

Arachidonic Acid Pathways in Nociception

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Inflammation may release or generate a diverse population of proinflammatory and/or pronociceptive mediators, including bradykinins, serotonin, histamine, nitric oxide, prostaglandins, and cytokines. These and other substances contribute to the classic clinical picture of redness, heat, swelling, soreness, and diminished function associated with inflammation and may produce pain, hyperalgesia, or allodynia.

Among the major substances that play a pivotal role in both inflammation and pain are the metabolites of arachidonic acid. These metabolites include both enzymatically generated products (thromboxanes, prostaglandins, leukotrienes, lipoxins, and epoxyeicosatrienoic acids [EETs]) and non-enzymatically produced substances (isoprostanes and cyclopentenone prostaglandins).^{1,2}

Arachidonic Acid Metabolism

As shown in Figure 1, arachidonic acid metabolism generally occurs via one of four major avenues³:

- the cyclooxygenase (COX) pathway, which produces prostanoids;
- the lipoxygenase pathway, which produces leukotrienes and lipoxins;
- non-enzymatic lipid peroxidation, which produces isoprostanes; and
- the cytochrome P₄₅₀ monooxygenase pathway, which produces EETs and other substances.

Oxidation reactions include epoxidation, allylic oxidation, and omega hydroxylation. In addition, arachidonic acid can undergo autooxidation to hydroperoxy acids.

Inflammation leads to the rapid biosynthesis

Abstract The metabolism of arachidonic acid may follow multiple, inter-related pathways, leading to the generation or release of a wide variety of biologically active substances (including bradykinins, serotonin, histamine, prostaglandins, and cytokines) that produce pain and/or inflammation. Therapeutic agents that directly affect one particular pathway, such as cyclooxygenase inhibitors (typically, nonsteroidal anti-inflammatory agents), may indirectly affect one or more other pathways, as well as the resolution of inflammation. Rather than taking the traditional approach to treating multiple types of pain by inhibiting a single pathway, future treatments of pain and inflammation may target specific pathways and pain mechanisms and/or address two or more pathways simultaneously. A thorough understanding and appreciation of the mechanisms contributing to various pain and inflammatory states, the metabolic fates of arachidonic acid, the functions of its many metabolites, and the interrelatedness of the various metabolic pathways involved in nociception may lead to more rational and optimal approaches to addressing patient suffering.

of lipid mediators generated de novo from arachidonic acid in response to multiple stimuli, such as mechanical trauma, cytokines, and growth factors. In most cells, arachidonic acid may be released at the endoplasmic reticulum and nuclear membrane, predominantly via the translocation of type IV cytosolic phospholipase A₂ (PLA₂). Arachidonic acid is subsequently metabolized to prostaglandin H₂, or PGH₂ (an intermediate arachidonic acid metabolite), by the action of prostaglandin H synthase (PGHS; also referred to as COX). Two distinct active catalytic sites exist on COX: the cyclooxygenase active site (CAS), which converts arachidonic acid to prostaglandin G₂ (PGG₂), and the peroxidase active site (PAS), which transforms PGG₂ to PGH₂. PGH₂ may then be acted upon by various enzymes to yield multiple prostanoids.

The prostanoid that has received the most attention—since it is thought to play the most important role in nociceptive processes—is prostaglandin E₂ (PGE₂). PGH₂ is acted upon by PGE synthase (PGES) to yield PGE₂. A sequence of three enzymatic reactions (PLA₂ → COX → PGES) leads to generation of PGE₂ from the cell membrane.

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Manuscript submitted March 29, 2005; accepted November 30, 2005.

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J Support Oncol 2006;4:277-287

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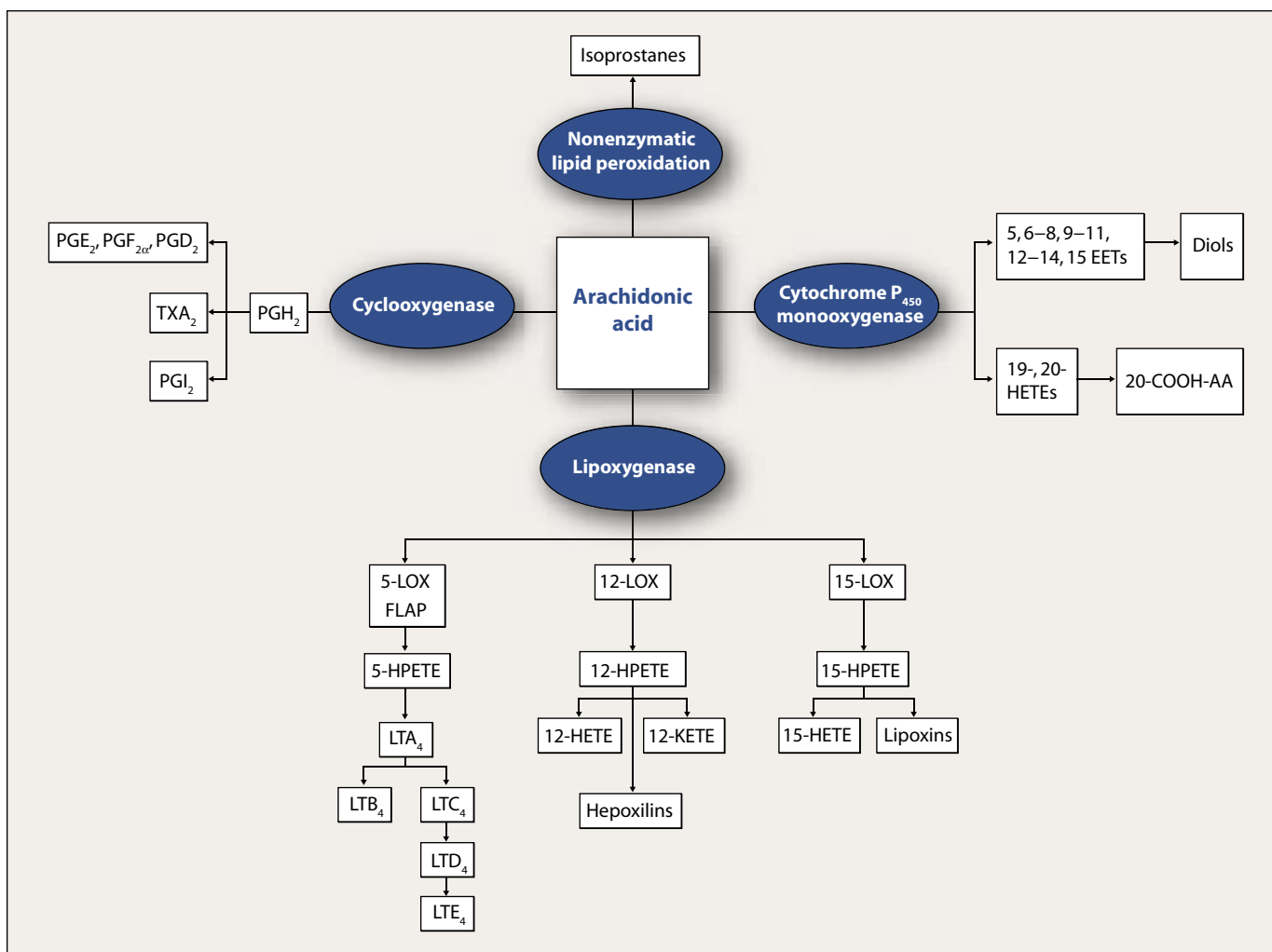


Figure 1 Four Major Pathways of Arachidonic Acid Metabolism

Abbreviations: EETs = epoxyeicosatrienoic acids; FLAP = 5-lipoxygenase-activating protein; HETEs = hydroxyeicosatetraenoic acids; HPETE = hydroxyperoxyeicosatetraenoic acid; 12-KETE = 12-ketoeicosatetraenoic acid; LOX = lipoxygenase; LT = leukotriene; PG = prostaglandin; TXA₂ = thromboxane A₂

PHOSPHOLIPASE A₂ (PLA₂)

There are three main classes of PLA₂. Each major class contains multiple family members, which carry out similar reactions but are regulated by different mechanisms. Different PLA₂ enzymes may act on different substrates, producing various pathways for phospholipid degradation, membrane remodeling, and eicosanoid biosynthesis.⁴

The three main classes of PLA₂ are secretory PLA₂ (sPLA₂), cytosolic PLA₂ (cPLA₂), and calcium-independent PLA₂ (iPLA₂).⁵

Secretory PLA₂. sPLA₂ is generally released extracellularly, has a low molecular weight (14–18 kDa), requires millimolar concentrations of calcium for catalytic activity, and shows little fatty acid specificity.⁵

In the heparin sulfate proteoglycan (HSPG)–shuttling pathway, group II subfamily sPLA₂s may bind to glypican in activated cells.⁶ Glypican is a glycosylphosphatidylinositol-anchored HSPG that facilitates the “sorting,” or trafficking,

of sPLA₂s into caveolae/raft-dependent vesicular routes, concentrating these enzymes into particular membrane compartments, where they lead to the release of arachidonic acid adjacent to perinuclear arachidonic acid–metabolizing enzymes, such as COX-2 and 5-lipoxygenase.⁶

Cytosolic PLA₂. cPLA₂ functions intracellularly, has a high molecular weight (40–110 kDa), requires micromolar levels of calcium for membrane translocation, exhibits a definite preference for the sn-2 arachidonic acid position, and is phosphorylated by mitogen-activated protein kinases (MAPKs).⁵ cPLA₂ appears to be especially important in regulating the release of arachidonic acid in human immune cells.⁵

The cPLA₂ family consists of three isoenzymes, cPLA_{2α}, cPLA_{2β}, and cPLA_{2γ}. cPLA_{2α} appears to play a key role in the evolution of various lipid mediators in certain immune cells. Compared with wild-type macrophage cells, macrophages obtained from cPLA_{2α} knockout mice do not give rise to significant levels of PGE₂, leukotriene B₄ (LTB₄), leukotriene C₄ (LTC₄), or platelet-activating

factor (PAF) in the A23187-induced immediate arachidonic acid response.⁶ Calcium binding to the C2 domain of cPLA_{2α} promotes its translocation to the phosphatidylcholine-rich perinuclear region, where it is activated/stabilized by phosphorylation of serine residues and/or binding to anionic phospholipids with subsequent “lid” removal, thereby exposing the active catalytic site resulting in the generation of arachidonic acid.

