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Excitable Membranes, Lipid Messengers, and Immediate-Early Genes

Alteration of Signal Transduction in Neuromodulation and Neurotrauma

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Introduction

Membranes serve as physical delimiting boundaries of cells, provide microenvironments for correct protein function, and provide phospholipid substrates for multiple signal transduction pathways. Components of signal transduction include the release of diacyl-glycerols and subsequent activation of C-type protein kinases, the hydrolysis of inositol phosphates from phosphatidyl precursors and the intracellular receptor-mediated Ca^{2+} ionization by inositol phosphates, and the oxidative metabolism of free arachidonic and other polyunsaturated fatty acids. Neural cell membrane lipids, by virtue of their intrinsic role as sites of second messenger synthesis, are susceptible to extensive hydrolysis during the unregulated neural stimulations that follow neurotrauma. These resulting physical and chemical changes in cell membranes contribute to neural damage and, ultimately, to loss of brain function. Under physiological conditions, lipid second messengers derived from membranes serve as intermediaries between the extracellular environment

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and the cytoplasmic and nuclear metabolic machinery. Transduction of neurotransmission to the nucleus to initiate synthesis of new proteins is an important mechanism in neural processes, including maintenance of neural integrity, memory formation and synaptic plasticity, and repair following trauma. An integral component of this transduction is the rapid stimulation of immediate-early genes following neural stimulation. The proteins encoded by these inducible genes regulate expression of the cellular proteins that effect long-term neural adaptations. The functioning of lipid messengers in transduction of surface-to-nucleus phenomena in neural cells make them important potential targets of unregulated neurotransmission following trauma and cognitive impairment, as well as integral components during learning and memory formation. This paper summarizes current information regarding the role of membrane-derived lipids, in particular platelet-activating factor, in the regulation of gene expression induced through neurotransmission during physiological and pathophysiological processes.

Membrane Phospholipid Hydrolysis Following Neurotrauma

Neural stimulation involves the degradation of membrane phospholipids by phospholipases through diverse and selective receptor-mediated events. Many products of these enzymes are intra- and extracellular mediators of the initiating receptor stimulation. Under pathological conditions (e.g., ischemia, convulsions, and other trauma), an overstimulation of receptors leads to the synthesis and accumulation of such biologically active lipid mediators in brain. A further consequence of pathological phospholipid hydrolysis is disruption of intrinsic membrane homeostasis and symmetry, which may leave cells susceptible to toxic alterations in membrane-associated biochemical events and may lead to the release of toxic levels of phospholipid metabolites.

Certain phospholipids of synaptic membranes are rapidly deacylated by phospholipase A_2 during convulsions, leading to the accumulation of free arachidonic and docosahexaenoic acids (Bazan, 1970; Bazan et al., 1986; Bazan and Birkle, 1987), the most prevalent polyunsaturated fatty acids (PUFAs) in neural tissue. Ischemia and other forms of brain injury, such as cryogenic brain edema, also lead to the accumulation of free PUFAs (Bazan, 1970; Aveldano and Bazan, 1975a,b; Cenedella et al., 1975; Bosisio et al., 1976; Yoshida et al., 1980; Seisjö et al., 1982; Lazarewicz et al., 1983; Ikeda et al., 1986; Politi et al., 1985). Moreover, the ischemia-induced accumulation of free fatty acids preferentially takes place in grey matter as compared with white matter (Bazan, 1971). In retina, similar responses occur during anoxia (Aveldano and Bazan, 1974; Giusto and Bazan, 1983) or depolarization (Birkle and Bazan, 1984). An enhanced production of lipoxygenase products takes place under these conditions in the retina as well as in brain synaptosomes. Analysis of subcellular fractions from the brains of rats undergoing bicuculline-induced status epilepticus shows that the synaptosome fraction, and not endoplasmic reticulum fractions, is the prevalent site of phospholipase A_2 activation and accumulation of lipoxygenase reaction products (Birkle and Bazan, 1987).

During reperfusion after ischemia, the renewed availability of oxygen in the presence of abnormally large PUFA pools leads to enzyme-mediated oxygenation of arachidonic acid and other PUFAs and to peroxidation of membrane lipids (Braughler and Hall, 1992). In the pathogenesis of brain damage, degradation of certain excitable membrane phospholipids, effects concomitant with accumulation of free PUFAs or their metabolites, may contribute to functional impairment (Siesjö et al., 1982; Bazan, 1976; Bazan and Rodriguez de Turco, 1980). These early responses of the nervous system may result from overstimulation of receptors and increased intracellular Ca^{2+}, an activator of cellular phospholipases (Bazan and Birkle, 1987; Bazan, 1976; Bazan and Rakowski, 1970). The membrane phospholipid

components that are primarily degraded by phospholipases are thought to be ethanolamine plasmalogen (Edgar et al., 1982), inositol lipids (Reddy and Bazan, 1987), and phosphatidylcholine (Marion and Wolfe, 1978). The ischemic involvement of inositol lipids is further documented, since these lipids decrease in ischemia and convulsions concomitant with the accumulation of stearoylarachidonoyl-*sn*-glycerol, the predominant diacylglycerol byproduct of inositol lipid hydrolysis in brain (Bazan, 1970; Aveldano and Bazan, 1975a; Aveldano de Caldironi and Bazan, 1979; Ikeda et al., 1986).

The ether-linked molecular species of phosphatidylcholine, 1-*O*-alkyl-2-acyl-*sn*-glycero-3-phosphocholine (alkyl-acyl-GPC) that is a precursor of platelet-activating factor (PAF; 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) may also function as a source of free PUFAs that accumulate in the nervous system under ischemic conditions and during epilepsy. The synthesis of PAF is triggered in several tissues in response to injury, inflammation, or during immune reactions through different biosynthetic routes (reviewed in Snyder, 1990; Fig. 1):

1. *A remodeling pathway*, initiated by phospholipase A_2 activation that releases the PAF precursor lyso-PAF from membrane alkyl-acyl-GPC, or
2. *A de novo pathway* involving choline transfer to 1-alkyl-2-acetyl-*sn*-glycerols.

It has been suggested that brain cells synthesize PAF through the latter *de novo* pathway, because acetyltransferase activity has not been detected at appreciable levels in brain tissue (Francescangeli and Goracci, 1989). However, brain does contain a Ca^{2+}-activatable lysophospholipase D (Kawasaki and Snyder, 1987; Lee et al., 1988) that will generate *de novo* pathway substrate 1-*O*-alkyl-2-*lyso-sn*-glycerol from lyso-PAF released by the action of phospholipase A_2 on membrane phospholipid. Lysophospholipase D may represent a "neural shunt" of PAF synthesis between remodeling and *de novo* pathways.

During pathological conditions such as seizure and ischemia, Ca^{2+}-evoked phospholipase A_2 activation (Bazan, 1970; Bazan and Rodriguez de Turco, 1980) is likely a major source of PAF liberated from cell membrane precursors (Bazan et al., 1991). In addition, the observations that Ca^{2+} inactivates all enzymes of *de novo* pathway of PAF synthesis (Lee et al., 1986; Woodard et al., 1987; Lee et al., 1988) suggest that phospholipid remodeling initiated through Ca^{2+}-activated phospholipase A_2 is the source of PAF synthesis during ischemic and epileptic Ca^{2+} fluxes.

Since the remodeling route involves an initiating phospholipase A_2 activation, it has been suggested that PAF synthesis may be accompanied by the generation of free PUFAs following ischemia or convulsions (Panetta et al., 1987; Birkle et al., 1988). Furthermore, the alkyl-acyl-GPC, on which phospholipase A_2 acts to release the PAF precursor *lyso*-PAF, is known in several cells and tissues to be enriched in arachidonoyl groups at the *sn*-2 position (Chilton et al., 1984; Ramesha and Pickett, 1986; Suga et al., 1990). Recently, synthesis of PAF through phospholipase A_2 digestion of ethanolamine plasmalogen has been demonstrated (Nieto et al., 1991). A CoA-independent transacylase functions to transfer arachidonic acid from alkyl-acyl-GPC to the *lyso*-plasmalogen product of the initiating phospholipase A_2 activity, generating the PAF precursor, *lyso*-PAF. Since ethanolamine plasmalogen is among the earliest phospholipids hydrolyzed by phospholipase A_2 activity following neurotrauma (Edgar et al., 1982), discovery of this route of PAF synthesis further suggests involvement of phospholipid remodeling as the major route of PAF synthesis following neurotrauma.

