Arachidonic acid in cell signaling

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Important advances have recently been made in our understanding of the arachidonic acid cascade. The molecular characterization of different forms of phospholipase A_2 indicates that multiple pathways are involved in the release of arachidonic acid evoked by physiological or pathological stimuli. Moreover, studies on the expression of enzymes that metabolize arachidonic acid reveal the potential participation of the eicosanoids in central aspects of cell regulation, such as control of mitogenesis. Finally, cloning of the first eicosanoid receptors is a major step towards elucidating the diverse cellular functions exerted by these bioactive lipids.

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Introduction

The many cellular actions exerted by arachidonic acid (AA), a polyunsaturated fatty acid, are initiated by its release from membrane phospholipids and by its subsequent conversion into a family of biologically active metabolites, collectively called 'eicosanoids'. Some of the enzymatic pathways involved in AA release and metabolism are shown in Fig. 1, where I have summarized the results of studies that cannot be adequately described within the limits of this review. Here, I will review what I consider to be the most significant recent advances in three subject areas related to the AA cascade: the mechanism underlying release of AA; the molecular characterization of AA-metabolizing enzymes; and the cellular functions exerted by the eicosanoids.

Pathways of arachidonic acid release

Cytosolic, arachidonic acid specific phospholipase A_2

Phospholipase A_2 (PLA_2) catalyzes the hydrolytic cleavage of glycerophospholipid at the *sn*-2 position (where AA is most often esterified) yielding free fatty acid and lysophospholipid (Fig. 1). A high molecular weight (85.2 kDa) cytosolic phospholipase A_2 ($cPLA_2$), which selectively hydrolyzes AA-containing phospholipids, has been purified from several sources, and a full-length cDNA that encodes it has been isolated and sequenced [1–3,4••,5]. *In vitro*, purified $cPLA_2$ is active at concentrations of free Ca^{2+} (0.1–1 μmol/l) similar to those reached intracellularly during receptor-dependent Ca^{2+} responses, and prefers AA-containing phospholipids in natural membranes about 20-fold more than phospholipids containing other fatty acids.

Raising levels of Ca^{2+} from 100 to 300 nmol/l causes the association of recombinant $cPLA_2$ with membrane vesicles, suggesting that, in response to receptor-stimulated rises in Ca^{2+}, $cPLA_2$ may translocate from the cytosol to the cell membrane, where both competent G proteins (see below) and phospholipid substrate are located [6••,7]. Ca^{2+}-dependent translocation is probably mediated by a region in the amino-terminal portion of $cPLA_2$ that shows significant sequence homology with the constant region 2 of protein kinase C (PKC). This domain, which is thought to be involved in Ca^{2+} -dependent binding of PKC to the membrane, is shared by other membrane-associated proteins including p65 (synaptotagmin), GTPase-activating protein and phosphoinositide-specific phospholipase C (PLC). By contrast, no sequence similarity appears to exist between $cPLA_2$ and any known low molecular weight (14 kDa) forms of PLA_2, which are not selective for phospholipids that contain AA [5].

Regulation of $cPLA_2$ activity by receptors, G proteins and protein phosphorylation

Several hormones, neurotransmitters, growth factors and cytokines evoke the rapid, receptor-dependent hydrolysis of AA-containing phospholipids. These include thyrotropin-releasing hormone [8•], serotonin acting at 5-HT_2 receptors [9], glutamate acting at metabotropic mGluR1 receptors [10•], basic fibroblast-derived growth factor [11] and α- and γ-interferons [12,13]. By contrast, neurotransmitters that do not stimulate AA release directly may amplify it when such release has been initiated by stimulating appropriate membrane receptors or by increasing intracellular Ca^{2+}. These facilitatory mediators include the action of dopamine at D_2 receptors [14•],

Abbreviations

AA—arachidonic acid; **CHO**—Chinese hamster ovary; **$cPLA_2$**—cytolitic phospholipase A_2; **DAG**—diacylglycerol; **EGF**—epidermal growth factor; **G protein**—GTP-binding protein; **PG**—prostaglandin; **PGHS**—cyclo-oxygenase (prostaglandin G/H synthase); **PKC**—protein kinase C; **PLC**—phospholipase C.