Calcium-independent PLA₂. iPLA₂ occurs intracellularly, has a high molecular weight (45–80 kDa), and exhibits activity that appears to be independent of calcium concentration. iPLA₂ generally has little acyl specificity, exhibits enhanced activity with ATP, and seems to have a role in regulating intracellular arachidonic acid levels in myocardium (iPLA₂[myocardium]) and macrophages (iPLA₂[macrophages]).⁵ It is conceivable that an ionic intracellular trigger to activation of iPLA₂ may be a drop in the intracellular concentration of potassium, whereas an increase in intracellular calcium may trigger activation of cPLA₂. During conditions when expression of cPLA₂ and sPLA₂ is suppressed, iPLA₂ may function as the primary arachidonic acid hydrolyzing phospholipase. iPLA₂ appears to have unique functional roles in apoptosis, myocardial ischemia, calcium homeostasis,⁶ and postsynaptic modulation of neurotransmission,⁷ as well as in the induction of arachidonic acid release by reactive oxygen species.⁸ Therefore, iPLA₂ may play a key role in pain states associated with high oxidative stress.

CYCLOOXYGENASE (COX) PATHWAY

COX, which converts arachidonic acid to endoperoxide-containing intermediates to produce prostaglandins and thromboxanes, exists in multiple isoforms.⁹ COX-1 and COX-2 share 60% amino acid sequence homology. Both enzymes are membrane bound; however, COX-2 is twice as abundant at the nuclear envelope than within the endoplasmic reticulum, whereas the concentration of COX-1 is equal at both locations.⁶

Differences in COX isoforms. COX-1 functions predominantly in the endoplasmic reticulum and COX-2 mostly in the nucleus.^{10,11} Therefore, it appears that COX-1 and COX-2 are two distinct prostanoid biosynthetic systems with separate biological functions for their products. COX-1 produces prostaglandins constitutively for secretion as extracellular mediators, and COX-2 produces prostaglandins predominantly within the nuclear fat. Convenient simplifications depict COX-1 as a homeostatic continuous regulator that is expressed constitutively and COX-2 as being an inducible enzyme, the expression of which is upregulated by various neurotransmitters, growth factors, proinflammatory cytokines, lipopolysaccharides, calcium, and small peptide hormones.^{12,13} However, COX-1 expression can be induced under certain conditions, such as neural insult,^{13–16} and various tissues, such as kidney and nerve tissue, express COX-2 constitutively.^{12,13} The clinical significance of the various isoforms remains incompletely appreciated; for example, studies reveal that mice deficient in COX-2 yield normal inflammatory responses with exogenous arachidonic acid but exhibit an increased incidence of suppurative peritonitis.¹⁷

A third isoform, denoted COX-3 (a variant of COX-1), has been identified in brain tissue. Its physiologic role remains uncertain. It has been purported to represent the long sought-after acetaminophen-sensitive COX activity that may play a key role in fever regulation^{9,18}; however, the clinical significance of the COX-3 isoform in humans is unclear.

Relation to neuropathic pain. COX-2 is known to be upregulated during inflammation; however, Ma and Eisenach¹⁹ have demonstrated (primarily in infiltrating macrophages) that COX-2 is universally upregulated following various types of peripheral nerve injury. The resulting overproduction of prostaglandins appears to contribute to the central plasticity and maintenance of neuropathic pain after nerve insult, due, in part, to facilitating the release of nociceptive neuropeptides, such as substance P and calcitonin gene-related peptide (CGRP), from primary afferent fibers with increased spinal dynorphin.²⁰

The development of allodynia associated with neural insult may be partly due to spinal prostaglandins synthesized by COX-1 as well as COX-2.^{21–25} This finding is in contrast to the predominant role of COX-2 in inflammatory pain.

Hefferan and colleagues²¹ found that spinal prostaglandins synthesized by COX-1 in the early period (4–8 hours) after neural insult appear to be important in the development of allodynia in rats. These researchers compared the effects of intrathecal administration of SC-560 (a selective COX-1 inhibitor) with that of S(+)-ibuprofen (which inhibits both COX-1 and COX-2²¹) and, since ibuprofen was better at reversing allodynia than SC-560, suggested that both COX-1 and COX-2 are important in the neuropathic pain that may emerge after neural insult.

In much of the peripheral nervous system, COX-1 is expressed constitutively; peripheral COX-2 is expressed to any physiologically significant degree only after tissue injury is induced secondary to inflammation.¹³ However, the majority of neurons and radial glia in the spinal cord (ie, the glia in the white matter, not gray-matter glia, such as microglia astrocytes and oligodendrocytes) express significant, detectable levels of constitutive COX-2.²⁶ The constitutive COX-2 in the spinal cord is important in generating PGE₂, leading to hyperalgesia.²⁶ Ghilardi et al²⁷ suggested that blocking constitutive spinal COX-2 before tissue injury may diminish sensitization (both peripheral and central) following tissue injury. Lashbrook et al²⁸ suggested that spinal prostanoids generated via both the COX-1 and COX-2 pathways may play a role in the hyperalgesia/allodynia seen in nerve-injured rats. Zhu and Eisenach,²³ working with partial sciatic nerve transection and L5-L6 spinal-nerve ligation models in male rats, suggested that COX-1 expression in the spinal cord is not static and changes, in a time- and laminar-dependent manner, after nerve injury. Further, these authors suggested that spinally administered specific COX-1 inhibitors may be useful in preventing and treating neuropathic pain.

Ma and Eisenach²⁴ provided morphological and pharmacological evidence of the role of peripheral prostaglandins in the pathogenesis of neuropathic pain. At 2 and 4 weeks following partial sciatic nerve ligation (PSNL), dramatic increases in COX-2 immunoreactive cell profiles were observed

at the injured site and in the adjacent region.²⁴ In addition, an increased number of COX-1 immunoreactive cell profiles were observed in the epidermis of the ipsilateral foot pad of PSNL rats. Local injection of ketorolac, a nonselective COX inhibitor, into the ipsilateral plantar side or into the injured region of the sciatic nerve reversed the mechanical allodynia induced by PSNL for more than 5 days and suppressed the PSNL-induced increase in the phosphorylation of a transcription factor cAMP (cyclic adenosine monophosphate) response element-binding protein in the ipsilateral spinal cord dorsal horn of L4 and L5.²⁴

Broom et al²⁵ reported similar results using the rat spared nerve injury model of neuropathic pain. They concluded that although COX-2 plays a key role in the development of inflammatory hypersensitivity induced by injection of complete Freund adjuvant into the rat hind paw, the pain hypersensitivity produced by this model is not significantly COX-2 dependent.

The clinical implications of these findings translate into the appropriate selection of selective COX-2 inhibitors for inflammatory pain—preferably one that crosses the blood-brain barrier reasonably well. However, in the setting of neural insult, it would appear reasonable to utilize agents that inhibit both COX-1 and COX-2 in order to diminish and/or prevent neuropathic pain. If this work is validated in human studies, clinical correlates may translate into the preferred use in certain subpopulations of traditional nonsteroidal anti-inflammatory agents that inhibit both COX-1 and COX-2 (perhaps with partial COX-1 selectiveness over COX-2) in situations where the potential for the development of neuropathic pain exists (eg, before treatment with vincristine).

PROSTAGLANDIN E SYNTHASE (PGES)

As mentioned earlier, PGES facilitates the conversion of PGH₂ to PGE₂. As with PLA₂ and COX, multiple forms of PGES exist with different enzymatic qualities, locations, and functions. Cytosolic PGES (cPGES) is constitutively expressed in many tissues and is functionally coupled to COX-1.²⁶ Microsomal (mPGES) is a membrane-associated enzyme that exists in two isoforms, mPGES-1 and mPGES-2.

Mabuchi et al²⁹ studied a neuropathic pain model created by L5 spinal nerve transection in mPGES-1 knockout (mPGES-1) mice. Although the mice retained normal nociceptive responses, they did not exhibit mechanical allodynia or thermal hyperalgesia for over a week following transection, suggesting that PGE₂ produced by mPGES-1 is involved in neuropathic pain.²⁹

Kamei et al,³⁰ also using mPGES-1 knockout mice, provided evidence that mPGES-1 plays a pivotal role in the production of PGE₂ involved in pain hypersensitivity and inflammation.

