TUTORIAL ON EICOSANOIDS

Introduction

I. Through this tutorial you will learn basic eicosanoid facts needed to appreciate:

- Physiological roles of eicosanoids
- How eicosanoid-related pathways can be manipulated by drugs for the benefit of patients.

II. Eicosanoids mediate a large number of physiological responses related to injury. These responses include

- perception of pain
- inflammation
- smooth muscle constriction and dilation
- blood clotting
- response to stomach acid
- cancer

III. Eicosanoid synthesis and eicosanoid signaling pathways are important targets for many drugs including:

- aspirin and other NSAIDS (non-steroidal anti-inflammatory drugs)
- some allergy medications
- some asthma preventatives
- glucocorticoid steroids (such as cortisol)

IV. Eicosanoids are 20-carbon compounds derived from the fatty acid arachidonic acid. There are three medically important classes of eicosanoids:

- **Prostanoids** *(prostaglandins and thromboxanes)*
  Prostanoids are mediators of pain, inflammation, fever, blood clotting, smooth muscle contraction and dilation, and protection of the gastrointestinal tract.

- **Leukotrienes**
  Leukotrienes participate in early stages of the immune response. They are also powerful bronchioconstrictors.

- **Lipoxins**
  Lipoxins participate in resolving the immune response.
V. Eicosanoids mediate paracrine and autocrine signaling.

**Paracrine signaling**

* but NOT endocrine signaling (eicosanoids are quickly degraded so they do not travel very far).

**Autocrine signaling**

**Endocrine signaling**
VI. Eicosanoids are synthesized primarily from arachidonic acid, an $\omega-6$ unsaturated fatty acid.

![Arachidonic acid](image)

- The rate limiting and highly regulated step in eicosanoid synthesis is the release of arachidonic acid from phospholipids in the E.R. or outer nuclear membrane.
- The type of eicosanoid that is produced from the arachidonic acid depends on which enzymes are present in the cell.

- **Arachidonic acid is a component of phospholipids in the E.R. and nuclear membranes.**
  - Arachidonic acid is released from the membranes primarily by “cytosolic” phospholipase A$_2\alpha$ (cPLA$_2\alpha$).
  - A small amount of arachidonic acid can be released by phospholipase C (the lipase that is activated by Gq-signaling)
  - Phospholipase A$_2\alpha$ is inactive when in the cytosol and active when associated with the E.R. or nuclear membrane.
  - Phospholipase A$_2\alpha$ activation requires Ca$^{2+}$ (e.g. as produced by Gq signaling) and is greatly stimulated when phosphorylated by MAP kinases.
  - Phospholipase A$_2\alpha$ hydrolyzes phospholipids at the 2 position, producing arachidonic acid

- **Glucocorticoids reduce inflammation in part by inhibiting eicosanoid production by macrophages.**
  - Phospholipase A$_2\alpha$ is inhibited by the protein Annexin-1
  - Annexin-1 synthesis by macrophages is stimulated by glucocorticoids such as cortisol.
  - Annexin-1 also works by other mechanisms, some of which are described later in the tutorial.
  - Glucocorticoids also work by other mechanisms, some of which are described later in the tutorial.
VII. Arachidonic acid gives rise to three classes of eicosanoids.
Prostanoids

I. Introduction

• The prostanoids are a class of compounds that affect many physiological processes including pain, inflammation, fever, blood clotting, and smooth muscle contraction and relaxation.

• Many drugs work by affecting prostanoid synthesis or action.

• Prostanoids induce physiological effects in nearly all cells, but each cell type responds differently to each prostanoid.

• Prostanoids are produced by nearly all cells, but different prostanoids are produced by different cell types.

• Prostanoids share a common precursor (prostaglandin H) that is produced from arachidonic acid by one of two enzymes.
  - cyclooxygenase 1 (COX-1)
  - cyclooxygenase 2 (COX-2)

• It is not necessary to learn the structures, but you should note that prostanoids have the following features:
  - a hydroxyl group on carbon 15
  - a 5-carbon, oxygen containing ring (one prostanoid, thromboxane has a 6-member ring)

• prostanoids that leave the cell are produced by modifying prostaglandin H.

II. Nomenclature

• The prostanoids include several classes of prostaglandins and thromboxane.

• The individual prostaglandins are named with the prefix PG followed by a letter, A to K. Prostaglandin I (PGI) is also termed prostacyclin.