PUFAs and PAF as Second Messengers or Mediators of Cerebral Ischemia and Seizure

Alkyl-acyl-GPC and ethanolamine plasmalogen may provide substantial sources of both PUFAs and PAF, following neural trauma. Production of PUFAs and PAF may have potent

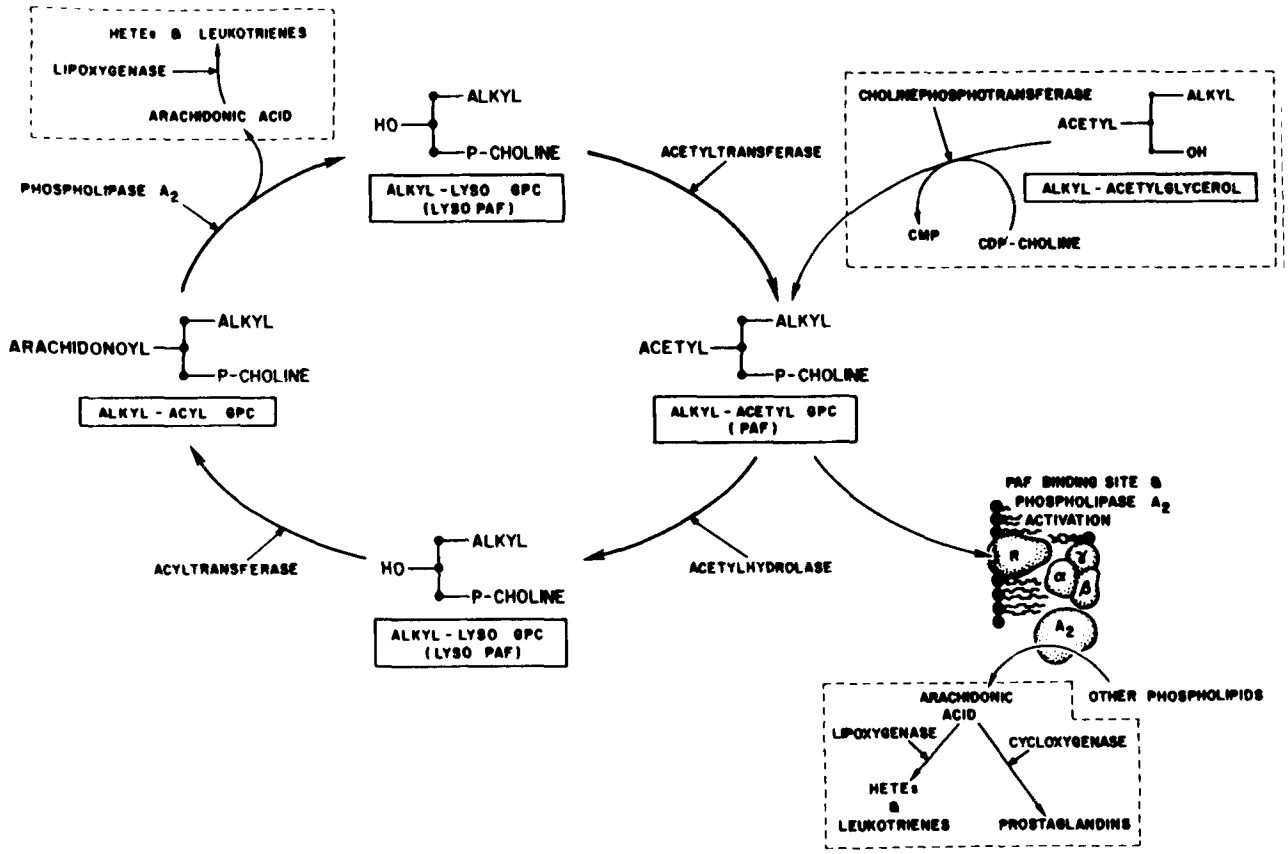

Fig. 1. The PAF synthetic cycle. Synthesis of PAF involves independent enzymatic pathways. The *remodeling* pathway is initiated by the activity of phospholipase A_2 on alkyl-acyl-glycerophosphocholine (alkyl-acyl-GPC), a species of membrane phospholipid that is enriched in arachidonoyl chains at the *sn*2 position. The products of the phospholipase A_2 reaction are free arachidonic acid, the precursor of bioactive eicosanoids, and the PAF precursor lyso-PAF. The subsequent activity of a specific acetyltransferase generates PAF (alkyl-acetyl-GPC). The *de novo* pathway of PAF synthesis involves the transfer of phosphocholine from CDP-choline to alkyl-acetyl-glycerol. PAF interaction with specific membrane binding sites results in the activation of phospholipases and other second messenger systems. PAF can be inactivated by the action of an acetylhydrolase followed by reacylation. The *de novo* route is believed to provide endogenous PAF required for physiological functions. Cellular stimulation during pathophysiological conditions is believed to generate PAF through the *remodeling* pathway.

biological consequences. PUFAs are substrates for oxygenating enzymes, which produce the most biologically potent mediators of cellular swelling and edema, the eicosanoids. Levels of free PUFAs and eicosanoids increase following seizure, ischemia reperfusion, and trauma (reviewed in Bazan and Birkle, 1987). One of the potential effects of PUFAs released by the activity of phospholipase A_2 is the uncoupling of oxidative phosphorylation (Hillered and Chan, 1990). Suppression of mitochondrial ATP production is associated with formation of free radicals, which damage membranes through the formation and propagation of membrane-disruptive lipid peroxides (Braughler et al., 1985; Braughler and Hall, 1992).

Although the metabolic fates of arachidonic acid and other PUFAs released during ischemia and convulsions are not completely known, some effects associated with PUFA release may be attributed directly to arachidonic acid, as opposed

to its bioactive oxygenated metabolites. Arachidonic acid is released from hippocampal slices through stimulation by NMDA and glutamate (Pellerin and Wolfe, 1991). Arachidonic acid potentiates channel current through NMDA receptors in rat cerebellar granule cells (Miller et al., 1992), and inhibits glutamate uptake in glial cells (Barbour et al., 1989). These effects are mediated by arachidonic acid itself and apparently not its lipoxygenase or cyclooxygenase metabolites. It is conceivable that arachidonic acid exerts these effects either through direct binding to glutamate receptors or through physical alteration of membrane fluidity surrounding these proteins. Thus, release of free arachidonic acid affects excitatory neurotransmission by enhancing glutamate activity at the synapse and may contribute to neuronal death during ischemia through this mechanism.

Massive free fatty acid release following ischemia-evoked phospholipase A_2 may affect other receptor systems. Phospholipase A_2 treatment of adult rat synaptic membranes increases the affinity of AMPA binding to neural membranes (Massicotte and Baudry, 1990; Baudry et al., 1991). Increased membrane polyunsaturation in retinoblastoma cells through enrichment of the culture medium with arachidonic or docosahexaenoic acids increases high affinity uptake mechanisms for choline and taurine (Hyman and Spector, 1982; Yorek et al., 1984). This is clear evidence that PUFAs released from phospholipid sequestration in cell membranes will affect ligand binding processes in cell membranes.

Docosahexaenoic acid is the other major fatty acid released following seizure and ischemia (Bazan, 1970; Bazan et al., 1986). Although the function of this PUFA is largely unknown, its release from phospholipids has been demonstrated to inhibit PAF-induced Ca^{2+}-influx (Weber et al., 1991). This inhibition of PAF activity is not observed with other PUFAs and may occur through a lipoxygenase metabolite of docosahexaenoic acid. Lipoxygenase metabolites of docosahexaenoic acid have previously been described in retina (Bazan et al., 1984).

PAF has been implicated as a modulator of neural activity, particularly during the initiation of various forms of neural trauma. PAF precursors are abundant in neural tissue (Blank et al., 1981). Convulsions, induced either electrically or through the administration of picrotoxin or bicuculline, are accompanied by PAF synthesis in brain (Kumar et al., 1988). PAF directly impairs brain metabolism (Kochanek et al., 1988), and PAF antagonists reduce cellular damage and improve metabolism in brain following ischemia (Panetta et al., 1987; Spinnewyn et al., 1987; Oberpichler et al., 1990; Lindsberg et al., 1990; Gilböe et al., 1991). PAF apparently does not traverse the blood-brain barrier (Kumar et al., 1988), and, hence, PAF accumulation in brain, following neural trauma, must be derived from neural metabolism.

PAF, the cognate phospholipase A_2 product of PUFA liberation, may exert a unique spectrum of detrimental effects during neural trauma. Choline lysophospholipids, including PAF, induce efflux of Ca^{2+} from mitochondria, and this may represent a mechanism by which phospholipase A_2, through lysophospholipid generation, and impaired mitochondrial function, may mediate ischemic tissue damage (Rustenbeck et al., 1991). Several PAF antagonists improve neural metabolism following ischemia (Panetta et al., 1987; Spinnewyn et al., 1987; Oberpichler et al., 1990; Lindsberg et al., 1990; Gilböe et al., 1991), suggesting an activity of PAF in tissue damage through disruption of metabolism.

PAF antagonists suppress the rapid accumulation of brain free fatty acids following ischemia and electrically or pharmacologically-induced seizure (Panetta et al., 1987; Birkle et al., 1988). The suppression of free fatty acid release by PAF antagonists suggests inhibition of a phospholipase A_2 activity, as evidenced by the fact that arachidonic and docosahexaenoic acids are the prevalent PUFAs liberated under these conditions (Bazan, 1970).

In addition, the ischemia-reperfusion-associated generation of free radicals appears to be sensitive to PAF antagonism (Braquet et al., 1990;

Gilböe et al., 1991). It is likely that neural free radicals generated during ischemia are metabolites of PUFAs, the phospholipase A_2-mediated release of which is sensitive to PAF antagonists.

From these biochemical events resulting from cerebral injury, the sensitivity to PAF antagonists implicate PAF as a mediator in component cellular processes. PAF, in addition, has been identified as a potent chemotactic agent for leukocytes, which are recruited to sites of injury and produce rapid and profound effects on the vascular system (Braquet et al., 1987), including alterations of blood-brain barrier permeability (Kumar et al., 1988). The protective effects of PAF antagonists on neural metabolism following trauma may reflect participation of endothelial and leukocytic cells.