Distinct subcellular localization with colocalization of terminal prostaglandin synthases affects enzyme functions and coupling. COX-1 is more enriched in the endoplasmic reticulum than in the perinuclear envelopes, whereas COX-2 is located predominantly in the perinuclear envelope.³¹ Coexpression studies utilizing both COX enzymes with certain terminal prostaglandin synthases have shown that synthases near the

perinuclear membrane, such as prostacyclin (PGI₂) synthase and mPGES-1, tend to be selectively coupled with COX-2,³² whereas PGES (complexed with cytosolic proteins [Hsp90 and casein kinase 2]) is coupled with COX-1.³² Hematopoietic PGD synthase (a cytosolic terminal enzyme) translocates to the endoplasmic reticulum as the immediate response to stimulation with A23187 (where it is preferentially coupled with COX-1) and translocates to the perinuclear envelope as the delayed response to cytokine stimulation (where it is preferentially coupled with COX-2).³²

“Traditional” nonselective COX inhibitors and COX-2-specific inhibitors not only block the formation of individual prostaglandins but also may knock out other “bystander” eicosanoids that may be needed to maintain homeostasis or may have the effect of “shunting” arachidonic acid metabolism toward other metabolic pathways.³³ Trebino and coworkers,³⁴ studying the pathogenesis of collagen-induced arthritis in mPGES-1-deficient mice, have defined the contributions of mPGES-1 and PGE₂ in chronic inflammation and pain, suggesting that agents that block mPGES-1 may be comparable in efficacy to COX-2 inhibitors against pain and inflammation but may be better tolerated and have fewer unfavorable side effects (eg, without knocking out bystanders).

Activation of MAPK induces increased expression of the transcription factor Egr-1 and subsequently activates Egr-1 (via phosphorylation).^{35,36} Activated Egr-1 binds to the proximal GC box in the mPGES-1 promoter, which promotes transcriptional activation of the mPGES-1 gene.³⁵

PROSTAGLANDINS

PGE₂, the “original prostaglandin,” has received the most attention among prostaglandins because of its contribution to nociception and inflammation. PGE₂ stereospecifically exerts potent (ie, within the nanomolar to micromolar range) tissue- and cell type-selective actions.³⁷

PGE₂ is not only thought to play a key role in nociception (eg, intradermal PGE₂ is largely responsible for hyperalgesia in the peripheral nervous system) but also appears to be involved in a wide variety of other functions, including vasodilation, altered microvascular permeability, and febrile responses.³⁸⁻⁴⁰ PGE₂ induces different cellular responses via interaction with specific receptors, known as EP1 to EP4, which have restricted patterns of expression and receptor-specific actions.⁴¹

Mode of action. The most widely accepted explanation for the effects of prostaglandins on nociception relates to the activation of the adenylyl cyclase/cAMP/protein kinase A (PKA) pathway sparked by the binding of PGE₂ to the EP2 receptor, which leads to enhanced, tetrodotoxin-resistant sodium currents (probably via phosphorylation of NaV 1.8), inhibition of voltage-dependent potassium currents, and increased voltage-dependent calcium influx in nociceptive afferent fibers.⁴² These changes in ion influx result in decreased firing thresholds, increased firing rates, and the release of excitatory amino acids, substance P, CGRP, and nitric oxide. Additionally, the PKA activated via PGE₂/EP2 binding may selectively block in-

hibitory (strychnine-sensitive) glycinergic neurotransmission onto superficial dorsal horn neurons.⁴³ This PGE₂-induced “disinhibition” may facilitate transmission of nociceptive input to higher areas of the central nervous system. Prostaglandins also facilitate membrane currents and the release of substance P and CGRP induced by protons, bradykinin, and capsaicin.⁴²

The primary mode of prostaglandin action is through specific G-protein–coupled receptor superfamilies of seven transmembrane-spanning proteins, or domains. The DP2 receptor is an exception, being a member of the chemoattractant receptor subgroup.⁴⁴

Differences in prostaglandin receptors. The receptors IP, DP1, EP2, and EP4 are considered one group, referred to as “relaxant” receptors. These receptors signal via G_s-mediated increases in intracellular cAMP via adenylate cyclase, with resultant activation of PKA and phosphorylation of sodium channels. EP1, EP3, and TP are considered a second group, known as “contractile” receptors, that signal through G_q-mediated increases in intracellular calcium.⁴⁴ The EP3 receptor is considered an inhibitory receptor that signals through G_i-mediated decreases in cAMP formation.⁴⁴ Although the mechanisms by which eicosanoids contribute to or facilitate nociception remain uncertain, it is apparent that both peripheral and central nervous system processes seem to be involved. It appears that in certain situations, greater sensitivity may be mediated by increased numbers of EP receptors.⁴⁵

Ma and Eisenach²⁴ found a dramatic increase in immunoreactivity for EP1–EP4 in injured nerve of PSNL rats and suggested that PGE₂ may be overproduced in injured nerve and facilitate upregulation of EP receptors.

A complex interplay of receptor subtypes may be involved in various hypersensitivity states, and different subtypes could be activated depending upon the amount of agonist present. Utilizing EP1 and EP3 receptor knockout mice, Minami et al⁴⁶ demonstrated that spinal EP1 receptors contribute to PGE₂-induced allodynia (apparently upstream from N-methyl-D-aspartate receptor activation and resultant nitric oxide generation). At low doses of PGE₂, however, it appears that spinal EP3 receptors may play a key role in PGE₂-induced hyperalgesia.⁴⁶ Nanomolar concentrations of PGE₂ reduce the responsiveness of postsynaptic inhibitory glycine receptors in the superficial layers of the spinal cord dorsal horn⁴³ and may also directly depolarize deep dorsal horn neurons.

PGI₂ and other prostaglandins. Although PGE₂ appears to be the key prostaglandin involved in nociceptive processes, PGI₂ may contribute to hyperalgesia under certain circumstances. PGI₂ levels are elevated in the central nervous system during the early phase of carrageenan-induced paw edema, and PGI₂ is thought to contribute to pain and inflammation because IP knockout mice show a reduction in pain and edema.⁴⁷ Ueno et al⁴⁸ implicated IP and EP3 receptors in mediating the enhanced acetic acid–induced writhing response in mice previously exposed to lipopolysaccharide. Bradykinin-evoked increases in excitability in isolated nodose neurons demonstrated the dependence on PGI₂ in the neuronal membrane, since inhibi-

tors of PGI₂ synthesis negated the effect of bradykinin.⁴⁹ PGD₂ may lead to hyperalgesia via DP effects on substance P release, with subsequent binding to neurokinin-1 receptors on small-diameter primary afferent fibers.⁴² DP effects have also been implicated in antagonizing PGE₂-induced allodynia.⁴² Further, PGF_{2α} has been shown to lead to allodynia via its effects on large-diameter (myelinated) fibers.⁴²

GLYCINE RECEPTORS

An international team⁵⁰ has discovered that the glycine receptor subtype α3 (GlyR α3) may be the receptor that spinal prostaglandins utilize to produce central inflammatory pain sensitization. This receptor may therefore represent an important potential therapeutic target in attempts to provide analgesia.

Harvey and coworkers⁵⁰ demonstrated that mice deficient in GlyR α3 lack the normally seen inhibition of glycinergic neurotransmission caused by spinal injection of PGE₂ or peripheral inflammation. They concluded that during inflammatory pain states, PGE₂ disinhibits the spinal transmission of nociceptive input from the spinal cord to higher brain areas via PKA-dependent phosphorylation and inhibition of GlyR α3, thereby facilitating the development of central thermal and mechanical hypersensitivity.

Glycine-mediated inhibition of the actions of neurons normally occurs when an agonist, such as glycine, binds to the GlyR α3 receptor and triggers the movement of chloride ions into the neuron.⁵¹ The addition of phosphate to GlyR α3 impedes or blocks chloride ion influx, which leads to facilitation (ie, disinhibition) of neuronal activity and nociceptive input.

Case Examples

The following are some hypothetical clinical vignettes that illustrate the potential utility of the preceding discussion in medical decision-making regarding targeted treatments for analgesia.

- A 73-year-old man returns to his oncologist with lung cancer and metastatic invasion into the right brachial plexus, with resultant right brachial plexopathy and severe right upper extremity pain. His physician suggested potent opioids, a selective COX-1 inhibitor, a 5-LOX inhibitor, and a combined-adenosine receptor antagonist/p38 MAPK/PDE₄ inhibitor.
- An 83-year-old woman with breast cancer and an ischemic left lower extremity is complaining of severe pain in the extremity despite taking high doses of potent opioids. Epoprostenol therapy, antioxidants, and isoprostane antagonists are added to her pharmacotherapy.
- A 39-year-old woman with pancreatic cancer and a chronic inflammatory lower extremity lesion, which waxes and wanes in its severity, is on high-dose opioid therapy and complaining of abdominal and leg pain. Her physician feels that she may have disturbances in the processes involved with the resolution of inflammation and prescribes lipoxin A₄, a lipoxygenase inhibitor, and an LTB₄ receptor antagonist.

Case Examples (continued)

- A 79-year-old woman with breast cancer and a preexisting peripheral neuropathy is about to undergo chemotherapy with vincristine as part of the CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) regimen. She is concerned about an exacerbation of neuropathic pain and asks her physician if there is any medication she could take prior to and throughout her chemotherapy to abort or lessen any potential exacerbation of her symptomatic peripheral neuropathy. She states that she has essentially no money or medical coverage to obtain medications and wonders if there are any other options. Her physician tells her to take a baby aspirin daily, since it functions as a selective COX-1 inhibitor and may ameliorate any potential exacerbation of neuropathic pain.

LIPOXYGENASE PATHWAY

In human tissues, arachidonic acid can be metabolized via three major lipoxygenases: 5-lipoxygenase (5-LOX), 12-lipoxygenase (12-LOX), and 15-lipoxygenase (15-LOX).