• A subscript, 1 to 3, indicates the number of double bonds in the molecule. Prostaglandins can be synthesized from 20-carbon fatty acids with 3, 4 or 5 double bonds to produce prostaglandins with 1, 2, or 3 double bonds, respectively. Prostaglandins with 2 double bonds (synthesized from arachidonic acid) are the most common.

• Prostaglandin F has an additional subscript, either α or β, that designates the configuration of the hydroxyl at carbon 9.

• The thromboxanes are termed TXA and TXB. TXA is the active thromboxane. TXB is a degradation product that is biologically inactive.
III. Common Prostanoids

It is NOT necessary to learn these structures.

- PGD$_2$
  - Aggravates asthma & allergy
  - Promotes inflammation

- PGE$_2$
  - Increases pain & fever
  - Promotes inflammation

- PGF$_{2\alpha}$
  - Mediates many aspects of reproduction

- PGI$_2$
  - Decreases clotting
  - Decreases platelet aggregation
  - Relaxes smooth muscles

- TXA$_2$
  - Promotes clotting
  - Promotes platelet aggregation
  - Constricts smooth muscles

IV. Cyclooxygenase-1 (COX-1) or cyclooxygenase-2 (COX-2) converts arachidonic acid to the common prostanoid precursor, prostaglandin H.

Other enzymes lead to leukotriene and lipoxin synthesis.
V. A knowledge of COX-1 and COX-2 is important for understanding the medical aspects of prostanoid action. The essential to know differences between COX 1 and COX 2 are:

**Distribution:**

- Most cells synthesize COX-1.
- COX-2 is present primarily in:
  - cells that mediate pain, fever and inflammation
  - endothelial cells that line blood vessels
  - the epithelial cells that line the gastrointestinal tract.

**Expression:**

- COX-1 and COX-2 are encoded by different genes.
- COX-1 is synthesized at a relatively constant rate; its synthesis is not induced.
- COX-2 synthesis is induced by extracellular signals; in cells not receiving these signals, COX-2 is not present or present in small amounts.
- Glucocorticoids suppress expression of the COX-2 gene.

**COX inhibitors:**

- NSAIDS (non-steroidal anti-inflammatory drugs) other than aspirin inhibit both COX-1 and COX-2.
- Inhibition of COX-2 provides relief from pain, fever and inflammation.
- Inhibition of COX-1 increases irritation of the stomach lining and decreases blood clotting.
- Some drugs specifically inhibit COX-2.

VI. Prostanoid receptors

- The prostanoids, as well as other eicosanoids, interact with their target cells primarily via G-protein-coupled receptors.
- There are one or more receptors for each prostanoid.
- Each prostanoid receptor activates a particular signaling cascade; Gs, Gi, or Gq
  - Different prostanoids may have different effects on a particular cell type. For example, thromboxane (TXA) and prostacyclin (PGI) have opposite effects on vascular smooth muscle cells: TXA promotes constriction while PGI promotes dilation.
  - The same prostanoid may have different effects on different cell types.
### Some properties of prostanoid receptors
(Examples only, not to be memorized)

<table>
<thead>
<tr>
<th>Receptor*</th>
<th>Signaling Pathway</th>
<th>Signal</th>
<th>Effect on smooth muscle/other effects</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PGD receptors</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DP1</td>
<td>Gs</td>
<td>Increase cAMP</td>
<td>Relaxation; inflammation; allergy</td>
</tr>
<tr>
<td>DP2</td>
<td>Gi</td>
<td>Decrease cAMP</td>
<td>Immune activation</td>
</tr>
<tr>
<td><strong>PGE receptors</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EP1</td>
<td>Gq</td>
<td>Increase Ca++</td>
<td>Constriction; mediates pain</td>
</tr>
<tr>
<td>EP2</td>
<td>Gs</td>
<td>Increase cAMP</td>
<td>Relaxation</td>
</tr>
<tr>
<td>EP3</td>
<td>Gi</td>
<td>Decrease cAMP</td>
<td>Constriction; fever</td>
</tr>
<tr>
<td>EP4</td>
<td>Gs</td>
<td>Increase cAMP</td>
<td>Relaxation</td>
</tr>
<tr>
<td><strong>PGF receptor</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FP</td>
<td>Gq</td>
<td>Increase Ca^{2+}</td>
<td>Uterine contraction</td>
</tr>
<tr>
<td><strong>PGI receptor</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IP</td>
<td>Gs</td>
<td>Increase cAMP</td>
<td>Relaxation; Anti-thrombotic</td>
</tr>
<tr>
<td><strong>TXA receptor</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TP</td>
<td>Gq</td>
<td>Increase Ca^{2+}</td>
<td>Constriction; Platelet aggregation</td>
</tr>
</tbody>
</table>

* Prostanoid receptors are named by the prostanoid letter (A-K), followed by the letter P with subscript to denote subtype.

