

Prostaglandin biosynthesis and functions

Introduction

Prostaglandins and related molecules are called eicosanoids as a class. The term eicosanoid is derived from “eicosa” meaning “twenty”, referring to the 20 carbons in most of the molecules. The eicosanoids are used as signaling molecules. They generally act locally, either affecting cell that makes them or nearby cells; in most cases, eicosanoids are not systemic hormones, because of their short half-lives.

Most prostaglandins are synthesized from arachidonic acid (20:4 $\Delta^{5,8,11,14}$). These are called “Series 2” products, because most have two double bonds. However, the triene fatty acid 20:3 $\Delta^{8,11,14}$ can also be used; the products have one fewer double bond than the arachidonic acid derivatives and are called Series 1 products.

Both of these potential precursor molecules are ω^6 fatty acids. In the absence of ω^6 fatty acids, the organism may attempt to produce eicosanoids from ω^9 fatty acids. These ω^9 -derivative compounds, regardless of the number of double bonds, are inactive.

In contrast, 20:5 $\Delta^{5,8,11,14,17}$, a fatty acid produced from diets high in seafood fatty acids (such as the typical Eskimo diet) is also a substrate for prostaglandin synthesis; the products from this compound have one more double bond than the series two products. The properties of the different series are somewhat different. Eskimos have a low incidence of heart disease in spite of an extremely high fat diet; one likely contributing factor is the higher degree of unsaturation in the fatty acid prostaglandin precursors and in the prostaglandins.

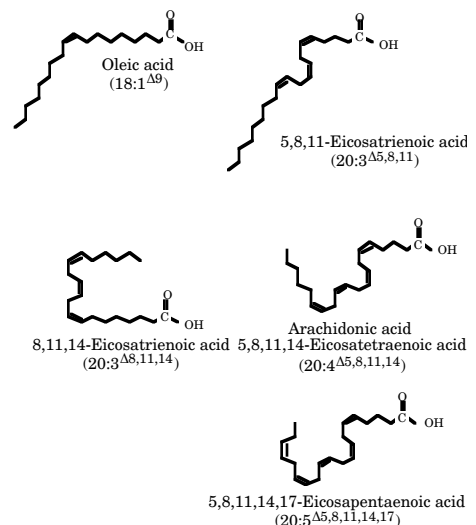
Reminder of ω nomenclature

Polyunsaturated fatty acids all have double bonds three carbons apart. This allows the first or the last carbon present as a double bond to be used in identifying the compound. It is possible therefore to count from the methyl-group end of the fatty acid; the Greek letter ω (the last letter in the Greek alphabet) is used to refer to the position of the double bond counting from the terminal methyl group.

Humans can synthesize ω^9 fatty acids such as oleic acid and its 20:3 $\Delta^{5,8,11}$ derivative. However, this is ordinarily a minor pathway, and the 20:3 $\Delta^{5,8,11}$ cannot be used to make functional prostaglandins.

Two ω^6 fatty acids, 20:3 $\Delta^{8,11,14}$, and arachidonic acid (20:4 $\Delta^{5,8,11,14}$) are substrates for most prostaglandin biosynthesis (producing the series one and series two products, respectively).

In addition, the 20:5 $\Delta^{5,8,11,14,17}$ fatty acid mentioned above, an ω^3 fatty acid, can also be used for prostaglandin biosynthesis.