Two isoforms of 15-LOX exist: 15-LOX type 1 and 15-LOX type 2.⁵² The two isoforms may have antagonistic effects, with type 1 providing anti-inflammatory effects, protection against bone loss, and perhaps analgesic effects via reduced expression of various cytokines (eg, interleukin 1 β , tumor necrosis factor, and growth factors)^{53,54} and type 2 yielding opposing effects (eg, aggravating bone loss).⁵³

Bradykinin (acting at B2 bradykinin receptors) activates transient receptor potential vanilloid receptor-1 (TRV1) via an intracellular second messenger pathway involving mobilization of arachidonic acid by PLA₂ and generation of a lipoxygenase product, 12-hydroperoxyeicosatetraenoic acid.⁵⁵ Aley and Levine⁵⁶ demonstrated in animal models that nordihydroguaiaretic acid (NDGA, a nonselective lipoxygenase inhibitor), baicalein (BAIC, a 12-lipoxygenase inhibitor), and 5,6-dehydroarachidonic acid (a 5-lipoxygenase inhibitor) inhibited epinephrine-induced hyperalgesia and μ RACK (a selective activator of protein kinase C ϵ [PKC ϵ])-induced hyperalgesia. NDGA and 5,6-dehydroarachidonic acid inhibited PGE₂-induced hyperalgesia mediated via PKA.⁵⁶ They suggested that products of the 5-LOX and 12-LOX pathways contribute to peripheral hyperalgesia induced by agents that act directly on primary afferent nociceptors (eg, epinephrine and PGE₂) at or downstream of PKA and PKC ϵ .⁵⁶

Leukotriene B₄. The most widely appreciated leukotriene involved in nociceptive processes may be the 5-LOX product LTB₄. LTB₄ can act on neutrophils through the LBT₁ receptor to elicit chemotaxis. LTB₄ does not induce pain itself but lowers the pain threshold for other stimuli. LTB₄ and PGE₂ may act synergistically to promote inflammation⁵⁷ and possibly nociception as well. The LOX products LTB₄, 12(S)- and 15(S)-hydroperoxyeicosatetraenoic acids, and 5(S)- and 15(S)-hydroxyeicosatetraenoic acids directly activate TRV1 in isolated membrane patches of sensory neurons.⁵⁸

LTB₄ is a rapidly synthesized neutrophil chemoattractant and activator of inflammatory cells (which also appears to mediate T-cell recruitment^{59,60}) that signals primarily its high-affinity, cell-surface G protein-coupled receptor BLT-1. BLT-1 receptor activation may result in IP3-mediated calcium release initially from intracellular stores and later via influx through cell-membrane channels with subsequent activation of PKA pathways and/or protein tyrosine kinase pathways.⁶¹ LTB₄ may play a role as a nociceptive mediator under certain circumstances.^{62,63}

Dual 5-LOX/COX-2 inhibition. Singh and colleagues⁶⁴ induced mechanical and thermal hyperalgesia by autologous nucleus pulposus in rat sciatic nerves. Zileuton (Zyflo), a 5-LOX inhibitor, significantly decreased mechanical as well as thermal hyperalgesia in a dose-dependent (25–100 mg/kg) manner when compared with the nucleus pulposus group.⁶⁴ Coadministration of zileuton and indomethacin enhanced the antihyperalgesic effects of zileuton, suggesting that leukotrienes and prostaglandins may both play a significant role in hyperalgesia induced by autologous nucleus pulposus in rats—and that dual inhibition of the LOX and COX pathways may provide a useful treatment approach under certain conditions.⁶⁴

These results have excited investigators in the pain arena as well as in oncology. Dual inhibition of 5-LOX and COX-2 (which may produce an efficacy similar to that of glucocorticoids but without their adverse effects) may yield synergistic effects, in part via downregulation of PGE₂ and LTB₄.⁶⁵ Such dual inhibitors would be especially useful in situations with leukotriene-mediated nociception, where inhibition of COX-2 may lead to shunting of arachidonic acid metabolism toward the leukotriene pathway.

As a result of this interest, multiple dual 5-LOX/COX-2 inhibitors are under development,⁶⁶ including S 19812 (N-hydroxy-N-methyl 4-[2,3-bis-methoxyphenyl]-thiophen-5-yl) butanamide; CAS 181308-68-9⁶⁷ and licofelone ([2,2-dimethyl-6-(4-chlorophenyl)-7-phenyl-2,3-dihydro-1H-pyrrolizine-5-yl]-acetic acid),⁶⁸ in an effort to provide novel therapeutic agents for the treatment of pain and inflammation. Licofelone is a competitive inhibitor of 5-LOX, COX-1, and COX-2, decreasing both leukotriene and prostaglandin expression with presumed low gastrototoxicity.⁶⁹ Studies in animal models suggest that licofelone may impede the progression of osteoarthritis without increasing the frequency or severity of gastrointestinal side effects when co-administered with low-dose aspirin⁶⁹; however, it may exert potent antiplatelet effects due to inhibition of COX-1 activity.⁶⁸

NONENZYMATIC LIPID PEROXIDATION PATHWAY

Isoprostanes (stereoisomers of prostaglandins) are formed in vivo primarily via nonenzymatic in situ peroxidation of arachidonic acid by reactive oxygen species.^{70,71} Evans et al,⁷² using isolated rat sensory neurons, demonstrated that 8-isoprostaglandin E₂ (8-iso PGE₂) lowers nociceptive thresholds to mechanical and thermal stimuli, showing that 10 μ M 8-iso PGE₂ stimulates peptide release directly from sensory neurons, whereas treatment with 1 μ M 8-iso PGE₂ augments the release of transmitters

evoked by bradykinin or capsaicin from sensory neurons.

Pretreatment of neuronal cultures with the nonsteroidal anti-inflammatory agent ketorolac, a COX inhibitor, did not affect the sensitizing action of 8-iso PGE₂ on peptide release, suggesting that the actions of 8-iso PGE₂ were not mediated by the production of prostaglandins via the COX pathway and that isoprostanes may be important mediators for producing or facilitating nociception in situations where oxidative stress modulates neural functions.⁷² A growing body of evidence suggests that reactive oxygen species may contribute to nociception under certain circumstances.⁷³⁻⁷⁷ It is conceivable that isoprostanes may facilitate nociception under conditions where reactive oxygen species are involved. Therefore, in pain states with high oxidative stress, such as ischemic pain, selective isoprostane antagonists may be particularly useful.

Epoprostenol therapy has been demonstrated to decrease oxidant stress, although it does not appear to affect thromboxane production.⁷⁸ Cyclopentenone isoprostanes contain highly reactive unsaturated carbonyl moieties on the prostane ring that readily adduct relevant biomolecules, such as thiols, via the chemical process of Michael addition.⁷⁹ Unlike cyclopentenone prostaglandins, cyclopentenone isoprostanes may be formed in large amounts in vivo.⁷⁹ Further, investigators have synthesized A-ring isoprostanes, such as 15-A(2)isoprostanes, and have examined the formation of cyclopentenone isoprostane-like compounds from fatty acids, such as linolenic acid and docosahexaenoic acid.⁷⁹ Although the potential role of these substances as modulators of pain and/or inflammation under certain circumstances remains uncertain, it is conceivable that they may represent a therapeutic target for future research.

MONOOXYGENASE CYTOCHROME P₄₅₀ EPOXYGENASE PATHWAY

EETs are synthesized from arachidonic acid via monooxygenases (eg, cytochrome P₄₅₀ epoxygenases). Monooxygenases are mixed-function oxidase enzymes of the cytochrome P₄₅₀ superfamily. Monooxygenases generally metabolize arachidonic acid into three major classes of eicosanoid products⁸⁰:

1. midchain conjugated dienols formed by LOX-like bis-allylic oxidation of arachidonic acid, generating six EETs;
2. C₁₆–C₂₀ alcohols formed by omega/omega-1 terminal hydroxylation of arachidonic acid omega-n arachidonate alcohols (known as the omega-oxygenase reaction), yielding five EETs; and
3. *cis*-EETs produced by olefin epoxidation of arachidonic acid (known as the epoxygenase reaction⁸¹), generating four EETs. Cytochrome P₄₅₀ epoxygenases produce four EET regioisomers: 5,6-EET, 11,12-EET, and 14,15-EET.

EETs generated from arachidonic acid may be taken up from the cytosol and/or circulation and re-tailored into the sn-2 position of the cell membrane glycerol phospholipids.^{79,81,82} Furthermore, depending upon the local environment, epoxygenases may be functionally inhibited, thereby leading to “effective shunting” to arachidonic acid metabolic pathways, utilizing other enzymes⁸¹ already “committed” to monooxygenase pathways. This

process may lead to an increase in LOX-like bis-allylic oxidation products and/or omega hydroxylase products.⁸¹

Functions of EETs. Various EETs may have numerous functions, with many involving effects on ion channels and/or ion influx. Efforts to delineate EET effects have focused largely on modulation of cardiovascular function via actions predominantly on the vasculature and in the kidneys.⁸³⁻⁸⁵ Wang et al⁸⁶ provide support for EETs functioning as endothelium-derived hyperpolarizing factors that can upregulate endothelial nitric oxide synthase via activation of MAPK and protein kinase C signaling pathways. Various isomers of EETs may contribute to endogenous antipyraxis,⁸¹ play a role in neuroimmunomodulation,⁸¹ and potentially stimulate the release of melanocyte stimulating hormone α (MSH α).⁸¹ 11,12-EET displays inhibitory effects on the generation of PGE₂; interleukin-6, however, facilitates the release of tumor necrosis factor- α .⁸¹

The exogenous addition of 11,12-EET or overexpression of CYP 2J2 decreases cytokine-induced endothelial cell adhesion molecule expression, thereby diminishing leukocyte adhesion to the endothelial lining.⁸⁷ Additionally, EETs induce polymorphonuclear leukocyte aggregation, and this also decreases leukocyte adhesion to endothelial cells,⁸⁸ thereby potentially modulating nociception in pain states where leukocytes may facilitate nociceptive signals. Nuclear transcription factor κ B (NF- κ B) is thought to play a critical role in cytokine-mediated inflammation. EETs appear to inhibit I κ B kinase (I κ B kinase is the enzyme that normally functions to prevent the degradation of I κ B), which produces persistent binding of I κ B to NF- κ B.⁸⁹ This maintains NF- κ B in the inactive state and inhibits NF- κ B-mediated gene transcription. Node et al⁸⁷ suggested that EETs may play an important nonvasodilatory role in vascular inflammation in part by the inhibition of NF- κ B-mediated vascular cell adhesion molecule-1 (VCAM-1) expression. The effects of EETs on NF- κ B may play a role in nociceptive processes in various pain and inflammatory states.