### VII. Example of Prostanoid Action: Blood Clotting

**Overview:**

1. **Thromboxane (TXA) is made by platelets in response to injury.**
   - Thromboxane stimulates platelet aggregation
   - Thromboxane stimulates vasoconstriction
2. **Prostacyclin (PGI) is made by endothelial cells in response to blood flow.**
   - Prostacyclin inhibits platelet aggregation
   - Prostacyclin stimulates vasodilation
3. **COX-inhibitors affect blood clotting.**
   - Low doses of aspirin inhibit thromboxane production thereby decreasing blood clotting
   - COX-2 inhibitors such as the drug Celebrex inhibit prostacyclin production, thereby increasing blood clotting
A blood clot forming at the site of a wound

Clotting involves interactions between:

<table>
<thead>
<tr>
<th>Smooth muscle cells which:</th>
<th>Endothelial cells which</th>
<th>Platelets which:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>synthesize prostacyclin (PGI)</td>
<td>synthesize thromboxane (TXA)</td>
</tr>
<tr>
<td></td>
<td>have thromboxane receptors (TP)</td>
<td>have thromboxane receptors (TP)</td>
</tr>
<tr>
<td></td>
<td>have PGI receptors (IP)</td>
<td>have PGI receptors (IP)</td>
</tr>
</tbody>
</table>
Thromboxane Stimulates clotting

- **Platelets produce Thromboxane (TXA)**
  - Factors that stimulate clotting activate receptors on platelets
  - Phospholipase A₂ is activated
  - PGH synthesized by COX-1 is converted to TXA

- **TXA stimulates platelet aggregation and clotting**
  - TXA acts via autocrine signaling or diffuses a short distance to neighboring platelets
  - TXA binds to the thromboxane receptor (TP), a Gq-linked G-protein coupled receptor
  - Ca²⁺ concentration increases
  - Ca²⁺ promotes platelet aggregation
Thromboxane Stimulates Vasoconstriction

- **Platelets produce Thromboxane (TXA) as above:**
  - Factors that stimulate clotting activate receptors on platelets
  - Phospholipase A\(_2\) is activated
  - PGH by COX-1 is converted to TXA

- **TXA diffuses a short distance to neighboring smooth muscle cells**
  - TXA binds to the thromboxane receptor (TP), a G\(\alpha\)-linked G-protein coupled receptor
  - Ca\(^{2+}\) concentration increases
  - Ca\(^{2+}\) causes phosphorylation of myosin light chain
  - Vasoconstriction results
Normal blood flow inhibits clotting

- **Prostacyclin (PGI) is produced by endothelial cells**
  - Shear forces of blood flow stimulates a mechanoreceptor
  - Phospholipase A_2_ is activated
  - PGH synthesized by COX-1 and COX-2 is converted to PGI

- **Prostacyclin reduces platelet aggregation**
  - PGI diffuses a short distance to neighboring platelets
  - PGI binds to a Gs-linked G-protein coupled receptor
  - cAMP concentration increases
  - cAMP reduces platelet aggregation
Blood flow induces vasodilation

- **Blood flow induces prostacyclin (PGI) synthesis as above**
  - Shear forces of blood flow stimulate a mechanoreceptor
  - Phospholipase A\textsubscript{2} is activated
  - PGH synthesized by COX-1 and COX-2 is converted to PGI

- **Prostacyclin relaxes smooth muscle cells**
  - PGI diffuses a short distance to neighboring smooth muscle cells
  - PGI binds to a Gs-linked G-protein coupled receptor
  - cAMP concentration increases
  - cAMP causes smooth muscle relaxation
VIII. Drugs and Blood Clotting

Aspirin works by irreversibly inhibiting COX-1 and modifying the activity of COX-2

Low doses of aspirin (one children's tablet per day for an adult) irreversibly inhibits COX-1
- In most cells one aspirin/day has no long term effect on COX-1. This is because COX-1 normally has a short half-life and is continually being synthesized
- In platelets one aspirin/day does have a long term effect on COX-1. This is because platelets have no nucleus and do not synthesize new COX-1.
- at low doses, aspirin has little effect on COX-2

