Overview

- Biological function of lipid mediators
- Arachidonic acid (AA) synthesis
- AA metabolites and related enzymes
  - Prostaglandins, thromboxanes, leukotrienes
    - EETS, Lipoxins, Isoprostanes
- Inhibition of AA metabolizing enzymes
- Receptors and Metabolism
Inflammation

• A protective response of the body

• Elicited by cell injury (microbes, toxins)
  – Mechanical, thermal, chemical, and bacterial insult

• Goal: eradication of harmful agents and initiation of the healing process

• Arachidonic acid (AA) metabolites participate in the pathogenesis of inflammation and fever

Inflammation

• Hallmarks:
  – Pain- PGE$_2$, LTB$_4$, LTC$_4$, LTD$_4$, PAF (hyperalgesia)
  – Fever- PGE$_2$
  – Vasodilation- PGE$_2$, PGI$_2$
  – Increased vascular permeability- PGE$_2$, PGD$_2$, PGI$_2$, LTC$_4$, LTD$_4$, PAF
  – Chemotaxis and Leukocyte activation- LTB$_4$, PAF
  – Cell Migration- LTB$_4$
  – Tissue damage

• Anaphylaxis:
  – LTC$_4$, LTD$_4$
### Other Effects of AA Metabolites

#### Bronchioconstrictor
- LTs, PAF
  - Asthma, acute insult

#### Platelet aggregation
- Promoted by TXA$_2$
- Inhibited by PGI$_2$

#### Gastric cytoprotection
- PGI$_2$, PGE$_2$, PGF$_2$, PAF
  - Smooth muscle contraction, inhibit acid secretion in stomach, enhance mucus secretion and mucosal blood flow

#### Renal function
- PGI$_2$, PGE$_2$
  - Modulate renal blood flow, urine formation

#### Reproduction
- PGE$_2$, PGF$_2$, PAF
  - Conception, labor, menstruation, male reproductive mechanism

#### Musculoskeletal
- PGs
  - Bone remodelling (osteoclast and osteoblasts)
  - Rheumatoid Arthritis
**Cellular Membranes: Phospholipid Bilayers**

Phosphatidylcholine is the major source of AA in the body; enriched in membranes of cells of myeloid origin and endothelium.

**Phospholipase A\textsubscript{2}**

- Secretory - first isolated
  - ~10 groups, only IIA, V, and X involved in PG synthesis
  - Low molecular weight (~14 kDa)
  - Restricted expression
    - IIA - small intestine
    - V - eye, heart, pancreas
    - X - testis, stomach
  - Have specific cell surface receptors
    - Are involved in signaling directly; can modulate PG production via receptor binding and internalization
Phospholipase A$_2$

- Cytosolic
  - 85 kDa major isoform (cPLA$_2$-$\alpha$), also $\beta$ (110 kDa) and $\gamma$ (61 kDa)
    - $\alpha$, $\beta$ Ca$^{2+}$-dependent
    - $\gamma$ Ca$^{2+}$-independent
  - Primary source of AA for PGs, etc.
  - Found in most tissues
  - Activated by Ca$^{2+}$ binding and membrane association
    - Activation also regulated by phosphorylation, phosphoinositides, scaffold proteins
  - Site of action:
    - Nuclear membrane
    - Also reported on ER, mitochondrial membranes

Structure of cPLA$_2$
Structure of cPLA$_2$

Active Site Serine
**Cyclooxygenase 1 & 2 (Cox 1/2)**

- Also known as Prostaglandin Endoperoxide H Synthase 1 & 2
- Bind AA with $K_m \approx 5 \, \mu M$, $O_2 \approx 5 \, \mu M$
- Cox 1
  - constitutive expression in most tissues
- Cox 2
  - Expressed in nervous, immune, renal tissue
  - Expression inducible by inflammatory and proliferative signals
  - May play a role in modulating endogenous cannabinoid signaling
    - $K_m$ for 2-arachidonylglycerol $\approx 5 \, \mu M$; for arachidonylethanolamide $\approx 24 \, \mu M$
**COX 1/2: One Enzyme, Two Activities**

- **Cyclooxygenase activity**-
  - Dependent on active site tyrosyl radical
  - Results in $\text{PGG}_2$
    - Can activate other COX enzymes

- **Peroxidase activity**-
  - Heme dependent
  - Leads to production of $\text{PGH}_2$
    - Short $t_{1/2}$
    - Rapidly metabolized to more stable PGs

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**COX1 Homodimer**

![Diagram of COX1 Homodimer]

- Epidermal Growth Factor Domain
- Membrane Binding Domain
- Catalytic Domain
- Flurbiprofen
- Heme
COX1 Monomer
• Cyclooxygenase activity dependent on tyrosyl radical
• Peroxidase activity dependent on heme group
**PGH2 Metabolizing Enzymes: Localization**

- **PGD₂ synthase (isomerase)**
  - Immune cells
- **PGE₂ synthase (isomerase)**
  - Most cells
- **PGF₂ synthase (reductase)**
  - Uterus, seminal vesicles
- **PGI₂ (Prostacyclin) synthase**
  - Endothelial cells
- **Thromboxane synthase**
  - Platelets
Cell activation: Generic cell cytokines, growth factors, mechanical trauma