At high concentrations, PAF is a membrane perturbant in vitro (Sawyer and Anderson, 1989) and is toxic to at least some neural cell types in vitro (Kornecki and Ehrlich, 1988; Lustig et al., 1992). Although it is not known if PAF could accumulate to such high perturbant or neurotoxic concentrations in vivo, even during ischemia and convulsions, it is easy to envision local membrane changes accompanying the synthesis of PAF. Both PAF and its remodeling pathway precursor, *lyso*-PAF, localize to the plasma membrane following their respective syntheses (Record et al., 1989; Vallari et al., 1990). Accumulation of PAF and *lyso*-PAF, owing to relatively small molecular densities at *sn*2, likely alter membrane homeostasis among the 2-acyl-*sn*-glycero-3-phospholipids (Harris et al., 1985). In this respect, transacylation of phospholipids and reacylation-inactivation of PAF function to preserve membrane integrity following stimulus-evoked hydrolysis of 2-acyl PAF precursors.

PAF and Neurotransmission

PAF is a potent activator and recruiter of leukocytes during the inflammatory response (reviewed in Braquet et al., 1987). Such actions imply that PAF derived from intracellular metabolism is made available externally. But the observation that leukocytes and endothelial cells retain a substantial mass of PAF following cell stimulation (reviewed in Henson, 1987) has led to speculation that PAF functions as an intracellular messenger. In these cell types, PAF antagonists will block the activities of endotoxin, chemotactic peptide (fMLP), and bradykinin, indicating that PAF mediates the intracellular activities of these cell stimulators (Stewart et al., 1989; Stewart and Phillips, 1989; Stewart et al., 1990). In neutrophils, exogenous PAF itself induces synthesis of intracellular PAF through the remodeling pathway (Tessner et al., 1989; Doebber and Wu, 1987).

Direct measurements of PAF synthesized in retina (Bussolino et al., 1986,1988), cerebellar granule cells (Yue et al., 1990), and fetal brain cells (Sogos et al., 1990), have provided indications that an intracellular mediator role for PAF also exists in neural cells. Stimulation of retina with acetylcholine and dopamine, but not glycine, GABA, or glutamate, induces *de novo* synthesis of PAF (Bussolino et al., 1986), an effect mediated through muscarinic cholinergic and D_2 dopamine receptors, respectively (Bussolino et al., 1989). The acetylcholine and dopamine effects are more profound following synapse maturation, indicating a physiological association of neurotransmitter-evoked PAF synthesis with the synapse (Bussolino et al., 1988). Acetylcholine is also an agonist of intracellular PAF synthesis in fetal brain cells (Sogos et al., 1990). Bradykinin-induced PAF synthesis in leukocytes is not without ramifications as a potential mechanism in neural tissue as well, since this mediator of inflammation is a potent stimulator of phospholipid hydrolysis in neural tissue, and may, in fact, participate in the progression of neurotrauma (Francel, 1992).

In addition to being a product of cholinergic and dopaminergic transmission, PAF is implicated in the functioning of other neurotransmitters. Exogenous PAF affects secretion of neurohormones from the hypothalamus (Junier et al., 1988; Rougeot et al., 1990; Blasquez et al., 1990), and excitatory neurotransmitters from

presynaptic hippocampal neurons (Clark et al., 1992). In addition, PAF induces secretion of PC12 cell adenosine triphosphate, which is stored and released with catecholamines during stimulation of PC12 cells, through a Ca^{2+}-dependent mechanism (Kornecki and Ehrlich, 1988). The nonbenzodiazepine PAF antagonist BN52021 (a ginkgolide terpene) enhances GABA-dependent chloride uptake in cortical synaptoneurosomes through a noncompetitive mechanism at the $GABA_A$ benzodiazepine site (Miller et al., 1991). Although activity of PAF at GABA receptors has not been demonstrated, triazole-substituted benzodiazepines are both potentiators of GABAergic neurotransmission and potent PAF antagonists (Kornecki et al., 1984).

In rat brain, two kinetically distinct high-affinity intracellular PAF binding sites are present on microsomal membranes, in addition to a lower affinity synaptic binding site (Marcheselli et al., 1990). A synaptic PAF receptor may be linked to presynaptic enhancement of excitatory neurotransmission (Clark et al., 1992). The piperidinothieno diazepine BN50730 (Braquet et al., 1990) exhibits high affinity and selectivity for the intracellular sites (Marcheselli and Bazan, 1991). Localization of high-affinity PAF binding sites to intracellular membranes in brain suggests that cellular retention of PAF is functionally linked to metabolic events subsequent to its stimulus-evoked synthesis.

The structure of PAF itself suggests its candidacy as an intracellular messenger, particularly during the phospholipase activations and membrane degradation that accompany neurotrauma. The acetyl group at *sn*2 does not make it a preferred substrate of phospholipase A_2, if it can be a substrate for that enzyme at all. Indeed, an acetylhydrolase highly specific for phospholipids with short chains at *sn*2 utilizes PAF as a substrate during PAF degradation (Blank et al., 1981; Wardlow et al., 1986). An ether linkage at *sn*1 imparts resistance to esterases such as phospholipase A_1. Although the significance of phospholipase A_1 activation in neural tissue is not fully understood despite its broad intracellular distribution (Bazan, 1971), the major fatty acids liberated from neural membranes following ischemia and seizure, in addition to arachidonic acid, are saturated fatty acids (Bazan, 1970), typically esterified at *sn*1 in membrane phospholipids. In addition, there is evidence that the phospholipase C metabolite of PAF, 1-alkyl-2-acetyl-*sn*-glycerol, retains PAF activity (Stoll et al., 1989). Thus, particular structural features of PAF, unique among cellular phospholipids, impart intrinsic resistance to phospholipase-mediated hydrolysis following cell stimulation and suggest a role as an intracellular mediator of signal transduction.

Although the identity and location of the initiating phospholipase A_2 in PAF synthesis is not clear, the other enzymes of PAF synthesis localize to intracellular membranes (Record et al., 1989; Vallari et al., 1990). Following synthesis, PAF relocalizes from the endoplasmic reticulum to the plasma membrane (Vallari et al., 1990). Although factors determining either intracellular retention of PAF or its liberation as an agonist are unknown, studies in erythrocyte membranes suggest that both membrane phospholipid asymmetry as well as presence of acceptor molecules inside and outside the cell determine the fate of intracellular PAF (Bratton et al., 1991).

Extracellular PAF acts as an agonist coupled to multiple phospholipases, including types A_2, C, and D (reviewed in Shukla, 1992). Intracellular PAF synthesized following cell stimulation may also be coupled to phospholipase A_2 activation. PAF antagonists block free radical and eicosanoid generation in leukocytes and endothelial cells following stimulation, suggesting its involvement as a second messenger (Stewart et al., 1989,1990; Stewart and Phillips, 1989). Generation of PAF through the phospholipase A_2 remodeling of neural membranes, in addition to activation of phospholipase A_2 by intracellular PAF, suggests an amplification circuit that may account for the protective effects of PAF antagonists against trauma-evoked free fatty acid release and cellular damage (Birkle et al., 1988; Spinnewyn et al., 1987).

PAF and Immediate-Early Genomic Responses

Cell stimulation is transduced to the nucleus though mechanisms involving the generation of second messengers and stimulation of target protein kinases. The immediate-early genomic response is the earliest transcriptional activity following cell stimulation (reviewed in Herschman, 1989; and Morgan and Curran, 1991). The stimulus-evoked transcription is both transient and independent of *de novo* protein synthesis. Nuclear machinery is poised for a burst of new gene expression following cell stimulation. The components of the immediate-early genomic response include members of the *fos, jun, fra, myc,* and TIS immediate-early gene (IEG) families (reviewed in Herschman, 1991). The structures of these IEG products suggest proteins of various inferred functions, but the majority of those discovered and by far the most well-studied are nuclear-localized, DNA-binding transcription factors. The transduction of extracellular stimuli to the generation of transcription factors suggests a long-term mechanism that mediates cellular fate, in the event of stimulus, through synthesis of new proteins.

The best-studied IEGs in neural tissue include *fos, jun,* and *zif/268. fos* and *jun* encode DNA-binding transcription factors with leucine-zipper structures that allow formation of DNA-binding dimers (reviewed in Ransone and Verma, 1990). Fos-Jun and Jun-Jun dimers constitute sequence-specific AP-1 transcription regulatory activity. Zif/268 is also a transcription factor, but it binds DNA as a monomer through zinc-finger structures at sequences distinct from those bound by AP-1 proteins (Christy et al., 1988; Christy and Nathans, 1989).

An immediate-early genomic response to PAF has been reported in leukocytes (Ho et al., 1987; Squinto et al., 1989; Mazer et al., 1991; Schulam et al., 1991), epidermal carcinoma cells (Tripathi et al., 1991), and neural cells (Squinto et al., 1989; Doucet and Bazan, in preparation). Further evidence of an immediate-early genomic response to PAF is provided by the cycloheximide-sensitive stimulation of prostaglandin E_2 in macrophage-like cells (Glaser et al., 1990). It is interesting to note that cyclooxygenase (Maier et al., 1990), and possibly prostaglandin endoperoxide synthase (Herschman et al., 1991), are encoded by immediate-early genes. In primed leukocytes, stimulated synthesis of PAF itself, likely through a phospholipase A_2-mediated pathway, is also dependent on new protein synthesis (Wirthmueller et al., 1990). Since exogenous PAF is an activator of phospholipase A_2 and intracellular *lyso*-PAF is a product of the enzyme, it is likely that *de novo* proteins are regulators of phospholipase A_2 stimulation.

