

Arachidonoyldiacylglycerol (20:4-DAG): The Significance of Diacylglycerol Kinase ε in Synaptic Plasticity

In addition to activating PLA_2, the excitatory neurotransmitter glutamate activates phospholipase C (PLC) through G protein-linked glutamate metabotropic receptors; consequently, the pool sizes of both diacylglycerol (DAG) and inositol-1,4,5-trisphosphate (IP3) increase. The DAGs are complex regarding their fatty acid composition, because their pool size is also modulated by phospholipase D (PLD)-mediated degradation of phosphatidyl choline (PC) to phosphatidic acid (PA), followed by lipid phosphate phosphatase-mediated generation of DAG. We now know that subcellular compartments contain scaffold proteins that are anchoring sites for multiple enzymes of convergent as well as divergent signal transduction pathways. Therefore, the fatty acid composition analyzed in a tissue or cell extract does not represent the subtleties and specificities of subcellular signaling. The prevalent molecular species of inositol lipid-derived DAGs is arachidonoyl–stearoyl–glycerol, which also corresponds to the major molecular species of the membrane reservoir, phosphatidylinositol-4,5-bisphosphate (PIP2), which is critical in inositol lipid signaling. Neurons synthesize inositol lipid synthesis by way of a *de novo* route through PA, which is replenished by DAG kinase following synaptic activity-induced PIP2 hydrolysis. Therefore, the DAGε, (which is specific for 20:4-DAG) is critical for the synthesis of PIP2 containing 20:4, or AA.

DAGs act as messengers modulating certain PKC isoforms, whereas IP3 releases Ca^{2+} from intracellular stores. DAG targets may discern DAG fatty acid composition *(12)*. Thus, DAG generated from PC through PLD has a different fatty acid composition from that generated from PIP2. PC at *sn*2 is mainly oleate (18:1, *n*-3; ref. *13*), 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 *(14–17)*; however, early and late DAG peaks display different fatty acid composition, strongly indicative of sources other than PIP2. Therefore, it is clear that the fatty acid composition of inositol lipids is important in the generation of 20:4-DAG. The enzymes for inositol lipid synthesis selectively employ 20:4-containing lipids as substrates to achieve enrichment in 20:4 in PIP2 *(18)*. A critical enzyme, DAG kinase, catalyzes the phosphorylation of DAG to PA. Nine genes encode these enzymes. Experimental deletion of DGKε, (which selectively phosphorylates 20:4-DAG) has made it possible to determine the biochemical and neurobiological activities of these lipid signals *(19)*.

Similarly to mGluR1, the cellular distribution of DGKε, in brain is localized in Purkinje cerebellar neurons, mitral cells of the olfactory bulb, hippocampal interneurons, and neurons of the thalamus and substantia nigra *(20,21)*. The neurotransmitter glutamate activates G protein-coupled mGluR1, promoting PIP2 degradation by PLC as well as the subsequent activation of PKCβ. We found that DGKε,-deficient mice displayed a marked downregulation of 20:4-inositol lipid signaling when exposed to electroconvulsive shock (ECS), as reflected by reduced PIP2 degradation and reduced accumulation of DAG and free AAs and stearic acids after ECS. These results suggest that the DGKε signaling pathway participates in synaptic function, LTP, seizures, and neuroprotection.

The implication that low-molecular-weight phospholipases elicit functions on the cell surface is supported by the presence of secretory PLA_2 ($sPLA_2$) receptors. $sPLA_2$s binding to neural- and muscle-type receptors were identified using purified snake and bee venom $sPLA_2$ as ligands *(22–25)*. When $sPLA_2$ receptors in primary neurons in culture were activated using purified $sPLA_2$ as a ligand, the result was a synergistic toxicity with glutamate, inducing neuronal cell death *(26)*. Moreover, AA metabolism *(26)*, COX-2 activation *(27)*, ischemic neuronal survival *(28)*, and cal-

cium influx *(29)* are clearly engaged in this response. $sPLA_2$ may be released along with neurotransmitters *(30)*, a notion that should be explored in specific synapses. In the intercellular space, $sPLA_2$ generates lipid mediators, and the secreted enzyme itself becomes a messenger. The consequences of $sPLA_2$ acting on cells is the release of lipid messengers, either on the cell surface or intercellularly. It is not yet clear how $sPLA_2$s act on astrocytes, microglia, and neurons or whether they are restricted to certain cell types.