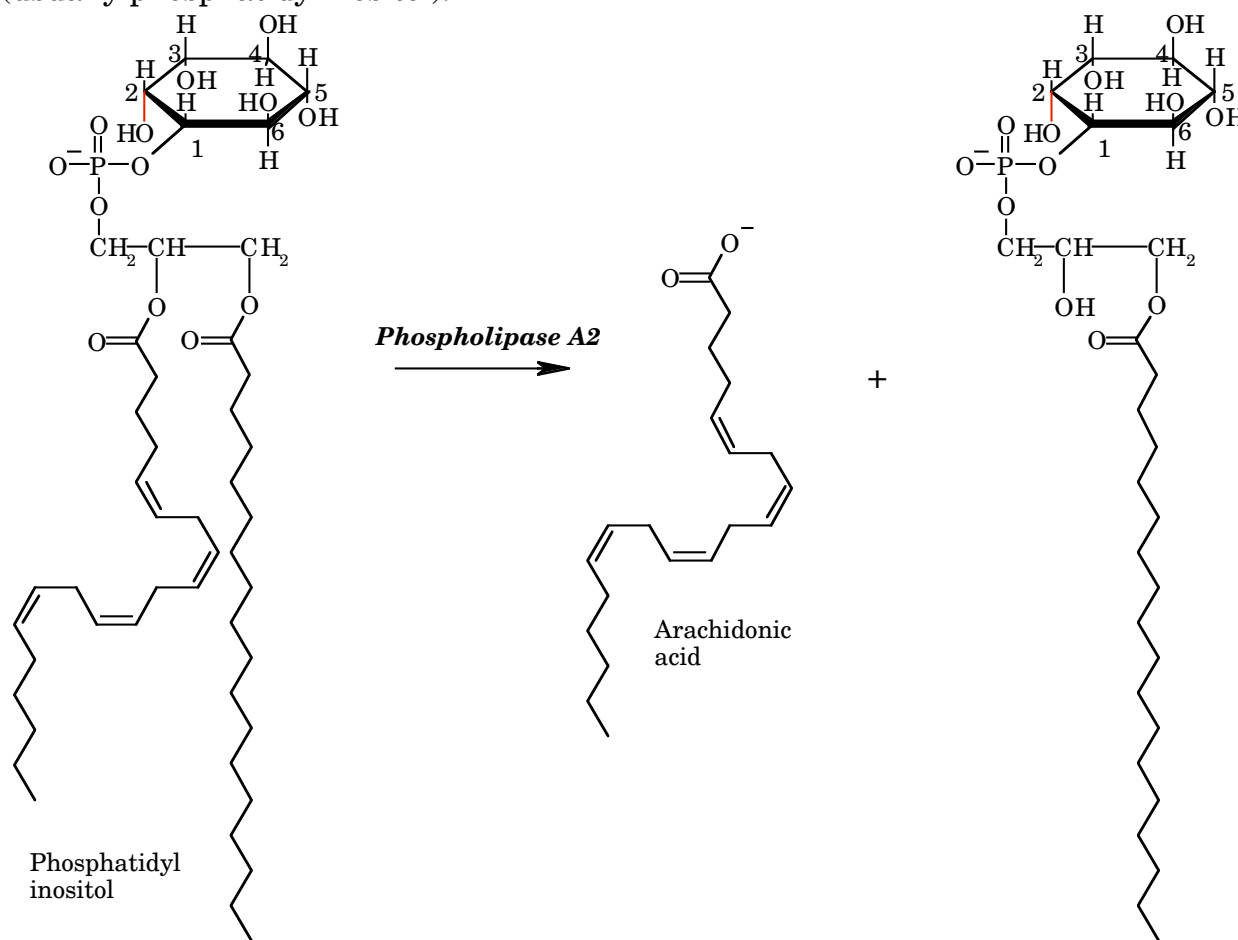


Synthesis

Prostaglandin biosynthesis has two control points.

Phospholipase A₂

The starting material for prostaglandin biosynthesis is a fatty acid. The fatty acid used is nearly always derived from the 2-position of a membrane phospholipid (usually phosphatidylinositol).