Case Examples (continued)

- A 74-year-old man with prostate cancer and painful osseous metastases comes to his physician complaining of widespread bone pain. He is told that he is not a candidate for external-beam radiation therapy or radiopharmaceuticals, and he has had significant adverse reactions to glucocorticoids. His physician suggested a dual 5-LOX/COX-2 inhibitor, a PDE₄ inhibitor, and potent opioids. The patient is having difficulty obtaining the combination medication, so his physician prescribes a 5-LOX inhibitor (eg, zileuton) and a COX-2 inhibitor. The “physician of the future” prescribes lumiracoxib, hoping that it may have a slight edge over other COX-2 inhibitors for the treatment of painful osseous metastases, since its pK_a (ionization constant) is such that it may work especially well in acidic environments. The microenvironment at the interface of the osteoclast with the bone surface is highly acidic, since the osteoclast uses a proton pump to help it “digest” bone; therefore, lumiracoxib may be a reasonable future choice.

Mechanisms of action. EETs and related products may contribute to the modulation of nociceptive processes under certain conditions. This could occur via multiple avenues, including the anti-inflammatory properties of EETs (via autocrine effects on endothelial cells),⁸⁶ EET effects on signaling pathways that may be involved in nociceptive processes (eg, Src), and EET-stimulated effects on PGE₂,⁹⁰ or the above-mentioned effects on NF- κ B.

In addition to possible inhibition of cytokine-induced inflammatory responses,^{87,89} potential EET effects on prostaglandin production,⁹⁰ as well as increased calcium influx,^{91,92} could possibly affect various nociceptive processes under certain conditions.

It appears that EETs may have numerous effects and therefore most likely work via a number of different mechanisms. It is conceivable that EETs bind to a receptor (which is then linked to a cyclic AMP and PKA signal transduction pathway). EETs may also activate potassium transport^{84,93} and may affect calcium channels.⁹⁴ Alternatively, EETs may bind to a guanine-binding protein (G-protein), thereby activating G α s with subsequent cyclic AMP production and PKA activation.⁹⁵

EETs may set in motion a series of protein phosphorylations that subsequently activate the epidermal growth factor (EGF) receptor and attract various scaffolding proteins and c-Src³⁵ signaling transduction pathways, possibly including phosphatidylinositol 3-kinase (PI3-K), MAPK, Akt, and extracellular signal-regulated kinase (ERK) 1/ERK 2, with subsequent effects on gene regulation.⁹⁶ Furthermore, EETs may potentiate nociception via effects on α MSH α , since the melanocortin system appears to be involved in nociceptive processes,^{97–101} particularly in activating sensory neuron-specific receptors.^{102,103}

Finally, it is possible that EETs may exert effects on sodium movement: Lee et al¹⁰⁴ demonstrated that 5 μ M EET reduced the open-state probability of sodium channels in rat ventricular myocardium by as much as 73% \pm 5%, which is similar to that seen with local anesthetics such as lidocaine.

Resolution of Inflammation

Inflammation does not simply wane as the concentration of inflammatory mediators begins to fall. Instead, endogenous local mediators of resolution play a key role in controlling inflammation and its organized coordinated “active” resolution.¹⁰⁵

Among them, lipoxins are the trihydroxytetraene-containing eicosanoids that are generated largely by transcellular biosynthesis and involved in a coordinated scheme to move from inflammation to its resolution.¹⁰⁵ These “stop signals” in inflammation may be involved in switching the cellular response from additional recruitment of polymorphonuclear leukocytes toward monocytes (in a non-phlogistic fashion), thereby facilitating resolution of the inflammation response.¹⁰⁵

15-Deoxy- Δ -^{12,14}-prostaglandin J₂ (15d-PGJ₂) is a cyclopentenone that is the dehydration end product of PGD₂. Dehydration of PGD₂ yields PGJ₂ and subsequently yields 15d-PGJ₂. There is no specific prostaglandin synthase yielding 15d-PGJ₂, and no specific 15d-PGJ₂ receptor has been identified.¹⁰⁶ 15d-

PGJ₂ exhibits anti-inflammatory and proresolution effects, which may be mediated in part via interaction with DP1 and DP2 receptors, intracellular interaction with peroxisome proliferator-activated receptors (PPARs) interfering with NF- κ B DNA binding, or by covalently binding to I κ K (thereby inhibiting I κ K function), among other mechanisms.¹⁰⁶

Aspirin inhibits COX-1 and converts COX-2 into an aspirin-triggered lipid mediator-generating system that produces an array of novel endogenous local autocoids from dietary omega-3 polyunsaturated fatty acids.^{107–109} Some of these local autocoids exhibit potent anti-inflammatory or antineutrophil recruitment activity and may also play a significant role in the resolution of inflammation, earning them the appellation “resolvins.”¹⁰⁷ Resolvins, docosatrienes, and neuroprotectins, as well as their aspirin-triggered counterparts, appear to be involved in post-inflammation clean-up activities, along with certain lipoxins.^{107–109}

Gilroy and colleagues¹¹⁰ suggested that mechanisms that “switch off” acute inflammation lead to the expression of pro-resolving COX-2-derived 15d-PGJ₂ during “active resolution,” which subsequently induces apoptosis of both neutrophils and macrophages. A failure of acute inflammation to resolve may facilitate persistence of the inflammatory response, thereby contributing to chronic inflammation.

These proresolution mediators—including lipoxins and cyclopentenones, as well as resolvins, docosatrienes, neuroprotectins, and their endogenous aspirin-triggered epimeric counterparts—could modulate nociceptive processes under certain circumstances and therefore may represent novel therapeutic targets for achieving analgesia.

Future Prospects

Alteration of arachidonic acid metabolism may modulate pain and inflammation and may also be involved in human carcinogenesis.^{111–116}

Inhibiting COX pathways may lead to a “shunting” of metabolic machinery toward the production of other arachidonic acid metabolic products (eg, leukotrienes). It is becoming more apparent that we can no longer think of inflammation in terms of a series of good and bad pathways, where blocking one avenue can promote beneficial effects without affecting other processes. Instead, inflammation must be viewed as a complex, interrelated network, and any perturbation of various enzymes and pathways may affect other processes and pathways involved in promoting or inhibiting inflammation or its resolution.

In the past, therapies aimed at modulation of LOX pathways received less attention than those aimed at COX pathways. LOX products and other products of arachidonic acid metabolism may represent potential therapeutic targets for pain and inflammation, as well as cancer prevention.^{115,117} The development of dual COX/LOX inhibitors may eventually have clinical utility in certain pain and/or inflammation states.

Leukotriene A₄ hydrolase (LTA₄H) catalyzes the hydrolysis of the epoxide LTA₄ to the diol LTB₄. Bestatin, an

