5-Hydroxy tryptamine

(5-HT ; Serotonin)

- 5-Hydroxytryptamine (5-HT) has important pharmacological and physiological role in the body:-

  - **Neurotransmitter in CNS**
  - **Regulator of smooth muscle in CVS,GIT**
  - **Regulation of platelet aggregation.**

Biosynthesis

Tryptophan (From diet)

↓ **Tryptophan hydroxylase**

5Hydroxytryptophan

↓ **Aromatic L-A.A decardoxylase**

5-Hydroxy tryptamine

↓ **Monoamine oxidase(MAO)**

5 Hydroxyindole acetaldehyde

↓ **Aldehyde dehydrogenase**

5Hydroxyindole acetic acid (5HIAA)

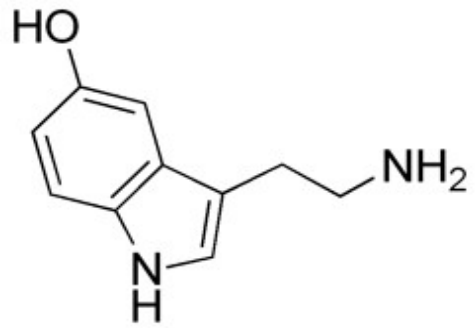
Distribution

- GIT enterochromaffin cells (90%)and myentric plexus.
- Platelets
  - diffuse inside from plasma by active transport , released at site of damage
- Lungs, bone marrow, pineal gland, CNS

Serotonin Receptors

- seven main types
- **(5-HT_1 to 5-HT_7).**
- **5-HT_1, 5-HT_2 subdivided**
- **Total 14 types of 5-HT receptors present.**

5HT present in ↑ concentrations in:



- **CNS** – midbrain acts as NT

  pineal gland as precursor of melatonin

- **Blood** - platelets
- **Gut wall -** mucosal enterochromaffin cells and neurons in myenteric plexus

Storage and release

- Stored in storage granules just like catecholamines
- Diffuses over a relatively large region and activate 5-HT receptors located on

  dendrites

  cell bodies

  presynaptic terminals of adjacent neurons

Reuptake

- Uptake of 5-HT from the synapse by **specific monoamine transporter** (5-HT reuptake transporter) on presynaptic neuron.
- *There are several different **monoamine transporters**.*

  dopamine transporter

  norepinephrine transporter

  serotonin transporter

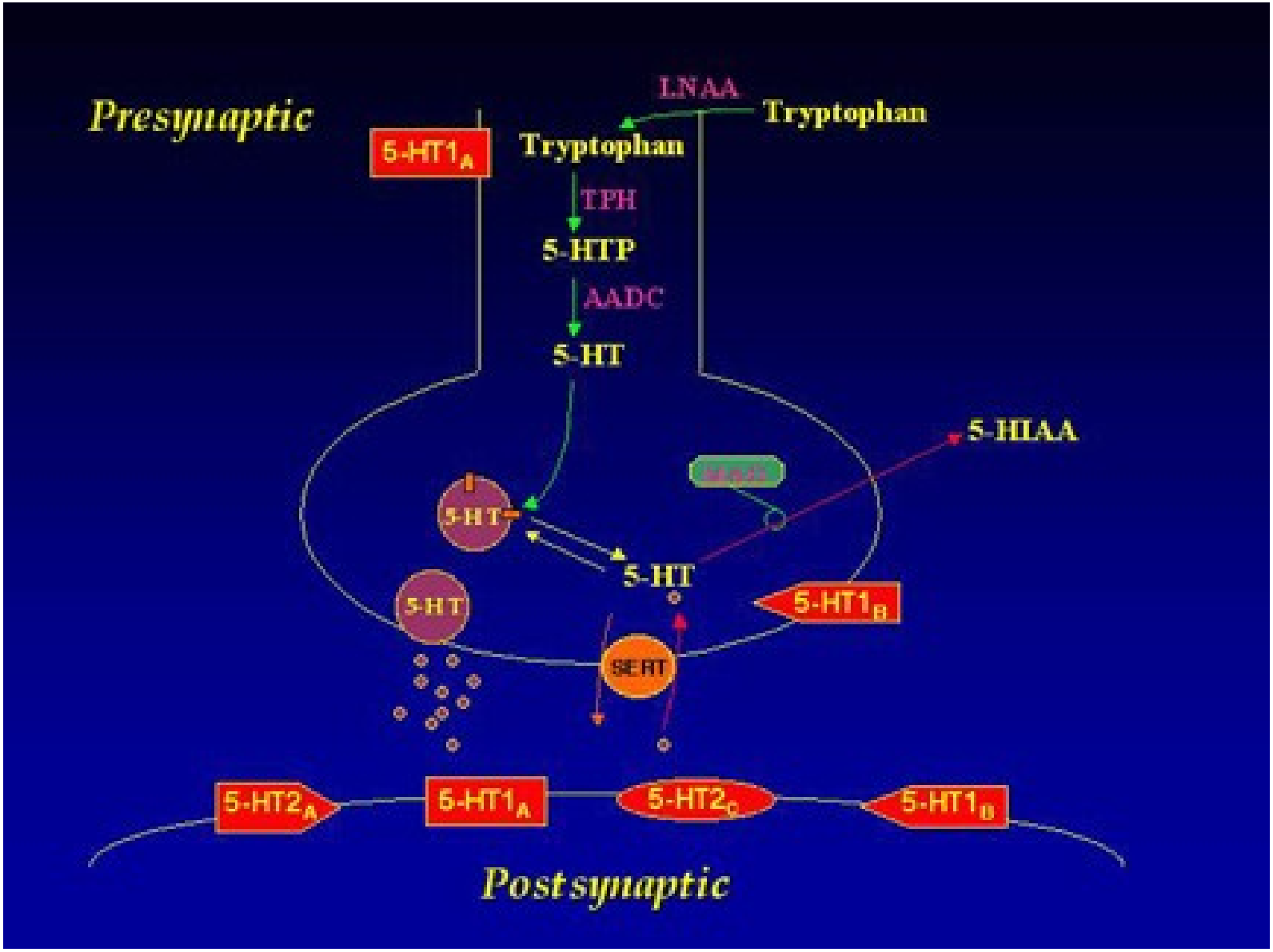
Various agents can inhibit 5-HT reuptake including

