Prostaglandins and Leukotrienes: Advances in Eicosanoid Biology

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Prostaglandins and leukotrienes are potent eicosanoid lipid mediators derived from phospholipase-released arachidonic acid that are involved in numerous homeostatic biological functions and inflammation. They are generated by cyclooxygenase isozymes and 5-lipoxygenase, respectively, and their biosynthesis and actions are blocked by clinically relevant nonsteroidal anti-inflammatory drugs, the newer generation coxibs (selective inhibitors of cyclooxygenase-2), and leukotriene modifiers. The prime mode of prostaglandin and leukotriene action is through specific G protein–coupled receptors, many of which have been cloned recently, thus enabling specific receptor agonist and antagonist development. Important insights into the mechanisms of inflammatory responses, pain, and fever have been gleaned from our current understanding of eicosanoid biology. A discovery chain culminating in one of the most important classes of lipid mediators, known as eicosanoids (from the Greek *eicosa* = twenty; for twenty carbon fatty acid derivatives), was initiated in 1930 with two seminal, though seemingly unrelated, laboratory observations (1–3). The first of these found that exclusion of fat from the diet of rats led to growth retardation, reproductive disturbances, scaly skin, kidney lesions, and excessive water consumption, which led to the discovery of essential fatty acids. The second identified a factor with fatty acid properties and vasodopressor and smooth muscle–stimulating activity that was termed “prostaglandin.” Bergström and Samuelsson, some 30 years later, linked these observations when they elucidated the structures of the “classical” prostaglandins and demonstrated that they were produced from an essential fatty acid, arachidonic acid (4). Thus began an era of eicosanoid research.

The diverse and potent biological actions of prostaglandins on almost all organs stimulated research on these fascinating molecules over the ensuing four decades. In 1971, Vane discovered that aspirin-like drugs, known for their analgesic, antipyretic, and anti-inflammatory actions, could inhibit prostaglandin biosynthesis (5). Soon thereafter, the platelet proaggregatory and vasoconstrictor molecule thromboxane A2 was elucidated, followed by the isolation of vascular wall-synthesized prostacyclin, which counteracts thromboxane action. Prostaglandins were found to induce labor or act as abortifacients. Prostaglandins were also found to induce labor or act as abortifacients. Prostaglandins were also found to induce labor or act as abortifacients. Prostaglandins were also found to induce labor or act as abortifacients.

**Biosynthesis of Prostaglandins**

Prostaglandins are formed by most cells in our bodies and act as autocrine and paracrine lipid mediators (i.e., they signal at or immediately adjacent to their site of synthesis). They are not stored but are synthesized de novo from membrane-released arachidonic acid when cells are activated by mechanical trauma or by specific cytokine, growth factor, and other stimuli [e.g., collagen and adenosine diphosphate (ADP) in platelets, bradykinin and thrombin in endothelium]. A host of enzymes exquisitely regulate cellular levels of arachidonic acid, keeping it esterified until mobilized by phospholipases (PLA2). The control of arachidonic acid release from membranes has undergone several paradigm shifts in recent years with the continuing identification of new PLA2 members (8). Despite this, type IV cytosolic PLA2 (cPLA2), remains the key player for eicosanoid production because cells lacking cPLA2 are generally devoid of eicosanoid synthesis. Cell-specific and agonist-dependent events coordinate translocation of cPLA2, to the nuclear envelope, endoplasmic reticulum (ER), and Golgi apparatus (9).

At the ER and nuclear membrane, arachidonic acid released by cPLA2 is presented to prostaglandin H synthase (PGHS, referred to colloquially as COX for cyclooxygenase) and is then metabolized to an intermediate prostaglandin PGH2 (1). PGHS exists as two isozymes referred to as PGHS-1 (COX-1) and PGHS-2 (COX-2) (10). In simplistic terms, COX-1 is the enzyme responsible for basal, constitutive prostaglandin synthesis, whereas COX-2 is important in various inflammatory and “induced” settings. There are notable exceptions to this over-simplification, but in general this classification has aided the rapid advancement in this field since the discovery of COX-2 10 years ago. The COX enzymes are monotonically inserted in the ER and nuclear membrane with the substrate-binding pocket precisely orientated to take up released arachidonic acid. The crystal structures of COX-1 and COX-2 are remarkably similar, with one notable amino acid difference that leads to a larger “side-pocket” for substrate access in COX-2 (10).