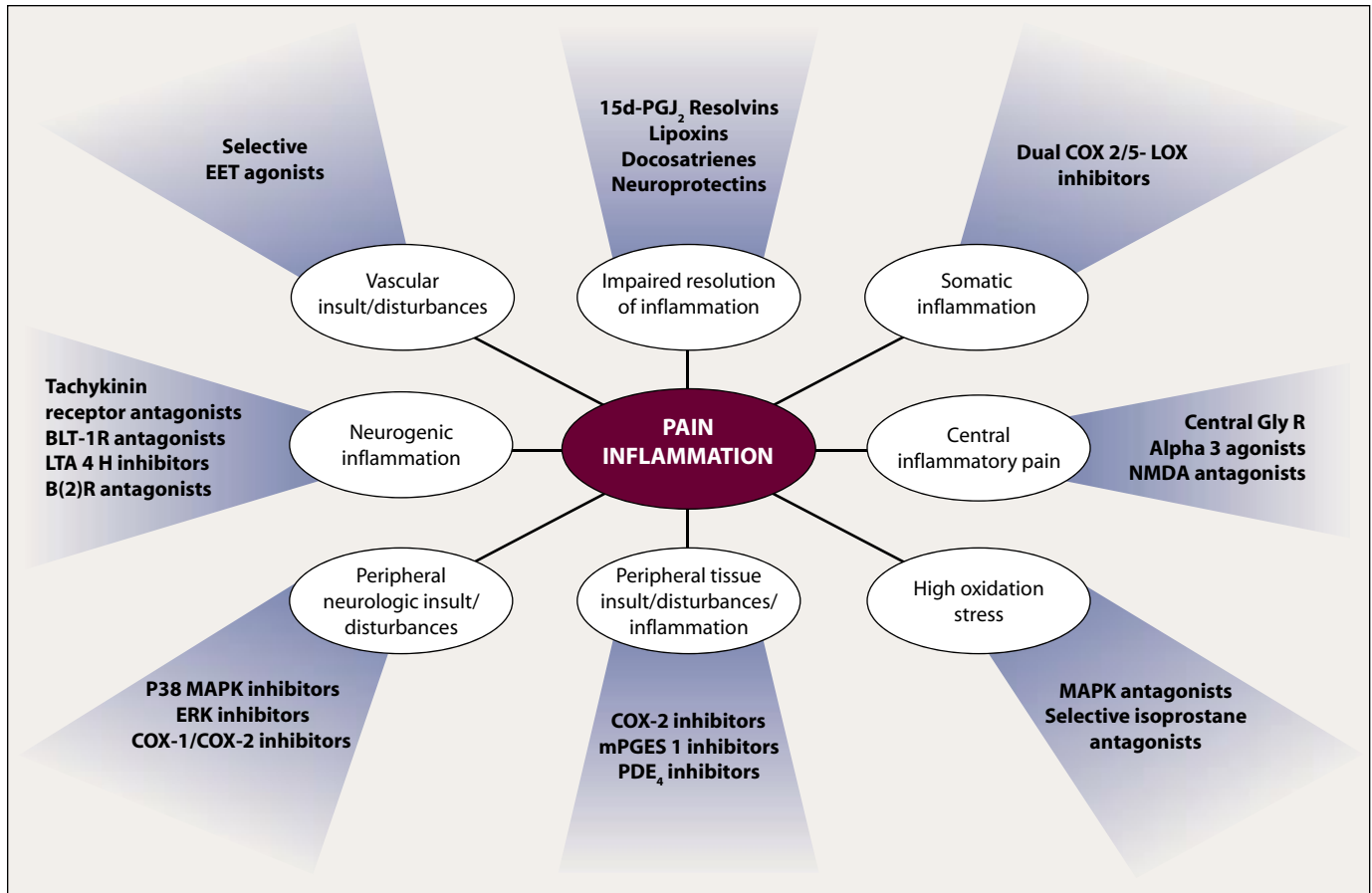


Figure 2 Hypothetical Analgesic and Anti-inflammatory Treatments Targeting Arachidonic Acid Metabolites and Pathways

LTA₄H inhibitor, suppresses tumorigenesis in animal models of esophageal carcinoma¹¹⁷ and may be potentially useful (in a manner similar to BLT-1 receptor antagonists) as a therapeutic agent in certain pain and/or inflammatory states. Native lipoxin A₄ and lipoxin B₄ and their synthetic analogs ATL₄, ZK-142, and ZK-994, retain broad anti-inflammatory activity after intravenous, oral, and topical administration.¹¹⁸ Further, interfering with second-messenger signaling may be a useful therapeutic adjunct to approaching modulation of pain and inflammation. Specific inhibitors of phosphodiesterase type 4 (PDE₄), such as cilomilast and roflumilast, appear not only to elevate intracellular concentrations of cAMP but also may inhibit neutrophil degranulation.^{119–121}

Future agents may possess multiple mechanisms aimed at combating pain and/or inflammation, such as CGH2466, a

combined adenosine receptor antagonist, p38 mitogen-activated protein kinase, and PDE₄ inhibitor.¹²²

Substances that promote the resolution of inflammation may potentially become useful clinical therapeutic agents. Different strategies to address pain or inflammation may be better suited or targeted to different pain and inflammatory states. Figure 2 is a hypothetical diagram meant only to partially illustrate the many mechanisms and avenues involved in the pathogenesis of pain and inflammation associated with arachidonic acid metabolites and to suggest potential treatment options. Many mechanisms are omitted, and there is considerable overlap among the various conditions depicted. Figure 2 is certainly not intended to guide treatment choices, at least at this stage; it is based only on the imagination of the author and not on any definitive studies.

References

1. Serhan CN, Gotlinger K, Hong S, et al. Resolvins, docosatrienes, neuroprotectins, novel omega-3-derived mediators, and their aspirin-triggered endogenous epimers: an overview of their protective roles in catabasis. *Prostaglandins Other Lipid Mediat* 2004;73:155–172.
2. Lawrence T, Willoughby DA, Gilroy DW. Anti-inflammatory lipid mediators and insights into the resolution of inflammation. *Nat Rev Immunol* 2002;2:787–795.
3. Smith HS. Nonsteroidal drugs: bedside. In: Smith HS, ed. *Drugs for Pain*. Philadelphia, Pa: Hanley and Belfus; 2003.
4. Serhan CN. Eicosanoids and related compounds. In: Koopman WJ, ed. *Arthritis and Allied Conditions. A Textbook of Rheumatology*. Baltimore, Md: Lippincott Williams and Wilkins; 2005.
5. Balsinde J, Winstead MV, Dennis EA. Phospholipase A(2) regulation of arachidonic acid mobilization. *FEBS Lett* 2002;531:2–6.
6. Bonventre JV, Huang Z, Taheri MR, et al. Reduced fertility and postischemic brain injury in mice deficient in cytosolic phospholipase A2. *Nature* 1997;390:622–625.
7. St-Gelais F, Menard C, Congar P, et al. Postsynaptic injection of calcium-independent phospholipase A₂

inhibitors selectively increases AMPA receptor-mediated synaptic transmission. *Hippocampus* 2004;14:319–325.

8. Martinez J, Moreno JJ. Role of Ca^{2+} -independent phospholipase A2 on arachidonic acid release induced by reactive oxygen species. *Arch Biochem Biophys* 2001;392:257–262.

9. Chandrasekharan NV, Dai H, Roos KL, et al. COX-3 a cyclooxygenase-1 variant inhibited by acetaminophen and other analgesic/antipyretic drugs: cloning, structure, and expression. *Proc Natl Acad Sci USA* 2002;99:13926–13931.

10. van der Donk WA, Tsai A-L, Kulmacz RJ. The cyclooxygenase reaction mechanism. *Biochemistry* 2002;41:15451–15458.

11. Schievella AR, Regier MK, Smith WL, et al. Calcium-mediated translocation of cytosolic phospholipase A₂ to the nuclear envelope and endoplasmic reticulum. *J Biol Chem* 1995;270:30749–30754.

12. O'Banion MK. Cyclooxygenase-2: molecular biology, pharmacology, and neurobiology. *Crit Rev Neurobiol* 1999;13:45–82.

13. Samad TA, Sapirstein A, Woolf CJ. Prostanoids and pain: unraveling mechanisms and revealing therapeutic targets. *Trends Mol Med* 2002;8:390–396.

14. Morteau O. Prostaglandins and inflammation: the cyclooxygenase controversy. *Arch Immunol Ther Exp (Warsz)* 2000;48:473–480.

15. Schwab JM, Brechtel K, Nguyen TD, Schluesener HJ. Persistent accumulation of cyclooxygenase-1 (COX-1) expressing microglia/macrophages and upregulation by endothelium following spinal cord injury. *J Neuroimmunol* 2000;111:122–130.

16. Hartner A, Pahl A, Brune K, Goppelt-Struebe M. Upregulation of cyclooxygenase-1 and the PGE2 receptor EP2 in rat and human mesangioliferative glomerulonephritis. *Inflamm Res* 2000;49:345–354.

17. Morham SC, Langenbach R, Loftin CD, et al. Prostaglandin synthase 2 gene disruption causes severe renal pathology in the mouse. *Cell* 1995;83:473–482.

18. Simmons DL, Botting RM, Robertson PM, et al. Induction of an acetaminophen-sensitive cyclooxygenase with reduced sensitivity to nonsteroid anti-inflammatory drugs. *Proc Natl Acad Sci U S A* 1999;96:3275–3280.

19. Ma W, Eisenach JC. Cyclooxygenase 2 in infiltrating inflammatory cells in injured nerve is universally upregulated following various types of peripheral nerve injury. *Neuroscience* 2003;121:691–704.

20. Ma W, Eisenach JC. Intraplantar injection of a cyclooxygenase inhibitor ketorolac reduces immunoreactivities of substance P, calcitonin gene-related peptide, and dynorphin in the dorsal horn of rats with nerve injury or inflammation. *Neuroscience* 2003;121:681–690.

21. Hefferan MP, O'Reilly DD, Loomis CW. Inhibition of spinal prostaglandin synthesis early after L5/L6 nerve ligation prevents the development of prostaglandin-dependent and prostaglandin independent allodynia in the rat. *Anesthesiology* 2003;99:1180–1188.

22. Zeilhofer HU, Brune K. A role for cyclooxygenase-1 in neuropathic pain? *Anesthesiology* 2003;99:1043–1044.

23. Zhu X, Eisenach JC. Cyclooxygenase-1 in the spinal cord is altered after peripheral nerve injury. *Anesthesiology* 2003;99:1175–1179.

24. Ma W, Eisenach JC. Morphological and pharmacological evidence for the role of peripheral prostaglandins in the pathogenesis of neuropathic pain. *Eur J Neurosci* 2002;15:1037–1047.

25. Broom DC, Samad TA, Kohno T, et al. Cyclooxygenase 2 expression in the spared nerve

injury model of neuropathic pain. *Neuroscience* 2004;124:891–900.

26. Tanioka T, Nakatani Y, Semmyo N, et al. Molecular identification of cytosolic prostaglandin E2 synthase that is functionally coupled with cyclooxygenase-1 in immediate prostaglandin E2 biosynthesis. *J Biol Chem* 2000;275:32775–32782.

27. Ghilardi JR, Svensson CI, Rogers SD, et al. Constitutive spinal cyclooxygenase-2 participates in the initiation of tissue injury-induced hyperalgesia. *J Neurosci* 2004;24:2727–2732.

