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 vasodepressor 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. From 1975 to 1980, the leukotriene biosynthetic pathway was compiled, which demonstrated that leukotrienes are potent lipid mediators associated with asthma and allergic reactions (6). Lastly, Nobel Prizes related to the eicosanoid field were awarded (7).

The composition of the eicosanoid family has expanded immensely in the past two decades to include virtually all long-chain oxygenated polyunsaturated products, whether formed enzymatically or nonenzymatically. Arachidonic acid (20:4ω6) is the premier eicosanoid precursor in mammalian cells. The quintessential properties of “true” eicosanoids are their stereochemoical precision in formation and recognition, their potency in the nanomolar range in vitro, and their bona fide biological activities. This review will cover our current knowledge of two eicosanoid members, the prostaglandins and leukotrienes.

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 (Fig. 1). PGHS exists as two isoforms 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 oversimplification, 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 monotypically 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 PGF synthase in uterus, and two types of PGI₂ 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 prostanooid 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 forms 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
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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 phosphatidyicholine-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 chemotactic 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 thromolysin and a COOH-terminal domain with armadillo-like repeats (17), points to an interesting evolutionary heritage. Because LTA4H is found in yeast (18), predating the appearance of other leukotriene biosynthetic enzymes, it may have had, at one time, exclusively aminopeptidase or other noneicosanoid related functions.

LTA4 conjugation with glutathione to form LTC4 at the nuclear envelope is catalyzed by LTB4 synthase (LTC4H) (19), another member of the MAPEG family. LTC4 is transported out of the cell by transporters such as the multidrug resistance-associated protein (MRP1), a process that apparently regulates dendritic cell migration to lymph nodes (20). The peptide moiety of LTC4 is subjected to extracellular metabolism, forming LTD4 and LTE4. These three leukotrienes comprise the cysteiny1 leukotrienes, or an entity described more than 60 years ago as “slow-reacting substance of anaphylaxia” for its slow and sustained muscle smooth contracting abilities (6).

Mechanisms of Prostaglandin Action

Prostaglandins are released from cells predominantly by facilitated transport through a known prostaglandin transporter (PGT) of the organic anion transporter polypeptide family, and potentially by other uncharacterized transporters (21). Due to the evanescent nature of thromboxane 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 receptor forms in mouse and man, as well as several additional splice variants with divergent carboxy termini (22). Four of the receptor subtypes bind PGE2 (EP1, EP2), two bind PGD2 (DP1 and DP2) (23–25), and the receptors that bind PGI2, TXA2, and LTC4 (FP, IP, and TP, respectively) each derive from a single gene. The prostaglandin receptors belong to three clusters (on the basis of homology and signaling attributes rather than by ligand-binding properties) within a distinct subfamily of the G protein-coupled receptor (GPCR) superfamily of seven-transmembrane spanning proteins. The lone exception is DP2, which is a member of the chemotactic receptor subgrouping. The “relaxant” receptors IP, DP1, DP2, and EP4 form one cluster, signaling through Gmediated increases in intracellular cyclic adenosine monophosphate (cAMP); the “contractile” receptors EP1, EP2, and TP form a second group that signals through Gmediated increases in intracellular calcium. The EP1 receptor is regarded as an “inhibitory” receptor that couples to G and decreases cAMP formation. Although most of the prostaglandin GPCRs are localized at the plasma membrane, some are situated at the nuclear envelope (26).

Mechanisms of Leukotriene Action

Leukotrienes also act at distinct GPCRs, four of which have been characterized to date (27–30).