The coupling of PGH2 synthesis to metabolism by downstream enzymes is intricately orchestrated in a cell-specific fashion. Thromboxane synthase is found in platelets and macrophages, prostacyclin synthase is found in endothelial cells and PGE synthase in uterus, and two types of PGG synthase are found in brain and mast cells. Microsomal PGE synthase (mPGES), a member of the MAPEG (membrane-associated proteins in eicosanoid and glutathione metabolism) family, is responsible for PGE2 synthesis (11). Coordinate induction of multiple enzymes in the prostanoïd pathway, in particular mPGES and COX-2, in inflammatory settings is a current concept being developed (12).

**Biosynthesis of Leukotrienes**

In contrast to prostaglandins, leukotrienes are made predominantly by inflammatory cells like polymorphonuclear leukocytes, macrophages, and mast cells. Cellular activation by immune complexes, bacterial peptides, and other stimuli elicit a sequence of events that include cPLA2 and 5-lipoxygenase (5-LO) translocations to the nuclear envelope (Fig. 2). 5-LO, a nonheme iron dioxygenase, is the key enzyme in this cascade and is located in the nucleus in some cell types and the cytosol of others (13). 5-LO possesses an NH2-terminal domain that binds two calcium ions, similar to the β-sandwich C2 domains of cPLA2 and protein kinase C, and a large catalytic domain that binds iron (14, 15). It transforms released arachidonic acid to the epoxide LTA4 with the concerted efforts of 5-lipoxygenase–activating protein (FLAP). There are questions as to how this cascade of events takes
place. The directed 5-LO translocation process could be governed by specific motifs in its structure (e.g., SH3 binding region) through protein-protein or protein-cytoskeleton interactions or by the intrinsic predilection of its NH2-terminal domain to seek out phosphatidylcholine-rich lipid domains. FLAP may retrieve the substrate and transfer it to 5-LO, but the mechanisms remain obscure.

LTA₄ undergoes transformation by one or more of three possible fates depending on the cellular context: hydrolysis, conjugation with glutathione, or transcellular metabolism to generate bioactive eicosanoids (16). Hydrolytic attack of LTA₄ by leukotriene A₄ hydrolase (LTA₄H) in the cytoplasm, and potentially in the nucleus, yields LTB₄, a potent neutrophil chemoattractant and stimulator of leukocyte adhesion to endothelial cells (6, 13). LTA₄H is a bifunctional zinc-containing enzyme with epoxide hydrolase and aminopeptidase activities. The three-domain crystal structure of LTA₄H, with a catalytic domain highly related to the cysteine proteinase domain to seek out phosphatidylcholine-rich lipid domains. FLAP may retrieve the substrate and transfer it to 5-LO, but the mechanisms remain obscure.

Mechanisms of Prostaglandin Action

Prostaglandins are released from cells predominantly by facilitated transport through a known anion transporter polypeptide family, and potentially by other uncharacterized transporters (21). Due to the evanescent nature of thromboxane A₂ and prostacyclin (which have half-lives on the order of seconds to a few minutes), these compounds must act near their sites of synthesis. There are at least 9 known prostaglandin receptors, four of which have been characterized to date (27–30).

