

Eicosanoids

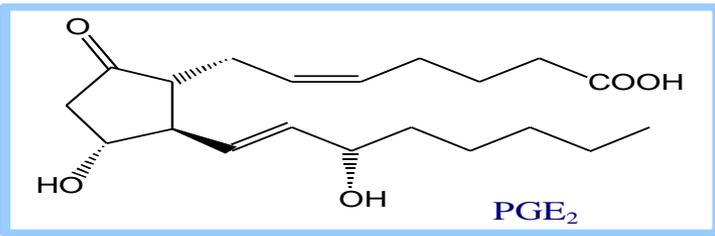
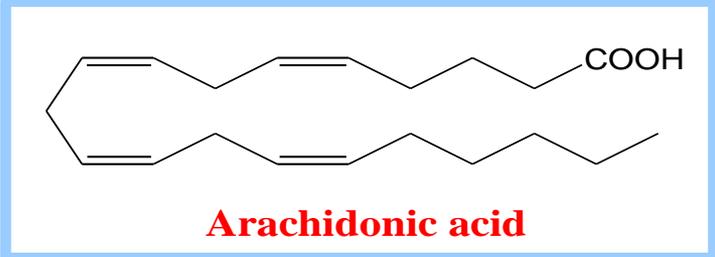
Eicosanoids

- Produced from arachidonic acid, a 20-carbon polyunsaturated fatty acid (5,8,11,14-eicosatetraenoic acid).
- The eicosanoids are considered "local hormones."
 - 1- They have specific effects on target cells close to their site of formation and not transported to distal sites within the body.
 - 2- Rapidly degraded (short half life).
 - 3- Active at very low concentrations.
 - 4- Autocrine and paracrine signaling.
 - 5- There are various eicosanoid molecules and of receptors causing different effects in the organism.
 - 6- Participate in intercellular signaling and intracellular signal cascades.

Types of eicosanoids:

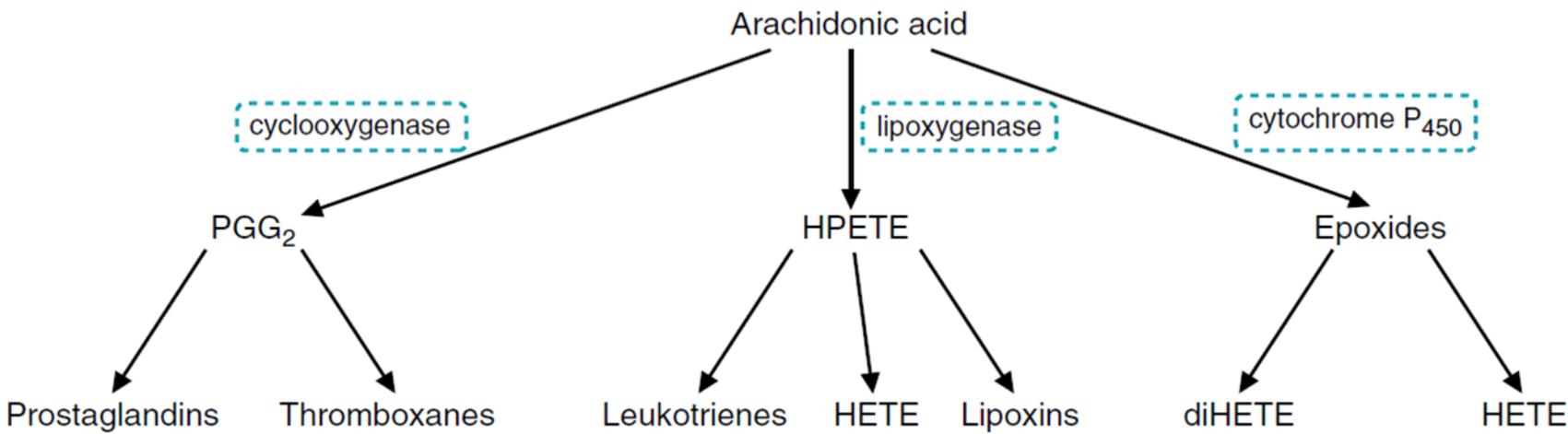
- Prostaglandins
- Prostacyclins
- Thromboxanes
- Leukotrienes
- Lipoxines.

- They have roles in:
 - Inflammation
 - Fever
 - Regulation of blood pressure
 - Blood clotting
 - Regulation of sleep/wake cycle.
 - Immune system modulation
 - Control of reproductive processes & tissue growth



- Prostaglandins all have a cyclopentane ring, while, thromboxanes have instead a 6-member ring.