In NG108-15 neurohybrid cells, picomolar PAF elicits expression of the *zif/268* (Egr-1, TIS 8, NGFI-A) immediate-early gene (Doucet and Bazan, in preparation). This potency of PAF suggests that, it is an important component of the immediate-early genomic response in neural cells. The effectiveness of low concentrations of PAF in eliciting an immediate-early genomic response may reflect the activity of low concentrations of PAF in *priming* cells for enhanced responses to subsequent stimuli, an effect described in leukocytes and epithelial cells (reviewed in Braquet et al., 1989) that may be dependent on *de novo* synthesis of proteins (Wirthmueller et al., 1990). The immediate-early genomic response to PAF in NG108-15 cells is suppressed by the potent intracellular PAF antagonist BN50730, suggesting that the immediate-early transcription is transduced through an intracellular PAF receptor. In addition, the immediate-early genomic response to acetylcholine in these cells is suppressed by the intracellular PAF antagonist.

In neuroblastoma cells (Squinto et al., 1989; Squinto et al., 1990), PAF elicits both (1) transcription of both *fos* and *jun* immediate-early genes and (2) stimulation of transcription from promoters containing multiple AP-1 binding sequences. This indicates that the immediate-early transcriptional stimulation by PAF is physiologically coupled to corresponding protein activity. In addition, deletion mutagenesis of the *fos* promoter has revealed the requirement of a calcium-response

sequence (CaRE) for PAF-stimulated *fos* expression (Squinto et al., 1989). The *fos* CaRE, coincident with the cyclic AMP-response sequence, locates upstream of the *fos* transcription initiation site and functions as a binding site for a transcription factor during cyclic AMP- and Ca^{2+}-stimulated *fos* expression (Sheng et al., 1988; Sheng et al., 1990). The finding that PAF utilizes the *fos* CaRE for transcriptional regulation is supported by studies demonstrating cytoplasmic influx of Ca^{2+} stimulated by PAF from both intracellular and extracellular stores in neural cells (Yue et al., 1991a,b). The presence of the CaRE in transcriptional promoter regions of other immediate-early genes, in particular *zif/268* (Christy et al., 1988), which is co-regulated with *fos* under many types of cell stimulations including neural cell depolarization (Sukhatme et al., 1988; Bartel et al., 1989), suggests that PAF and other stimulators of Ca^{2+} mobilization may regulate multiple components of the immediate-early genomic response through a common mechanism.

Although the mechanism through which PAF elicits genomic effects is not completely understood, certainly a role for Ca^{2+} influx stimulated by exogenous PAF is implicated in the stimulation of *fos*. The finding that PAF stimulates histone H1 kinase ($p34^{cdc2}$-cyclin) activity in nonproliferating cells (Samiei et al., 1991) suggests a broader mechanism of transcriptional control for PAF. Histone H1 regulates genomic transcription by affecting chromatin compactation and maintaining a transcriptionally repressed state (reviewed in Zlatanov, 1990). In addition, histone H1 kinase phosphorylates the product of the *fos* immediate-early gene (Fos) on residues important for negative regulation of transcription by the protein (Abate et al., 1991).

Immediate-Early Genomic Response to Neurotrauma

In the nervous system, the immediate-early genomic response is elicited by physiological and pathological conditions (reviewed in Doucet et al., 1990; and Morgan and Curran, 1991). These eliciting events include ischemia, seizure, injury, and establishment of long-term potentiation. The existence of stimulus-evoked expression of transcription factors in neural tissue suggests that long-term changes in cellular activity and function, including reparative changes following neurotrauma and changes during memory formation, derive from primary, short-term genomic responses.

PAF is implicated in the regulation of the immediate-early genomic response to seizure. Expression of *fos* and several other immediate-early genes is stimulated by pharmacologically- or electrically-induced seizure (Morgan et al., 1987; Saffen et al., 1988; Cole et al., 1990; reviewed in Doucet et al., 1990; and Morgan and Curran, 1991). The potent intracellular PAF antagonist BN50730 (Marcheselli and Bazan, 1991) suppresses *fos* and *zif/268* expression induced by electroconvulsive shock (Marcheselli et al., 1991; Marcheselli and Bazan, 1992). This suggests that (1) the endogenous PAF generated through seizure is functionally coupled to the stimulation of a immediate-early genomic response and that (2) the coupling of PAF to IEG expression may be an intracellular event in neural tissue.

Both the activation of phospholipase A_2 following ischemic onset (Bazan, 1970; Bazan and Rodriguez de Turco, 1980) and the protective effects of PAF antagonists against cellular damage in brain (Panetta et al., 1987; Spinnewyn et al., 1987; Birkle et al., 1988; Oberpichler et al., 1990; Frerichs et al., 1990; Gilböe et al., 1991) implicate PAF as a mediator of ischemic damage. Immediate-early genomic responses to ischemia have been described (Onodera et al., 1989; Jorgenson et al., 1989; Kindy et al., 1991). In both experimentally evoked seizures and ischemia, increased immediate-early gene activity in dentate gyrus and hippocampal neurons is particularly pronounced, and specific PAF binding sites are concentrated in these brain structures (Domingo et al., 1988).

In vitro PAF exerts intrinsic cytotoxicity in certain neural cells (Kornecki and Ehrlich, 1988; Lustig et al., 1992). As a neural Ca^{2+} agonist (Yue et al., 1991a,b), PAF may contribute to neural

damage by altering Ca^{2+} homeostasis. Hippocampal neurons are sensitive to Ca^{2+} fluxes during brain injury (Dienel, 1984; Siesjö and Bengtsson, 1989; Siesjö, 1990; Young, 1992), and the accumulation of PAF during pathological conditions may affect extracellular and cytoplasmic Ca^{2+} mobilizations through mechanisms mediated by possibly both specific PAF receptors and by membrane perturbations (Sawyer and Anderson, 1989; Young, 1992).

The protective effects of PAF antagonists in neurotrauma suggest that PAF effects deleterious metabolic changes in traumatized neural cells. However, as an inducer of immediate-early genomic responses, it is presumed that PAF stimulates new protein synthesis as a means of cellular repair and maintenance. The cellular logic of a mediator of damage evoking mechanisms of repair is not apparently clear. The participation of PAF in these two apparently disparate mechanisms is reflected in studies of NG108-15 cells (Kornecki and Ehrlich, 1988), where high concentrations of PAF exhibit neurotoxicity in these cells, while low concentrations stimulate neuronal maturation. Interestingly, in cerebral cortical cultures sensitive to glutamate toxicity, PAF exerted no toxic effects (Lustig et al., 1992). The magnitude of PAF synthesis and membrane hydrolysis following neurotrauma may, on a physiological scale, reflect homeostatic changes during normal cell metabolism. In this respect, it is the over- or unregulated production of PAF during neurotrauma that contributes to cellular damage. The PAF synthesis following normal neurotransmission and during the initial phases of injury are likely promoters of the new protein synthesis that direct molecular changes and cellular plasticity.

Role of PAF, PUFAs, and IEGs in Long-Term Potentiation

One of the long-term events that may be established physiologically, through the activity of immediate-early transcription factors, is memory formation. Long-term potentiation (LTP) is an experimental model of memory that involves activity-dependent changes in synaptic morphology and circuitry. Long-term structural and functional modulations of synaptic connections are believed to require *de novo* synthesis of proteins (Davis and Squire, 1984; Montarolo et al., 1986). LTP induces immediate-early genomic responses, particularly in cells of the dentate gyrus (reviewed in Abraham et al., 1992; Kaczamarek, 1992). The activity of the *zif/268* transcription factor may be most specifically linked to LTP, as its expression is particularly correlative with the method of LTP induction and its duration.

A role for PAF in the formation of LTP-mediated neuronal changes is suggested because PAF antagonist inhibition hippocampal synaptic responses during establishment of LTP (del Cerro et al., 1990; Arai and Lynch, 1992). In addition, PAF facilitates excitatory neurotransmission at a presynaptic, antagonist-sensitive site (Clark et al., 1992). Cell-generated PAF may therefore mediate plastic responses in the neuron, and this plasticity may involve the synthesis of new proteins mediated by products of a PAF-mediated immediate-early genomic response.

Neuronal expression of *zif/268* is induced by picomolar concentrations of PAF in vitro (Doucet and Bazan, in preparation), suggesting that small changes in PAF synthesis are sufficient to stimulate an immediate-early genomic response. In addition, low concentrations of PAF induce morphological differentiation of neural cells into mature neuronal (Kornecki and Ehrlich, 1988), and astrocytic (Kentroti et al., 1991) phenotypes, alluding to a role for PAF during neural cell development and formation of cellular associations.

Several lines of evidence support a role for phospholipase A_2 in the maintenance of LTP. Phospholipase A_2 inhibitors block the maintenance of LTP in certain cell populations (Linden et al., 1987; Massicotte et al., 1990; Williams and Bliss, 1989; Okada et al., 1989). Arachidonic acid directly affects the maintenance of LTP (Kato et al., 1991), possibly deriving from a post-synaptic site (Clements et al., 1991; Barbour et al., 1989).