Aspirin's ability to reduce pain, fever and inflammation at moderate to high doses, is due to modification of COX-2 as well as COX-1.
- Pain, fever and inflammation are mediated by PGD and PGE, and in most cells, COX-2 is the major producer of PGD and PGE.
- COX-2 synthesis is induced by conditions that lead to pain fever and inflammation, making it the predominant cycloxygenase under these conditions
- When modified by aspirin, COX-2 does not make prostaglandins (e.g. PGD and PGE). Instead, it makes anti-inflammatory eicosanoids that you will meet in later sections.
Normal blood clotting

In the absence of aspirin there is a delicate balance between vasodilation, vasoconstriction, and platelet aggregation. This balance is regulated, in part, by the ratio of PGI to TXA. The effects of PGI and TXA are explained in the previous sections.
Clotting in the presence of aspirin

Daily low dose aspirin inhibits COX-1 in platelets (endothelial COXs are unaffected since they can be resynthesized).

TXA synthesis is reduced. The clot promoting effects of TXA are diminished.
The vasoconstriction effects of TXA are also diminished.

Selective COX-2 inhibitors such as Celebrex work by inhibiting COX-2 with very little effect on COX-1.

COX-2 inhibitors are able to reduce pain, fever and inflammation without some of the side effects of aspirin. (COX-1 inhibition by high dose aspirin can cause stomach distress. This will be discussed in the eicosanoids lecture).

Selective COX-2 inhibitors do have the adverse side effect of promoting clot formation. The adverse side effect of promoting clot formation can be understood in terms of alterations to the PGI/TXA balance.
COX-2 in endothelial cells is inhibited. COX-1 is not affected.

PGI synthesis is reduced. The clot inhibiting effects of PGI are reduced.
The vasodilation effects of PGI are also reduced.

**IX. Leukotrienes**

**Introduction**

- **Leukotrienes are important mediators of inflammation**
  - They increase vascular permeability
  - They increase immune cell chemotaxis
  - They increase immune cell activation
  - They increase immune cell production

- **Leukotrienes are powerful bronchioconstrictors:**
  - leukotrienes signal via G-protein coupled receptors that lead to smooth muscle contraction
    - Gq receptors (increase IP3 leading to increased Ca$^{2+}$ leading to myosin light chain phosphorylation)
    - Gi receptors (decrease cAMP; cAMP stimulates myosin light chain dephosphorylation)
Leukotrienes are derived from arachidonic acid through the action of 5-lipoxygenase

Activation of leukotriene synthesis
Both phospholipase A₂ and 5-lipoxygenase are inactive in the cytosol. Activation (via increased cytosolic Ca²⁺ and phosphorylation) brings them to the nuclear membrane where they join FLAP (5-lipoxygenase activating protein).
- Cyclooxygenases also associate with FLAP
- FLAP brings the enzymes that produce and use arachidonic acid together into one complex.
5-lipoxygenase makes leukotriene A (LTA). LTA is converted to other leukotrienes.

It is not necessary to learn the structures. They are only presented here so that you can see what they look like.

Leukotrienes can be produced from polyunsaturated fatty acids other than arachidonic acid to produce LTA with a different number of double bonds.
LTA is converted to Leukotrine B, and the cysteine-leukotrienes (cysLT): LTD and LTE. It is not necessary to learn the structures. They are only presented here so that you can see what they look like.

**Aspirin Sensitivity**

In some patients, moderate to high doses of aspirin may induce bronchioconstriction and accentuate immune responses.

This can have serious consequences during an asthma attack or in some types of allergic reactions.

**The mechanism is as follows:**

- Aspirin decreases prostaglandin production.
- Arachidonic acid produced by activation of phospholipase A₂ is funneled into leukotriene production.
- The excess leukotrienes that are produced inappropriately stimulate the immune response.
• Leukotrienes are bronchio-constrictors. The excess production of leukotrienes may narrow the airways to a dangerous degree.

Leukotriene Receptors
• Leukotrienes signal via Gi and Gq G-protein coupled receptors. Hence they lead to smooth muscle contraction.