Synthesis of eicosanoids: Overview

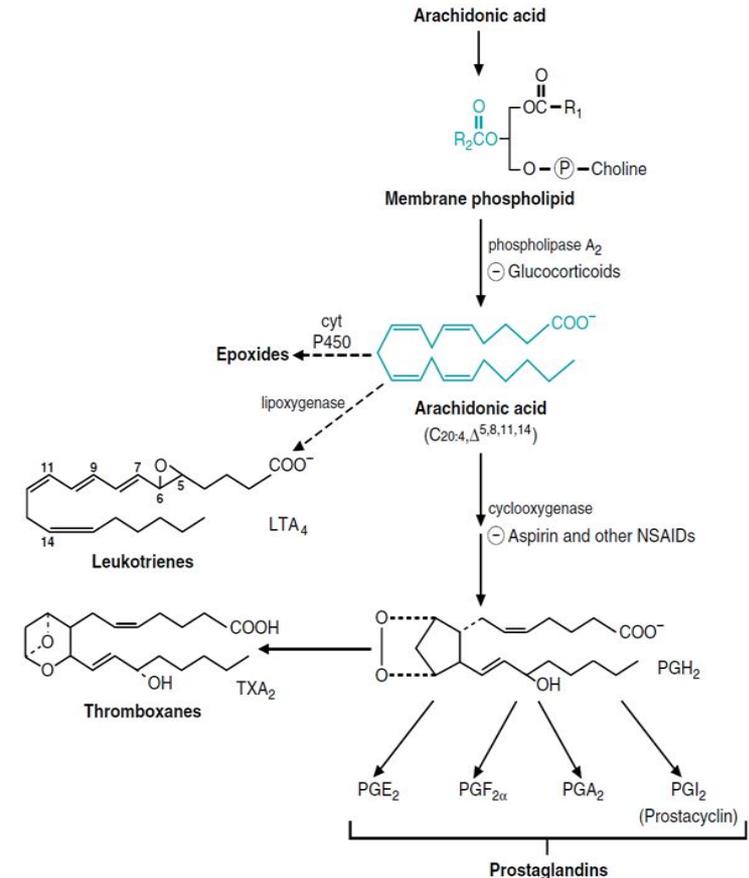


Main eicosanoid production sites

- Endothelial cells
- Leukocytes
- Platelets
- Kidneys
- Unlike e.g. histamin, eicosanoids are not synthesized in advance and stored in granules
- In case of an emergent need, they are rapidly produced from a released arachidonate
- Eicosanoid biosynthesis takes place in every cell type except red blood cells

Main steps of eicosanoid production

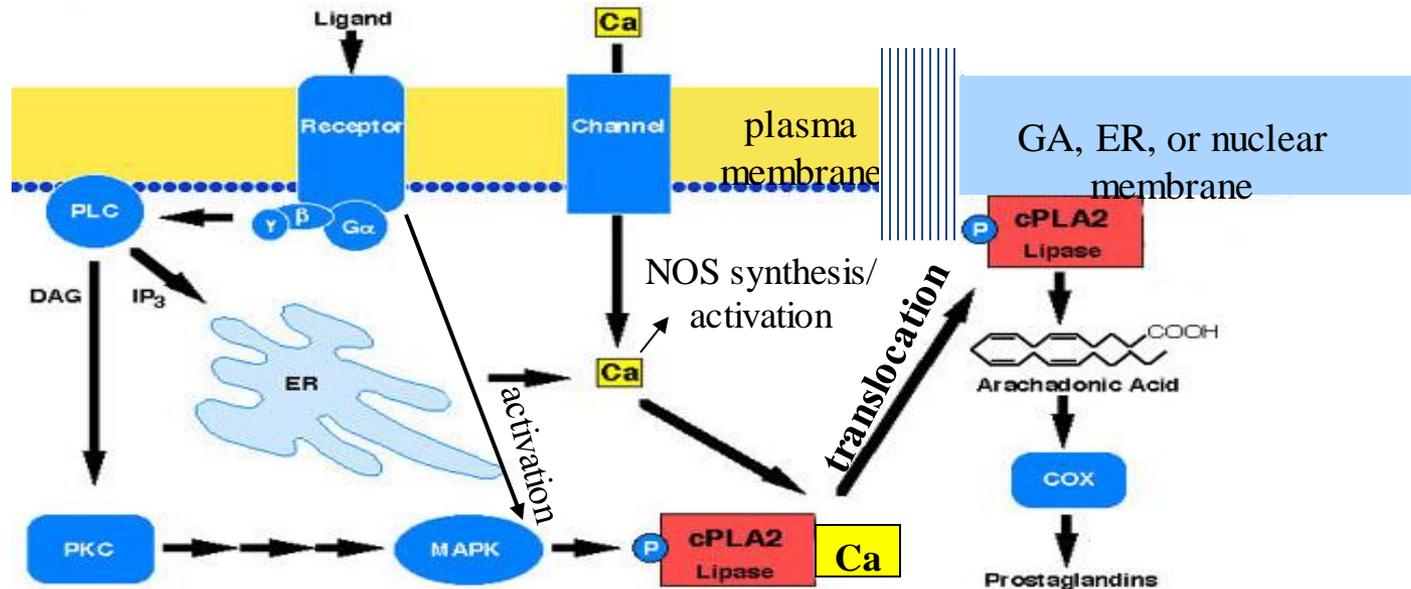
- 1- Activation of phospholipase A₂ (PLA₂)
- 2- Release of arachidonate from membrane phospholipids by PLA₂
- 3- Eicosanoids synthesis from arachidonate: COX or LO pathway further modifications by synthases/ isomerases depending on cell type.