Mechanisms of Leukotriene Action

Leukotrienes also act at distinct GPCRs, four of which have been characterized to date (27–30).
The high-affinity B-LT₁ receptor on leukocytes binds LTB₄ in the subnanomolar range and elicits a pertussis toxin–sensitive G_i–linked chemotactic response. High concentrations of LTB₄ and signaling through G_i coupling stimulates neutrophil secretion. A recently characterized B-LT₂ receptor that binds LTB₄ with much lower affinity than B-LT₁ displays a widespread tissue distribution pattern, and its function is presently unknown. Interestingly, the genes for B-LT₁ and B-LT₂ reside intertwined, one within the promoter of the other (28). Two subtypes of cysteinyl leukotriene receptors, CysLT₁ and CysLT₂, mediate the actions of LTC₄ and LTD₄. CysLT₁ is found on airway smooth muscle cells (29) and vascular endothelial cells (31) promoting bronchoconstriction and up-regulation of cell adhesion molecules, respectively. CysLT₂ may also masquerade as a pyrimidinergic (UDP) receptor but the significance of this finding remains to be determined (32). CysLT₂, originally found in pulmonary vein preparations by pharmacological assays, is detected in spleen, Purkinje fibers of the heart, and discrete regions of the adrenal gland by molecular methods. Leukotriene functions in these tissues are unknown, so fruitful avenues for future research will arise.

**GPCR, PPARs, or Both?**

Do prostaglandins and leukotrienes exert their actions solely through GPCR? Peroxiosomal proliferator-activated receptors (PPARs) can bind and be activated by a variety of eicosanoids; PPAR-α by LTB₄ and 8(S)-HETE, PPAR-γ by 15-deoxy-delta-12,14-PGJ₂ (a dehydration metabolite of PGD₂), and PPAR-δ by prostacyclin analogs (33–37). However, the approach for ligand discovery was not based on known eicosanoid biochemistry. Problematic issues relate to cell-specific eicosanoid biosynthetic patterns, whether certain ligands are formed in vivo (e.g., 15-deoxy-delta-12,14-PGJ₂), and the concentrations of ligand needed to activate responses (micromolar range versus nanomolar for conventional eicosanoid GPCR) (22). Whether eicosanoids are bona fide endogenous PPAR ligands has yet to be resolved with rigorous analytical methods and testing of COX, 5-LO, and other eicosanoid-deficient mice.

**Drugs Affecting Prostaglandin and Leukotriene Formation and Action**

Nonsteroidal anti-inflammatory drugs (NSAIDs; e.g., aspirin, indomethacin, ibuprofen), known to block PGHS-derived prostaglandin synthesis, are firmly entrenched in the common man’s armamentarium of analgesics and anti-inflammatories. Although the mechanism of COX inhibition by NSAIDs is rarely disputed, some NSAIDs affect the transcription factors nuclear factor kappa B (NF-κB) and PPAR family members; however, higher concentrations are required than those that effectively block COX activity (38, 39). Aspirin remains the sole member of this class of drugs with a unique mechanism of action on COX by covalently acetylating a serine residue. This blocks proper substrate access and orientation at the active site. The coxibs (selective inhibitors of COX-2), celecoxib (Celebrex) and rofecoxib (Vioxx), are newer COX-2 specific drugs that have been used clinically for the past 2 years in arthritis and other pain symptom management (40). The second-generation coxibs, valdecoxib and etoricoxib, are undergoing clinical development.