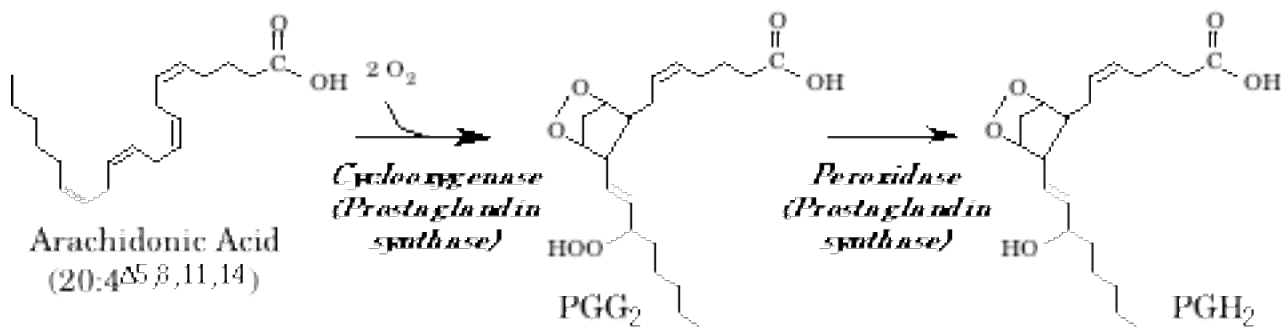


Release of the fatty acid from the phospholipid is the first control point in the prostaglandin biosynthetic pathway. One function of glucocorticoids is inhibition of phospholipase A₂ and therefore of eicosanoid synthesis.

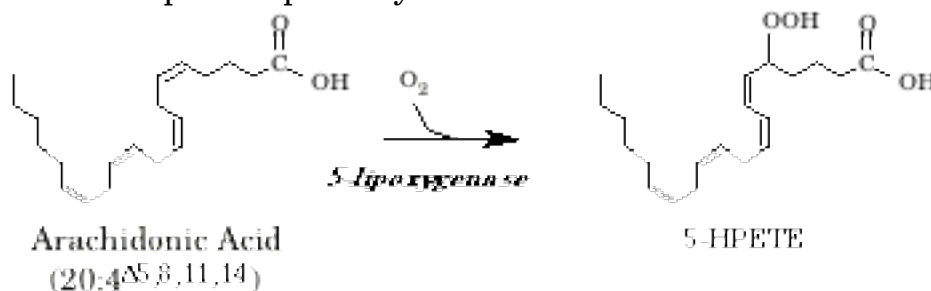
COX and lipoxygenase

The second control point is the enzyme responsible for converting the fatty acid to the first molecule in the relevant pathway. Two enzymes are primarily involved in eicosanoid biosynthesis. **Prostaglandin synthase** and **5-lipoxygenase**. Prostaglandin synthase is a complex enzyme that catalyzes the first two steps in the prostaglandin synthesis pathway. It is often called **cyclooxygenase** (referring to the first of the two reactions it mediates); cyclooxygenase is abbreviated COX.

The two reactions catalyzed by COX are shown below:



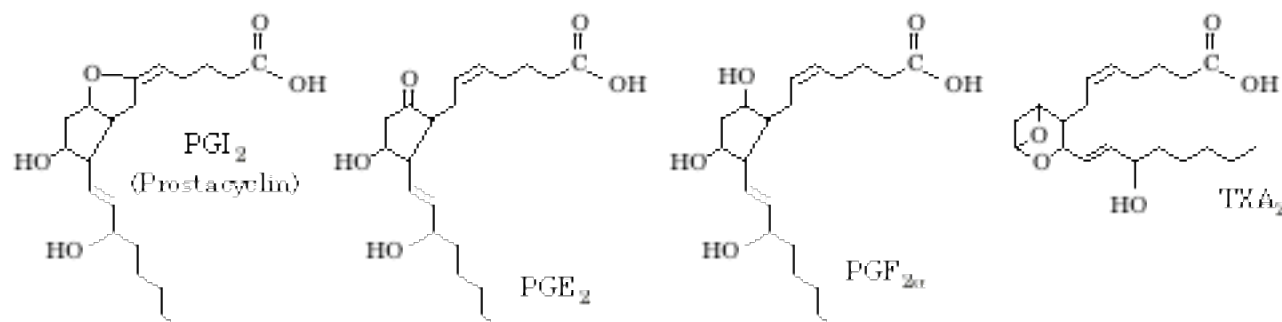
5-Lipoxygenase is one type of lipoxygenase; 5-Lipoxygenase catalyzes the first step in one of the more important pathways.



Physiological Eicosanoids

Prostaglandins and Thromboxanes

The product of the COX reactions can then be converted to the physiologically active compounds. A number of biologically active compounds are known to exist. Some of the more important ones are shown below.