- Cocaine
- Tricyclic antidepressants
- Selective serotonin reuptake inhibitors(SSRIs)

  e.g. Fluoxetine

Elimination

- Metabolized by MAO and then
  Aldehyde dehydrogenase to form
  5-hydroxyindole acetic acid(5-HIAA)
- 5-HIAA is excreted in urine


Presynaptic
LNAA
Tryptophan
5-HT1A
Tryptophan
TPH
5-HTP
AADC
5-HT
5-HIAA
MAO
5-HT
5-HT
5-HT
5-HT1B
SERT
5-HT2A
5-HT1A
5-HT2C
5-HT1D
Postsynaptic

Receptors

- 5-HT receptors are located on the cell membrane of nerve.

- 5-HT3 receptor a ligand gated ion channel,

  all other 5-HT receptors are G-protein coupled receptors

Receptor Sites

- CNS – All
- Cerebral vessels – $5HT_{1D}$
- Nerve endings (PNS) -Nociceptive $5HT_3$

  Enteric mucosal $5HT_3$

  Myenteric plexus $5HT_4$
- Smooth muscles – $5HT_{2A}$ (vessels, bronchi)
- Gastric fundus – $_{2B}$
- Platelets – $_{2A}$

Receptor sites & actions

- 5-HT_{1A} – CNS Neuronal inhibition (auto receptor)

  Behavioral effects

  $_{1B}$ – CNS Presynaptic inhibition(auto receptor)

  $_{1D}$ – CNS Behavioral effects

  Cerebral Vasoconstriction

  Locomotion

- $5HT_{2A}$- CNS **Neuronal excitation**

  $_{2C}$– choroid plexus CSF secretion

- $5HT_3$ – CNS — Excitation, Anxiety
  - Sensory neuron — Pain and itching
  - Enteric neurons — Emesis

- $5HT_4$ – CNS — Neuronal excitation
  - Myenteric neurons — GI motility

- $5HT_{5-6}$ – CNS

- $5HT_7$ – CNS, GI tract, blood vessels

Systemic effects of 5-HT

- **CNS** – As Neurotransmitter

  Functions associated with 5HT pathways are:

  Behavioral responses

  Feeding behavior

  Mood and emotion control

  Sleep / wakefulness control

  Emetic reflex (esp. chemical triggered)

  Control of sensory pathways

- **CVS** –

  Vasodilation in skeletal ms., coronary bed, arterioles

  Vasoconstriction in all other vessels

B P Triphasic response :

fall – Coronary chemoreflex

rise – Vasoconstriction,↑co

fall - Vasodilation in skeletal M.& arterioles

- **GIT –**
  - Stimulation of smooth ms. contraction via ($5HT_{2A}$) receptors on the muscles
    ($5HT_4$) receptors in myenteric plexus
  - Contraction of stomach fundus ($5HT_{2B}$)
  - Inhibition of gastric acid-pepsin secretion

- **Bronchi** - Bronchoconstriction ($5HT_{2A}$)
- **Platelets** – platelet aggregation ($5HT_{2A}$)

Agonists & Antagonists

- $5HT_{1A}$ – Buspirone — Ergotamine
- $5HT_{1D}$ – Sumtriptan — Ergotamine
- $5HT_{2A}$ – LSD — Ketanserin
  Methisergide
  Cyproheptadine
- $5HT_{3}$ – α-methyl-5HT — Ondansetron
  Granisetron
- 5HT - Cisapride — SB207266

Clinical utility

- Migraine – Sumatriptan Methisergide
  Ergotamine Cyproheptadine
- Anxiety - Buspirone
- Control of vomiting - Ondansetron
- Gastric stasis - Cisapride
  & GERD
- Raynaud's disease - Ketanserin
- Carcinoid tumor - Methisergide
  Cyproheptadine

There are indications that

5-HT_{1A} and 5-HT_4 agonists as well as

5-HT_2, 5-HT_3 antagonists and 5-HT uptake

inhibitors may have a role in treatment of

Alzheimer's disease & Amnesia

Serotonin reuptake inhibitors are useful as antidepressant drugs (non-selective Tricyclic antidepressants or SSRIs)

Cisapride

- Has peripheral $5HT_4$ agonist action
- Useful in GERD, Diabetic gastroparesis
- Releases Ach from cholinergic neurones in myenteric plexus
- Oral bioavailabilty ~30%
- T ½ 10 hrs
- Reported to cause serious ventricular arrhythmias
- Others are Renzapride, Mosapride

Sumatriptan

- Selective agonist for $5HT_{1D}$ and $5HT_{1B}$
- Useful in acute migraine attack
- Bioavailability ~ 15%
- Half life 2-3 hrs
- Can be given orally, S/C or as nasal spray
- Can cause chest pain in 5% patients
- Zolmitriptan, Naratriptan can be given orally, longer acting, safer

Buspirone

- Partial agonist at presynaptic $5HT_{1A}$ receptors
- Weak D_2 blocker
- Useful as anxiolytic
- Rapidly absorbed , undergoes extensive first pass metabolism
- t ½ 2-4 hrs
- Excreted in urine and faeces

Ketanserin

- Selective $5HT_2$ blocker (Stronger for $_{2A}$)
- No partial agonistic activity
- Weak α_1, H_1, Dopaminergic blocker
- Useful in Raynaud's diasease
- Has antihypertensive activity
- Congener is Ritanserin which is more selective for $5HT_{2A}$

Cyproheptadine

- $5HT_{2A}$ antagonist
- Has additional H_1 blocking as well as anticholinergic activity
- Useful in