- VSMC
- Vasodilation
- Platelets
- Aggregation
- Declumping
- Vasoconstriction
- Uterine smooth muscle
- Contraction, parturition
- Chemotaxis
- TH2 lymphocyte
- Allergic asthma
- Lung epithelial cell
- Bone resorption
- Fever Generation
- Neurons of OVLT of PDA
- Maturation for ovulation and fertilization
- Ovarian CO cells
- Pain response

X = NSAIDS
celecoxib
rofecoxib

PLA₂ 

Arachidonic Acid

COX 1/2
lipoxygenase

Prostaglandins

Prostacyclin

Thromboxanes

Leukotrienes
Lipoxygenase

- 5-Lipoxygenase → 5-HPETE → Leukotrienes
  - 5,12-DHETE
- 8-Lipoxygenase → 8-HPETE → 8-HETE
- 11-HPETE → 11-HETE
- 12-lipoxygenase → 12-HPETE → 12-HETE
- 5,12-DHETE
- 15-HETE
- 5S,12S-THETE
- 16,15-DHETE
- 8,15-DHETE

DHETE, HETE - Leukocytes, eosinophils, platelets = muscle contraction, cell degranulation, superoxide production, chemotaxis

AA Metabolism by 5-Lipoxygenase
Leukotriene (LT) Synthesis

- **LTA₄** - 5-Lipoxygenase
  - Neutrophils, basophils, eosinophils, mast cells, monocytes, macrophages
- **LTB₄** - LTA₄ hydrolase
  - Most cells
- **LTC₄** - LTC₄ synthase (glutathione transferase)
  - Basophils, eosinophils, mast cells, monocytes/macrophages, platelets
- **LTD₄** - γ-glutamyl transpeptidase, γ-glutamyl leukotrienenase
  - Extracellular metabolite of LTC₄
  - LTE₄ - aminopeptidase activity towards LTD₄
LTA₄ Synthesis Step 1

LTA₄ Synthesis Step 2
Leukotriene synthesis

LT Synthesis

- LTA₄ - 5-Lipoxygenase
  - Neutrophils, basophils, eosinophils, mast cells, monocytes, macrophages
- LTB₄ - LTA₄ hydrolase
  - Most cells
- LTC₄ - LTC₄ synthase (glutathione transferase)
  - Basophils, eosinophils, mast cells, monocytes/macrophages, platelets
  - mGST 2 or mGST 3 (testis)
- LTD₄ - γ-glutamyl transpeptidase, γ-glutamyl leukotrienenase
  - Extracellular metabolite of LTC₄
LTA₄ Hydrolase

LTA₄-H active site
LT Synthesis

- LTA$_4$ - 5-Lipoxygenase
  - Neutrophils, basophils, eosinophils, mast cells, monocytes, macrophages
- LTB$_4$ - LTA$_4$ hydrolase
  - Most cells
- LTC$_4$ - LTC$_4$ synthase (glutathione transferase)
  - Basophils, eosinophils, mast cells, monocytes/macrophages, platelets
  - mGST 2 or mGST 3 (testis)
- LTD$_4$ - $\gamma$-glutamyl transpeptidase, $\gamma$-glutamyl leukotrienenase
  - Extracellular metabolite of LTC$_4$

LTA4 Metabolites

- Neutrophil Chemotaxis
- Most Tissues
- Heart, adrenal, brain, spleen
- Bronchioconstriction, edema
- Airway SMC, Postcapillary venul endothelium
Lipoxins

- Cell-cell interactions
- Endogenous anti-inflammatory substance
- Inhibits neutrophil activation, migration, and chemotactic signals
- Inhibits TNF, NFκB, cytoprotective for enterocytes, stimulates macrophage phagocytosis of neutrophils
Other enzymes that metabolize AA

- Cytochrome P450 enzymes
  - ER resident enzymes
  - Main actions in renal and vascular systems
  - Action via receptor binding and modulation of intracellular signaling pathways

Epoxyeicosatrienoic acid

- Mechanism of Action: Activate smooth muscle large conductance Ca\(^{2+}\) activated K\(^+\) channels (BKC\(_{Ca}\)) which hyperpolarizes the smooth muscle causing vasorelaxation.