1- Activation of phospholipase A₂

- PLA₂ is activated by ↑ intracellular Ca²⁺ concentration.
- PLA₂ phosphorylation by MAPK and CAMKII plays a stimulating role.
- Ligand binding to a receptor → phospholipase C activation : PIP₂ → DAG + IP₃, which opens Ca²⁺ channels in ER.
- By the action of Ca²⁺ and phosphorylation, PLA₂ is translocated to the membranes of GA, ER and/or nucleus, from which arachidonate is released.

Ligand: e.g. ATP released from dying cells



- PLA₂ hydrolyzes the ester linkage between a fatty acid and the OH at C2 of the glycerol, releasing the fatty acid & lysophospholipid.
- Corticosteroids are anti-inflammatory because they prevent the expression of PLA₂ → ↓ arachidonate release.
- There are multiple PLA₂ enzymes, subject to activation via different signal cascades, platelet activating factor is involved in activating some PLA₂ variants.

- Success to develop drugs that can inhibit particular isoforms of PLA₂ for treating inflammatory diseases has been limited by the diversity of PLA₂ enzymes, and the fact that arachidonate may give rise to inflammatory or anti-inflammatory eicosanoids in different tissues.

- After PI is phosphorylated to PIP₂, cleavage via phospholipase C yields diacylglycerol and IP₃.
- Arachidonate release from diacylglycerol is then catalyzed by diacylglycerol lipase.

PLA₂ expression / activity stimulate:

- Interleukin-1
- Angiotensin II
- Bradykinin
- Thrombin
- Epinephrine...

2- Arachidonate mobilization for eicosanoid synthesis from membrane phospholipids, mostly by the action of PLA₂

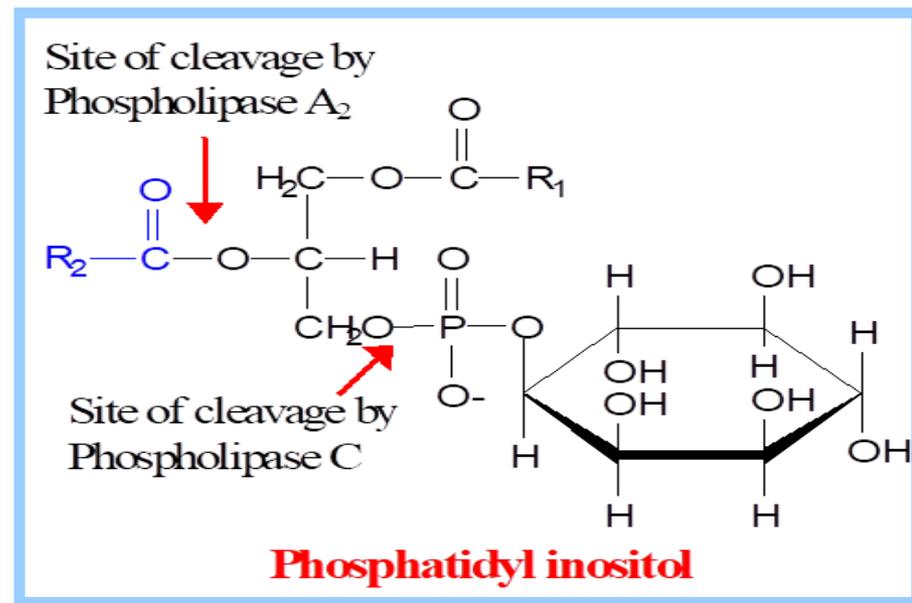
3- Eicosanoid biosynthesis

By 3 pathways:

