The Arachidonic Acid Cascade

Sandra P. Welch, Ph.D.
Professor
Pharmacology & Toxicology
Smith 734, 828-8424, swelch@hsc.vcu.edu

Arachidonic Acid Metabolism: Synthesis and Action of Prostaglandins (PGs), Thromboxane (TxA2), Leukotrienes (LTs), Epoxyeicosatrienoic Acids (EETs) and Anandamide

Major Goal: Know this diagram

Learning Objectives: After studying this material, the student should:

1. Know the fatty acid precursor from which the 2-series of prostaglandins (PG2) is made.
2. Know the 4 major enzyme pathways for production of arachidonic acid metabolites.
3. Understand the actions of the enzymes phospholipase A(2), cyclooxygenase and how steroids, aspirin and aspirin substitutes affect these enzymes.
4. Know the difference between how aspirin and aspirin substitutes affect cyclooxygenase enzyme activity.
5. Understand the difference between cyclooxygenase 1 (COX-1) and cyclooxygenase 2 (COX-2).
6. Know the importance of and the point at which oxygen radicals are formed in the arachidonic acid metabolic pathway.
7. Know the major cyclooxygenase products (no structures required) and give the major known biological activity or activities of the more important cyclooxygenase products.
8. Be able to describe the major effects of prostaglandins in the CNS.
9. Understand how PGE2, PGF2α, PGI2 and TxA2 can affect vascular and non-vascular smooth muscle tone.
10. Know how prostaglandins affect gastric function.
11. Understand how cyclooxygenase inhibitors and PGI2 (prostacyclin) affect platelet function and thus affect hemostasis.
12. Be able to provide three lines of reasoning which support the importance of prostaglandins in inflammation.
13. Know the name of the enzyme or enzyme systems responsible for the synthesis of leukotrienes and HETEs and know the major biological effects of the products of these enzyme systems.

14. Be able to describe the role of lipoxygenase products in immediate hypersensitivity reactions and asthma. Also know the major lipoxygenase enzyme inhibitor and the major antagonist of the leukotriene receptor.

15. Be aware of the possible role and function of anandamide (arachidonyl ethanolamide) on cannabinoid (marijuana) receptors.

I. History

1930's Discovered by Kurzok & Lieb
Characterized and named by Goldblatt & von Euler, thought substances came from prostate gland, hence the name prostaglandin (abbreviated PG)

1960 Bergstrom - elucidated chemical structure of PGs

1971 Vane - discovered that MOA of aspirin is inhibition of PG formation
mid 1970s - 80s Samuelsson - elucidated structure of thromboxane and lipoxygenase metabolites
1982 Bergstrom, Vane & Samuelsson share Nobel Prize for work in elucidation of the "Arachidonic Acid Cascade"
1980's Epoxygenase pathway elucidated and functions studied
1992 Anandamide (arachidonyl ethanolamide) discovered

II. Function

Modulation of cell function Arachidonic acid metabolites are found in virtually all cells and tissues.

Each cell type appears to have a characteristic balance of metabolites.

Compounds are synthesized locally, on demand, and are not stored for future release.

They act locally in the area in which they are found and in general do not have distant sites of action, as do many other types of chemical modulators or hormones.

III. Substrates

Phospholipases release the PUFA precursors from phospholipids.

These PUFA can then be metabolized by cyclooxygenase, lipoxygenase enzymes or P450 "epoxygenase".

IV. Synthesis of Arachidonic Acid Metabolites

A. Four General Pathways of Arachidonic Acid Product Formation

20:3 (di-homo-g-linolenic acid), not very predominant in nature, forms PG3

20:4 (arachidonic acid, eicosatetraenoic acid) forms PG2, 2 double bonds, predominant in nature. Metabolites of eicosatetraenoic acid are referred to as 'eicosanoids'.

20:5 (eicosapentaenoic acid) forms PG3, 3 double bonds. Eicosapentaenoic acid (EPA) abundant in fish oil and increased in those who consume increased amounts of fish.

Eicosapentaenoic acid can compete with arachidonic acid for enzyme metabolism. Increased EPA reduces formation of the PG2 series.
Anandamide Metabolism: Synthesis and Action of Prostaglandins (PGs), Thromboxane (Tx), Leukotrienes (LTs), Epoxyeicosatrienoic Acids (EETs) and Anandamide

1. Cyclooxygenase – 2 isoforms, cyclooxygenase 1 (COX-1) and cyclooxygenase 2 (COX-2). COX-1 is normally present in most tissues. COX-2 is normally present in brain and kidney and is induced in most tissues during inflammation and injury.