  Carcinoid tumor

  Post-gastrectomy dumping syndrome

  Pruritis

  Allergies

  ↑Appetite in children

Ondansetron

- Selective $5HT_3$ antagonist
- Useful as antiemetic agent
- Others are

Granisetron

Tropisetron

Methysergide

- Chemically related to ergot alkaloids
- Potent $5HT_{2A/2C}$ antagonist
- Acts on $5HT_1$ receptors also
- Has agonist activity in some tissues
- Useful in

  Migraine prophylaxis

  Carcinoid tumor

  Post-gastrectomy dumping syndrome

  Prolonged use endocardial, pulmonary fibrosis

Ergotamine

- $5HT_1$ and $5HT_2$ antagonist
- α-adrenergic antagonist
- Partial agonist activity at both types of receptors
- Useful in acute migraine attack
- Has emetic and oxytocic action as well


Thank You

Migraine

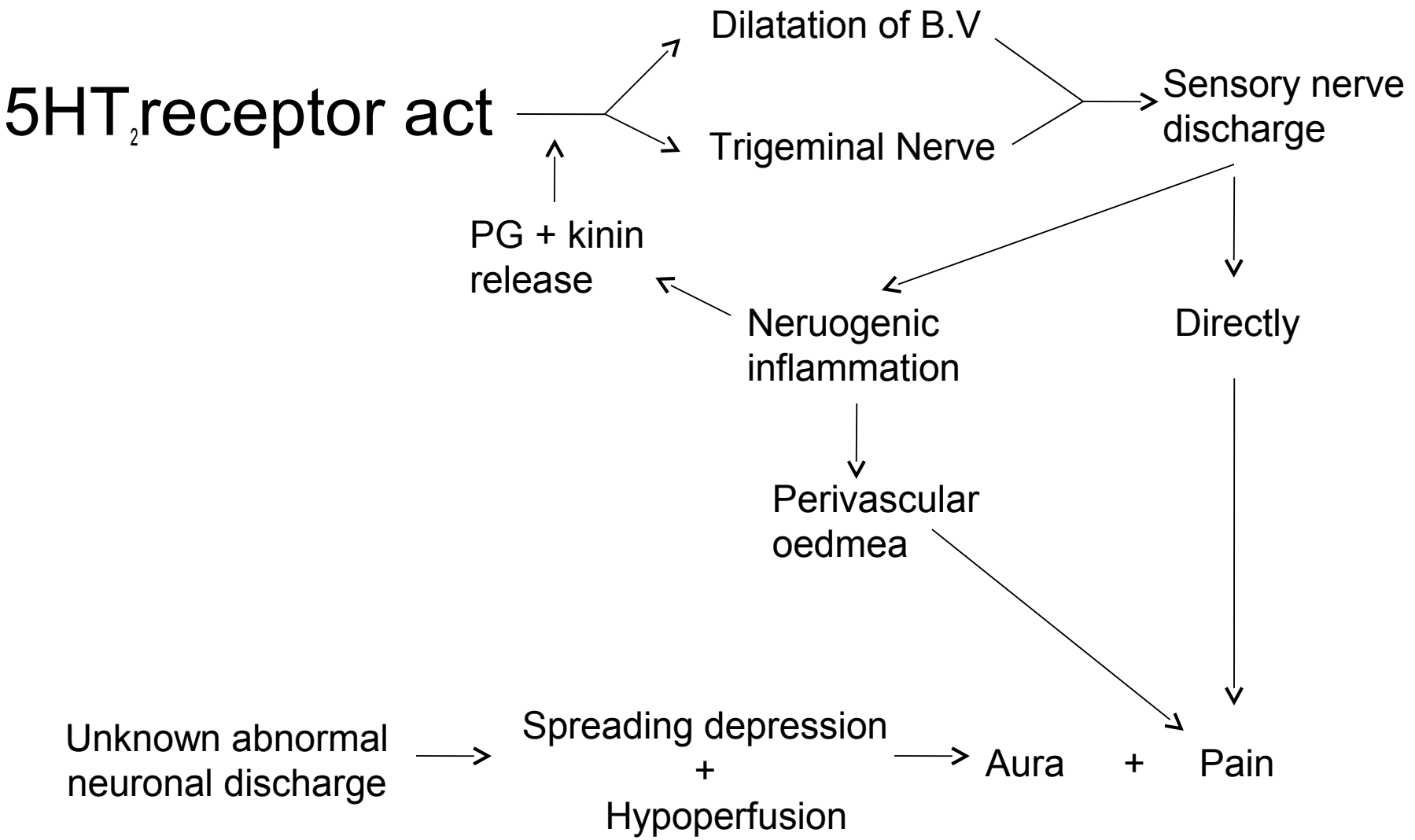
- Clinical Presentations:
  - Often accompanied by brief aura (visual scotomas, hemianopia)
  - Severe, throbbing, usually unilateral headache (few hours to a few days in duration)
- Migraine Pathophysiology:
  - Vasomotor mechanism -- inferred from:
    - increased temporal artery pulsation magnitude
    - pain relief (by ergotamine) occurs with decreased artery pulsations
  - Migraine attack associated with (based on histological studies):
    - sterile neurogenic perivascular edema
    - inflammation (clinically effective antimigraine medication reduce perivascular inflammation)

Migraine: Drug Treatment

- **Ergotamine:** best results when drug administered prior to the attack (prodromal phase) -- less effective as attack progresses
  - combined with caffeine: better absorption
  - potentially severe long-lasting Vasoconstriction.
- **Dihydroergotamine** (IV administration mainly): may be appropriate for intractable migraine
- **Nonsteroidal antiinflammatory drugs** (NSAIDs)
- **Sumatriptan**: alternative to ergotamine for acute migraine treatment; not recommended for patients with coronary vascular disease risk.
  - formulations: subcutaneous injection, oral, nasal spray
  - selective serotonin-receptor agonist (short duration of action)
  - probably more effective than ergotamine for management of acute migraine attacks (relief: 10 to 15 minutes following nasal spray)