$sPLA_2$ receptor activation in neurons induces sustained modifications in neuronal metabolism of arachidonoyl-phospholipids, although the specific messengers formed have not yet been defined. $sPLA_2$ receptor activation and glutamate synergize AA mobilization and neurotoxicity; therefore, excitotoxicity may involve not only glutamate (as widely believed) but also $sPLA_2$ *(26)*. It is also possible that $sPLA_2$ modulates physiological phenomena at the synapse and other sites in the nervous system.

Docosahexaenoic Acid of Membrane Phospholipids Is a Target of Peroxidation and a Precursor of Neuroprotective Docosanoids

Docosahexaenoic acid (DHA) is a major PUFA in the central nervous system (CNS), and brain and retina contain the highest DHA content of any tissues. Nevertheless, the significance of DHA is largely unknown. Some aspects of DHA in brain and retina may offer clues to its participation in cell function and its role in neurodegenerations. DHA is continuously required for biogenesis and maintenance of neuronal and photoreceptor membranes. This supply is met by the liver, where DHA is incorporated into plasma lipoproteins and delivered to the retina through the choriocapillaris *(31–35)*. DHA uptake by the retina occurs in the retinal pigment epithelium (RPE), which then deliver it to photoreceptors *(32–34,36–38)*. During the daily process of photoreceptor renewal, active recycling by the RPE of phagosome-derived DHA through the interphotoreceptor matrix retains DHA within photoreceptors *(37,39,40)*. Endothelial cells and RPE *(33–34)* can synthesize DHA from dietary precursors, but levels of *n*-3 fatty acids, such as 18:3 *n*-3 and 20:5 *n*-3, are very low in plasma; therefore, the retina must largely rely on DHA supplied by the liver, either derived from dietary sources or synthesized in the liver from 18:3 *n*-3. A postulated signal generated in brain or retina to control DHA delivery from the liver *(31)* could allow activation of DHA export during development, when DHA is required for active synapse and photoreceptor outer segment formation. This type of signal might also operate when replenishment of DHA is needed following loss of DHA from excitable membranes resulting from injury or neurodegenerations *(31,36)*.

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 *(37)*. The most frequently reported phenotype is low plasma and red blood cell levels of DHA *(41–44)*. An autosomal recessive RP associated with hearing loss and lower plasma levels of 22:6- and 20:4-phospholipids are also demonstrated in Usher's Syndrome *(45)*. 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 *(46)*.

Low plasma level of 22:6 *n*-3 in patients with RP has also been interpreted as a mechanism to protect the retina from oxidative damage resulting from photochemical function of rhodopsin *(47)*. Stress signals originating from retinas undergoing degeneration (e.g., RP) may shut the communication between the retina and the liver *(31)*, reducing the systemic liver supply of 22:6 *n*-3. Low levels of DHA in photoreceptor phospholipids occur in dogs with progressive rod-cone degeneration (*prcd*; ref. *48*) as well as in other retinal degeneration models *(47)*. Moreover, the synthesis of DHA

from 20:5 *n*-3 in RPE cultures from *prcd* is not affected by the retinal degeneration *(49)*, and dietary supplementation with DHA failed to prevent the loss of photoreceptor DHA and the *prcd* phenotype *(48)*. Moreover, cultured hepatocytes from dogs with *prcd* display higher accumulation of 22:6-phospholipids in the liver and impaired hepatocyte secretion of DHA-containing, very low-density lipoproteins *(45)*; this supports the hypothesis that in retinal degenerations, a systemic DHA metabolic defect occurs that leads to reduced liver DHA supply to the retina. It is not known whether DHA dietary supplementation protects the retina of patients with RP.