The interrelationship of PAF in phospholipase A_2 activity, the enzyme being both the initiator of PAF synthesis through the remodeling pathway and a target for activation by PAF, is a possible explanation of the effect of both PAF antagonists and phospholipase A_2 inhibitors in blocking LTP. In addition, Ca^{2+} antagonists block LTP formation (Obenaus et al., 1989), and PAF is linked to increased intracellular cellular Ca^{2+} through both a plasma membrane Ca^{2+} channel and through the phospholipase C-catalyzed generation of the intracellular Ca^{2+} agonist IP_3 (Yue et al., 1991a,b). Furthermore, Ca^{2+} is a stimulator of phospholipase A_2 activity (Lapetina and Crouch, 1989) and may mediate the activation of phospholipase A_2 through NMDA receptors (Dumuis et al., 1988) which mediate formation of LTP (Collingridge et al., 1983). The maintenance of LTP is likely comprised of a selective, self-limiting stabilization of phospholipase A_2 metabolites in cell membranes that affects membrane structure at the synapse and physically and biochemically alters transport mechanisms and ion channels available at the plasma membranes of the synapse.

Summary

The physical nature of neuronal cells, particularly in the functional and morphological segregation of synapse, soma, and dendrites, imparts special importance on the integrity of their cell membranes for the localization of function, generation of intrinsic second messengers, and plasticity required for adaptation and repair. The component phospholipids of neural membranes are important sources of bioactive mediators that participate in such diverse phenomena as memory formation and cellular damage following trauma. A common role for PAF in these processes is established through the suppressive effects of its antagonists. Furthermore, being both an extracellular and intracellular agonist of phospholipase activation, in addition to being a product of phospholipase activity, PAF assumes a centralized role in the cellular metabolism following neural stimulation. The linkage of PAF to neural immediate-early gene expression, both in vitro and in vivo, suggests that its effects are initiating to long-term formative and reparative processes. Such a common link between destructive and plastic responses provides an important view of cellular and tissue maintenance in the nervous system.

References

Abate C., Marshak D., and Curran T. (1991) Fos is phosphorylated by $p34^{cdc2}$ cyclic AMP-dependent protein kinase and protein kinase C at multiple sites clustered within regulatory regions. *Oncogene* **6,** 2179–2185.

Abraham W., Dragunow M., and Tate W. (1992) The role of immediate-early genes in the stabilization of long-term potentiation. *Mol. Neurobiol.* **5,** 1–15.

Arai A. and Lynch G. (1992) Antagonists of the platelet-activating factor receptor block long-term potentiation in hippocampal slices. *Eur. J. Neur.* **4,** 411–419.

Aveldano de Caldironi M. I., and Bazan N. G. (1979) α-methyl-*p*-tyrosine inhibits the production of free arachidonic acid and diacylglycerols in brain after a single electroconvulsive shock. *Neurochem. Res.* **4,** 213–221.

Aveldano M. I. and Bazan N. G. (1974) Displacement into incubation medium by albumin of highly unsaturated retina free fatty acids arising from membrane lipids. *FEBS Lett.* **40,** 53–56.

Aveldano M. I. and Bazan N. G. (1975a) Rapid production of diacylglycerols enriched in arachidonate and stearate during early brain ischemia. *J. Neurochem.* **25,** 919–920.

Aveldano M. I. and Bazan N. G. (1975b) Differential lipid deacylation during brain ischemia in a homeotherm and a poikilotherm. Content and composition of free fatty acids and triacylglycerols. *Brain Res.* **100,** 99–110.

Bartel D., Sheng M., Lau L., and Greenberg M. (1989) Growth factors and membrane depolarization activate distinct programs of early response gene expression: dissociation of *fos* and *jun* induction. *Genes Dev.* **3,** 304–313.

Barbour B., Szatkowski M., Ingledew N., and Attwell D. (1989) Arachidonic acid induces a prolonged inhibition of glutamate uptake into glial cells. *Nature* **342,** 918–920.

Baudry M., Massicote G., and Hauge S. (1991) Opposite effects of phospholipase A_2 on [^{3}H]-AMPA binding in adult and neonatal membranes. *Brain Res. Dev. Brain Res.* **61,** 265–267.

Bazan N. G. (1971) Phospholipases A_1 and A_2 in brain subcellular fractions. *Acta Phys. Lat. Amer.* **21,** 101–106.

Bazan N. G. (1970) Effects of ischemia and electroconvulsive shock on free fatty acid pool in the brain. *Biochim. Biophys. Acta* **218,** 1–10.

Bazan N. G. (1971) Free fatty acid production in cerebral white and grey matter of the squirrel monkey. *Lipids* **6,** 211,212.

Bazan N. G. (1976) Free arachidonic acid and other lipids in the nervous system during early ischemia and after electroshock, in *Functional and Metabolism of Phospholipids in the Central and Peripheral Nervous System* (Porcellati G., Amaducci L., and Galli C., eds.), **vol 72,** Plenum, New York, pp. 317–335.

Bazan N. G., Birkle D. L., and Reddy T. S. (1984) Docosahexaenoic acid (22:6, n-3) is metabolized to lipoxygenase reaction products in the retina. *Biochem. Biophys. Res. Comm.* **125,** 741–747.

Bazan N. G. and Birkle D. L. (1987) Polyunsaturated fatty acids in inositol phospholipids at the synapse in neuronal responsiveness, in *Molecular Mechanisms of Neuronal Responsiveness* (Ehrlich Y., Lennox R., Kornecki E., Berry W., eds.), Plenum, New York, pp. 45–68.

Bazan N. G. and Rakowski H. (1970) Increased levels of brain free fatty acids after electroconvulsive shock. *Life Sci.* **9,** 501–507.

Bazan N. G. and Rodriguez de Turco E. B. (1980) Membrane lipids in the pathogenesis of brain edema: phospholipids and arachidonic acid, the earliest membrane components changed at the onset of ischemia, in *Brain Edema, Advances in Neurology* (Cervos-Navarro J. and Ferszt R., eds.), **vol 28,** Raven, New York, pp 197–205.

Bazan N. G., Birkle D. L., Tang W., and Reddy T. S. (1986) The accumulation of free arachidonic acid and the formation of prostaglandins and lipoxygenase reaction products in the brain during experimental epilepsy, in *Basic Mechanisms of the Epilepsies. Molecular Cellular Approaches Advances in Neurology,* **vol 47** (Delgado-Escueta A., Ward A., and Woodbury D., eds.), Raven, New York, pp. 209–217.

Bazan N. G., Squinto S., Braquet P., Panetta T., and Marcheselli V. L. (1991) Platelet-activating factor and polyunsaturated fatty acids in cerebral ischemia or convulsions: intracellular PAF binding sites and activation of Fos/Jun/AP-1 transcriptional signalling systems. *Lipids* **26,**1236–1242.

Birkle D. L., Kurian P., Braquet P., and Bazan N. G. (1988) The plateletactivating factor antagonist BN 52021 decreases accumulation of free polyunsaturated fatty acid in mouse brain during ischemia and electroconvulsive shock. *J. Neurochem.* **51,** 1900–1905.

Birkle D. L. and Bazan N. G. (1984) Effects of K^+ depolarization on the synthesis of prostaglandins and hydroxyeicosatetra(Δ5,8,11,14)enoic acids (HETEs) in the rat retina. Evidence for esterification of 12-HETE in lipids. *Biochim. Biophys. Acta* **795,** 564–573.

Birkle D. L. and Bazan N. G. (1987) Effect of bicuculline-induced status epilepticus on prostaglandins and hydroxyeicosatetraenoic acids in rat brain subcellular fractions. *J. Neurochem.* **48,** 1768–1178.

Blank M., Lee T., Fitzgerald V., and Snyder F. (1981) A specific acetylhydrolase for 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (a hypotensive and platelet-activating lipid). *J. Biol. Chem.* **256,** 175–178.

Blasquez C., Jegou S., Delarue C., Delbende C., Bunel D., Braquet P., and Vaudry, H. (1990) Effect of platelet-activating factor on hypothalamic and hypophyseal pro-opiomelanocortin-related peptides and hypothalamopituitary-adrenal axis in the rat. *Eur. J. Pharmacol.* **177,** 145–153.

Bosisio E., Galli C., Galli G., Nicosia S., Spagnuolo C., and Tosi L. (1976) Correlation between release of free arachidonic acid and prostaglandin formation in brain cortex and cerebellum. *Prostaglandins* **11,** 773–781.

Bratton D., Kailey J., Clay K., and Henson P. (1991) A model for the extracellular release of PAF: influence of plasma membrane phospholipid assymetry. *Biochim. Biophys. Acta* **1062,** 24–34.

Braquet, P., Laurent J., Rolland A., Martin C., Pommier J., Hosford D., and Esanu A. (1990) From ginkgolides to N-substituted piperidinothieno diazepines, a new series of highly potent PAF antagonists. *Adv. Prost. Thromb. Leuk. Res.* **21,** 929–937.

Braquet P., Paubert-Braquet M., Bourgain R., Bussolino F., and Hosford, D. (1989) PAF/cytokine auto-generated feedback networks in microvascular immune injury: consequences in shock, ischemia, and graft rejection. *J. Lipid Med.* **1,** 75–112.

