The Arachidonic Acid Cascade

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

"BOUND" PHOSPHOLIPID - ARACHIDONIC ACID

neurotransmitters
ACh, NE, etc.
bradykinin
thrombin
trauma
others

antinflammatory steroids

Binds to
marijuana (Δ9-THC) receptor

biochemical/
physiological/
behavioral
effects

PHOSPHOLIPASE

MAJOR GOAL!

KNOW THIS DIAGRAM:

Epoxyeicosatrienoic Acids (EETs)

Ca²⁺ influx
vasoconstriction

5,6-EET
7-EET
1,12-EET
4,15-EET

PG Synthase -
cyclooxygenase plus
epoxygenase

6-keto-PGF₁α
vasculature - I₂ synthase

PGH₂
hydroperoxidase
oxygen radicals
biochemical effects

PGF₂α
PGD₂

increases, reduces
vaccination
vascular permeability
sensation to pain
body temperature
uterine contraction

PGI₂ (t₁/₂ = 5 min)

PGD₂ (t₁/₂ = 30 sec)

"FREE"

5,6-EET
7-EET
1,12-EET
4,15-EET

t (COX-2) inducible

selective COX-2 inhibitors, e.g. celecoxib (Celebrex®)

blocks only COX-2, therefore
less GI and platelet effects

PG synthase -
cyclooxygenase plus
epoxygenase

vasoconstriction

thromboxane A₂ (t₁/₂ = 30 sec)

platelet aggregation
smooth muscle contraction

Thromboxane A₂

PG Synthase

5,6-EET
7-EET
1,12-EET
4,15-EET

enzyme uncertain

PG Synthase -
cyclooxygenase plus
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vasoconstriction

Biochemical/physiological/behavioral

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PGD₂ (t₁/₂ = 30 sec)
**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 and 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 PGE$_2$, PGF$_{2\alpha}$, PGI$_2$ and TxA$_2$ can affect vascular and non-vascular smooth muscle tone.

10. Know how prostaglandins affect gastric function.

11. Understand how cyclooxygenase inhibitors and PGI$_2$ (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.

16. **Know the mechanism of action of the drugs on the drug list at the end of this lecture.**
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  Epoxxygenase 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**

Formed from polyunsaturated fatty acids (PUFA).

Phospholipases release the PUFA precursors from phospholipids.

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

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III. **Substrates**

20:3 (di-homo-g-linolenic acid), not very predominant in nature, forms PG₁

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.
IV. Synthesis of Arachidonic Acid Metabolites

A. Four General Pathways of Arachidonic Acid Product Formation
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. lipoxygenase
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Note: LTC4 and D4 are primary components of SRS-A = “slow-reacting substance of anaphylaxis”

HPETE = hydroperoxyeicosatetraenoic acid
HETE = hydroxylated HPETE
3-lipoxygenase is major form and acts at five position of AA

3. P-450 monooxygenase “epoxygenase”
4. **anandamide**
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BOUND PHOSPHOLIPID - ARACHIDONIC ACID

neurotransmitters
ACh, NE, etc.
bradykinin
thrombin
trauma
others
antinflammatory steroids

"Bliss"
Anandamide
(arachidonyl
ethanolamide)

Binds to
marihuana (Δ⁹-
THC)
Receptor CB 1
and CB 2

biochemical/
physiological/
behavioral
effects

NOTE: Enzyme PLD removes anandamide from membrane PL's.