- Endothelium-derived hyperpolarizing factor.
Isoprostanes

- Non-cyclooxygenase isomers of prostaglandins: auto-oxidation products of polyunsaturated fatty acids.
- Stimulate the TP receptors
- Markers of lipid peroxidation, stimulate platelet aggregation, cell proliferation and vasoconstriction.
Inhibitors of PG Synthesis

- COX inhibitors-
  - Non-Steroidal Anti-Inflammatory Drugs (NSAIDS)
    - Salicylates
    - Arylpropionic acid derivatives
    - Acetaminophen
  - Selective COX 2 inhibitors

History

- The bark of willow was prescribed as far back as 400B.C by Hippocrates as a pain reliever and fever reducer

- First purified in 1829 by Leroux

- Introduced as “aspirin” by Bayer and Co. of Germany as an antiinflammatory in 1900
Mechanisms of Action

- NSAIDS were used for years without any knowledge of how it worked

- In 1971 Vane et al demonstrate that low concentrations of aspirin inhibit the enzymatic production of prostaglandins

Salicylates

- Aspirin
- Salicylic acids
- Diflunisal (arthritis)
Salicylates

- Mechanism of action
  - Acetylates proteins including COX-1 & 2
  - Irreversible inhibition

Aspirin-Triggered Lipoxin (ATL)
Inhibitors of PG Synthesis

- COX inhibitors-
  - Non-Steroidal Anti-Inflammatory Drugs (NSAIDS)
    - Salicylates
    - Arylpropionic acid derivatives
      - Ibuprofen (Motrin, Nuprin, Advil)
      - Naproxin (Aleve, Naprosyn, Anaprox)
      - Flurbiprofen (Ansaid)
      - Ketoprophen (Orudis)
      - Oxaprozin (Daypro) - long half-life
  - Acetaminophen???

- Selective COX 2 inhibitors

Arylpropionic Acid Derivatives

- Mechanism of Action
  - Competitive inhibitors
  - Block cyclooxygenase activity
  - Reversibly bind to COX 1 & 2 inhibiting interaction with arachidonic acid
**Proprionic Acid Derivatives:**

*Competitive COX Inhibitors*

Ibuprofen in AA binding site of COX

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**NSAID & Propionic acid derivative Activities**

- **Antiinflammatory**
  - Treatment of musculoskeletal disorders (rheumatoid arthritis, osteoarthritis, and ankylosing spondylitis)
  - Symptomatic relief only

- **Antipyretic**
  - Reduce body temperature in febrile states

- **Analgesic**
  - Low to moderate pain caused by inflammation
Acetaminophen

- Analgesic
- Antiinflammatory
- Not antipyrogenic
- Doesn’t inhibit COX1/2; may inhibit COX3
- No longer considered an NSAID
- Hepatotoxicity when combined with alcohol
  - Glutathione depletion

Inhibitors of PG Synthesis

- COX inhibitors-
  - Non-Steroidal Anti-Inflammatory Drugs (NSAIDS)
    - Salicylates
    - Arylpropionic acid derivatives
    - Acetaminophen???
      - Selective COX 2 inhibitors
COX-2 Inhibitors

- Celecoxib (Celebrex)
- Rofecoxib (Vioxx)
- Valdecoxib (Bextra)

• Mechanism of action:
  - Selectively inhibits COX-2 which is induced locally at the site of inflammation
  - Does not interfere with constitutive form that protects the gastrointestinal tract
  - Drug companies thought they had a safer drug to treat forms of osteoarthritis
    • Vioxx recall-
      - COX-2 metabolites important for platelet deaggregation and vascular healing
      - Major cardiovascular complications
        » Heart attack
        » Stroke
Merck Announces Voluntary Worldwide Withdrawal of VIOXX®

WHITEHOUSE STATION, N.J., Sept. 30, 2004—Merck & Co., Inc. today announced a voluntary worldwide withdrawal of VIOXX® (rofecoxib), its arthritis and acute pain medication. The company’s decision, which is effective immediately, is based on new, three-year data from a prospective, randomized, placebo-controlled clinical trial, the APPROVe (Adenomatous Polyp Prevention on VIOXX) trial.
Platelet Activating Factor

- Lipid mediator derived from phosphatidylcholine
- Platelet and leukocyte activation
  - Cell polarization
  - Integrin activation
  - Priming (for degranulation)
  - Redistribution of surface ligands
- Displayed on cell surface of endothelial cells

PAF Synthesis
Lipid Mediators elicit their effects through GPCRs

- G Protein-Coupled Receptors
  - Also called 7-transmembrane (TM) receptors

- Couple to trimeric G-proteins that are activated by ligand binding

- Have complex biology; not simply on-off switches

PAF Receptor
TXA$_2$ Receptor

PGE Receptor & Isoforms
### Prostaglandin Signaling: Receptors and 2nd Messengers

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**Leukotriene Receptors in the Human Lung**

- **Airways**
  - Epithelium
  - Smooth muscle
  - Contraction

- **Pulmonary Veins**
  - Endothelium
  - Contractile Factor
  - Relaxation
  - Contraction
EET Receptor system

Metabolism of Lipid Mediators

• Cytochrome P450 4A, 4F
  – ω-oxidation
  – β-oxidation

• Peptidase activity
• Make compounds less hydrophobic → excretion in urine
References

• Phospholipases

• COX1/2 & PGs
  – Rouzer CA, LJ Marnett Structural and functional differences between cyclooxygenases: Fatty acid oxygenases with a critical role in cell signaling Biochim Biophys Res Comm 338 (2005) 34-44

• Thromboxane

• Lipoxigenases and LTs

• PAF

• Inhibitors

• Receptors

• CYP 450

• PG/LT/TX function