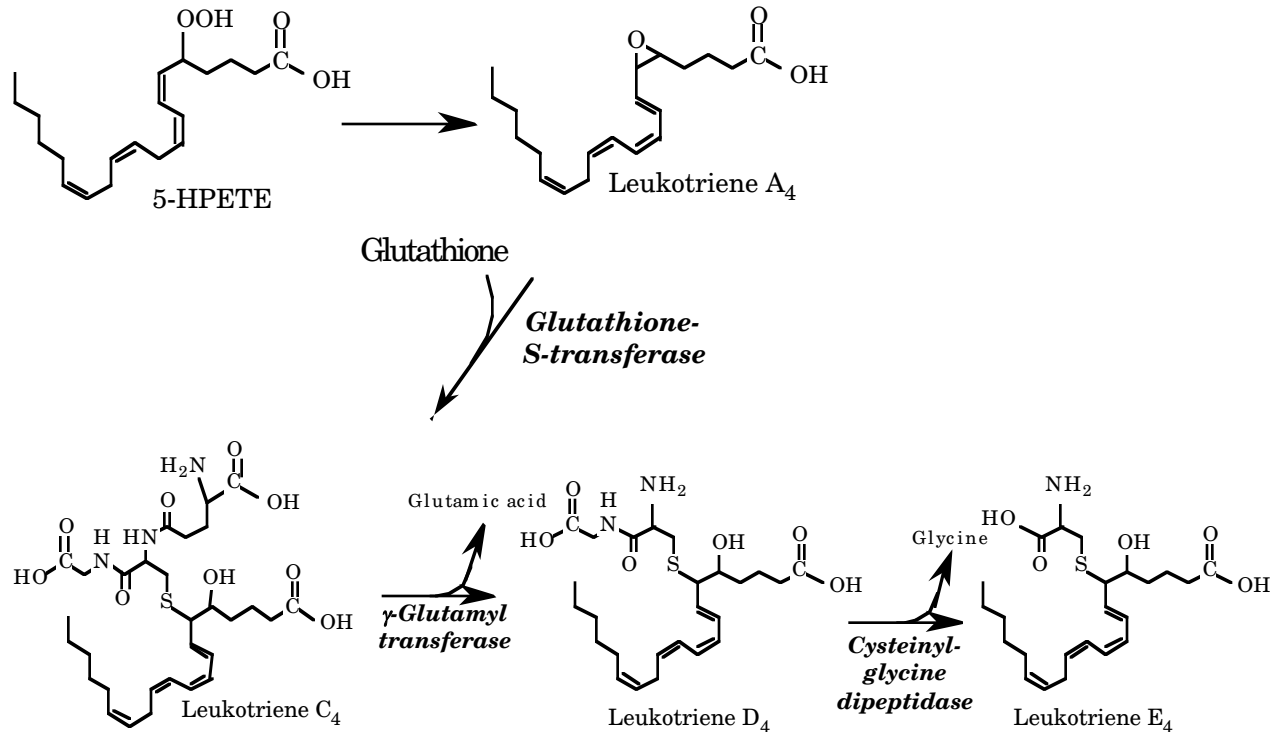
In the abbreviations, “PG” = “prostaglandin” and “TX” = “thromboxane”. The letters (*e.g.*, the “I” in “PGI₂”) indicate the structure and substituents of the ring, while the number refers to the number of double bonds present. The structures shown above are series 2 compounds, with two double bonds; series one compounds such as PGE₁ lack the double bond closest to the carboxylate.

Leukotrienes

The product of the 5-lipoxygenase reaction, HPETE (= Hydroperoxyeicosatetetraenic

acid) is usually converted to leukotrienes. (Note: the word leukotriene implies three double bonds; however, leukotriene derivatives of arachidonic acid have four double bonds.)

Leukotrienes C₄, D₄, and E₄ are usually present as a mixture of the three compounds. This mixture is known as the Slow Reacting Substance of Anaphylaxis, and is a powerful inflammatory agent that is responsible for some forms of allergic reactions.



Mechanism of action

Physiological functions of prostaglandins

Prostaglandins are rapidly degraded, and have such short half-lives that their functions are usually considered to be limited to actions on nearby cells. Prostaglandins seem to act via two separate mechanisms. **Secreted prostaglandins** bind to specific cell surface **G-protein coupled receptors**, and generally increase cAMP levels. Prostaglandins may also **bind to nuclear receptors** and alter gene transcription.