2. Lipooxygenase
3. **P-450 monooxygenase “epoxygenase”**

4. **anandamide**

**B. Synthesis and Action of Prostaglandins, Thromboxane, Leukotrienes, Epoxyeicosatrienoic Acids and Anandamide**

**V. Inhibitors of Arachidonic acid (AA) Metabolism**
A. Cyclooxygenase Pathway

Non-steroidal anti-inflammatory drugs (NSAIDs) inhibit cyclooxygenase metabolism.

Aspirin - irreversibly acetylates cyclooxygenase 1 and 2

Most other NSAIDs - reversibly, competitively inhibit cyclooxygenase 1 and 2

Selective cyclooxygenase 2 (COX-2) inhibitors - Celecoxib (Celebrex®) - reversibly, competitively, inhibit COX-2, which is normally present in brain and kidney and is induced in other tissues during inflammation. Relatively little effect on COX-1, which is present in most tissues and important to protect GI mucosa and induce platelet aggregation.

Summary of the Action of Non-selective vs. COX-2 Selective Inhibitors

<table>
<thead>
<tr>
<th>COX-1 &amp; COX-2 inhibitors</th>
<th>COX-2 selective inhibitors, e.g. Celecoxib (Celebrex®) Rofecoxib (Vioxx®)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin (irreversible) &amp; other (reversible) nonselective NSAIDs</td>
<td>Constitutive PGs (protective)</td>
</tr>
<tr>
<td></td>
<td>Tissue (pro-inflammatory)</td>
</tr>
</tbody>
</table>

B. Lipoxygenase Pathway

Agents which have been tested and found effective at reducing the occurrence of asthmatic symptoms and the need for beta-agonists to produce bronchiolar dilation include zileuton (Zyflo®), a 5-lipoxygenase enzyme inhibitor; and zafirlukast (Accolate®) and montelukast (Singulair®), competitive leukotriene receptor blockers.
VI. Biological Actions of Metabolites of Arachidonic Acid

A. Nervous System

PGs are thought to be modulators of neuronal activity. They can increase or decrease release of neurotransmitters and cause changes in behavior.

PGs can also sensitize pain receptors. Anandamide binds to marijuana receptors and causes analgesia and decreased locomotion.

PGs given intraventricularly into the brain can induce fever.

B. Smooth Muscle

1. Vascular
   a. PG\(_4\) is the predominant PG produced by vascular tissue, mainly by endothelium.
   b. PG\(_1\) and PGE\(_2\) relax muscle.
   c. Thromboxane A\(_2\) (TxA\(_2\)) and LTC\(_4\) contract muscle, vasoconstriction.

B. Bronchial and Tracheal

LTCA\(_4\) and LTD\(_4\) are very potent contractors of airway smooth muscle.

The release of these compounds by leukocytes, resident macrophages or mast cells is important in generation of the increased airway resistance in asthma and immediate hypersensitivity reactions.

3. GI

Generally PGs increase contraction and motility. This is often a side effect following the administration of PGs in other body areas.

4. Uterine

PGs cause contraction of uterine smooth muscle.

PGs are approved for induction of abortion and also for induction of full-term labor.

Normal uterine production of PGs is thought to contribute to menstrual cramping.

The NSAID ibuprofen is effective in reducing cramping.

The MOA of this agent is likely the inhibition of formation of contractile PGs, in addition to ibuprofen’s analgesic effect.
C. Hemostasis

1. Blood platelets are prolific metabolizers of AA.

PG endoperoxides (PGG<sub>2</sub>, PGH<sub>2</sub>) and TxA<sub>2</sub>, are produced by platelets and these compounds induce platelets to adhere to one another, thus inducing platelet aggregation.

Aspirin and other NSAIDs (except selective COX-2 inhibitors) double bleeding time because they inhibit the formation of these pro-aggregatory cyclooxygenase enzyme metabolites.

2. Vasculature

PGI<sub>2</sub> is a very potent inhibitor of platelet aggregation induced by ADP, collagen or epinephrine.

Since PGI<sub>2</sub> is formed by the vascular wall PGI<sub>2</sub> is hypothesized to be an important in vivo inhibitor of platelet aggregation.

There is evidence that very low doses of oral aspirin (1/8 - 1/4 tablet) may achieve a semi-selective inhibition of platelet but not arterial cyclooxygenase.

This would maximize the anti-thrombotic effect of aspirin since PGI<sub>2</sub> inhibits platelet aggregation.