Braquet P., Touqui L, Shen T., and Vargaftig B. (1987) Perspectives in platelet-activating factor research. *Pharmacol. Rev.* **39,** 97–145.

Braughler J., Duncan L., and Goodman T. (1985) Calcium enhances in vitro free radical-induced damage to brain synaptosomes, mitochondria, and cultured spinal cord neurons. *J. Neurochem.* **45,** 1288–1293.

Braughler J. and Hall E. (1992) Involvement of lipid peroxidation in CNS injury. *J. Neurotrauma* **9**(S1), S1–S7.

Bussolino F., Gremo F., Tetta C., Pescarmona G.P., and Camussi G. (1986) Production of platelet-activating factor by chick retina. *J. Biol. Chem.* **261,** 16,502–16,508.

Bussolino F., Pescarmona G., Camussi G., and Gremo F. (1988) Acetylcholine and dopamine promote production of platelet-activating factor in immature cells of chick embryonic retina. *J. Neurochem.* **51,** 1755–1759.

Bussolino F., Torelli S., Stephanini E., and Gremo F. (1989) Platelet-activating factor production occurs through stimulation of cholinergic and dopaminergic receptors in the chick retina. *J. Lipid Med.* **1,** 283–288.

Cenedella R., Galli C., and Paoletff R. (1975) Brain free fatty acid levels in rats sacrificed by decapitation versus focused-microwave irradiation. *Lipids* **10,** 290–293.

Chilton F., Marshall Ellis J., Olson S., and Wykle R. (1984) 1-*O*-Alkyl-2-arachidonyl-*sn*-glycero-3-phosphocholine. A common source of platelet-activating factor and arachidonate in human polymorphonuclear leukocytes. *J. Biol. Chem.* **259,** 12,014–12,019.

Christy B. and Nathans D. (1989) DNA binding site of the growth-factor inducible protein Zif/268. *Proc. Natl. Acad. Sci. USA* **86,** 8737–8741.

Christy B., Lau L., and Nathans D. (1988) A gene activated in mouse 3T3 cells by serum growth factors encodes a protein with "zinc finger" sequences. *Proc. Natl. Acad. Sci. USA* **85,** 7857–7861.

Clark G., Happel L., Zorumski C., and Bazan N. G. (1992) Enhancement of hippocampal synaptic transmission by platelet-activating factor. *Neuron,* in press.

Clements M., Bliss T., and Lynch M. (1991) Increase in arachidonic acid concentration in a postsynaptic membrane fraction following induction of long-term potentiation in the dentate gyrus. *Neurosci.* **45,** 379–389.

Cole A., Abu-Shakra S., Saffen D., Baraban J., and Worley, P. (1990) Rapid rise in transcription factor mRNAs in rat brain after electroshock-induced seizures. *J. Neurochem.* **55,** 1920–1927.

Collingridge G., Kehl S., and McLennan H. (1983) Excitatory amino acids in synaptic transmission in the Schaffer-commissural pathway of the rat hippocampus. *J. Physiol. (London)* **334,** 33–46.

Davis H. and Squire, L. (1984) Protein synthesis and memory. *Psychol. Bull.* **96,** 518–559.

del Cerro S., Arai A., and Lynch, G. (1990) Inhibition of long-term potentiation by an antagonist of platelet-activating factor receptors. *Behav. Neural Biol.* **54,** 213–217.

Dienel G. (1984) Regional accumulation of calcium in post-ischemic brain. *J. Neurochem.* **43,** 913–925.

Doebber T. and Wu M. (1987) Platelet-activating factor (PAF) stimulates the PAF-synthesizing enzyme acetyl-CoA: 1-alkyl-*sn*-glycero-3-phosphocholine O^2-acetyltransferase and PAF synthesis in neutrophils. *Proc. Natl. Acad. Sci. USA* **84,** 7557–7561.

Domingo M., Spinnewyn B., Charbrier P., and Braquet P. (1988) Presence of specific binding sites for platelet-activating factor (PAF) in brain. *Biochem. Biophys. Res. Comm.* **151,** 730–736.

Doucet J., Squinto S., and Bazan N. G. (1990) Fos-Jun and the primary genomic response in the nervous system: possible physiological role and pathophysiological significance. *Mol. Neurobiol.* **4,** 27–55.

Dumuis A., Sebben M., Haynes L., Pin J.-P., and Bockaert J. (1988) NMDA receptors activate the arachidonic acid cascade-system in striatal neurons. *Nature* **336,** 68–70.

Edgar A. D., Strosznajder J., and Horrocks L. A. (1982) Activation of ethanolamine phospholipase A_2 in brain during ischemia. *J. Neurochem.* **39,** 1111–1116.

Francel P. (1992) Bradykinin and neuronal injury. *J. Neurotrauma* **9**(S1), S27–S45.

Francescangeli E. and Goracci G. (1989) The *de novo* synthesis of platelet-activating factor in rat brain. *Biochem. Biophys. Res. Comm.* **161,** 107–112.

Frerichs K., Lindsberg P., Hallenbeck J., and Feuerstein G. (1990) Platelet-activating factor and progressive brian damage following focal brain injury. *J. Neurosurg.* **73,** 223–233.

Gilböe D., Kintner D., Fitzpatrick J., Emoto S., Esanu A., Braquet P., and Bazan N. G. (1991) Recovery of postischemic brain metabolism and function following treatment with a free radical scavenger

and platelet-activating factor antagonists. *J. Neurochem.* **56,** 311–319.

Giusto N. and Bazan N. (1983) Anoxia-induced production of methylated and free fatty acids in retina, cerebral cortex, and white matter: comparison with triglycerides and with other tissues. *Neurochem. Pathol.* **1,** 17–41.

Glaser K, Asmis R., and Dennis, E. (1990) Bacterial lipopolysaccharide priming of P338D, macrophage-like cells for enhanced arachidonic acid metabolism: PAF receptor activation and regulation of phospholipase A_2. *J. Biol. Chem.* **265,** 8658–8664.

Harris R., Clay K., Murphy R., and Henson P. (1985) Effects of PAF and related lipids on membrane physical properties. *Fed. Proc.* **44,** 858.

Henson P. M. (1987) Extracellular and intracellular activities of PAF, in *Platelet-Activating Factor and Related Lipid Mediators* (Snyder F., ed.), Plenum, New York, pp. 255–271.

Herschman H. (1989) Extracellular signals, transcriptional responses and cellular specificity. *Trends Biochem. Sci.* **14,** 455–458.

Herschman H. (1991) Primary response genes induced by growth factors and tumor promotors. *Ann. Rev. Biochem.* **60,** 281–319.

Hillered L. and Chan P. (1990) Effects of arachidonic acid on brain mitochondrial function, in *Lipid Mediators in Ischemic Brain Damage and Experimental Epilepsy, New Trends in Lipid Mediators Research* (Braquet P., ed.), vol. 4, Basel, Karger, pp. 190–202.

Ho Y.-S., Lee W. M. F., and Snyderman R. (1987) Chemoattractant-induced activation of c-*fos* gene expression in human monocytes. *J. Exp. Med.* **165,** 1524–1538.

Hyman B. and Spector A. (1982) Choline uptake in cultured human Y79 retinoblastoma cells: effect of polyunsaturated fatty acid compositional modification. *J. Neurochem.* **38,** 650–656.

Ikeda M., Yoshida S., Busto R., Santiso M., and Ginsberg M. (1986) Polyphosphoinositides as a probable source of brain free fatty acids accumulated at the onset of ischemia. *J. Neurochem.* **47,** 123–132.

Jorgenson M., Deckert J., Wright D., and Gehlert D. (1989) Delayed c-*fos* protooncogene expression in the rat hippocampus induced by transient global cerebral ischemia: an in situ hybridization study. *Brain Res.* **484,** 393–398.

Junier M., Tiberghien C., Rougeot C., Fafeur V., and Dray F. (1988) Inhibitory effect of PAF on lutenizing hormone-releasing hormone and somatostatin release from rat medain eminence *in vitro* correlated with characterization of specific PAF receptor sites. *Endocrinology* **123,** 72–80.

Kaczamarek L. (1992) Expression of c-*fos* and other genes encoding transcription factors in long-term potentiation. *Behav. Neur. Biol.* **57,** 263–266.

Kato K., Uruno K., Saito K., and Kato H. (1991) Both arachidonic acid and 1-oleoyl-2-acetyl glycerol in low magnesium solution induce long-term potentiation in hippocampal CA1 neurons *in vitro*. *Brain Res.* **563,** 94–100.

Kawasaki T. and Snyder F. (1987) The metabolism of lyso-platelet-activating factor (1-*O*-alkyl-2-*lyso-sn*-glycero-3-phosphocholine) by a calcium-dependent lysophospholipase D in rabbit kidney medulla. *Biochim. Biophys. Res. Comm.* **920,** 85–93.

Kentroti S., Baker R., Lee K., Bruce C., and Vernadakis A. (1991) Platelet-activating factor increases glutamine synthetase activity in early and late passage C-6 glioma cells. *J. Neurosci. Res.* **28,** 497–506.

Kindy M., Carney J. P., Dempsey R., and Carney J. M. (1991) Ischemic induction of protooncogene expression in gerbil brain. *J. Molec. Neurosci.* **27,** 217–228.