28. Lashbrook JM, Ossipov MH, Hunter JC, et al. Synergistic antiallodynic effects of spinal morphine with ketorolac and selective COX-1- and COX-2-inhibitors in nerve-injured rats. *Pain* 1999;82:65–72.

29. Mabuchi T, Kojima H, Abe T, et al. Membrane-associated prostaglandin E synthase-1 is required for neuropathic pain. *Neuroreport* 2004;15:1395–1398.

30. Kamei D, Yamakawa K, Takegoshi Y, et al. Reduced pain hypersensitivity and inflammation in mice lacking microsomal prostaglandin synthase-1. *J Biol Chem* 2004;279:33684–33695.

31. Morita I, Schindler M, Regier MK, et al. Different intracellular locations for prostaglandin endoperoxide H synthase-1 and -2. *J Biol Chem* 1995;270:10902–10908.

32. Ueno N, Murakami M, Tanioka T, et al. Coupling between cyclooxygenase, terminal prostanoid synthase, and phospholipase A2. *J Biol Chem* 2001;276:34918–34927.

33. Serhan CN, Levy B. Success of prostaglandin E₂ in structure-function is a challenge for structure-based therapeutics. *Proc Natl Acad Sci U S A* 2003;100:8609–8611.

34. Trebino CE, Stock JL, Gibbons CP, et al. Impaired inflammatory and pain responses in mice lacking an inducible prostaglandin E synthase. *Proc Natl Acad Sci U S A* 2003;100:9044–9049.

35. Murakami M, Kudo I. Recent advances in molecular biology and physiology of the prostaglandin E2-biosynthetic pathway. *Prog Lipid Res* 2004;43:3–35.

36. Naraba H, Yokoyama C, Tago N, et al. Transcriptional regulation of the membrane-associated prostaglandin E2 synthase gene: essential role of the transcription factor Egr-1. *J Biol Chem* 2002;277:28601–28608.

37. Samuelsson B, Dahlen SE, Lindgren JA, et al. Leukotrienes and lipoxins: structures, biosynthesis, and biological effects. *Science* 1987;237:1171–1176.

38. Williams TJ, Peck MJ. Role of prostaglandin-mediated vasodilation in inflammation. *Nature* 1977;270:530–532.

39. Raud J, Dahlen SE, Sydbom A, et al. Enhancement of acute allergic inflammation by indomethacin is reversed by prostaglandin E2: apparent correlation with in vivo modulation of mediator release. *Proc Natl Acad Sci U S A* 1988;85:2315–2319.

40. Dinarello CA, Bernheim HA, Duff GW, et al. Mechanisms of fever induced by recombinant human interferon. *J Clin Invest* 1984;74:906–913.

41. Narumiya S, FitzGerald GA. Genetic and pharmacological analysis of prostanoid receptor function. *J Clin Invest* 2001;108:25–30.

42. Vanegas H, Schaible HG. Prostaglandins and cyclooxygenases in the spinal cord. *Prog Neurobiol* 2001;64:327–363.

43. Ahmadi S, Lippross S, Neuhuber WL, et al. PGE(2) selectively blocks inhibitory glycinergic neurotransmission onto rat superficial dorsal horn neurons. *Nat Neurosci* 2002;5:34–40.

44. Funk CD. Prostaglandins and leukotrienes: advances in eicosanoid biology. *Science* 2001;294:1871–1875.

45. Burgess JK, Ge Q, Boustany S, et al. Increased

sensitivity of asthmatic airway and smooth muscle cells to prostaglandin E2 might be mediated by increased numbers of E-prostanoid receptors. *J Allergy Clin Immunol* 2004;113:876–881.

46. Minami T, Nakano H, Kobayashi T, et al. Characterization of EP receptor subtypes responsible for prostaglandin E₂-induced pain responses by use of EP1 and EP3 receptor knockout mice. *Br J Pharmacol* 2001;133:438–444.

47. Murata T, Ushikubi F, Matsuoka T, et al. Altered pain perception and inflammatory response in mice lacking prostacyclin receptor. *Nature* 1997;388:678–682.

48. Ueno A, Matsumoto, Naraba H, et al. Major roles of prostanoid receptors IP and EP(3) in endotoxin-induced enhancement of pain perception. *Biochem Pharmacol* 2001;62:157–160.

49. Weinreich D, Koschorke GM, Udem BJ, et al. Prevention of the excitatory actions of bradykinin by inhibition of PGI₂ formation in the nodose neurons of the guinea-pig. *J Physiol* 1995;483:735–746.

50. Harvey RJ, Depner UB, Wassle H, et al. GlyR $\alpha 3$: an essential target for spinal PGE₂-mediated inflammatory pain sensitization. *Science* 2004;304:884–887.

51. Marx J. Locating a new step in pain's pathway. *Science* 2004;304:811.

52. Kuhn H, Walther M, Kuban RJ. Mammalian arachidonate 15-lipoxygenases structure, function, and biological implications. *Prostaglandins Other Lipid Mediat* 2002;68–69:263–290.

53. Serhan CN. Clues for new therapeutics in osteoporosis. *N Engl J Med* 2004;350:1902–1903.

54. Klein RF, Allard J, Avnur Z, et al. Regulation of bone mass in mice by the lipoxigenase gene Alox15. *Science* 2004;303:229–232.

55. Shin J, Cho H, Hwang SW, et al. Bradykinin-12-lipoxygenase-VR1 signaling pathway for inflammatory hyperalgesia. *Proc Natl Acad Sci U S A* 2002;99:10150–10155.

56. Aley O, Levine JD. Contribution of 5- and 12-lipoxygenase products to mechanical hyperalgesia induced by prostaglandin E(2) and epinephrine in the rat. *Exp Brain Res* 2003;148:482–487.

57. Takano T, Clish CB, Gronert K, et al. Neutrophil-mediated changes in vascular permeability are inhibited by topical application of aspirin-triggered 15-epi-lipoxin A₄ and novel lipoxin B₄ stable analogues. *J Clin Invest* 1998;101:819–826.

58. Hwang SW, Cho H, Kwak J, et al. Direct activation of capsaicin receptors by products of lipoxygenases: endogenous capsaicin-like substances. *Proc Natl Acad Sci U S A* 2000;97:6155–6160.

59. Tager AM, Bromley SK, Medoff BD, et al. Leukotriene B4 receptor BLT1 mediated early effector T cell recruitment. *Nat Immunol* 2003;4:982–990.

60. Luster AD, Tager AM. T-cell trafficking in asthma: lipid mediators grease the way. *Nat Rev Immunol* 2004;4:711–724.

61. Sabirsh A, Bristulf J, Owman C. Exploring the pharmacology of the leukotriene B4 receptor BLT1, without the confounding effects of BLT2. *Eur J Pharmacol* 2004;499:53–65.

62. Cunha JM, Sachs D, Canetti CA, et al. The critical role of leukotriene B4 in antigen-induced mechanical hyperalgesia in immunised rats. *Br J Pharmacol* 2003;139:1135–1145.

63. Trang T, McNaull B, Quirion R, et al. Involvement of spinal lipoxygenase metabolism hyperalgesia and opioid tolerance. *Eur J Pharmacol* 2004;491:21–30.

64. Singh VP, Patil CS, Kulkarni SK. Effect of zileuton in radicular pain induced by herniated nucleus pulposus in rats. *Inflammopharmacology* 2004;12:189–195.