Migraine: Prophylaxis

- **Methysergide**
  - **effective in about 60% of patients**
  - **NOT effective in treating an active migraine attack or even preventing an impending attack.**
  - **Methysergide toxicity: retroperitoneal fibroplasia, subendocardial fibrosis. Recommend 3-4 week drug holiday every six months**
- **Propranolol - Most common for continuous prophylaxis**
  - **best established drug for migraine attack prevention.**
- **Amitriptyline (TCA)**
  - **most frequently used among the tricyclic antidepressants**
- **Valproic acid (Antiepileptic)**
  - **effective in decreasing migraine frequency.**
- **Nonsteroidal antiinflammatory drugs (NSAIDs)**
  - **used for attack prevention and aborting acute attack**


5HT2receptor act
Dilatation of B.V
Trigeminal Nerve
Sensory nerve discharge
PG + kinin release
Neruogenic inflammation
Directly
Perivascular oedmea
Unknown abnormal neuronal discharge
Spreading depression
+
Hypoperfusion
Aura
+
Pain

Serotonin in Migraine

- Neurogenic vs. Vascular theories
- Several drugs that modulate the serotonin system are effective in migraine:

1. Cyproheptadine/methysergide - prophylaxis
2. Sumatriptan, ergotamine - acute
3. MAO inhibitors and TCA – both
4. Caffeine (↑ cAMP?)
5. Reserpine worsens migraine

Pharmacology of Autacoids

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MSc. Physiology
University of Baghdad
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Classically, the autacoids known as Tissue hormones or local hormones. In other word autacoids are substances with biologic activity that are synthesized at the site of action and exert primarily localized effects. They are not stored and released from neither gland, nor do they need to circulate to the site of action like "classical" hormones.

INFLAMMATION

Factors involved in the inflammatory response
A. **Increased blood flow and vascular permeability**

- produces **redness**, **heat** and **swelling** at the site of inflammation

B. **Production of Chemical Mediators**
1) Vasoactive amines - produce an immediate response to tissue damage

- histamine
- serotonin

2) The kinnin system

- bradykinnin - the most potent vasodilator; increases permeability of veinoules, increases sensitivity to pain
- **B_1** and **B_2** receptors; B_1 are induced by inflammation.

3) **Arachadonic acid derivatives -** products of cyclooxygenase and lipoxygenase pathways: prostaglandins, prostacyclin, throboxane, leukotienes

4) **The Complement system** - complement can be directly involved in cell/tissue damage.

C. **Leukocyte chemotaxis/phagocytosis**
1) migration of large numbers of white blood cells to the site of the injury

2) Release of lysosomal enzymes

D. **Nitric Oxide (NO) is produced by the conversion of arginine to citrulline by nitric oxide synthase (NOS)** NO produced from endothelial cells causes vasodilation

- NO may protect against inflammation by scavenging free radicals
- NO may mimic the cytoprotective effects of prostaglandins in the gastric mucosa

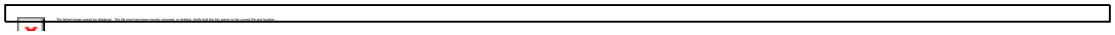
- NO that increases inflammatory cytokines
- NO released during airway inflammation

D. **Immune Response**

1) Humoral - antigens produced to host tissues.

2) Cellular

- Tissue toxicity from cytolytic T cells.
- Delayed hypersenstivity reactions



Histamine Drugs that interact with Histaminergic Receptors

Histamine (beta-aminoethylimidazole)

Receptors

- **H_1**, **H_2** and **H_3** receptors

Effects on Smooth muscle

- contract (gut intestinal muscle, bronchi), or relax (blood capillaries)
- hypotension from vasodilatation is a combined H_1 and H_2 response
- effects on vasculature produce a flushing, decreased peripheral resistance
- Histamine can produce shock through hypotension, reduced blood volume by increased vascular permeability, and decreased venous return

Bronchi

- H_1 contracts bronchi, predominant response; H_2 relaxes bronchi
- Histamine produces bronchospasm in asthmatics

Heart

- H_2 : increases inotropic (by promoting Ca^{++} flux) and chronotropic response (increase diastolic depolarization of the SA node)
- H_1 : slows AV conduction, increases automaticity

Stomach

- Histamine targeting **parietal cells** is a potent gastric secretagogue,

Stimulation of sensory nerves

- Cause pruritis in the epidermis; can produce pain coupled with pruritis in the dermis.

Histamine agonists

H_2 agonists used for diagnostic procedures in assessing gastric secretory response

1. **Betazole** - some residual **H_1** activity
2. **Impromidine** - 10,000 fold greater selectivity for **H_2** receptors

Histamine Antagonists

H_1 antihistamines

A) Ethylenediamine derivatives

B) Ethanolamine derivatives (aminoalkyl ethers)

- **diphenhydramine** - commonly used as a sedative; its anticholinnergic effects are of benefit in antiemetics (phenothiazines); it also possesses antitussive activity
- **doxylamine** - a hypnotic effect
- **clemastine** - common in allergy preparations

C) Propylamine derivatives (alkylamines)

Cause less sedation, commonly used in cold/allergy preparations

- **Brompheniramine**
- **Chlorpheniramine**
- **triprolidine**

D) Phenothiazine derivatives

Principal usage as antipsychotic; as anti-emetics and antipruritics

promethazine

1. **Anticholinergic**, α_1 antagonist (produce hypotension),
2. **Dopamine** antagonist - increases **prolactin** release, decreased **corticotrophin** and **growth hormone** release
3. **Local anesthetic** effect,
4. **Antiarrhymthic** action,
5. Used as a **sedative** and an **antiemetic,**

E) Piperazine derivatives

- **buclizine, cyclizine and meclizine** anticholinergic and CNS depressant effects.
- **hydroxyzine** is a sedative, antipruritic, and has some anxiolytic effects

F) Second Generation Agents

Antihistamines are more specific for histamine receptors and are primarily used for rhinitis. (Less anti-cholinergic effects; less sedation) and have longer durations of action.

- **terfenadine, astemizole, loratidine,cetirizine, acrivastine**,
- **terfenadine and astemizole** can cause arrhythmias, particularly with drugs that compete with their hepatic metabolism.

- **levocabastine** - an H_1-specific topical ophthalmic agent.