Action of anandamide terminated by FAAH (fatty acid amide hydrolase)

Endogenous Cannabinoid System

Phosphatidylethanolamine

Phospholipase D

Acyl-transferase

Anandamide

Fatty acid
Amide hydrolase

Arachidonic acid

Δ⁹-THC
Receptor mechanisms:
  GPCR’s
  Multiple 2nd messengers
  Complexity of subtypes = complexity of activity

PGI2, PGE1, PGD2 = activate AC = increase c-AMP = increase Ca intracellular
PGE2 (EP1) = PLC = IP3 activation
PGE2 (EP2) = AC increased activity = increase c-AMP
PGE2 (EP3) = AC decreased activity = decreased c-AMP

TXA2 = PLC to increase IP3 = increases calcium

LTB4 = PLC to increase IP3 = increase calcium

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

COX-1 & COX-2 inhibitors
Aspirin (irreversible) & other (reversible) nonselective
Non-steroidal anti-inflammatory drugs (NSAIDs)

Cyclooxygenase 2 (COX-2)
Inducible, i.e. not present all the time (except brain & kidney), is induced to form in inflammatory cells during inflammation

Cyclooxygenase 1 (COX-1)
Constitutive - meaning enzyme always there. Found in virtually all cells, including platelets and GI mucosa

Thromboxane A2 (platelets → aggregation)

COX-2 selective inhibitors, e.g. Celecoxib (Celebrex®)

 Constitutive PGs (includes GI → protective)
 Induced PGs (includes pro-inflammatory PGs)

Structures of Cyclooxygenase (COX) Isoenzymes

**COX-1**
- Active site
- Hydrophobic channel

**COX-2**
- Active site
- Hydrophobic channel
- "Side pocket"
No Inhibition of COX-1 by Celecoxib at Therapeutic Concentrations

Inhibition of COX-2 by Celecoxib
**Celecoxib (Celebrex)**

Selective COX-2 inhibitor
approved by the FDA for osteoarthritis and rheumatoid arthritis; **not** approved for relieving post-surgical dental pain
Not approved for patients < 18 years of age

pharmacokinetics:
rapidly absorbed; peak serum levels in about 3 h
metabolized by CYP2CP in liver and excreted in urine and feces
half-life = 11 hr.

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**Celecoxib (Celebrex): Adverse Effects and Drug Interactions**

abdominal pain
Diarrhea
indigestion
also rare cases stomach bleeding
7.1% patients discontinued Celebrex vs. 6.1% of patients on placebo

Interactions with other CYP2C9 inhibitors
(e.g. fluconazole (Diflucan) fluvastatin (Lescol))

Increased serum levels of drugs metabolized by 2D6
(e.g., some beta blockers, antidepressants, and antipsychotic drugs).
Celecoxib (Celebrex): FDA Alert: 3/2005

Based on emerging information, including preliminary reports from one of several long term National Institutes of Health (NIH) prevention studies, the risk of cardiovascular events (composite endpoint including MI, CVA and death) may be increased in patients receiving Celebrex. FDA will be analyzing all available information from these studies to determine whether additional regulatory action is needed.

Other COX-2 Inhibitors

Rofecoxib (Vioxx)
September 2004: Merck & Co., Inc. announced a voluntary withdrawal of Vioxx (rofecoxib) from the U.S. and worldwide market due to safety concerns of an increased risk of cardiovascular events (including heart attack and stroke) in patients on Vioxx. Vioxx is a prescription COX-2 selective, non-steroidal anti-inflammatory drug (NSAID) that was approved by FDA in May 1999 for the relief of the signs and symptoms of osteoarthritis, for the management of acute pain in adults, and for the treatment of menstrual symptoms. Vioxx was later approved for the relief of the signs and symptoms of rheumatoid arthritis in adults and children.

Valdecoxib (Bextra)
On April 7, 2005, the Food and Drug Administration (FDA) asked Pfizer to voluntarily remove from the market.
Merk Voluntarily Removes Vioxx (rofecoxib) From Market!
September 30, 2004

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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 CB receptors and produces analgesia and decreased locomotion.

PGs given intraventriculatly into the brain can induce fever.
Pain

NSAIDs are effective when inflammation sensitizes pain receptors.

I.V. administration of PGs can cause headaches and vascular pain

- lowers threshold for firing of the polymodal nociceptor C fibers.