Prostaglandin action is incompletely understood.

Known actions include:

Induction of inflammation

Mediation of pain signals

Induction of fever

Smooth muscle contraction (including uterus) – (especially PGF_{2α})

Smooth muscle relaxation -- especially PGE series

Protection of stomach lining

Simulation of platelet aggregation (thromboxanes)

Inhibition of platelet aggregation (prostacyclin)

COX-1, COX-2, and COX-3

Humans, and most other mammals have two genes for cyclooxygenase.

The products of the genes, COX-1 and COX-2, are structurally quite similar, with only subtle differences. They catalyze the same reactions, although COX-2 works with a wider range of substrates. COX-1 is constitutively expressed in nearly all tissues. In contrast, COX-2 is inducible, especially by inflammatory stimuli.

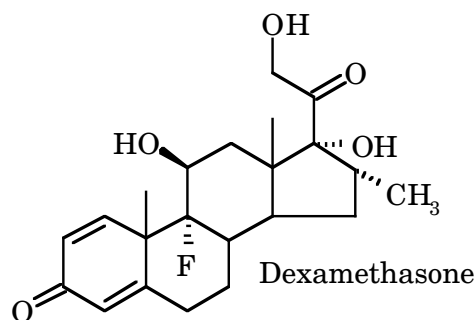
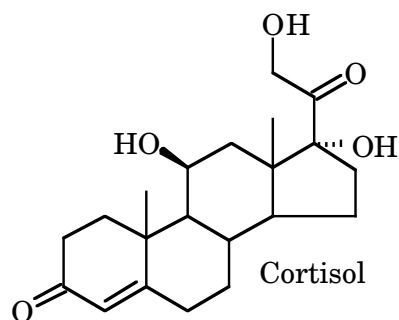
Some evidence suggests that COX-1 is responsible for generating the prostaglandins required for protection of the gastrointestinal tract, while COX-2 is responsible for the increased prostaglandin synthesis associated with inflammation, fever, and pain responses. This has led to attempts to find specific inhibitors of COX-2. On the other hand, some evidence suggests that the roles of the two isozymes may not be quite that clearly defined.

A new isozyme, COX-3 was discovered in 2002; it is thought to be an intron-splice variant of COX-1. It has a similar sequence, but not identical amino acid sequence to that of COX-1, but has some functional differences. The role of COX-3 is the subject of considerable interest, but much remains to be learned about the role of all of the isozymes.

Inflammation

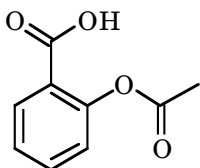
The inflammatory response involves the migration of immune system cells into a damaged tissue. In some cases, this is beneficial (especially for fighting infection); in many cases, however, the inflammatory response actually increases the damage to the tissue. This is true for asthma, several forms of arthritis, and for muscle and connective tissue damage associated with sprains and similar injuries; in addition, there is evidence that inflammation may be a step on the pathway toward certain cancers (especially colon cancer).

Inflammation can be treated with two major classes of antiinflammatory drugs: steroids, and non-steroids. The steroids are compounds with glucocorticoid activity, and include the physiological glucocorticoid, cortisol, and synthetic glucocorticoid analogs such as dexamethasone.

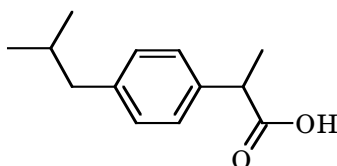


Glucocorticoids inhibit inflammatory responses by several mechanisms, and are more powerful drugs than NSAIDs. One mechanism is phospholipase A₂ inhibition; this **inhibits both prostaglandin and leukotriene** synthesis, and therefore has a stronger effect than COX inhibition alone. In addition, glucocorticoids have other effects, unrelated to eicosanoid pathways.

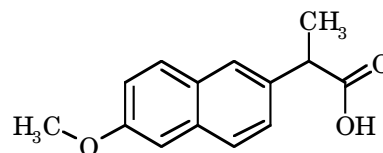
The non-steroidal compounds are called NSAIDs (Non-Steroidal Anti-Inflammatory Drugs). The NSAIDs are COX inhibitors; some of the most widely used drugs, including aspirin, ibuprofen, and naproxen fall into this class.