G) Inhibitors of mast cell degranulation and histamine release

- **Cromolyn sodium** - prophylaxis for asthma believed to **inhibit calcium influx**
- **Nedocromil** - **mechanism similar to cromolyn**
- **Lodoxamide tromethamide** - prevents **antigen-induced calcium influx into mast cells** that causes histamine release. Used topically for inflammatory diseases of the eye.

H_2 Receptor Antagonists

A remarkable degree of specificity for Gastric H_2 receptors

H_2 antagonists can inhibit responses to gastric secretagogues

- **Cimetidine** Initially these agents maintained the imidazole of histamine. ***cimetidine*** result from this drug's ability to bind to cytochrome P-450 and thereby inhibit the metabolism of drugs that use mixed-function oxidases.
- **Ranitidine**
- **Famotidine**

Serotonin (5HT)

Drugs that interact with the Serotonergic System

Pharmacologic actions

Serotonin has profound effects on gastrointestinal, cardiovascular, respiratory, and central and peripheral nervous system function

Pharmacologic actions include:

--Effects respiratory minute volume and rate
--Bronchoconstriction
--Increase in motility of small intestine
--Vasoconstriction
--Vasodilatation of skeletal muscle beds
--The positive inotropic and chronotropic
--Stimulation of sensory nerves, can contribute to pain responses
--Stimulation of autonomic ganglia
--Stimulation of catecholamine release from the Adrenal gland
--5-HT is a neurotransmitter in the CNS and is responsible for diverse
--psychoneurologic effects

Serotonin receptor subtype pharmacology and therapeutic agents

1. ***5-HT$_1$ Receptors***
2. ***5-HT$_{1A}$ receptors***

    - **Buspirone** - partial-agonist for 5-HT$_{1A}$ receptor,
    - **Ibogaine**

3. ***5-HT$_{1D}$ Receptor***
    - **Sumatriptan** Produces contraction of the large intracranial arteries.
    - **Zolmetriptan, Naratriptan** and **Rizatriptan**.
4. ***5-HT$_{2A}$ and 5-HT$_{2B}$ receptors***

These receptors are present in smooth muscle (vasculature, gut and uterus)

Ergot Alkaloids and derivatives group

From the fungus *Claviceps purpurea* that grows on rye and other grains, they have long been recognized as an abortifacient

- **Ergotamine and dihydroergotamine**

    1. Have high affinity antagonizing 5-HT$_1$ receptors.
    2. Alpha$_1$ receptors stimulating at low concentrations
    3. Block the reuptake of norepinephrine
    4. Stimulates the chemoreceptor trigger zone (CTZ) and may require an antiemetic.
- **Methysergide** a semi-synthetic ergot alkaloid
- **Ergonovine and methyl ergonovine** produce uterine contractions and contractions of other smooth muscles.
- **Ketanserin**
- **Cyproheptadine**
- **Risperidone**

5. ***5-HT$_3$ Receptors***

    - **Ondansetron and Granisetron** as an anti-emetic. (serotonergic, dopaminergic, adrenergic, histaminergic)

6. ***5-HT$_4$ receptors***: **Cisapride** is a specific 5-HT$_4$ receptor agonist, similar to metoclopramide

5-HT Reuptake Inhibitors group: fluoxetine, sertraline. dexfenfluramine Sibutramine,

Arachidonic Acid Metabolites:

Prosdtaglandins, Prostacyclin, Thromboxane A$_2$, Leukotrienes

Eicosanoids - derived essential fatty acids, arachidonic acid is the most common precursor of eicosanoids, eicosanoids mediated activation of phospholipase A_2.

Classification of Prostanoid Receptors

- **PGD_2:** vasodilatation and myometrial relaxation
- **PGE_2:** smooth muscle relaxation and inhibitons of granulocytes
- **PGF_{2a}:** smooth muscle contraction (uterus)
- **PGI_2:** vasodilatation
- **TBX:** vasoconstriction, bronchoconstriction and platelet aggregation

Vasculature

- **PGEs** are potent vasodilators.
- **Prostacyclin** (PGI_2) is a potent vasodilator producing hypotension
- **Thromboxane A_2** is a potent vasoconstrictor.
- **Leukotrienes LTC_4 and LTD_4** cause capillary leakiness.

****Alprostadil** (PGE_1) dilates the ductus arteriosus

Platelets

- **PGI_2** inhibits platelet aggregation,
- **TXA_2** is a platelet activator
- **LTB_4** chemotaxis of eosinophils, monocytes, neutrophils
- **prostaglandins** inhibit cellular and humoral immunity

Lung

- **prostaglandins** have mixed effects on bronchial muscle
- **TXA2** Inhibitors of thromboxane reduce bronchoconstrictive response.
- **LTC4 and LTD4** potent bronchoconstrictors

Uterus

Prostaglandins cause uterine contraction in pregnancy, clinically used as abortifacients or to induce labor

- **Dinaprostone (PGE_2)**
- **Carboprost (15-methyl-PGF_{2a})**

GIT

PGEs and PGI_2 inhibits gastric acid secretion, stimulated by feeding, histamine or gastrin. Maintenance of the gastric mucosa - stimulation of mucus secretion

PGE_2 and PGI_2 increase renal blood flow,

Plasma Kinnins

- Bradykinin named for its ability to produce a slow ("brady") contraction of the gut.
- kinnins degraded by kininase II, angiotensin converting enzyme

Pharmacologic effects of kinnins

1. Plasma kinins are the most potent vasodilator autacoids
2. large arteries and most veins are contracted by bradykinnin
3. increase capillary permeability, produce edema
4. involved in pain responses
5. Contract bronchioles

Blocking kinnin action : *aprotinin* it work on

1. Receptor antagonists
2. Inhibitors of kallikrien - no kinnin production

INHIBITORS OF CYCLOXYGENASE

1. Non-steroidal Anti-inflammatory Drugs (NSAIDS)
2. Inhibit the production of eicosanoids by inhibiting cyclooxygenase

COX-1 - present in blood vessels, platelets, stomach and kidney

COX-2 - induced by inflammatory cytokines and mediators; expressed in tumors and regulates angiogenesis, vascular proliferation in rheumatic joints

Common Pharmacologic Actions of NSAIDS

1. Analgesia -
2. Anti-inflammatory
3. Inhibition of Platelet Aggregation
4. Anti-pyretic

Other autacoids agent:

Nitric oxide (EDRF) Have many various action in the body involved vasodilatation, hormonal modulator, activation immune system, growth booster, stimulate sexual and performance of male and regulate female reproductive system and other works in the tissue of the body.