Leukotriene modifiers or antileukotrienes constitute 5-lipoxygenase inhibitors [zileuton (Zyflo)] and CysLT₁ receptor antagonists [zafrilukast (Accolate) and montelukast (Singulair)] used clinically in long-term maintenance of asthma control (41). Are leukotriene modifierns an important asthma therapy? There is much debate about their clinical efficacy (42). There currently known. LTB₄ may also act intracellularly on the nuclear transcription factor PPAR-α to induce target genes like those involved in β-oxidation. This would cause a negative feedback loop resulting in LTB₄ metabolic degradation, thus limiting its proinflammatory actions. LTD₄ can also be converted to LTC₄ by LTC₄ synthase, a FLAP-like protein found in the nuclear envelope. The multidrug resistance-associated protein (MRP1) can facilitate transfer of LTC₄ out of the cell, where it is metabolized by extracellular-localized γ-glutamyl transpeptidase (GGT) or γ-glutamyl leukotrienate (GGLT) to LTD₄. On airway smooth muscle cells (SMC) and postcapillary venule endothelial cells, LTD₄ can activate CysLT₂ receptors to cause bronchoconstriction and edema. The clinically important drugs in asthma, montelukast (Singulair) and zafrilukast (Accolate), block this binding step (Y marks the site of inhibition). LTC₄ or LTD₄ may also bind CysLT₂ receptors found in a variety of tissues.
is well-documented effectiveness in exercise-induced asthma and aspirin-intolerant asthma. Clinical trials show bronchodilatory effects beyond those provided by β agonists, as well as reduced eosinophil numbers in sputum. But there are also definite nonresponder patients, which might be explained by nonleukotriene dependent asthma mechanisms or by pharmacogenetic factors. In fact, the therapeutic response to a 5-LO inhibitor can vary with 5-LO promoter polymorphisms containing variable numbers of GC-boxes capable of binding Sp1 and Egr-1 transcription factors. (43, 44). Leukotriene modifiers provide a steroid-sparing benefit in mild to moderate asthmatics, although questions remain regarding the additional advantage of adding an antileukotriene to traditional therapies (β agonists, corticosteroids, theophyllines) in chronic persistant asthma (42, 43, 45).

**The Multifaceted Roles of Eicosanoids**

The renewed growth and rapid advance in the eicosanoid field over recent years is attributed in part to studies carried out with knockout mice (22, 47, 48). Virtually all genes encoding enzymes and receptors in the prostaglandin and leukotriene pathways have been disrupted by gene targeting. The studies are unraveling novel eicosanoid actions, confirming long-held viewpoints, and providing provocative data for further in-depth research. Eicosanoids are implicated in functions in practically every organ, tissue, and cell in our bodies (see some examples in Figs. 1 and 2). One prominent area of interest is the role of eicosanoids in pain, fever, and inflammation.

**Inflammation.** Scientists have been grappling for years over the specific mechanisms of how prostaglandins mediate their effects on the cardinal signs of acute inflammation: pain, vasodilation (swelling and redness), and fever. COX-1 is expressed in nearly all tissues, whereas COX-2 is absent in most (some exceptions are the glomerulus and certain brain regions) until induced by various inflammatory insults in monocytes or mast cells or by shear stress in endothelium. In most instances, COX-1 expression is marginally affected by inflammatory stimuli. However, exceptions to the “constitutive” mode of COX-1 prostaglandin synthesis are known (e.g., both COX-1 and COX-2 are expressed in the inflamed synovia of joints). Most of the traditional NSAIDs do not distinguish between the two COX isoforms. Coxibs, however, were developed specifically with the promise that they would selectively block synthesis of “proinflammatory” prostaglandins derived from the induced COX-2 enzyme while leaving intact the COX-1-derived “homeostatic” prostaglandins involved in renal water and electrolyte balance, gastric cytoprotection, and platelet aggregation (40). Two years of clinical use in pain management indicate that coxibs are as effective as traditional nonselective NSAIDs and also reveal a 50% reduction in adverse gastrointestinal events (49). Although indications of potentially deleterious actions of COX-2 inhibitors (e.g., causing acute tubulointerstitial nephritis or decreased cardioprotection) have been reported (49, 50), the case to support an increased incidence of adverse events compared with traditional NSAIDs has not been developed.

Vasodilation and increased permeability of postcapillary venules, early events in the inflammatory response, reflect the effects of COX-2-derived prostaglandins and leukotrienes at sites of inflammation. Prostaglandins synergize with other mediators (e.g., bradykinin, histamine) to elicit enhanced vascular permeability and edema. These molecules can be viewed within the context of a complex milieu of parenchymal and inflammatory cells, an array of cytokine and other non-eicosanoid mediators, and extracellular matrix interactions combined with the overall physiological status of the host. To complicate matters, prostaglandins may act as both proinflammatory and anti-inflammatory mediators depending on the context, which is due in part to the array of EP-type prostaglandin receptors with opposing signal transduction pathways. How tissues and cells sort out the mixed signals has been reviewed recently (48). The temporal sequence of events in acute inflammation may be governed by eicosanoid profile switching such that eicosanoids made during the initial phase are gradually replaced by other lipid mediators in the resolution phase (51). In vitro evidence indicates that monocytes and/or macrophages can undergo a shift in eicosanoid products (52, 53), a process perhaps mediated by altered gene expression of the synthases downstream of COX-1 and COX-2 or by specific compartmentalization of the enzymes to various stimuli. Combined data from several murine inflammation models support a complex regulatory network in eicosanoid signaling (48, 51, 54).