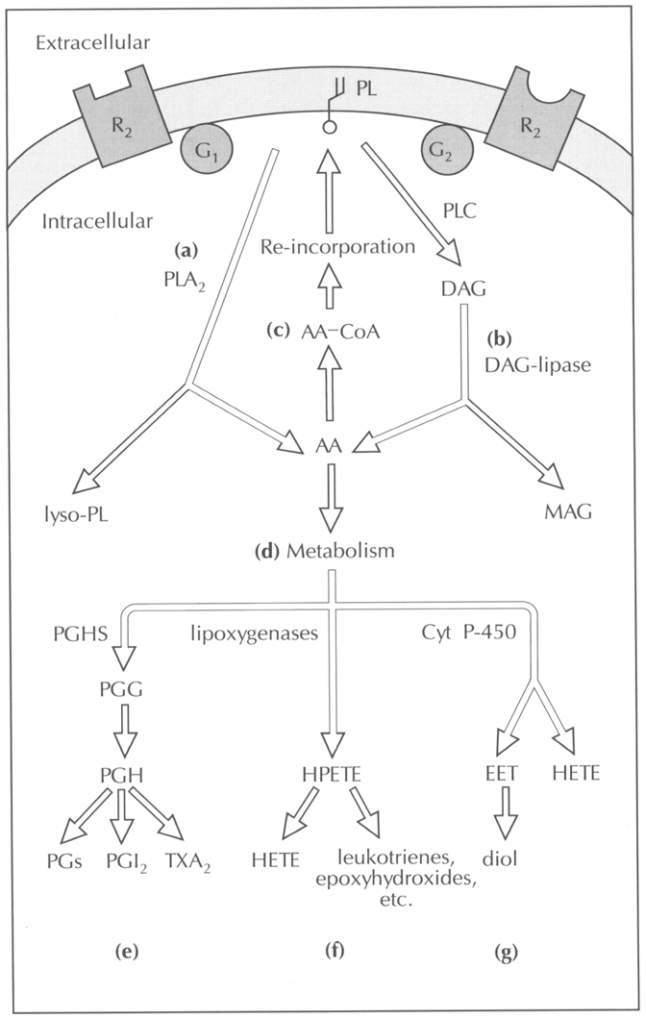

Fig. 1. Release of AA from membrane phospholipids occurs by two main pathways. **(a)** Cleavage of the glycerophospholipid (PL) backbone at the *sn*-2 position, catalyzed by various forms of PLA_2, yields free AA and lysophospholipid. **(b)** Alternatively, activation of PLC forms DAG, which is subsequently hydrolyzed by DAG-lipase into free AA and monoacylglycerol (MAG). Further hydrolysis of MAG by MAG-lipase yields additional fatty acid and glycerol (not shown). Either pathway may be stimulated independently by occupation of G protein-linked membrane receptors (R_1 and R_2). **(c)** After release, free AA may be reincorporated into membrane PL. This reaction requires conversion of the fatty acid into arachidonoyl–coenzyme A (AA–CoA) by arachidonoyl–CoA synthetase, and its subsequent esterification by arachidonoyl-lysophospholipid transferase. **(d)** Alternatively, AA may be metabolized by one of three pathways: **(e)** PGHS catalyzes the conversion of AA into two reactive intermediates, PGG and PGH, which are, in turn, precursors of the prostaglandins, prostacyclin (PGI_2) and thromboxane (TXA_2); **(f)** lipoxygenases form hydroperoxyeicosatetraenoic acids (HPETE) as primary products, which can undergo a complex metabolism including reduction to corresponding hydroxyacids (HETE), or conversion into leukotrienes, epoxyhydroxides, etc; **(g)** cytochrome P-450 catalyzes the conversion of AA into epoxyeicosatrienoic acids (EET), which are hydrolyzed to corresponding diols by epoxide hydrolase.

adenosine at A_1 [15•], norepinephrine at α_2 [16•], acetylcholine at muscarinic m_2 and m_4 [16•] and serotonin at 5-HT_1 [17•]. It is likely that stimulation of $cPLA_2$ activity mediates at least some of these responses. In agreement, when Chinese hamster ovary (CHO) cells overexpressing $cPLA_2$ are stimulated with ATP or thrombin, release of AA is dramatically enhanced compared with wild-type CHO cells or cells overexpressing a low molecular weight form of PLA_2 [18••].