- A- Cyclooxygenase (COX): produces prostaglandins, thromboxanes and prostacyclines.
- B- Lipoxygenase (LO): produces leukotrienes, lipoxins, hepoxilins and 12- and 15-HETE (hydroxyeicosatetraenoic acids)
- C- Cytochrome P450 enzyme (monooxygenase): produces HETE, e.g. 20-HETE; it is a main pathway in kidney proximal tubules.

PLA₂ expression / activity block:

- Dexametason (synt. corticoid)
- Annexin 1 (lipocortin) – protein inducible by glucocorticoids
- Caspase-3

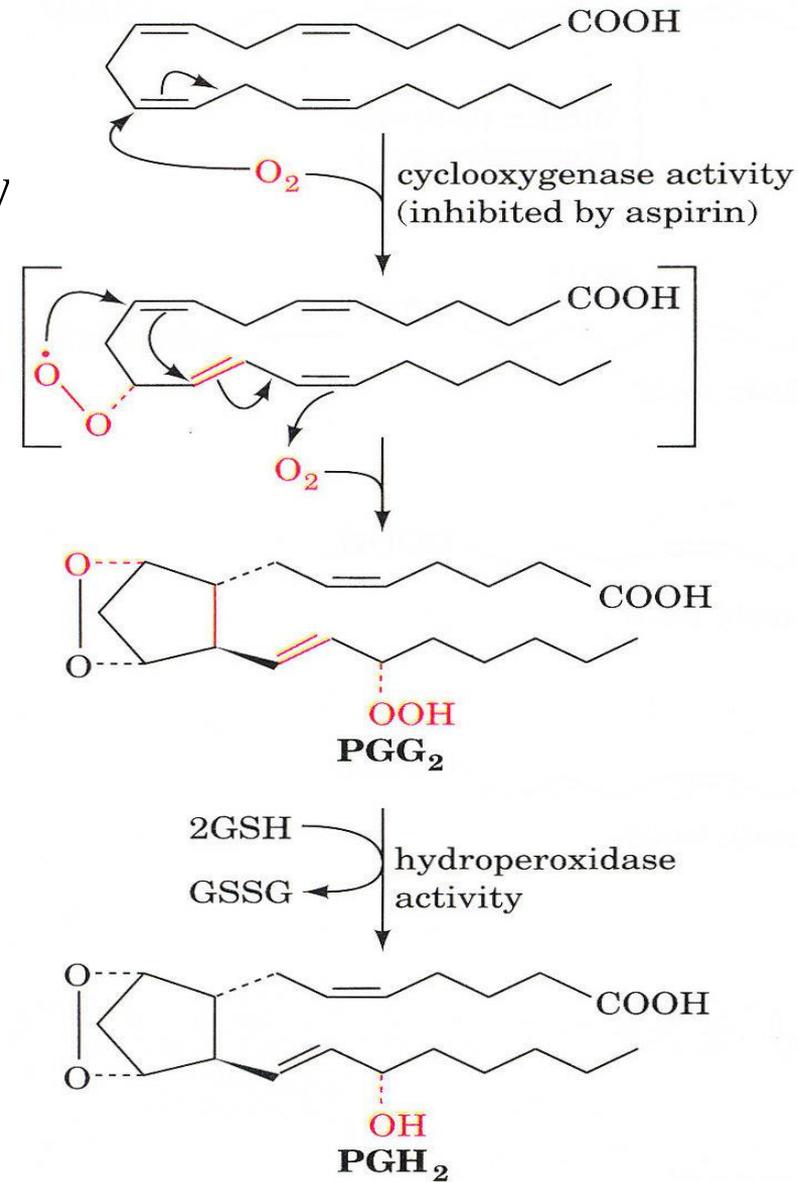


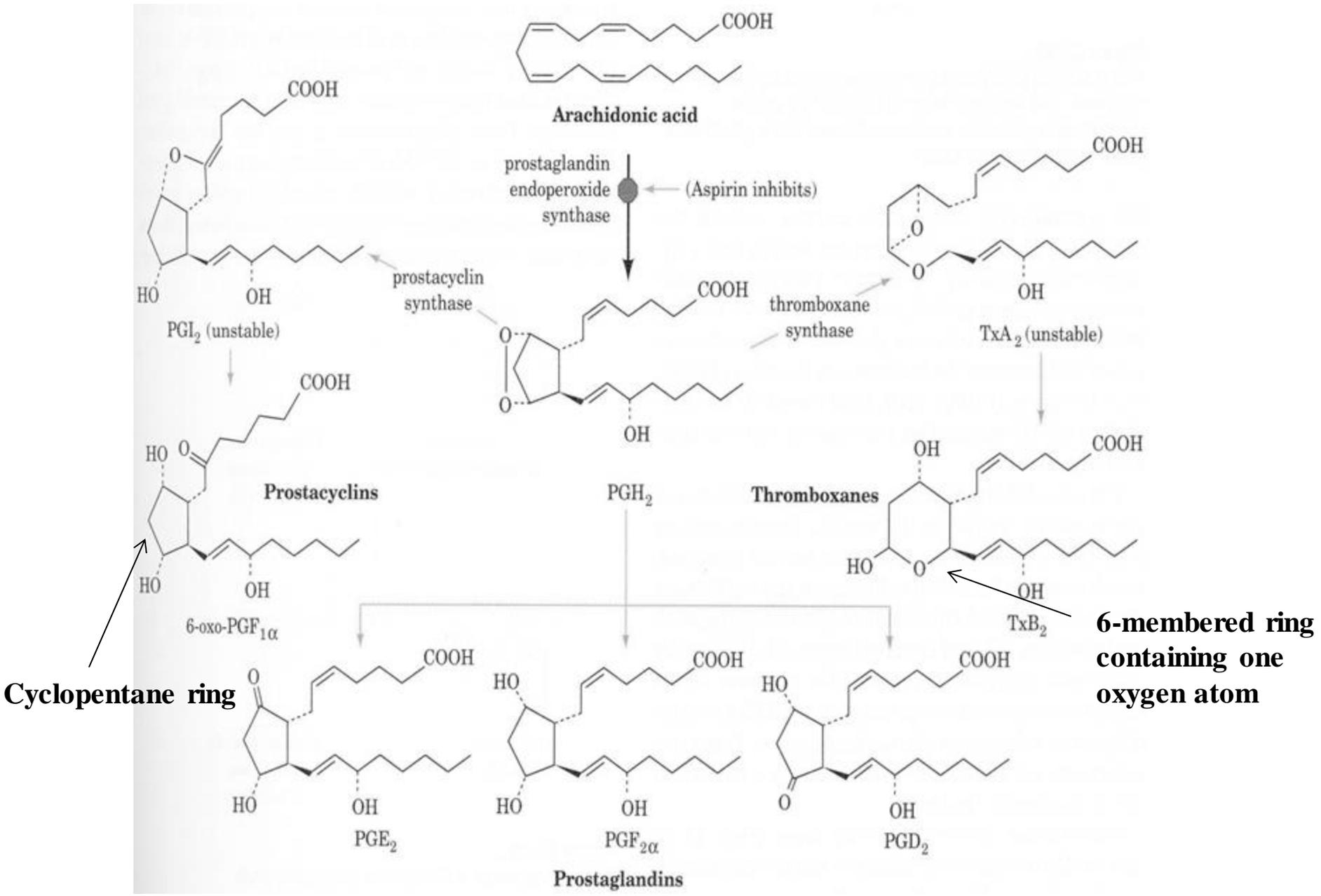
A- COX pathway

- PGH synthase (PGHS), catalyzes the committed step in the “cyclic pathway” that leads to the production of PGH_2 , a precursor for prostaglandins, prostacyclins & thromboxanes
- Different cell types convert PGH_2 to different compounds.
- PGHS exists in 2 isoforms (PGHS-1/COX-1, PGHS-2/COX-2) and has two different activities:
 - 1- Cyclooxygenase (COX): catalyses addition of two molecules of O_2 into arachidonate molecule forming PGG_2
 - 2- Hydroperoxidase: uses glutathione to convert PGG_2 into PGH_2
- A particular cell type produces mostly one particular prostanoid type: platelets produce almost exclusively thromboxanes (because of containing thromboxane synthase); vascular endothelial cells produce prostacyclins (because of containing prostacyclin synthase); myocardium cells produce mainly PGI_2 , PGE_2 , $\text{PGF}_{2\alpha}$