Aspirin
[2-acetoxybenzoic acid]
[salicylic acid acetate]



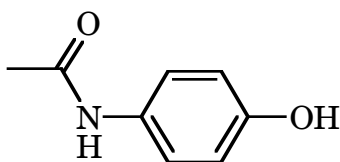
Ibuprofen
[α-methyl-4-(2-methylpropyl)benzene-acetic acid]
[2-(4-isobutylphenyl)propionic acid]



Naproxen
[2-(6-methoxynaphthyl)propionic acid]

Most currently available NSAID compounds, such as aspirin, ibuprofen, and naproxen are inhibitors of both COX isozymes. Aspirin covalently modifies the enzymes; this abolishes cyclooxygenase activity (although it leaves peroxidase activity intact). In contrast, ibuprofen and naproxen are reversible inhibitors of COX.

Acetaminophen is often classed with the NSAIDs. Although the structure of acetaminophen is similar to the NSAIDs mentioned above, and although acetaminophen inhibits some prostaglandin-mediated responses, probably via specific inhibition of COX-3, it does **not** inhibit COX-1 or COX-2, and does not have anti-inflammatory actions. It is therefore not an NSAID. The actual mechanism of acetaminophen action remains controversial.



Acetaminophen
[N-(4-hydroxyphenyl)acetamide]
[p-hydroxyacetanilide]
[p-acetaminophenol]

COX inhibition and the stomach

Indomethacin, a high affinity inhibitor of COX (and in some individuals, aspirin, and to a lesser extent ibuprofen) induces ulceration; some anti-ulcer drugs appear to function by increasing prostaglandin synthesis.

COX inhibition and the kidney

Normal kidneys do not appear to require prostaglandins. However, kidneys in individuals with chronic liver, heart, or kidney disease do require prostaglandin biosynthesis in the kidney. In these individuals, COX inhibitors can severely damage the kidney.

Prostaglandins and pregnancy

Prostaglandins are required for normal implantation of the fertilized oocyte. In addition, prostaglandins are involved in initiation of labor. Prostaglandins are used for labor induction (and for RU-486 induced abortions); COX-inhibitors (probably via COX-2) delay onset of labor. COX-2 seems to be required for ovulation.

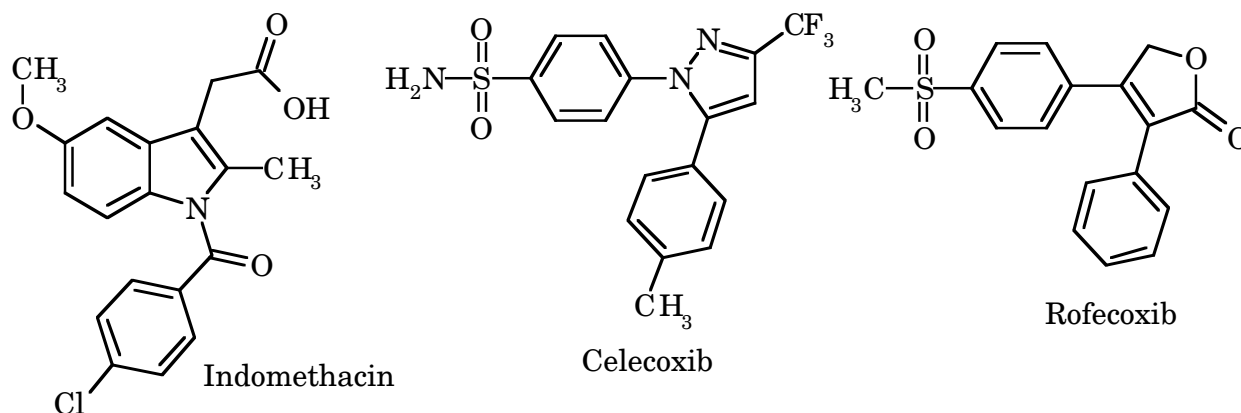
Prostaglandins and fever and pain

Prostaglandins appear to form a major part of the signaling pathway in fever induction. COX inhibitors are thought to exert their anti-pyretic actions by interrupting this pathway. Prostaglandins appear to be involved in some pain pathways; inhibition of COX (probably COX-2) is thus analgesic.