65. Ye YN, Wu WK, Shin VY, et al. Dual inhibition of 5-LOX and COX-2 suppresses colon cancer formation promoted by cigarette smoke. *Carcinogenesis* 2005;26:827–834.
66. Pommery N, Taverne T, Telliez A, et al. New COX-2/5-LOX inhibitors: apoptosis-inducing agents potentially useful in prostate cancer chemotherapy. *J Med Chem* 2004;47:6195–6206.
67. Tordjman C, Sauveur F, Droual M, et al. Synthesis of the butanamide derivative S 19812, a new dual inhibitor of cyclooxygenase and lipoxygenase pathways. *Arzneimittelforschung* 2003;53:774–779.
68. Rotondo S, Krauze-Brzosko K, Manarini S, et al. Licofelone, an inhibitor of cyclooxygenase and 5-lipoxygenase, specifically inhibits cyclooxygenase-1-dependent platelet activation. *Eur J Pharmacol* 2004;488:79–83.
69. Brune K. Safety of anti-inflammatory treatment—new ways of thinking. *Rheumatology (Oxford)* 2004;43(suppl 1):16–20.
70. Morrow JD, Awad JA, Boss HJ, et al. Non-cyclooxygenase-derived prostanoids (F2-isoprostanes) are formed in situ on phospholipids. *Proc Natl Acad Sci U S A* 1992;89:10721–10725.
71. Morrow JD, Minton TA, Mukundan CR, et al. Free radical-induced generation of isoprostanes in vivo: evidence for the formation of D-ring and E-ring isoprostanes. *J Biol Chem* 1994;269:4317–4326.
72. Evans AR, Junger H, Southall MD, et al. Isoprostanes, novel eicosanoids that produce nociception and sensitize rat sensory neurons. *J Pharmacol Exp Ther* 2000;293:912–920.
73. Kim HK, Park SK, Zhou JL, et al. Reactive oxygen species (ROS) play an important role in a rat model of neuropathic pain. *Pain* 2004;111:116–124.
74. Chung JM. The role of reactive oxygen species (ROS) in persistent pain. *Mol Interv* 2004;4:248–250.
75. Wang ZQ, Porreca F, Cuzzocrea S, et al. A newly identified role for superoxide in inflammatory pain. *J Pharmacol Exp Ther* 2004;309:869–878.
76. Twining CM, Sloane EM, Milligan ED, et al. Periscaptic proinflammatory cytokines, reactive oxygen species, and complement induce mirror-image neuropathic pain in rats. *Pain* 2004;110:299–309.
77. Muscoli C, Mollace V, Wheatley J, et al. Superoxide-mediated nitration of spinal manganese superoxide dismutase: a novel pathway in N-methyl-D-aspartate-mediated hyperalgesia. *Pain* 2004;111:96–103.
78. Robbins IM, Morrow JD, Christman BW. Oxidant stress but not thromboxane decreases with epoprostenol therapy. *Free Radic Biol Med* 2005;38:568–574.
79. Milne GL, Musiek ES, Morrow JD. The cyclopentamine (A2/J2) isoprostanes—unique, highly reactive products of arachidonate peroxidation. *Antioxid Redox Signal* 2005;7:210–220.
80. Capdevila JH, Falck JR, Estabrook RW. Cytochrome P450 and the arachidonate cascade. *FASEB J* 1992;6:732–736.
81. Kozak W, Fraifeld V. Non-prostaglandin eicosanoids in fever and anapnyxia. *Front Biosci* 2004;9:3339–3355.
82. VanRollins M, Kaduce TL, Fang X, et al. Arachidonic acid diols produced by cytochrome P-450 monooxygenases are incorporated into phospholipids of vascular endothelial cells. *J Biol Chem* 1996;271:14001–14009.
83. Harder DR, Campbell WB, Roman RJ. Role of cytochrome P-450 enzymes and metabolites of arachidonic acid in the control of vascular tone. *J Vasc Res* 1995;32:79–92.
84. Campbell WB, Harder DR. Endothelium-derived hyperpolarizing factors and vascular cytochrome P450 metabolites of arachidonic acid in the regulation of tone. *Circ Res* 1999;84:484–488.
85. Roman RJ. P-450 metabolites of arachidonic acid in the control of cardiovascular function. *Physiol Rev* 2002;82:131–185.
86. Wang H, Lin L, Jiang J, et al. Up-regulation of endothelial nitric-oxide synthase by endothelium-derived hyperpolarizing factor involves mitogen-activated protein kinase and protein kinase C signaling pathways. *J Pharmacol Exp Ther* 2003;307:753–764.
87. Node K, Huo Y, Ruan X, et al. Anti-inflammatory properties of cytochrome P450 epoxygenase-derived eicosanoids. *Science* 1999;285:1276–1279.
88. Pratt PF, Rosolowsky M, Campbell WB. Effects of epoxyeicosatrienoic acids on polymorphonuclear leukocyte function. *Life Sci* 2002;70:2521–2533.
89. Campbell WB. New role for epoxyeicosatrienoic acids as anti-inflammatory mediators. *Trends Pharmacol Sci* 2000;21:125–127.
90. Fang X, Moore SA, Stoll LL, et al. 14,15-Epoxyeicosatrienoic acids inhibits prostaglandin E2 production in vascular smooth muscle cells. *Am J Physiol* 1998;275:H2113–H2121.
91. Graier WF, Simecek S, Sturek M. Cytochrome P450 mono-oxygenase-regulated signaling of C₂+ entry in human and bovine endothelial cells. *J Physiol* 1995;482:259–274.
92. Mombouli JV, Holzmann S, Kostner GM, et al. Potentiation of C₂+ signaling in endothelial cells by 11,12-epoxyeicosatrienoic acid. *J Cardiovasc Pharmacol* 1999;33:779–784.
93. Fisslthaler B, Popp R, Kiss L, et al. Cytochrome P450 2C is an EDHF synthase in coronary arteries. *Nature* 1999;401:493–497.
94. Zeldin DC. Epoxygenase pathways of arachidonic acid metabolism. *J Biol Chem* 2001;276:36059–36062.
95. Node K, Ruan XL, Dai J, et al. Activation of G alpha s mediates induction of tissue-type plasminogen activator gene transcription by epoxyeicosatrienoic acids. *J Biol Chem* 2001;276:15983–15989.
96. Spector AA, Fang X, Snyder GD, et al. Epoxyeicosatrienoic acids (EETs): metabolism and biochemical function. *Prog Lipid Res* 2004;43:55–90.
97. Starowicz K, Bilecki W, Sieja A, et al. Melanocortin 4 receptor is expressed in the dorsal root ganglions and down-regulated in neuropathic rats. *Neurosci Lett* 2004;358:79–82.
98. Starowicz K, Przewlocka B. The role of melanocortins and their receptors in inflammatory processes, nerve regeneration and nociception. *Life Sci* 2003;73:823–847.
99. Beltramo M, Campanella M, Tarozzo G, et al. Gene expression profiling of melanocortin system in neuropathic rats supports a role in nociception. *Brain Res Mol Brain Res* 2003;118:111–118.
100. Vrinten DH, Adan RA, Groen GJ, et al. Chronic blockade of melanocortin receptors alleviates allodynia in rats with neuropathic pain. *Anesth Analg* 2001;93:1572–1577.
101. Vrinten DH, Kalkman CJ, Adan RA, et al. Neuropathic pain: a possible role for the melanocortin system? *Eur J Pharmacol* 2001;429:61–69.
102. Grazzini E, Puma C, Roy MO. Sensory neuron-specific receptor activation elicits central and peripheral nociceptive effects in rats. *Proc Natl Acad Sci U S A* 2004;101:7175–7180.
103. Bertorelli R, Fredduzzi S, Tarozzo G, et al. Endogenous and exogenous melanocortin antagonists induce anti-allodynic effects in a model of rat neuropathic pain. *Behav Brain Res* 2005;157:55–62.
104. Lee HC, Lu T, Weintraub NL, et al. Effects of epoxyeicosatrienoic acids on the cardiac sodium channels in isolated rat ventricular myocytes. *J Physiol* 1999;519:153–168.
105. Serhan CN, Chiang N. Novel endogenous small molecules as the checkpoint controllers in inflammation and resolution: entree for resolomics. *Rheum Dis Clin North Am* 2004;30:69–95.
106. Sher JU, Pillinger MH. 15d-PGJ₂: the anti-inflammatory prostaglandin? *Clin Immunol* 2005;114:100–109.
107. Serhan CN. A search for endogenous mechanisms of anti-inflammation uncovers novel chemical mediators: missing links to resolution. *Histochem Cell Biol* 2004;122:305–321.
108. Serhan CN. Novel eicosanoid and docosanoid mediators: resolvins, docosatrienes and neuroprotectins. *Curr Opin Clin Nutr Metab Care* 2005;8:115–121.
109. Serhan CN. Novel omega-3-derived local mediators in anti-inflammation and resolution. *Pharmacol Ther* 2005;105:7–21.
110. Gilroy DW, Colville-Nash PR, McMaster S, et al. Inducible cyclooxygenase-derived 15-deoxy (Delta) 12-14 PGJ2 brings about acute inflammation resolution in rat pleurisy by inducing neutrophil and macrophage apoptosis. *FASEB J* 2003;17:2269–2271.
111. Shureigi I, Lippman SM. Lipoxygenase modulation to reverse carcinogenesis. *Cancer Res* 2001;61:6307–6312.
112. Tong WG, Ding XZ, Witt RC, et al. Lipoxygenase inhibitors attenuate growth of human pancreatic cancer xenografts and induce apoptosis through the mitochondrial pathway. *Mol Cancer Ther* 2002;1:929–935.
113. Tong WG, Ding XZ, Hennig R, et al. Leukotriene B4 receptor antagonist LY293111 inhibits proliferation and induces apoptosis in human pancreatic cancer cells. *Clin Cancer Res* 2002;8:3232–3242.
114. Tsujii M, Kawano S, DuBois RN. Cyclooxygenase-2 expression in human colon cancer cells increases metastatic potential. *Proc Natl Acad Sci U S A* 1997;94:3336–3340.
115. Hoque A, Lippman SM, Wu TT, et al. Increased 5-lipoxygenase expression and induction of apoptosis by its inhibitors in esophageal cancer: a potential target for prevention. *Carcinogenesis* 2005;26:785–791.
116. Shureiqi I, Jiang W, Zuo X, et al. The 15-lipoxygenase-1 product 13-S-hydroxyoctadecadienoic acid down-regulates PPAR-delta to induce apoptosis in colorectal cancer cells. *Proc Natl Acad Sci U S A* 2003;100:9968–9973.
117. Chen X, Wang S, Wu N, et al. Leukotriene A4 hydrolase as a target for cancer prevention and therapy. *Curr Cancer Drug Targets* 2004;4:267–283.
118. Bannenberg G, Moussignac RL, Gronert K, et al. Lipoxins and novel 15-epi-lipoxin analogs display potent anti-inflammatory actions after oral administration. *Br J Pharmacol* 2004;143:43–52.
119. Jones NA, Boswell-Smith V, Lever R, et al. The effect of selective phosphodiesterase isoenzyme inhibition on neutrophil function in vitro. *Pulm Pharmacol Ther* 2005;18:93–101.
120. Banner KH, Trethick MA. PDE4 inhibition: a novel approach for the treatment of inflammatory bowel disease. *Trends Pharmacol Sci* 2004;25:430–436.
121. Lipworth BJ. Phosphodiesterase-4 inhibitors for asthma and chronic obstructive pulmonary disease. *Lancet* 2005;365:167–175.
122. Trifilieff A, Keller TH, Press NJ, et al. CGH2466, a combined adenosine receptor antagonist, p38 mitogen-activated protein kinase and phosphodiesterase type 4 inhibitor with potent in vitro and in vivo anti-inflammatory activities. *Br J Pharmacol* 2005;144:1002–1010.