DHA-containing phospholipids are a target for lipid peroxidation; for example, peroxidation catalyzed by free radicals results in F4-isoprostane formation *(50,51)*. F2-isoprostanes are derived from free-radical-catalyzed peroxidation of AA, rather than DHA *(52)*. F4-isoprostanes are found esterified in phospholipids, and their content is increased in brains of patients with Alzheimer's disease *(53)*.

Docosanoids (enzyme-derived DHA metabolites) were first identified in retina *(54)* and have been proposed to elicit neuroprotection *(55)*. The synthesis of DHA-oxygenation messengers during brain ischemia–reperfusion was recently detected by lipidomic analysis by tandem liquid chromatography–photodiode array–electrospray ionization–mass spectrometry *(56)*. On one hand, two DHA-oxygenation pathways give rise to 10,17*S*-docosatriene; on the other hand, these two DHA-oxygenation pathways give rise to the synthesis of resolvin-type messengers (17*R*-DHA), which have also been identified outside the nervous system as a response to aspirin treatment *(57)*. Brain resolvins are also synthesized in response to aspirin treatment *(56)*. Both DHA-oxygenation pathways generate messengers that behave as counter-proinflammatory signals *(57–59)*. The docosanoid 10,17*S*-docosatriene potently inhibits brain ischemia–reperfusion-induced infiltration of polymorphonuclear leukocytes as well as nuclear factor-κB and COX-2 expression *(56)*. When 10,17*S*-docosatriene was infused into the third ventricle during ischemia–reperfusion, there was marked reduction of the stroke volume *(56)*. Discovery of these docosanoid messengers has revealed some of the ways the brain modulates its response to inflammatory injury, as well as potential targets for new drugs to treat neurological 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.

Cell Signaling in Synaptic Plasticity and Neuronal Survival: How Does the Bioactive Phospholipid PAF Modulate Cell Function?

Excitatory synaptic neurotransmission promotes activation of phospholipases that, in turn, cleave synaptic membrane phospholipid reservoirs, releasing biologically active lipid messengers. One such reservoir is phosphatidylcholine phospholipids, which 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 have a phosphorylcholine ester. The membrane phosphatidylcholines are the target of PLA_2s, which cleave the fatty acid linked to C2, producing lyso-PAF and a free PUFA—either AA or DHA. Subsequently, lyso-PAF is acetylated, and the product, PAF, is a potent phospholipid messenger (remodeling pathway; Fig. 1). Therefore, similarly to prostaglandins and other lipid messengers, PAF is not stored in membranes as a preformed structure but is rapidly synthesized upon specific stimulation.

As a physiological mediator, PAF modulates glutamate release and acts as a retrograde messenger of LTP, a model of learning and memory *(60–63)*. PAF modulates neuronal calcium ionization through the PAF receptor *(64)* and is also generated by NMDA receptor activation. Modulation of the function of PAF is appar-