Kochanek P., Nemoto E., Melick J., Evans R., and Burke D. (1988) Cerebrovascular and cerebrometabolic effects of intracarotid infused platelet-activating factor in rats. *J. Cereb. Blood Flow Metab.* **8,** 546–551.

Kornecki E. and Ehrlich Y. (1988) Neuroregulatory and neuropathological actions of the ether-phospholipid platelet-activating factor. *Science* **240,** 1792–1794.

Kornecki E., Ehrlich Y., and Lenox R. (1984) Platelet-activating factor-induced aggregation of human platelets specifically inhibited by triazolobenzodiazepines. *Science* **226,** 1454–1456.

Kumar R., Harvey S. A. K., Kester M., Hanahan D. J., and Olson M. S. (1988) Production and effects of platelet-activating factor in the rat brain. *Biochim. Biophys. Acta* **963,** 375–383.

Lapetina E. and Crouch M. (1989) The relationship between phospholipases A_2 and C in signal transduction, in *Arachidonic Acid Metabolism in the Nervous System,* vol. 59 (Baraki A., and Bazan N., eds.), Academy of Science, New York, pp. 153–157.

Lazarewicz J. W., Leu V., Sun G. Y., and Sun A Y. (1983) Arachidonic acid release from K^+-evoked depolarization of brain synaptosomes. *Neurochem. Int.* **5,** 471–478.

Lee T., Malone B., and Snyder F. (1986) A new *de novo* pathway for the formation of 1-alkyl-2-acyl-*sn*-glycerols, precursors of platelet-activating factor. Biochemical characterization of 1-alkyl-2-lyso-*sn*-glycero-3-P:acetyl-CoA acetyltransferase in rat spleen. *J. Biol. Chem.* **261,** 5373–5377.

Lee T., Malone B., and Snyder F. (1988) Formation of 1-alkyl-2-acyl-*sn*-glycerols via *de novo* biosynthetic pathway for platelet-activating factor. Characterization of 1-alkyl-2-*lyso-sn*-glycero-3-phosphate phosphohydrolase in rat spleens. *J. Biol. Chem.* **263,** 1755–1760.

Linden D., Sheu F., Murakami K., and Routtenberg A. (1987) Enhancement of long-term potentiation by cis-unsaturated fatty acid. Relation to protein kinase C and phospholipase A_2. *J. Neurosci.* **7,** 3783–3792.

Lindsberg P., Yue T-L., Frerichs K., Hallenbeck J., and Feuerstein G. (1990) Evidence for platelet-activating factor as a novel mediator in experimental stroke in rabbits. *Stroke* **21,** 1452–1457.

Lustig H., Chan J., and Greenberg D. (1992) Comparative neurotoxic potential of glutamate, endothelins, and platelet-activating factor in cerebral cortical cultures. *Neurosci. Lett.* **139,** 15–18.

Maier J., Hla T., and Maciag T. (1990) Cyclooxygenase is an immediate-early gene induced by interleukin-1 in human endothelial cells. *J. Biol. Chem.* **265,** 10,805–10,808.

Marcheselli V. L. and Bazan N. G. (1992) ECS-induced zif-268 in hippocampus is inhibited by a PAF antagonist. *Trans. Amer. Soc. Neurochem.* **23,** 329.

Marcheselli V. L. and Bazan N. G. (1991) A specific antagonist for intracellular platelet-activating factor (PAF) binding sites lacks activity on synaptic membranes. *Trans. Amer. Soc. Neurochem.* **22,** 187.

Marcheselli V. L., Doucet J. P., and Bazan N. G. (1991) Platelet-activating factor is a mediator of *fos* expression induced by a single seizure in rat hippocampus. *Soc. Neurosci. Abs.* **17,** 349.

Marcheselli V. L., Rossowska M., Domingo M. T., Braquet P., and Bazan N. G. (1990) Distinct platelet-activating factor binding sites in synaptic endings and in intracellular membranes of rat cerebral cortex. *J. Biol. Chem.* **265,** 9140–9145.

Marion J. and Wolfe L. (1978) Increase in vivo of unesterified fatty acids, prostaglandin $F_{2\alpha}$, but not thromboxane B_2 in rat brain during drug induced convulsions. *Prostaglandins* **16,** 99–110.

Massicotte G. and Baudry M. (1990) Modulation of DL-alpha-amino-3-hydroxy-5-methylisoxazile-4-proprionate (AMPA)/quisqualate receptors by phospholipase A_2 treatment. *Neurosci. Lett.* **118,** 245–248.

Massicote G., Oliver M., Lynch G., and Baudry M. (1990) Effect of bromphenacyl bromide, a phospholipase A_2 inhibitor, on the induction and maintenance of LTP in hippocampal slices. *Brain Res.* **537,** 49–53.

Mazer B., Domenico J., Sawami H., and Gelfand E. (1991) Platelet-activating factor induces an increase in intracellular calcium and expression of regulatory genes in human B lymphoblastoid cells. *J. Immunol.* **146,** 1914–1918.

Miller B., Sarantis M., Traynelis S., and Attwell D. (1992) Potentiation of NMDA receptor currents by arachidonic acid. *Nature* **355,** 722–725.

Miller L, Bazan N. G., Roy R., Clostre F., Gaver A., and Braquet P. (1991) Platelet-activating factor antagonists interact with $GABA_A$ receptors. *Res. Comm. Chem. Path. Pharmacol.* **74,** 253–256.

Montarolo P., Goelet P., Castellucci V., Morgan J., Kandel E., and Schracher S. (1986) A critical period for macromolecular synthesis in long-term heterosynaptic facilitation in *Aplysia. Science* **234,** 1249–1254.

Morgan J., Cohen D., Hempstead J., and Curran T. (1987) Mapping patterns of c-*fos* in the central nervous system after seizure. *Science* **237,** 192–197.

Morgan J. and Curran T. (1991) Stimulus-transcription coupling in the nervous system: involvement of the inducible protooncogenes *fos* and *jun*. *Ann. Rev. Neurosci.* **14,** 421–451.

Nieto M., Venable M., Baudry S., Greene D., Kennedy M., Bass D., and Wykle R. (1991) Evidence that hydrolysis of ethanolamine plasmalogens triggers synthesis of platelet-activating factor via a transacylation reaction. *J. Biol. Chem.* **266,** 18,699–18,706.

Obenaus A., Mody I., and Baimbridge K. (1989) Dantrolene-Na (Dantrium) blocks induction of long-term potentiation in hippocampal slices. *Neurosci. Lett.* **98,** 172–178.

Oberpichler H., Sauer D., Rosberg C., Mennel H., and Krieglstein, J. (1990) PAF antagonist ginkgolide-B reduces postischemic neuronal damage in the rat-brain hippocampus. *J. Cereb. Blood Flow Metab.* **10,** 133–135.

Okada D., Yamagishu S., and Sugiyama, H. (1989) Differential effects of phospholipase inhibitors in long-term potentiation in the rat hippocampal mossy fiber synapses and Schaffer/commissural synapses. *Neurosci. Lett.* **100,** 141–146.

Onodera H., Kogure K., Ono Y., Igarashi K., Kiyota Y., and Nagaoka A. (1989) Protooncogene c-*fos* is transiently induced in the rat cerebral cortex after forebrain ischemia. *Neurosci. Lett.* **98,** 101–104.

Panetta T., Marcheselli V. L., Braquet P., Spinnewyn B., and Bazan N. G. (1987) Effects of a platelet-activating factor antagonist (BN 52021) on free fatty acids, diacylglycerols, polyphosphoinositides and blood now in the gerbil brain: Inhibition of ischemia-reperfusion induced cerebral injury. *Biochem. Biophys. Res. Commun.* **149,** 580–587.

Pellerin L. and Wolfe L. (1991) Release of arachidonic acid by NMDA-receptor activation in the rat hippocampus. *Neurochem. Res.* **16,** 983–989.

Politi L. E., Rodriguez de Turco E. B., and Bazan N. G. (1985) Dexamethasone effect on free fatty acid and diacylglycerol accumulation during experimentally induced vasogenic brain edema. *Neurochem. Pathol.* **3,** 249–269.

Ramesha C. and Pickett W. (1986) Platelet-activating factor and leukotriene biosynthesis is inhibited in polymorphonuclear leukocytes depleted of arachidonic acid. *J. Biol. Chem.* **261,** 7592–7596.

Ransone L. and Verma I. (1990) Nuclear protooncogenes *fos* and *jun*. *Ann. Rev. Cell Biol.* **6,** 539–557.

Record M., Ribbes G., Terce F., and Chap H. (1989) Subcellular localization of phospholipids and enzymes involved in PAF-acether metabolism. *J. Cell Biochem.* **40,** 353–358.

Reddy T. S. and Bazan, N. G. (1987) Arachidonic acid, stearic acid and diacylglycerol accumulation correlates with the los of phosphatidylinositol 4,5-bisphosphate in cerebrum 2 seconds after electroconvulsive shock. Complete reversion of changes 5 minutes after stimulation. *J. Neurosci. Res.* **18,** 449–455.

Rougeot C., Junier M., Minary P., Weidenfeld J., Braquet P., and Dray F. (1990) Intracerebroventricular injection of platelet-activating factor induces secretion of adrenocorticotropin, beta-endorphin and corticosterone in conscious rats—a possible link between the immune and nervous systems. *Neuroendocrinology* **51,** 315–319.