Beside Ca^{2+} levels, two additional elements appear to play important roles in the receptor-dependent activation of $cPLA_2$: an activated G protein and the activity of a serine protein kinase. In transfected CHO cells, pertussis toxin, which ADP-ribosylates and inhibits G proteins belonging to the G_i/G_o families, prevents both receptor-operated AA release [19] and facilitation of such release by stimulation of D_2 or 5-HT_1 receptors [14•,17•]. Pertussis toxin inhibits receptor-dependent stimulation of AA release in a similar manner in many other cell types [20], as well as facilitation of AA release produced by A_1 receptors in striatal glial cells [15•]. Phorbol esters enhance $cPLA_2$ activity in many tissues, whereas non-selective protein kinase inhibitors, such as staurosporine, inhibit it [16•,18••]. In addition, stimulation of $cPLA_2$ activity by ATP or phorbol ester in CHO cells, or by PDGF or EGF in Rat-2 cells, is accompanied by increased phosphorylation of $cPLA_2$ on a serine residue [18••]. The protein kinase responsible for phosphorylating $cPLA_2$ has been recently identified as mitogen-activated protein (MAP), which in turn may be activated by PKC-dependent protein phosphorylation [21]. Underscoring the differences existing between $cPLA_2$ and other types of PLA_2, the activity of membrane-bound PLA_2 in a preparation of rat brain synaptic endings is not affected by incubation with either phorbol esters or purified PKC [22•].

Low molecular weight forms of PLA_2

Based on primary structure, mammalian low molecular weight secreted and membrane-bound forms of PLA_2 (14 kDa) may be classified into two types, group I (PLA_2-I) and group II (PLA_2-II). Both groups may exert multiple cellular functions, participating in the non-selective release of fatty acids from phospholipids (including, beside AA, other substrates for oxydative metabolism, such as linoleic acid), in phospholipid remodelling (particularly important in preserving membrane integrity and in cell motility), or in a series of newly described actions mediated via high affinity membrane-binding sites, which have recently been the focus of several studies.

A high affinity binding site for PLA_2-I has been characterized in various cell types [23], and is thought to mediate the effects of PLA_2-I on proliferation of Swiss 3T3 fibroblasts and on stimulation of migration of embryonic smooth muscle and contraction of isolated cerebral arteries [24,25,26•]. It is not known whether the phospholipid-hydrolyzing activity of PLA_2-I is crucial for these actions. Like PLA_2-I, PLA_2-II may also be secreted from cells, and bind to specific membrane receptors. Several agents have been shown to stimulate expression and secretion of PLA_2-II, in parallel with AA release and metabolism. These include pro-inflammatory factors, such as tumor necrosis factor, interleukin I and lipopolysaccharide. By contrast, anti-inflammatory gluco-

corticoids inhibit expression of PLA_2-II, suggesting that regulation of expression and secretion of this lipase may play a role in the pathogenesis of inflammation [27•,28•].

Phospholipase C/diacylglycerol lipase

Activation of PLA_2 provides a direct pathway of AA release. Alternatively, release may be initiated by the activation of phosphoinositide-specific PLC, which cleaves the phospholipid at the phosphate ester bond producing 1,2-diacylglycerol (DAG). This intermediate is in turn broken down by a DAG-lipase to yield free fatty acid and monoacylglycerol (Fig. 1). The PLC/DAG-lipase pathway, originally described in blood platelets, has now been unequivocally shown to mediate bradykinin-induced release of AA in dorsal root ganglion neurons in primary culture [29••].

Molecular characterization of enzymes that metabolize arachidonic acid

After release, free AA has several possible fates. It can diffuse out of the cell. Alternatively, it can be either converted into arachidonoyl–coenzyme A and reincorporated into phospholipids, or metabolized. Most enzymes involved in the metabolism of AA (cyclooxygenase, PGD synthase, PGF synthase, 5-lipoxygenase, leukotriene A_4 hydrolase, 15-lipoxygenase and 12-lipoxygenase) have been purified from various sources, and cDNAs encoding them have been isolated and characterized. The interested reader is referred to a recent review [30••], to which only a few developments need to be added here.

Cyclooxygenase

Cyclooxygenase (prostaglandin G/H synthase, PGHS) catalyzes the stepwise conversion of AA into the reactive intermediates PGG and PGH, which are in turn the precursors of prostaglandins, prostacyclin and thromboxanes. Two cDNAs encoding PGHS have been isolated. One, obtained by screening cDNA libraries of sheep seminal vesicles, hybridizes with a 2.8–3.0 kb mRNA on northern blots [30••]. This mRNA may undergo alternative splicing, resulting in a transcript that is 111 base pairs shorter [31]. An additional 4.1 kb cDNA encoding for a PGHS-related protein has been recently cloned, and shown to confer PGHS activity to transfected cells. Unlike the 2.8 kb PGHS mRNA, levels of the 4.1 kb mRNA rapidly increase in human monocytes stimulated with pro-inflammatory agents, such as interleukin 1β, and decrease in monocytes stimulated with anti-inflammatory agents, such as dexamethasone. The results suggest that induction of this transcriptionally regulated PGHS species, termed glucocorticoid-regulated inflammatory (gri) PGHS, may participate in the inflammatory response [32,33••]. The functions of griPGHS are unlikely to be limited to inflammation, however. Evidence indicates that griPGHS is identical to TIS10, the product of a primary response gene whose expression is superinduced by the mitogenic phorbol ester, 12-O-tetradecanoylphorbol-13-acetate, in a cell type restricted fashion [34••,35]. It is known that mitogenic signals induce a transient enhancement of prostaglandin release, and the discovery that a primary response gene encodes an active PGHS provides further support for a role of AA metabolism in the regulation of cell division (see below).