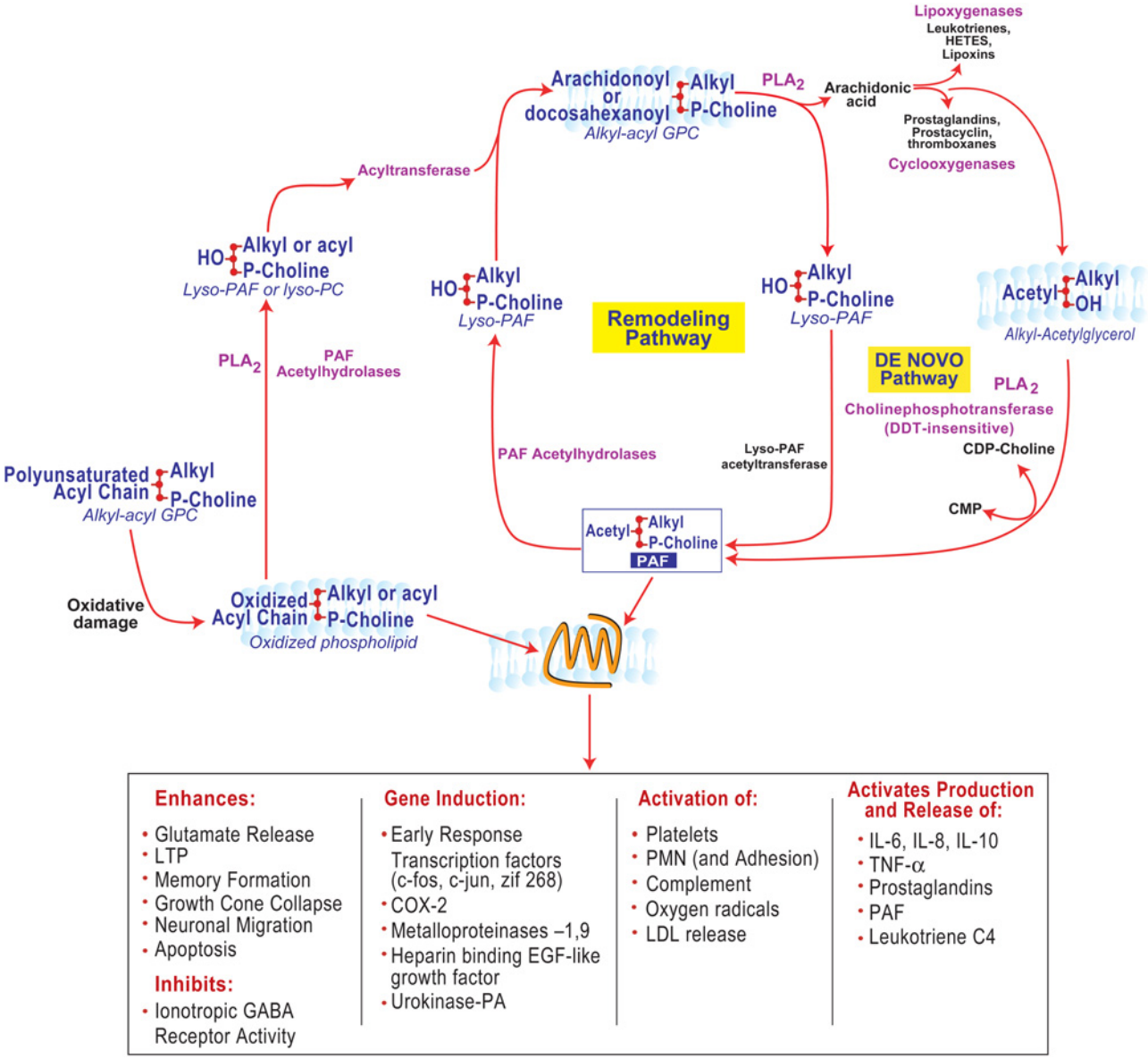

Fig. 1. PAF synthesis and degradation. The remodeling pathway or "PAF cycle" from the membrane phospholipid PAF precursor, alkyl-acyl-glycerophosphorylcholine (GPC; left) to the the biologically active PAF (right). The remodeling route includes the production of lyso-PAF, which is generated from the PAF precursor alkyl-acyl-GPC either directly by the action of phospholipase A2 or by the transfer of the *sn*-2 acyl moiety to a "donor" lyso-plasmalogen (not shown), which is mobilized from membrane plasmalogen by phospholipase action. The *de novo* route of PAF synthesis involves the direct transfer of a choline moiety to alkyl-acetylglycerol. Note that PAF-AH inactivates all PAF molecules—regardless of their biosynthetic route—and also inactivates oxidatively damaged phospholipids (shorter peroxidated acyl group at C2) that possess biological activity at the PAF receptor.

ently effected by its synthesis and degradation by tightly regulated enzymes. In brain, there are several PAF acetylhydrolases (PAF-AHs; ref. *65*), which rapidly remove the acetate moiety from C2 and render PAF biologically inactive. The existence of these "off-signal"

enzymes emphasizes the fact that PAF is a potent messenger *(7)*.