COX-2 inhibitors

The current hypotheses regarding prostaglandin action suggest that inhibitors specific for COX-2 should have many useful effects, including anti-inflammatory actions, analgesic effects, and anti-pyretic effects, without altering platelet function or damaging the gastrointestinal tract. The first generation compounds were discovered by searching for effective compounds with minimal stomach irritation; new compounds are in trials based on direct assays on COX-1 and COX-2, and on analyses of the crystal structures of the two isozymes.

Aspirin and indomethacin both have higher affinity for COX-1 and COX-3 than COX-2 (although both compounds bind to all three enzymes). Indomethacin is about 100-fold more potent than aspirin, and is rarely used as a drug as a result of its toxic effects.



COX-2 specific inhibitors such as celecoxib and rofecoxib have not been nearly as heavily tested as aspirin (aspirin is consumed at the rate of several thousand tons each year!); some unknown side effects of the COX-2 inhibitors may therefore exist. For example, some evidence indicates that COX-2 mediated prostaglandin synthesis is important in wound healing; in addition, little testing has been done on the

effects of these compounds on fertility or on fetal development. Studies using mice with COX-2 gene deletions suggest that COX-2 products are important for ovulation and for early development. Early studies with COX-2 inhibitors have suggested a greatly reduced incidence of stomach damage. However, aspirin induces stomach damage only in a small subset of individuals; it is therefore possible that the studies on the COX-2 inhibitors have not been large enough to detect the potentially significant side-effects.

Aspirin and heart disease

Platelet aggregation is regulated by eicosanoids (among a number of other stimuli). Thromboxane A₂ is produced in platelets and stimulates aggregation. Prostacyclin (PGI₂) is synthesized in the vascular endothelium, and inhibits aggregation. Aspirin irreversibly inhibits cyclooxygenase in both platelets and endothelial cells; however the endothelial cells can synthesize new enzyme, while the platelets, which lack protein biosynthetic machinery, cannot. Platelets normally circulate for 8-10 days; aspirin therefore has a significant antithrombosis effect. Clinical studies have found strong evidence suggesting that ~75 mg/day of aspirin (a small fraction of the normal 325 mg aspirin tablet) reduces risk of heart disease and stroke by reducing blood clot formation.

Note: aspirin increases clotting time, but is not a true anti-coagulant. COX-1 knockout mice exhibit changes in their platelets associated with aspirin administration, but do not exhibit symptoms of severe anti-coagulation that are observed with warfarin administration; warfarin (an indirect inhibitor of synthesis of some clotting factors via interference with the Vitamin K cycle) induces life-threatening internal and external hemorrhages.

COX inhibition and cancer

Colon cancer is a major life-threatening cancer. Aspirin has been shown to have an apparent protective effect against colon cancer; some evidence suggests that inhibition of colon tumor induction is due to inhibition of COX-2. Breast and stomach cancer growth may also be inhibited by COX inhibitors.

COX and Alzheimer's disease

The brain damage associated with Alzheimer's disease appears to be largely mediated by inflammatory responses; some epidemiological data have suggested a reduced incidence of Alzheimer's disease in individuals taking COX inhibitors.

Summary

Eicosanoids are important signaling molecules. Eicosanoids are synthesized from twenty-carbon polyunsaturated fatty acids that most animals cannot synthesize from acetyl-CoA. The precursors for these molecules are therefore called essential fatty acids.

Synthesis of any of the eicosanoid signaling molecules is controlled by two enzymes. The first enzyme, phospholipase A₂, is required for the synthesis of all of these molecules. The second enzyme depends on the type of molecule. Cyclooxygenase is the main regulated enzyme for prostaglandin and thromboxane synthesis, while leukotriene synthesis is regulated by 5-lipoxygenase.

Eicosanoids have a wide variety of actions, including mediating some pain pathways, many types of inflammation, and fever responses.

Phospholipase A₂ is inhibited by glucocorticoids. Cyclooxygenase is inhibited by aspirin and a number of other widely used drugs.