• There are two classes of LT receptors
  • LTB activation promotes inflammation.
    o LTB receptors are present on Immune cells (e.g. neutrophils)
  • CysLT receptors promote inflammation and bronchioconstriction. They are present on:
    o Immune cells (e.g. mast cells)
    o Smooth muscle cells

• In the airways, smooth muscle contraction leads to bronchioconstriction. The allergy and asthma drug montelukast (Singulair) is a cysLT receptor antagonist
X. Lipoxins

Lipoxins mediate the resolution of an inflammatory response

Lipoxins
- Block cysLT receptors
  - thereby inhibiting inflammatory leukotriene action
- Activate lipoxin receptors
  - inactivates inflammatory cells
  - stimulate phagocytosis of dead cells
  - stimulate proliferation of wound-healing cells

Lipoxin synthesis requires an interaction between leukocytes and other cells, most notably epithelial cells and platelets. This requirement for a close interaction means that lipoxin synthesis is maximal late in infection when lymphocytes are plentiful.

Example: Interaction between epithelial cell and leukocyte

1. During an inflammatory response, epithelial cells convert arachidonic acid to 15-hydroxyarachidonic acid. (The arachidonic acid is produced via phospholipase A₂).
2. 15-hydroxyarachidonic acid is transferred to interacting leukocytes, which convert it to lipoxins. (During an inflammatory response, 5-lipoxygenase is associated with FLAP and is active).

3. Lipoxins are produced and secreted into the blood.

**Aspirin and COX-2**

Aspirin modifies COX-2 so that it synthesizes lipoxins rather than prostanoids
- Normally, COX-2 leads to the synthesis of pro-inflammatory prostanoids.
- Aspirin modifies the active site of COX-2. The modified COX-2 leads to synthesis of anti-inflammatory lipoxins rather than prostanoids.

(epi-LXA and LXA have different configurations of the 15-OH, but they both function as lipoxins)
Lipoxin Action

- The lipoxins function by:
  - blocking the cysLT1 leukotriene receptor.
  - activating the lipoxin receptor ALXR. ALXR also responds to a peptide hormone, annexin-1, that is released from cells in response to glucocorticoids (described later).
- Lipoxins act to:
  - inhibit activation of inflammatory cells (e.g. polymorphonuclears and eosinophils).
  - stimulate macrophages to increase phagocytosis of immune cells that are dying by apoptosis.
  - stimulate proliferation of cells that are involved in wound healing such as fibroblasts, endothelial cells, gastrointestinal epithelial cells and renal mesangial cells.

XI. Leukotriene and lipoxin action during an allergic reaction

Early Phase (5-30 min.)
1. antigen-IGE complex binds to receptor on mast cell
2. phospholipase and 5-lipoxygenase activated
3. leukotrienes made
4. leukocytes activated

Late Phase (3-12 hrs.)
1. Leukotrienes recruit leukocytes
   and increase leukocyte production
2. The recruited leukocytes produce more leukotrienes
3. Inflammatory response is heightened

Resolution phase
1. Many leukocytes are present
2. The leukocytes frequently interact with other cells
3. Lipoxin synthesis starts
4. Lipoxins block LT receptors
5. Lipoxins activate LX receptors on many cell types
Commonly used drugs target leukotriene pathways

Zileuton: a 5-lipoxygenase inhibitor
1. Block Leukotriene synthesis
2. Leukotrienes are not able to amplify inflammatory reaction.

Montelukast: a cysLT receptor antagonist
1. Leukotriene receptors inhibited
2. Intracellular signaling reduced
3. Ability to stimulate an inflammatory response reduced

Aspirin sensitivity: see above
**Glucocorticoids:** Glucocorticoids such as cortisone are powerful anti-inflammatory drugs. They work, in part, by blocking eicosanoid production, and in part by blocking other inflammatory pathways.

**Glucocorticoids affect eicosanoid synthesis and eicosanoid action:**

- Glucocorticoids activate the synthesis of annexin-1 in leukocytes.
- Within the leukocyte, annexin-1 is a potent inhibitor of phospholipase A2 (cPLA2).
- Decreased cPLA2 activity decreases arachidonic acid production.
- Decreased arachidonic acid decreases both prostanoid and leukotriene production.
- Annexin-1 is also released from leukocytes.
- The annexin-1 that is released activates the lipoxin ALXR receptor.
- Activation of ALXR promotes the resolution of the inflammatory response.
- Glucocorticoids also decrease the amount of COX-2 that is synthesized, further lowering prostanoid synthesis.

**Glucocorticoids also block other inflammatory pathways.**
Resolvins and Protectins

- Resolvins and protectins are compounds that resemble lipoxins in that they help to resolve the immune response.
- Resolvins are derived from the omega-3 polyunsaturated fatty acids DHA (docosahexaenoic acid) or EPA (eicospentahexenoic acid), and have slightly different chemical structures than the lipoxins.
- The synthesis and mechanism of action of resolvins is new and currently very active area of investigation.