PAF and the free polyunsaturated fatty acids released during PAF synthesis accumulate in brain when stimulated *(66)*. In the retina and in neurons in culture, neurotransmitters trigger Ca^{2+}-independent *de novo* PAF synthesis through cytidine 5′-diphosphocholine *(67,68)*. Because PAF stimulates adenosine triphosphate release from PC12 cells, it has been implicated in vesicular release of neurotransmitters *(69)*, perhaps through a mechanism analogous to that engaged in vesicular release of vasoactive substances from platelets and eosinophils *(70)*.

PAF is rapidly degraded by deacetylation to the biologically inactive lipid lyso-PAF. Several PAF-AHs are expressed in the CNS *(61)*. 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 *(62)*. This study also demonstrated that the actions of PAF are mediated by presynaptic receptors and provided support for the notion that PAF is a second messenger in the CNS. Additional mc-PAF enhanced the amplitude of evoked postsynaptic currents and diminished the size of presynaptic action potentials. Interestingly, the effect of mc-PAF on excitatory synaptic transmission is very selective, because it does not increase general presynaptic function *(62)*. Additionally, mc-PAF elicts these actions independently of postsynaptic glutamate receptors. Moreover, mc-PAF augments the frequency of spontaneous miniature excitatory synaptic events, sparing their amplitude and their time course.

Retrograde messengers of LTP are likely candidates for the regulation of memory formation. When retrograde messengers released from the postsynaptic neuron enhance excitatory neurotransmitter release from the presynaptic neuron, the synaptic efficiency of LTP should be increased. 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 might be retrograde messengers; AA, nitric oxide, carbon monoxide, and PAF all have been suspected. Although released AA increases excitatory synaptic transmission when coupled with presynaptic stimulation, it requires relatively high concentrations *(71)*, and in CA1 neurons, AA effects are blocked by DL-aminophosphonovaleric acid *(72,73)*, which consequently limits the potential of AA as a retrograde messenger. On the other hand, PAF 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 following training enhanced retention scores. However, PAF infused 60 min posttraining had no effect on retention *(74)*. Marked attenuation of LTP was observed in PAF receptor-deficient mice. Infusion of PAF into brain regions enhanced memory formation in a time-dependent, neuro-anatomically specific fashion. A presynaptic PAF receptor antagonist had a clear amnesic effect in several memory task tests. Posttraining intrahippocampal infusion of intracellular forms of the PAF receptor did not modify memory for an inhibitory avoidance task *(74,75)*. However, posttraining injection of PAF into either amygdala, hippocampus, or entorhinal cortex enhanced memory in an inhibitory avoidance task *(74)*, whereas injection of a PAF receptor antagonist that selectively targets the presynaptic PAF receptor impaired memory in this task. Moreover, posttraining intrahippocampal and intradorsal striatal injections of either PAF or PAF receptor antagonists modulated memory in hidden and visible platform water maze tasks, respectively *(8,76,77)*. 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 blocked 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 *(78)*.

PAF receptor antagonists block LTP in rat hippocampal CA1 neurons, dentate gyrus, and medial vestibular nuclei. PAF increases excitatory postsynaptic responses and the frequency of spontaneous EPSCs and enhances the receptor-mediated release of the excitotoxic amino acid glutamate from presynaptic terminals. Excitatory synaptic transmission is modulated by various signaling systems, including the inhibitory neurotransmitter γ-aminobutyric acid (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 and enhanced it in approx 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; alternatively, 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 and decreases inhibitory GABAergic postsynaptic activity *(79)*.