Substance P

Capsaicin

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.

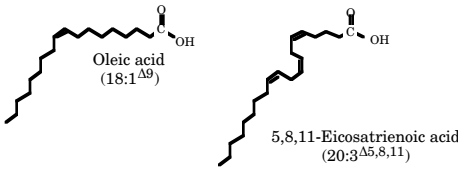
Both of these potential precursor molecules are ω^6 fatty acids. In the absence of ω^6 fatty acids, the organism may attempt to produce eicosanoids from ω^9 fatty acids. These ω^9-derivative compounds, regardless of the number of double bonds, are inactive.

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.

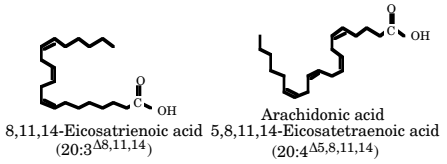
Reminder of ω nomenclature

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.

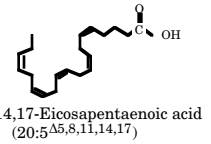
Humans can synthesize ω^9 fatty acids such as oleic acid and its 20:3 $\Delta^{5,8,11}$ derivative. However, this is ordinarily a minor pathway, and the 20:3 Δ5,8,11 cannot be used to make functional prostaglandins.



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.

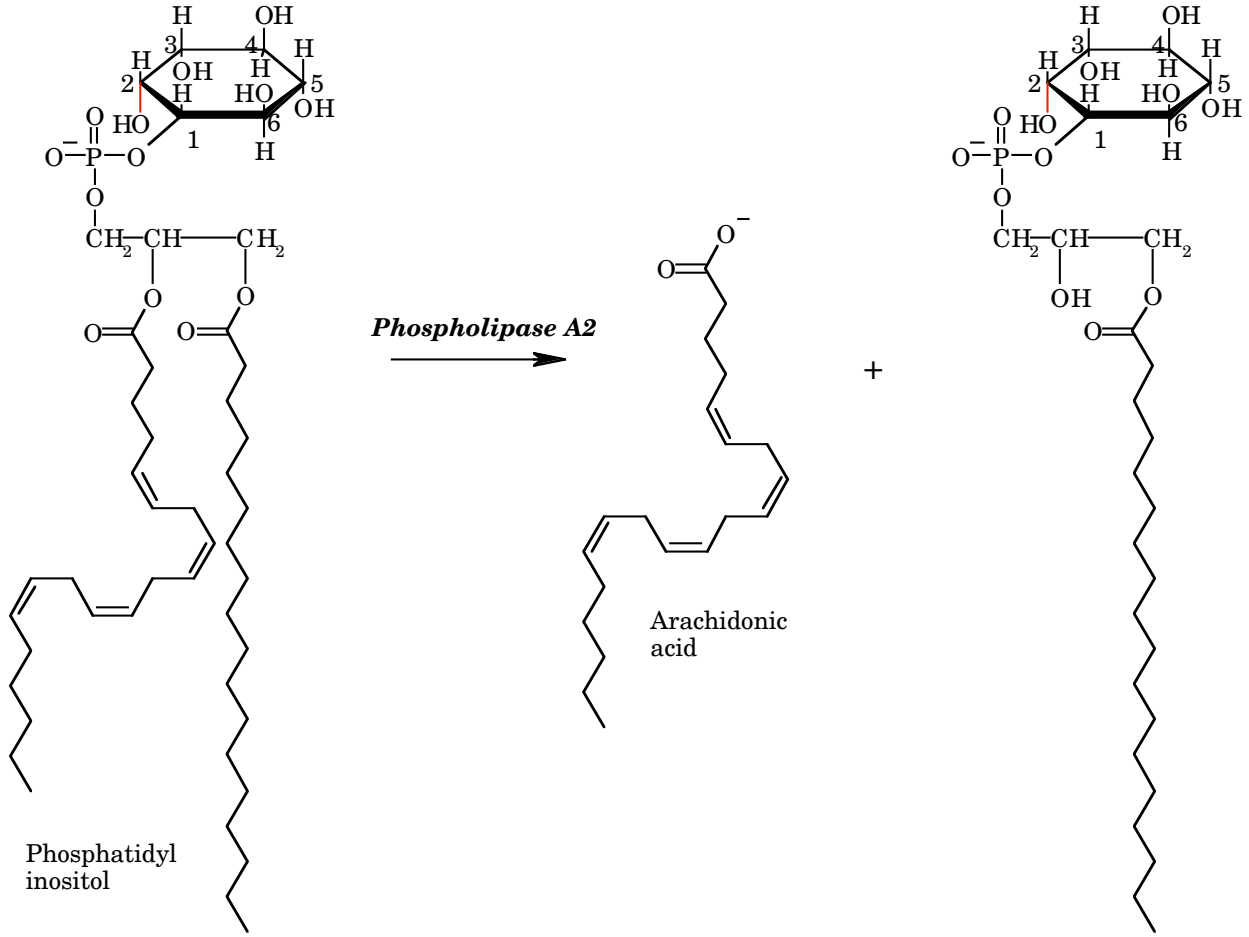


Synthesis

Prostaglandin biosynthesis has two control points.

Phospholipase A_2

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).