Cellular actions of the eicosanoids

The eicosanoids may act both as intracellular second messengers and as local mediators (autacoids): like second messengers, they may modify the activity of intracellular enzymes and ion channels; like local mediators, the eicosanoids may be released outside the cell of origin, and act on neighbouring cells by binding to high-affinity membrane receptors.

Regulation of ion channels and secretion

Both free AA and its metabolites can influence the activity of many membrane ion channels, either directly or by regulating intracellular protein kinases. Two brief reviews on this subject may be consulted for studies published before 1991 [36•,37•].

Recent reports support the involvement of lipoxygenase metabolites of AA in the activation of K^+ channels in a variety of cells ranging from molluscan neurons to mammalian platelets [38•–40•], and in the modulation of hormone and neurotransmitter secretion [41•,42•,43]. In secretory cells, including neurons, the activation of K^+ channels, by decreasing cell excitability, is expected to result in reduced Ca^{2+}-dependent secretion. In agreement, in a preparation of synaptic nerve endings, 12-lipoxygenase metabolites of AA inhibit Ca^{2+}-dependent glutamate release, possibly through activation of K^+ channels [41•]. In addition to this effect, lipoxygenase products may regulate secretion by inhibiting Ca^{2+}/calmodulin-dependent protein phosphorylation [37•]. Ca^{2+}/calmodulin-dependent protein kinase II plays an important role in the regulation of neurosecretion. Experiments with isolated pancreatic islets suggest that free AA, generated by physiological concentrations of glucose, may be part of a negative feedback loop that prevents excess insulin secretion by inhibiting Ca^{2+}/calmodulin-dependent protein kinase II activity [42•].

The actions of AA on neurosecretion are not limited to the inhibitory effects described above. Evidence indicates that AA and other polyunsaturated fatty acids may participate in certain forms of long-term potentiation, a model of synaptic plasticity and information storage that is thought to require, at least partly, enhanced release of glutamate from presynaptic terminals [44]. It was recently shown that stimulation of presynaptic glutamate receptors may enhance Ca^{2+}-dependent release of glutamate if free AA is also provided at low micromolar

concentrations [45••]. It is not known for certain where AA comes from; one suggestion is that it may be generated by stimulating postsynaptic glutamate receptors, and diffuse as a 'retrograde messenger' to the presynaptic terminal [44]. Other effects of AA also support a participation of this fatty acid in long-term potentiation, for example, its ability to potentiate ion currents at the glutamate N-methyl-D-aspartate receptor channel, which is central to the expression of long-term potentiation [46].

Modulation of Na^+/K^+ ATPase activity

The activity of Na^+/K^+ ATPase supplies the driving force for transcellular transport of electrolytes and organic solutes, playing a central role in the reabsorptive capacity of the kidney and in the maintenance of ion gradients in neural cells. Recent studies suggest the eicosanoids may participate in regulating Na^+/K^+ ATPase. Metabolites of AA via the epoxygenase (cytochrome P450) pathway are potent inhibitors of Na^+/K^+ ATPase activity in kidney tissue [47•]. Other metabolic pathways may also be involved, however [48–50]. For example, the inhibition of Na^+/K^+ ATPase activity produced by interleukin-1 in kidney collecting duct may be mediated by the prostaglandin, PGE_2. In agreement, interleukin-1 stimulates formation of PGE_2, while application of exogenous PGE_2 inhibits Na^+/K^+ ATPase activity. Moreover, PGHS blockers prevent Na^+/K^+ ATPase inhibition induced by interleukin-1 [48]. The actions of the eicosanoids on Na^+/K^+ ATPase may not be limited to the kidney, as AA also affects the enzyme activity in brain tissue [51].