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Fig. 1. Prostaglandin synthesis and actions. A “generic” cell when activated by mechanical trauma, cytokines, growth factors, or various inflammatory stimuli triggers signaling, including type IV cytosolic phospholipase (cPLA2) translocation to ER and nuclear membranes, arachidonic acid release from membrane lipids and metabolism by COX-1 or COX-2 to the intermediate PGH2. Other PLA2 subtypes could be involved in arachidonic acid release for eicosanoid synthesis but are not shown here. De novo COX-2 enzyme synthesis can be induced by a host of factors (top) to reinforce prostaglandin (PG) formation. In a cell-type restricted fashion, a heterogeneous family of PGH metabolizing enzymes can form PGE2, PGD2, PGF2α, PGJ2 (prostacyclin) and TXA2 (thromboxane). These prostaglandins may undergo facilitated transport from the cell through a known prostaglandin transporter (PGT) or other carrier to exert autocrine or paracrine actions on a family of prostaglandin receptors, EP1, EP2, EP3, EP4, DP1, DP2, DP3, FP, IP, TP1, and TP, on the cell types indicated. Only a few of the many diverse activities of prostaglandins are shown here. Prostaglandins could potentially enter the nucleus and activate nuclear hormone receptors such as PPARγ, PGE2, PGF2α synthase; PGDS, PGD synthase; PGFS, PGF synthase; PGIS, prostacyclin synthase; TXS, thromboxane synthase. VSMC is vascular smooth muscle cell. OVLT in POA is the organum vasculosum laminae terminals at the midline of the preoptic area. CO cells are cells of the cumulus oophorh. X marks the site of inhibition by NSAIDs (aspirin, ibuprofen, indomethacin) and the coxibs celecoxib (Celebrex) and rofecoxib (Vioxx).
GPCR, PPARs, or Both?
Do prostaglandins and leukotrienes exert their actions solely through GPCR? Peroxi-
somal proliferator-activated receptors (PPARs) can bind and be activated by a
variety of eicosanoids; PPAR-α by LTB4, and a family; however, higher con-
centrations are required than those that effec-
tively 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 inhibi-
tors of COX-2), celecoxib (Celebrex) and rofe-
coxib (Vioxx), are newer COX-2 specific drugs
that have been used clinically for the past 2
years in arthritis and other pain symptom man-
agement (40). The second-generation coxibs,
valdecoxib and etoricoxib, are undergoing clin-
ical development.

Leukotriene modifiers or antileukotrienes
constitute 5-lipooxygenase inhibitors [zileuton
(Zyflo)] and CysLT1 receptor antagonists
[zafirlukast (Accolate) and montelukast (Singu-
laire)] used clinically in long-term maintenance
of asthma control (41). Are leukotriene modifi-
ers an important asthma therapy? There is much
debate about their clinical efficacy (42). There

Fig. 2. Leukotriene synthesis and actions. Upon cellular activation of a
mast cell or macrophage by immunoglobulin E (IgE)-antigen complexes,
ionophores, or other stimuli, a cascade of cell activation events lead-
ing to leukotriene biosynthesis occurs. cPLA2 translocates to the nuclear enve-
lope, as does S-LO. The latter can translocate from either the nuclear or
cytoplasmic compartment. FLAP, a small resident nuclear envelope integ-
ral protein, acts as an apparent arachidonic acid transfer protein for the
facilitates presentation to S-LO for conversion to LTA4. S-LO activity is
inhibited by zileuton (Zyflo) (X marks the site of inhibition). LTA4 and
LTB4 may be formed on either side of the nuclear envelope by nuclear-
or cytosolic-localized pools of S-LO and LTA4 hydrolase. An uncloned transporter can facilitate efflux of LTB4 out of the cell, where it
may act on neutrophils through the B-LT1 receptor to elicit chemotaxis,
or at the recently cloned B-LT2 receptor to evoke functions that are not
currently known. LTB4 may also act intracellularly on the nuclear trans-
section 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. LTA4
may also be converted to LTC4 by LTC4 synthase, a FLAP-like protein
found in the nuclear envelope. The multidrug resistance-associated pro-
tein (MRP1) can facilitate transfer of LTC4 out of the cell, where it is
metabolized by extracellular-localized γ-glutamyl transpeptidase (GGT) or
γ-glutamyl leukotriene reductase (GGLT) to LTD4. On airway smooth muscle
cells (SMC) and postcapillary venule endothelial cells, LTD4 can activate
CysLT1 receptors to cause bronchoconstriction and edema. The clinically
important drugs in asthma, montelukast (Singulair) and zafirlukast (Ac-
colate), 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 persistent asthma (42, 45, 46).

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 prostanoid 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 (40). 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. LTß4 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 another 5-LO-derived eicosanoid, 5-oxo-EET, are eosinophil chemotactants. The use of 5-lipoxygenase, FLAP-, LTA4H-, and LTC4S-deficient mice has enabled a detailed examination of the leukotrienes in murine models, firmly establishing their roles in allergic inflammation.

Fever. The potent antipyretic 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 laminae terminalis (OVLT), at the midline of the preoptic area, mediates the febrile response. This occurs through the EP3 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 cPLA2 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
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), lysophosphatidylcholine (LPC), sphingosylphosphoryl choline (SPC), and sphingosine 1-phosphate (SIP). However, in contrast to the eicosanoids, whose critical roles in normal physiology and disease are underscored by the current widespread clini-