PAF receptor antagonists elicit neuroprotection in the gerbil brain ischemia–reperfusion model *(80)*, suggesting that PAF may exert its effects 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 *(80)*. The ischemia-induced PUFA release is believed to result from synaptic PLA_2 activity *(76)*. Additionally, the PAF antagonist shown to be neuroprotective in the gerbil ischemia–reperfusion model also selectively displaced radiolabeled PAF binding from synaptic membranes *(81)*. Therefore, this synaptic membrane-binding site was suggested to be the modulator of glutamate neurotransmitter release *(62)*. Moreover, intracellular PAF-binding sites identified in a microsomal fraction were distinct from those at the synaptic membrane fraction regarding responses to several antagonists *(81)*.

At that time, it was known that PAF activates early response genes *(82–84)*, thus the intracellular receptor was believed to be the signaling linkage to the nucleus. Cloning of the seven-transmembrane domain PAF receptor *(85–87)* and the discovery of specific PAF receptor-mediated Ca^{2+} influx into neurons *(64)* 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 EPSPs) were attenuated in PAF receptor-knockout mice, compared to LTP in wild-type mice. Moreover, PAF receptor-deficient mice displayed a marked attenuation of LTP after stimulation of the lateral, but not the medial, perforant path *(88)*. In another study, PAF-deficient mice had unaltered LTP; however, there were substantial differences between the experimental conditions of the two studies *(89)*. 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 *(88)*.

Most recently, the intracellular PAF receptors were further characterized. One form is confined to the endosomes *(90)*, and the other is confined to the nuclear membrane *(91)*. Both intracellular forms of the receptor may be part of the intracellular microsomal form described earlier *(81)*. 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 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 the transcriptional actions of PAF, 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 pathological 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 proinflammatory messenger and a mediator of neurotoxicity. Therefore, PAF activates COX-2 expression *(92)* as well as that of several early response genes that encode transcription factors *(82,83)*. PAF activates apoptosis and polymorphonuclear leukocytes and their adhesion to the microvasculature *(70)*. 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-alpha, and other mediators of the inflammatory response. PAF activates phospholipases through its receptor (resulting in additional PAF synthesis) along with those of prostaglandins and leukotrienes. Overall, PAF is a potent neuronal injury messenger. PAF also plays a prominent role in astrocytes and microglial cells. Many of these actions have been studied in non-neural 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 *(80,93–97)*.

Multiple PAF-degrading enzymes in the CNS further highlight the importance of maintaining a well-controlled PAF pool size. These include tissue PAF-AHs *(65)* as well as plasma-type PAF-AHs *(70)*. 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 *(7)*. 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, further implicating the phospholipid messenger in developmental neurobiology *(62)*.

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 does elicit neuroprotection *(98)*. The human form of PAF-AH is effective in inhibiting inflammation in non-neural cells *(99)*. rPAF-AH elicits concentration-dependent neuroprotection against NMDA-induced apoptosis in hippocampal neurons in primary culture *(98)*. The criteria used in this study included electron microscopy to monitor nuclear condensation, histone release, TUNEL, and DNA laddering. Therefore, rPAF-AH may be an alternative to PAF antagonists to regulate pathological 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 bio-availability after administration. Addition-

ally, rPAF-AH can hydrolyze oxidized phospholipids, which are also recognized by the PAF receptor *(70)*. PAF-like oxidized phospholipids accumulate in several pathological conditions that involve oxidative stress and that have not yet been clearly defined in neuropathological conditions.

In conclusion, the newly discovered docosahexaenoic acid signaling pathways, as well as other lipid messengers related to synaptic activation, may lead to the clarification of clinical issues relevant to stroke, age-related macular degeneration, spinal cord injury, Alzheimer's disease, and other diseases that include neuroinflammatory components.

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