The 5-LO pathway leading to leukotriene formation has long been recognized as a proinflammatory cascade. LTβ promotes neutrophil chemotaxis and adhesion to vascular endothelium through specific integrins. The cysteinyl leukotrienes cause plasma leakage from postcapillary venules and enhance mucus secretion. LTD₄ and receptor antagonists could be useful in pain management. Whether they offer any benefit over coxibs or NSAIDs remains to be discovered.

**Fever.** The potent antipyrogenic effects of NSAIDs have provided strong evidence for a role of prostaglandins in fever, but the mechanisms have remained obscure until recently (55, 58). Bacterial lipopolysaccharides and other marauding challenges induce cytokine networks that cause fever. Subsequently, these stimulate the neural pathways that raise body temperature. In response to both exogenous and endogenous pyrogens, cytokine-released PGE₂ derived from COX-2 in the organum vasculosum lamina terminalis (OVLT), at the midline of the preoptic area, mediates the febrile response. This occurs through the EP₁ receptor expressed in neurons surrounding the OVLT, a region exquisitely sensitive to exogenous PGE₂-induced pyrexia.

**Prospects**

Where is the eicosanoid field heading in the next few years? Interest in the area tends to undergo cyclical periods of grandeur and demise. A strong resurgent period is upon us, after the introduction of COX-2 inhibitors into the clinics, the cloning of four leukotriene receptor subtypes, and the characterization of prostaglandin receptor knockout mice. What lies on the horizon? Sorting out the events in the regulation of prostaglandin and leukotriene biosynthesis is definitely a priority. Are their specific microdomains to which ePLA₂ and 5-LO target? The compartmentalization of the enzymes within the eicosanoid pathways has received considerable attention but requires further refinement (10, 13). 5-LO undergoes dynamic movement in and out of the nucleus in a cell- and stimulus-dependent manner (48).
Lysophospholipids—Receptor Revelations

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Upon cell activation, membrane phospholipids are metabolized into potent lysophospholipid (LP) mediators, such as sphingosine 1-phosphate and lysophosphatidic acid. LPs fulfill signaling roles in organisms as diverse as yeast and humans. The recent discovery of G protein–coupled receptors for LPs in higher eukaryotes, and their involvement in regulating diverse processes such as angiogenesis, cardiac development, neuronal survival, and immunity, has stimulated growing interest in these lipid mediators. LP receptor biology has generated insights into fundamental cellular mechanisms and may provide therapeutic targets for drug development.

Glycerol-based and sphingosine-based phospholipids are abundant structural components of cellular membranes; however, they are metabolized into polar metabolites such as eicosanoids and lysophospholipids (LPs). The latter includes lysophosphatidic acid (LPA), lysosphosphatidylcholine (LPC), sphingosylphosphorylcholine (SIPC), and sphingosine 1-phosphate (S1P).

References and Notes
7. Samuelsson, Vane, and Bergström were awarded the prize in medicine or physiology in 1982 and E. J. Corey was awarded the Nobel prize in chemistry in 1990 (see www.nobel.se/medicine/laureates/1982/index.html and www.nobel.se/chemistry/laureates/1990/index.html).
25. DP1 and DP2 designations have been used informally, but this nomenclature has not yet been approved.
67. I regret being unable to cite all relevant references, due to space constraints. Supported by NIH grants HL58464, HL53558, and GM63130.

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