Inhibition of COX pathway

- Aspirin inhibits irreversibly COX activity of PGHS-1 and PGHS-2 (by acetylation of enzyme serine).
- Other non-steroidal anti-inflammatory drugs inhibit COX activity (ibuprofen competes with arachidonate).
- Anti-inflammatory corticosteroids block PGHS-2 transcription, so, they inhibit formation of prostaglandins involved in fever, pain and inflammation.
- They inhibit blood clotting by blocking thromboxane formation in platelets.
- Thromboxane A_2 stimulates platelets aggregation for blood clotting.
- Aspirin effect is long-lived because platelets lack a nucleus and do not make new enzyme.





-The two isoforms of PGH₂ Synthase:

1- COX-1 is constitutively expressed at low levels in many cell types.

2- COX-2 expression is highly regulated.

- Transcription of the gene encoding for COX-2 is stimulated by growth factors, cytokines, and endotoxins.

- COX-2 expression may be enhanced by cAMP, and in many cells PGE₂ produced as a result of COX-2 activity itself leads to changes in cAMP levels.

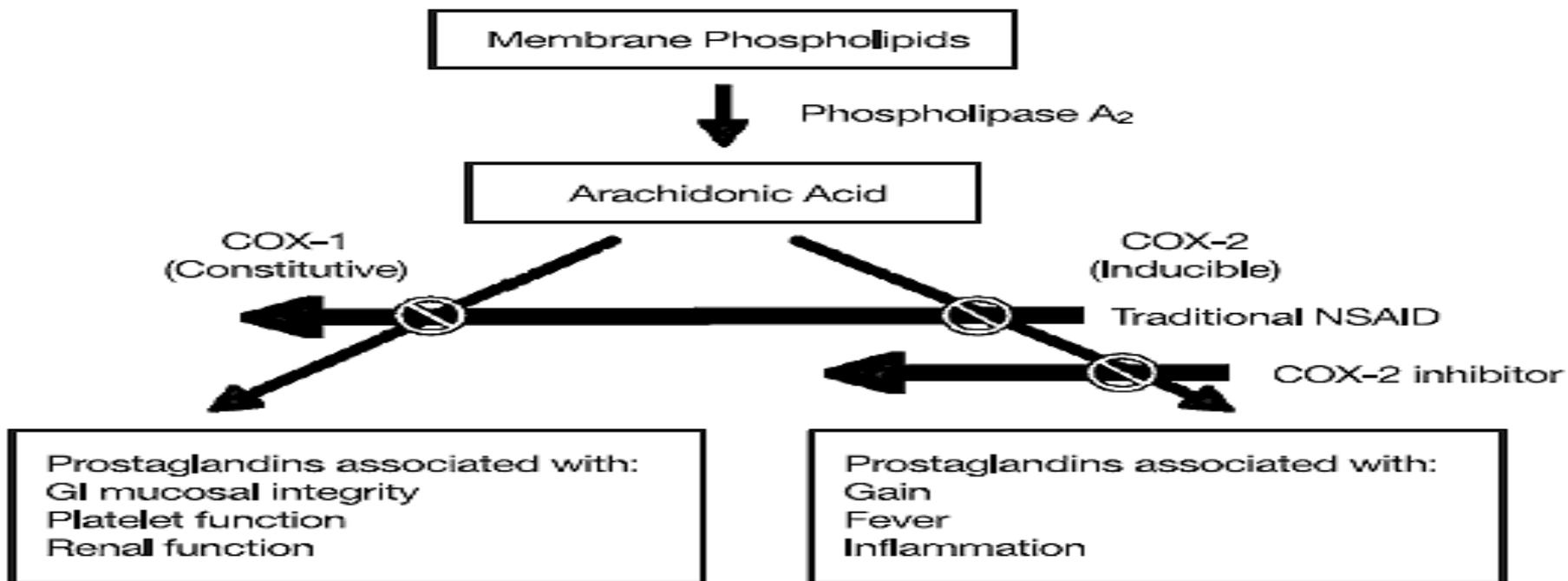
- Both catalyze PGH₂ formation, but differing localization within a cell of enzymes that convert PGH₂ into particular prostaglandins/ thromboxanes, may result in COX-1 and COX-2 yielding different ultimate products.