Rustenbeck I., Eibl H., and Lenzen S. (1991) Structural requirements of lysophospholipid-regulated mitochondrial Ca^{2+} transport. *Biochim. Biophys. Acta* **1069,** 99–109.

Saffen D., Cole A., Worley P., Christy B., Ryder K., and Baraban J. (1988) Convulsant-induced increase in transcription factor messenger RNAs in rat brain. *Proc. Natl. Acad. Sci. USA* **85,** 7795–7799.

Samiei M., Daya-Makin M., Clark-Lewis I., and Pelech S. (1991) Platelet-activating factor- and thrombin-induced stimulation of $p34^{cdc2}$-cyclin histone H1 kinase activity in platelets. *J. Biol. Chem.* **266,** 14,889–14,892.

Sawyer D. and Anderson, O. (1989) Platelet-activating factor is a general membrane perturbant. *Biochim. Biophys. Acta* **987,** 129–132.

Schulam P. G., Kuruvilla A., Putcha G., Mangus L., Johnson J., and Shearer W. T. (1991) Platelet-activating factor induces phospholipid turnover calcium flux, arachidonic acid liberation, eicosanoid generation, and oncogene expression in a human B-cell line. *J. Immunol.* **146,** 1642–1647.

Sheng M., Dougan S., McFadden G., and Greenberg M. (1988) Calcium and growth factor pathways of c-*fos* transcriptional activation require distinct upstream regulatory sequences. *Mol. Cell Biol.* **8,** 2787–2796.

Sheng M., McFadden G., and Greenberg M. (1990) Membrane depolarization and calcium induce c-*fos* transcription via phosphorylation of transcription factor CREB. *Neuron* **4,** 571–582.

Shukla S. (1992) Platelet-activating factor and signal transduction mechanisms. *FASEB J.* **6,** 2296–2301.

Siesjö B. (1990) Calcium in the brain under physiological and pathological conditions. *Eur. Neurol.* **30,** 3–9.

Siesjö B. K. and Bengtsson F. (1989) Calcium fluxes, calcium antagonists, and calcium-related pathology in brain ischemia, hypoglycemia, and spreading depression: a unifying hypothesis. *J. Cereb. Blood Flow Metab.* **9,** 127–140.

Siesjö B. K., Ingvar M., and Westerberg Z. (1982) The influence of bicuculline-induced seizures on free fatty acid concentrations in cerebral cortex, hippocampus and cerebellum. *J. Neurochem.* **39,** 796–802.

Snyder F. (1990) Platelet-activating factor and related acetylated lipids as potent biologically active cellular mediators. *Am. J. Physiol.* **259,** C697–C708.

Spinnewyn B., Blavet N., Clostre F., Bazan N., and Braquet P. (1987) Involvement of platelet-activating factor (PAF) in cerebral post-ischemic phase in mongolian gerbils. *Prostaglandins* **34,** 337–349.

Squinto S., Block A., Braquet P., and Bazan N. G. (1989) Platelet-activating factor stimulates a fos/jun AP1 transcriptional signalling system in human neuroblastoma cells. *J. Neurosci. Res.* **24,** 558–566.

Squinto S. P., Braquet P., Block A., and Bazan N. G. (1990) Platelet-activating factor activates HIV

promoter in transfected SH-SYSY neuroblastoma cells and MOLT-4 T-lymphocytes. *J. Mol. Neurosci.* **2,** 79–84.

Sogos V., Bussolino E., Pilia E., Torelli S., and Gremo F. (1990) Acetylcholine-induced production of platelet-activating factor by human fetal brain cells in culture. *J. Neurosci. Res.* **27,** 706–711.

Stewart A. and Phillips W. (1989) Intracellular platelet-activating factor regulates eicosanoid generation in guinea-pig resident peritoneal macrophages. *Br. J. Pharmacol.* **98,** 141–143.

Stewart A., Dubbin P., Harris T., and Dusting G. (1990) Platelet-activating factor may act as a second messenger in the release of icosanoids and superoxide anions from leukocytes and endothelial cells. *Proc. Natl. Acad. Sci. USA* **87,** 3215–3222.

Stewart A., Dubbin P., Harris T., and Dusting G. (1989) Evidence for an intracellular action of platelet-activating factor in bovine cultured aortic endothelial cells. *Br. J. Pharmacol.* **96,** 503–505.

Stoll L., Figard P., Yerram N., Yorek M., and Spector A. (1989) 1-*O*-alkyl-2-acetyl-*sn*-glycerol: a platelet-activating factor metabolite with biological activity in vascular smooth muscle cells. *Cell Regulation* **1,** 13–25.

Suga K., Kaurasaki T., Blank M., and Snyder F. (1990) An arachidonoyl (polyenoic)-specific phospholipase A_2 activity regulates the synthesis of platelet-activating factor in granulocytic HL-60 cells. *J. Biol. Chem.* **265,** 12,363–12,371.

Sukhatme V., Cao X., Chang L., Tsai-Morris C., Stamenkovich D., Ferreira P., Cohen D., Edwards S., Shows T., Curran T., Le Beau M., and Adamson E. (1988) A zinc finger-encoding gene coregulated with c-fos during growth and differentiation, and after cellular depolarization. *Cell* **53,** 37–43.

Tessner T., O'Flaherty J., and Wykle, R. (1989) Stimulation of platelet-activating factor synthesis by a nonmetabolizable bioactive analog of platelet-activating factor and influence of arachidonic acid metabolites. *J. Biol. Chem.* **264,** 4794–4799.

Tripathi Y., Kandala J., Guntaka R., Lim R., and Shukla, S. (1991) Platelet-activating factor induces expression of early response genes c-fos and TIS-1 in human epidermoid carcinoma cells. *Life Sci.* **49,** 1761–1767.

Vallari D., Record M., and Snyder F. (1990) Conversion of alkylacetylglycerol to platelet-activating factor in HL-60 cells and subcellular localization of the mediator. *Archiv. Biochem. Biophys.* **276,** 538–542.

Wardlow M., Cox C., Meng K., Greene D., and Farr R. (1986) Substrate specificity and partial characterization of the PAF-acylhydrolase in human serum that rapidly inactivates platelet-activating factor. *J. Immunol.* **136,** 3441–3446.

Weber C., Aepfelbacher M., Lux I., Zimmer B., and Weber P. (1991) Docosahexaenoic acid inhibits PAF and LTD_4 stimulated $[Ca^{2+}]_i$-increase in differentiated monocytic U937 cells. *Biochim. Biophys. Acta* **1133,** 38–45.

Williams J. and Bliss T. (1989) An *in vitro* study of the effects of lipoxygenase and cyclo-oxygenase inhibitors of arachidonic acid on the induction and maintenance of long-term potentiation in the hippocampus. *Neurosci. Lett.* **107,** 301–306.

Wirthmueller U., de Weck A., and Dahinden C. (1990) Studies of the mechanism of platelet-activating factor production in GM-CSF primed neutrophils: involvement of protein synthesis and phospholipase A_2 activation. *Biochem. Biophys. Res. Comm.* **170,** 556–562.

Woodard, D., Lee T., and Snyder F. (1987) The final step in the *de novo* biosynthesis of platelet-activating factor. Properties of a unique CDP-choline:1-alkyl-2-acetyl-*sn*-glycerol choline phosphotransferase in microsomes from the renal inner medulla of rats. *J. Biol. Chem.* **262,** 2520–2527.

Vallari D., Record M., and Snyder F. (1990) Conversion of alkylacetylglycerol to platelet-activating factor in HL-60 cells and subcellular localization of the mediator. *Archiv. Biochem. Biophys.* **276,** 538–542.

Yorek, M. Strom D., and Spector A. (1984) Effect of membrane polyunsaturation on carrier-mediated transport in cultured retinoblastoma cells: alterations in taurine uptake. *J. Neurochem.* **42,** 254–260.

Yoshida S., Inoh S., Asano T., Sano K., Kubota M., Shimazaki H., and Ueta N. (1980) Effect of transient ischemia on free fatty acids and phospholipids in the gerbil-brain. *J. Neurosci.* **53,** 323–331.

Young W. (1992) Role of calcium in nervous system injuries. *J. Neurotrauma* **9,** S9–S25.

Yue T.-L., Gleason M., Gu J.-L., Lysko P.G., Hallenbeck J., and Feuerstein G. (1991a) Platelet-activating factor (PAF) receptor-mediated calcium mobilization and phosphoinositide turnover in neurohybrid NG108-15 cells: studies with BN50739, a new PAF antagonist. *J. Pharmacol. Exp. Therap.* **257,** 374–381.

Yue T.-L., Gleason M., Hallenbeck J., and Feuerstein G. (1991b) Characterization of platelet-activating factor-induced elevation of cytosolic free-calcium

level in neurohybrid NCB-20 cells. *Neurosci.* **41,** 177–185.

Yue T.-L., Lysko P. G., and Feuerstein G. (1990) Production of platelet-activating factor from rat cerebellar granule cells in culture. *J. Neurochem.* **54,** 1809–1811.

Zlatanov J. (1990) Histone H1 and the regulation of transcription of eukaryotic genes. *TIBS* **15,** 273–276.