- NSAIDs do not block the pain or hyperalgesia caused by the direct action of PG

- NSAIDs block pain by blocking prostaglandin synthesis

Chemicals involved in pain that liberate PG’s and other mediators (substance P, CGRP) bradykinin (from plasma) cytokines (TNF, IL-1 and -8) to produce hyperalgesia

Fever

NSAIDs promote the return to normal temperature.

fever mediators = increases in cytokines including IL-1beta, IL-6, TNF

- These cytokines increase the synthesis of PGE2 in the circumventricular organs -- close to the preoptic area in the hypothalamus

- increases in c-AMP in the hypothalamus elevates body temperature by promoting increases in heat generation and decreases in heat loss.

- NSAIDs inhibit this by inhibiting synthesis of PGE2.

NSAIDS are not effective for:

1) fever caused by prostaglandins directly.
2) fever in response to exercise or ambient temperature.
B. Smooth Muscle

1. Vascular
   a. $\text{PGI}_2$ is the predominant PG produced by vascular tissue, mainly by endothelium.

   b. $\text{PGI}_2$ and $\text{PGE}_2$ - relax muscle, vasodilation.

   c. Thromboxane A$_2$ (TxA$_2$) and LTC$_4$ - contracts muscle, vasoconstriction.

2. Bronchial and Tracheal

   LTC$_4$ 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 (PGG₂, PGH₂) and TxA₂ 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 -

PGI₂ is a very potent inhibitor of platelet aggregation induced by ADP, collagen or epinephrine.

Since PGI₂ is formed by the vascular wall PGI₂ 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) inhibit (TXA2) but not arterial (PGI2) cyclooxygenase. This would maximize the anti-thrombotic effect of aspirin since PGI₂ inhibits platelet aggregation.
D. Kidney Function

The kidney papilla is rich in AA. $\text{PGI}_2$ and $\text{PGE}_2$ given into the renal artery produce diuresis and increase $\text{Na}^+$ and $\text{K}^+$ excretion. [thought to be via vasodilation, decrease ADH activity. Loop diuretics (furosemide) stimulate COX1.

Note: Inflammation in kidney, kidney transplant rejection = increased TXA2 = vasoconstriction and loss of renal function, increased ADH.

**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.

NSAIDs decrease activity of loop diuretics since loop diuretics (furosemide) stimulate COX1.

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E. GI Function

$\text{PGE}_2$ and $\text{PGI}_2$ 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®) 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 and bone formation [PG’s produced by osteoblasts].

Contribute to bone loss in menopause.

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. Used to maintain a patent (open) ductus arteriosus.

   For **delayed** closing of the ductus arteriosus in infants leading to respiratory distress, **indomethacin** is the drug of choice.

   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.

   Injectable
3. **Dinoprost (PGF$_{2a}$) tromethamine** is used intra-amniotically to induce abortion, usually in pregnancy of longer than 15 weeks.

4. **Dinoprostone (PGE$_2$)** is used in suppository form to induce abortion in pregnancies of less than 28 weeks. It is also used to induce full-term labor.

   Note: Both stimulate uterine contraction, break down collagen in cervix, synergize with oxytocin. Given vaginally the drugs enter mother’s circulation, but only low levels get to fetus.

5. **Misoprostol (PGE$_1$)** [Cytotec] and numerous PG analogs (arbaprostil, enprostil, 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

   See Dr. Ishac's notes for mechanism of action.
7. The French abortion pill **Mifepristone (RU-486)** 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 **Mifeprestone** the woman is given a prostaglandin **Misoprostol** 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.

**Drugs to Remember:**

- Aspirin
- Montelukast – Singulair®
- Misoprostol®
- Celecoxib - Celebrex®
- Dinoprost (PGF$_2$$_\alpha$)
- Dinoprostone (PGE$_2$)
- tromethamine
- Zafirlukast - Accolate®
- Zileuton - Zyflo®
**Recommended Reading:**