- COX-1 is essential for thromboxane formation in blood platelets, and for maintaining integrity of the gastrointestinal epithelium.

- COX-2 levels increase in inflammatory diseases such as arthritis.

- Inflammation is associated with up-regulation of COX-2 → increase amounts of particular prostaglandins.

- COX-2 expression is increased in some cancer cells, it enhances angiogenesis which is essential for tumor growth by increasing the expression of vascular endothelial growth factor (VEGF).
- Regular use of NSAIDs has been shown to decrease the risk of developing colorectal cancer.
- Most NSAIDs inhibit both COX I and COX II.
- Some evidence suggests the existence of a third isoform of PGH2 synthase, designated COX-3, with roles in mediating pain and fever.



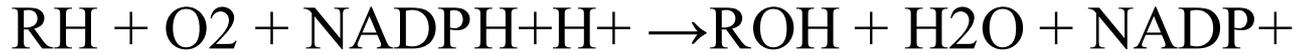
NSAID = nonsteroidal anti-inflammatory drug; COX = cyclooxygenase; GI = gastrointestinal.

B- LO pathway

- 3 different lipoxygenases introduce oxygen to position 5, 12 or 15 in arachidonate; a primary product is hydroperoxy-eicosatetraenoic acid (HPETE)
- Only 5-lipoxygenase produces leukotrienes; it requires protein FLAP (5-lipoxygenase activating protein).
- Many of the products have signal roles.
- Leukotrienes have roles in inflammation, produced in inflammation areas of blood vessel walls as part of the pathology of atherosclerosis.
- Leukotrienes are also implicated in asthmatic constriction of the bronchioles.
- Some leukotrienes act via specific G-protein coupled receptors (GPCRs) in the plasma membrane.
- Anti-asthma medications include:
 - 1- Inhibitors of 5-lipoxygenase
 - 2- Drugs that block leukotriene-receptor interactions (block binding of leukotrienes to their receptors on the plasma membranes of airway smooth muscle cells).

- **FLAP** binds arachidonate, facilitating its interaction with the enzyme.
- Translocation of 5-lipoxygenase from the cytoplasm to the nucleus, and formation of a complex including 5-lipoxygenase, FLAP and PLA₂ in association with the nuclear envelope has been observed during activation of leukotrienes synthesis in leukocytes.

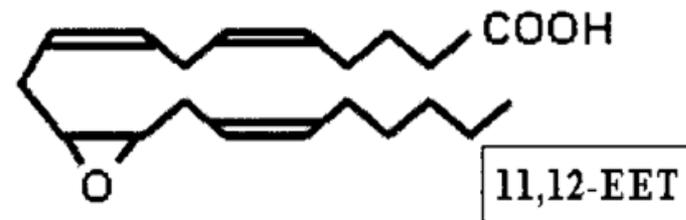
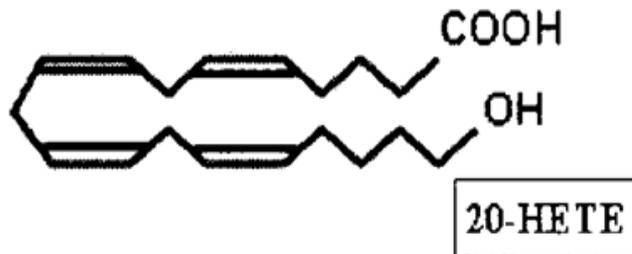
C- **Synthesis of eicosanoids by enzymes CYP450** (monooxygenase)



- Two types of compounds are produced:

1- **Epoxygenases** catalyze production of **epoxyeicosatrienoic acids (EETs)**, which are metabolized by epoxid-hydrolases into almost inactive dihydroxyeicosatrienoic acids (DiHETEs)

2- **Hydroxylases** catalyze production of **HETEs** (20-HETE, 13-HETE etc.).



Prostaglandin receptors:

- Prostaglandins and related compounds are transported out of the cells that synthesize them.
- Most affect other cells by interacting with plasma membrane G-protein coupled receptors.
- Depending on the cell type, the activated G-protein may stimulate or inhibit formation of cAMP, or may activate a phosphatidylinositol signal pathway leading to intracellular Ca^{++} release.
- Another prostaglandin receptor, designated **PPAR γ** , is related to a family of **nuclear receptors** with transcription factor activity.
- Different receptors for a particular prostaglandin may activate different signal cascades.
- Effects of a particular prostaglandin may vary in different tissues, depending on which receptors are expressed. e.g., in different cells PGE_2 may activate either G_s or G_i proteins, leading to either \uparrow or \downarrow in cAMP formation.