Regulation of cell proliferation

Stimulation of mitogenesis by growth factors is accompanied by marked changes in lipid turnover, including release and metabolism of AA. As discussed above, the primary response gene, *TIS10*, which can be superinduced by mitogenic stimuli, encodes an active PGHS, suggesting an involvement of prostaglandins in mitogenic control [34••]. Several studies lend further support to this idea. For example, in BALB/c3T3 fibroblasts, epidermal growth factor (EGF) stimulates formation of PGE_2 and $PGF_{2\alpha}$. These prostaglandins, in turn, enhance EGF-dependent DNA synthesis and c-*myc* expression [52]. Moreover, PGHS inhibitors prevent EGF-induced mitogenesis in BALB/c3T3 cells, an effect which may be overcome by the addition of exogenous prostaglandins [52]. Non-PGHS metabolites of AA may also play a role in modulating proliferation induced by various factors, including EGF in Syrian hamster embryo cells and rat mesangial cells [53–55], and angiotensin II in bovine adrenocortical cells [56]. The enzymatic pathways involved in these responses are not well characterized yet, but pharmacological experiments suggest that either lipoxygenases (both 12- and 15-lipoxygenase) or cytochrome P450 may participate. Interestingly, AA may not be the only substrate utilized. Because substrate requirement is less strict for lipoxygenases than for PGHS, other polyunsaturated fatty acids liberated by PLA_2 activation, such as linoleic acid, may be converted into products that modulate mitogenesis [53].

Eicosanoid receptors

Many transcellular actions of the eicosanoids are mediated by specific membrane receptors. A high-affinity receptor for thromboxane A_2 (TXA_2) was recently purified to apparent homogeneity, and its cDNA was cloned and characterized [57••]. The cDNA encodes a protein of 343 amino acids with seven putative transmembrane domains, which is linked to activation of phosphotidylinositol turnover and Ca^{2+} mobilization when expressed in *Xenopus* oocytes. Subsequently, polymerase chain reaction based on the sequence of this TXA_2 receptor has revealed a cDNA clone encoding a prostaglandin E receptor EP_3 subtype, and shown it to consist of 365 amino acids with seven putative transmembrane domains. When expressed in CHO cells, the EP_3 receptor is linked to G_i-mediated inhibition of adenylyl cyclase activity [58].

Conclusions

The last 2 years have helped in clarifying several long-standing problems related to AA-mediated signaling. The central position of $cPLA_2$ in receptor-operated AA release is now beginning to be appreciated, as are the different roles played by free Ca^{2+}, G proteins and protein phosphorylation in regulating activity of this enzyme. At the same time, the importance of additional pathways of AA release (low molecular weight forms of PLA_2, PLC/DAG-lipase) has been reinforced. This emerging diversity in the control of AA release underscores, on the one hand, the multiplicity of functions of this signaling cascade, and indicates, on the other, potential sites of action for novel therapeutic agents, directed at specific aspects of AA-related pathologies. Likewise, molecular biological studies are revealing the existence, not only of important diversities at the level of AA-metabolizing enzymes, which were once thought to be single entities, but also of exciting differences in expression and function of these various enzyme isoforms.

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Focus on the ability of polyunsaturated fatty acids, including AA, to modulate the activity of membrane ion channels directly (that is, without metabolism or intervention of other signaling pathways). See [37•].

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Along with [36•], this review also deals with ion channel regulation by PLA_2-derived second messengers. Here, the authors describe the possible roles of lipoxygenase metabolites of AA in neuronal signal transduction, suggested by their actions on ion channel and protein kinase activities.

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See [40•].

39. • NAKAJIMA T, SUGIMOTO T, KURACHI Y: **Platelet-Activating Factor Activates Cardiac G_K via Arachidonic Acid Metabolites.** *FEBS Lett* 1991, **289**:239–243.
See [40•].

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This paper and [38•,39•] provide three different examples of K^+ channel modulation by lipoxygenase-derived eicosanoids. In identified neurons of the mollusc, *Helisoma*, a lipoxygenase product may act as second messenger in the modulation of K^+ channel activity by the inhibitory tetrapeptide, FMRF-amide. In cardiac myocytes, metabolites of AA formed through the 5-lipoxygenase pathway may mediate the stimulation of muscarinic K^+ channels caused by application of platelet-activating factor. Finally, a 12-lipoxygenase product may participate in the osmotic volume control in human platelets, by regulating permeability to K^+ ions.

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This paper describes one of the two distinct mechanisms that may underlie the modulatory effects of AA and its metabolites on secretion (see [42•]). In a preparation of synaptic endings, the 12-lipoxygenase product, 12-HETE, may reduce Ca^{2+}-dependent glutamate release through activation of K^+ channels and reduction of cell excitability.

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This paper describes one of the two distinct mechanisms that may underlie the modulatory effects of AA and its metabolites on secretion (see [41•]). In pancreatic islets, AA may prevent glucose-induced insulin secretion through inhibition of type II Ca^{2+}/calmodulin-dependent protein kinase activity. The mechanisms documented in this paper and [41•] are not mutually exclusive, however, and they may cooperate within the same cell.

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