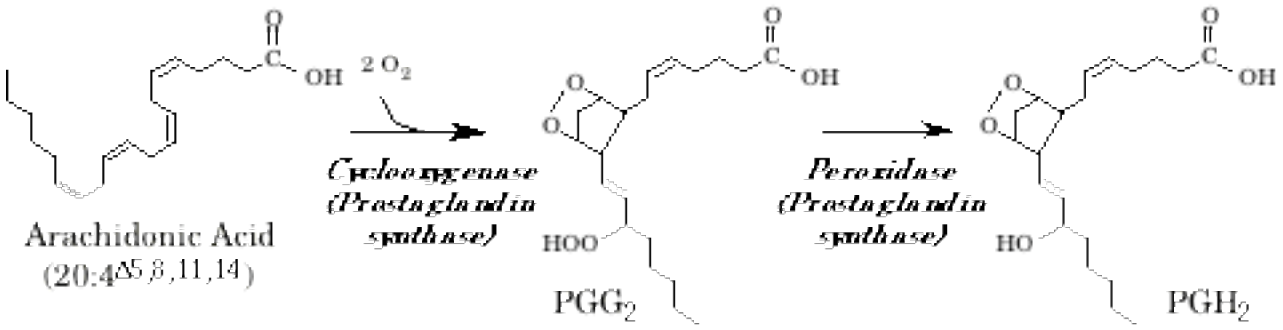


Release of the fatty acid from the phospholipid is the first control point in the prostaglandin biosynthetic pathway. One function of glucocorticoids is inhibition of phospholipase A_2 and therefore of eicosanoid synthesis.

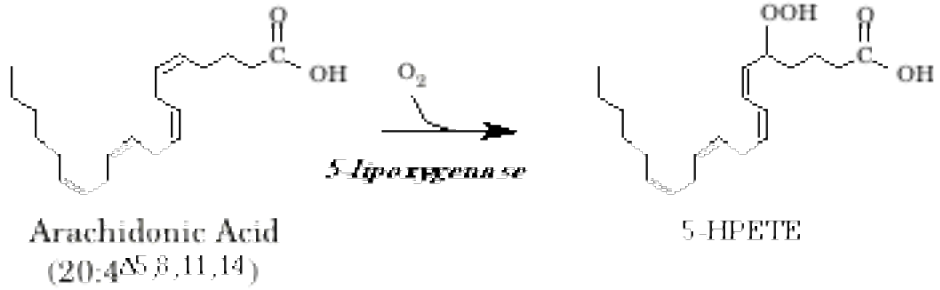
COX and lipoxygenase

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:



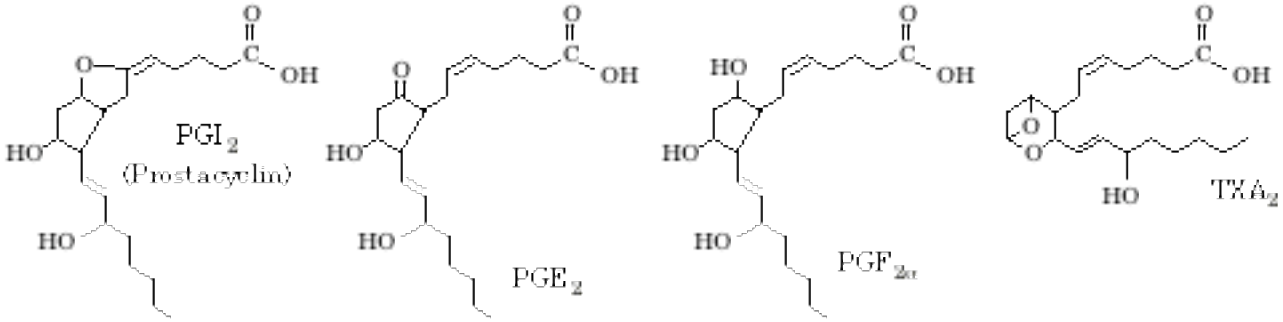
5-Lipoxygenase is one type of lipoxygenase; 5-Lipoxygenase catalyzes the first step in one of the more important pathways.



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.



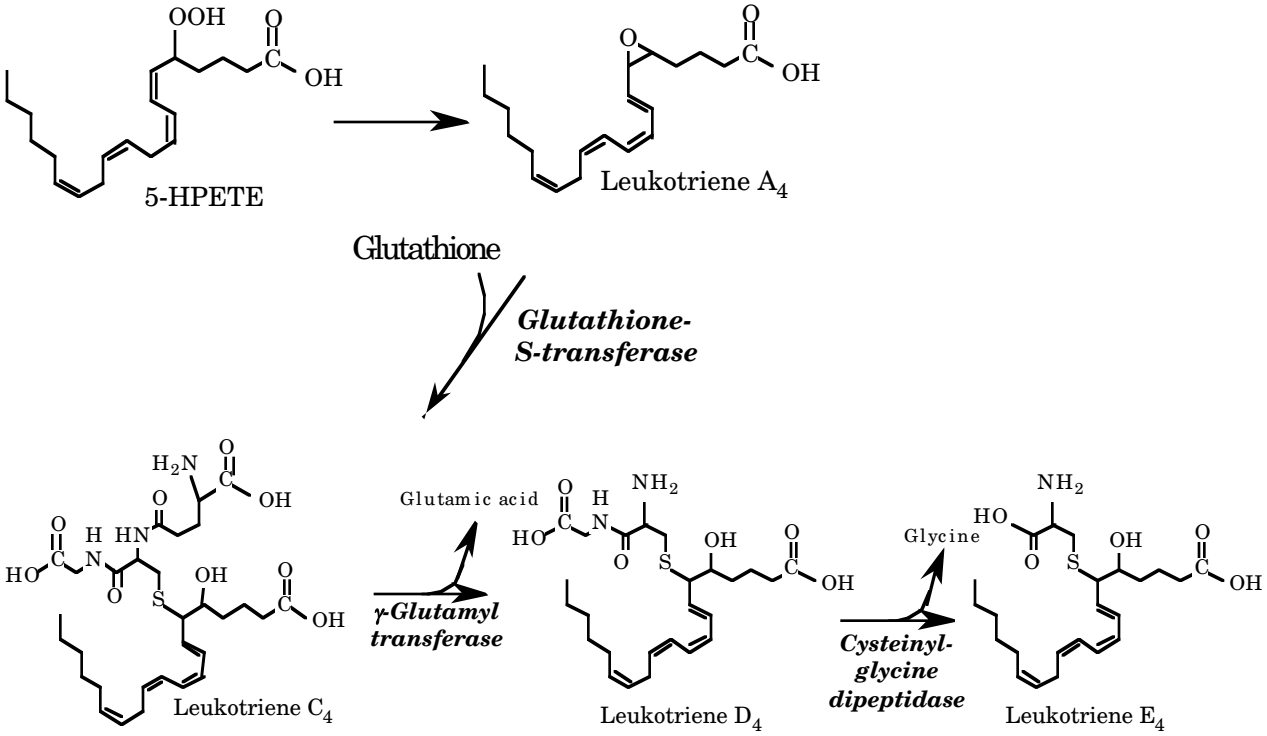
In the abbreviations, "PG" = "prostaglandin" and "TX" = "thromboxane". The letters (*e.g.*, the "I" in "PGI_2") indicate the structure and substituents of the ring, while the number refers to the number of double bonds present. The structures shown above are series 2 compounds, with two double bonds; series one compounds such as PGE_1 lack the double bond closest to the carboxylate.