D. Kidney Function

The kidney papilla is rich in AA. PGI<sub>2</sub> and PGE<sub>2</sub>, given into the renal artery produce diuresis and increase Na<sup>+</sup> and K<sup>+</sup> excretion.

NSAIDs can alter kidney function. The mechanisms by which PGs alter renal function are not certain, but likely involve redistribution of intrarenal blood flow and a change in tubular transport.

E. GI Function

PGE<sub>2</sub> and PGI<sub>2</sub> inhibit gastric acid secretion induced by feeding, histamine or gastrin.

PGs also increase GI mucous secretion.

Inhibition of PGs by NSAIDs (except selective COX-2 inhibitors) will therefore have pro-ulcerogenic effects (increased acid, decreased mucous).

For this reason, stable PG analogs (Misoprostol<sup>®</sup>) are now used in anti-ulcer therapy.

Also, selective COX-2 inhibitors, which do not decrease GI synthesis of protective PGs by COX-1, are available.

F. Endocrine System

Exogenous PGs can stimulate the release of several hormones. However, the exact role of PGs in endocrine function has not been adequately explored. PGs are known, however, to stimulate calcium metabolism and bone resorption.

G. Inflammation

There is little doubt that arachidonic acid metabolites are important in inflammation. Evidence supporting this conclusion includes that:

1. exogenous PGs and LTs can promote inflammation.

2. PGs and LTs are found in inflammatory exudates.

3. drugs which inhibit cyclooxygenase, reduce inflammation.
Arachidonic acid metabolites contribute to inflammation by:

1. increasing capillary permeability
2. inducing local vasodilation and thus redness
3. promoting infiltration of inflammatory cells
4. production of tissue injuring oxygen free radicals during the synthesis of PGs and LTs
5. producing inflammation-associated hyperalgesia (increased pain)

H. Commercial Preparations

1. Aloprostadil (PGE₁)

Can be used in infants with congenital heart defects in order to increase pulmonary blood flow until definitive surgery can be performed.

It is also marketed as Caverject to treat penile erectile dysfunction of neurogenic, vasculogenic or psychogenic origin. It causes erection by causing arterial dilation and occlusion of venous outflow.

2. Carboprost (15-methyl PGF₂α)

Induces second trimester abortion. It is a more powerful uterine contractor than oxytocin. The methyl group is present to prevent oxidation.

3. Dinoprost (PGF₂α) tromethamine

Is used intra-amniotically to induce abortion, usually in pregnancy of longer than 15 weeks.

4. Dinoprostone (PGE₂)

Is used in suppository form to induce abortion in pregnancies of less than 28 weeks. It is also used to induce full-term labor.

5. Misoprostol (PGE₁) and numerous PG analogs (arbaprostil, ensprostil, enisoprost, deprostil, rioprostil, trimoprostil)

Inhibit gastric acid and stimulate gastric mucous secretion; this may be inversely related to the mechanism of the gastrointestinal damage that follows exposure to non-selective nonsteroidal anti-inflammatory drugs (NSAIDs).
These protective agents are used for the treatment of gastrointestinal ulceration by virtue of their cytoprotective effects on the gastric mucosa.

a. Misoprostol is rapidly absorbed and metabolized in the liver and excreted in the urine. It has a half-life of less than 30 minutes.

b. When administered chronically, misoprostol can prevent gastric ulceration caused by NSAIDs.

c. Dose-related diarrhea can occur in as many as 40% of patients taking misoprostol.

d. Misoprostol is contraindicated for ulcers during pregnancy. It causes bleeding in 40% of women and, in a lower percentage, partial or complete expulsion of the products of conception.

6. **Latanoprost** - used for treatment of glaucoma. Acts by decreasing production of intraocular fluid. Works as well as timolol, but has side effect of turning blue eyes brown! Effectiveness and side effects of long term use is less certain.

7. The French abortion pill **Mifepristone (RU-486)**, “morning after pill”) must be taken within 9 weeks of the last menstrual period. **RU-486 blocks progesterone receptors** causing the uterus to reject the implanted egg.

Two days after taking Mifepristone the woman is given a prostaglandin which causes the cervix to soften and dilate and the uterus to contract and expel the embryo. PGs are also used similarly to induce uterine expulsion of the embryo after giving the cytotoxic agent methotrexate.

**Recommended Reading:**

**Drugs to Remember:**
- Aloprostadil® Montelukast – Singulair®
- Misoprostol® Celecoxib – Celebrex®
- Zileuton – Zyflo® Rofecoxib – Vioxx®
- Zafirlukast – Accolate®