Leukotrienes

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

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 C_4, D_4, and E_4 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 $PGF_{2\alpha}$)
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. The 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.

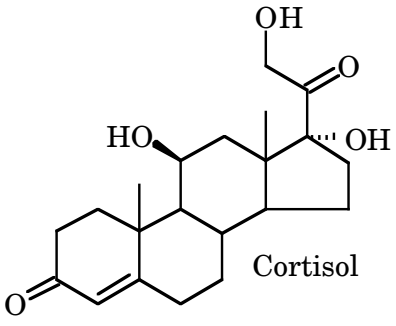
Some evidence suggests that COX-1 is responsible for generating the prostaglandins required for protection of the gastrointestinal tract, while COX-2 is responsible for the increased prostaglandin synthesis associated with inflammation, fever, and pain responses. This has led to attempts to find specific 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 a 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.

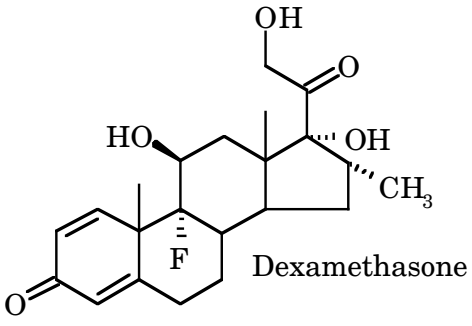
Inflammation

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 dexamethasone.



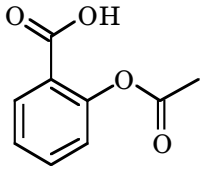
Cortisol



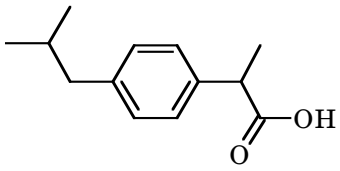
Dexamethasone

Glucocorticoids inhibit inflammatory responses by several mechanisms, and are more powerful drugs than NSAIDs. One mechanism is phospholipase A_2 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.

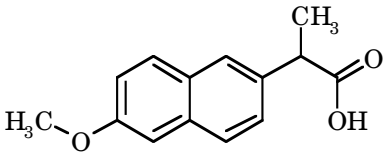
The non-steroidal compounds are called NSAIDs (Non-Steroidal Anti-Inflammatory Drugs). The NSAIDs are COX inhibitors; some of the most widely used drugs, including aspirin, ibuprofen, and naproxen fall into this class.



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



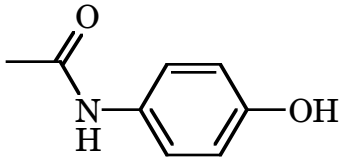
Ibuprofen
[α-methyl-4-(2-methylpropyl)benzene-acetic acid]
[2-(4-isobutylphenyl)propionic acid]



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 inhibtion 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.

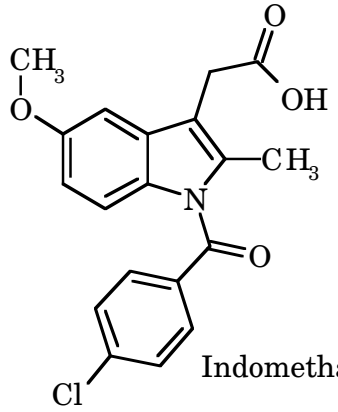
Prostaglandins and fever and pain

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.

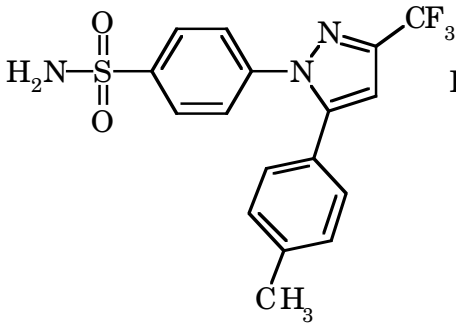
COX-2 inhibitors

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.

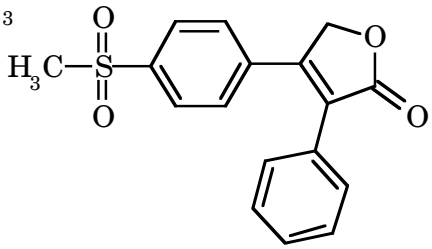
Aspirin and indomethacin both have higher affinity for COX-1 and COX-3 than COX-2 (although both compounds bind to all three enzymes). Indomethacin is about 100-fold more potent than aspirin, and is rarely used as a drug as a result of its toxic effects.



Indomethacin



Celecoxib



Rofecoxib

COX-2 specific inhibitors such as celecoxib and refecoxib 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 A_2 is produced in platelets and stimulates aggregation. Prostacyclin (PGI_2) 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 the 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 A_2, 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 A_2 is inhibited by glucocorticoids. Cyclooxygenase is inhibited by aspirin and a number of other widely used drugs.