Prostanoid signaling

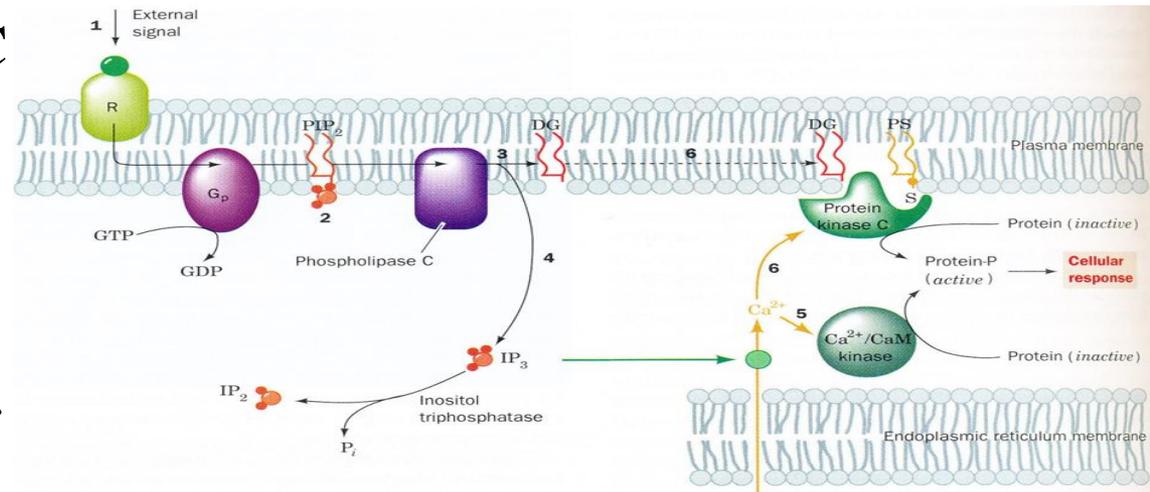
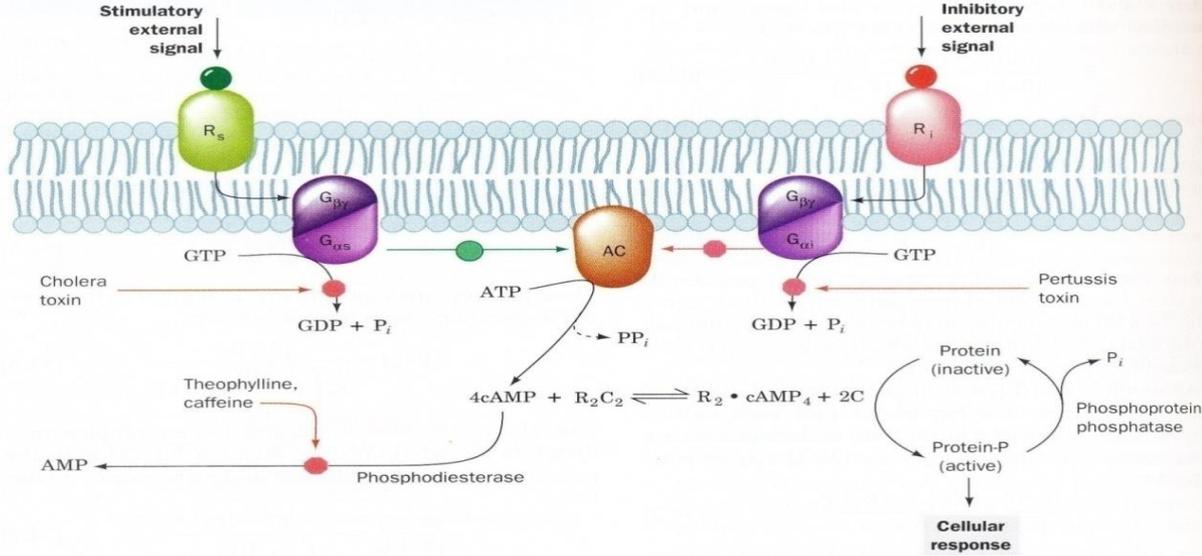
-Through G-protein-coupled receptors:

A- G_{α_s} activate adenylate cyclase $\rightarrow \uparrow$ cAMP \rightarrow activates PKA

B- G_{α_i} inhibit adenylate cyclase

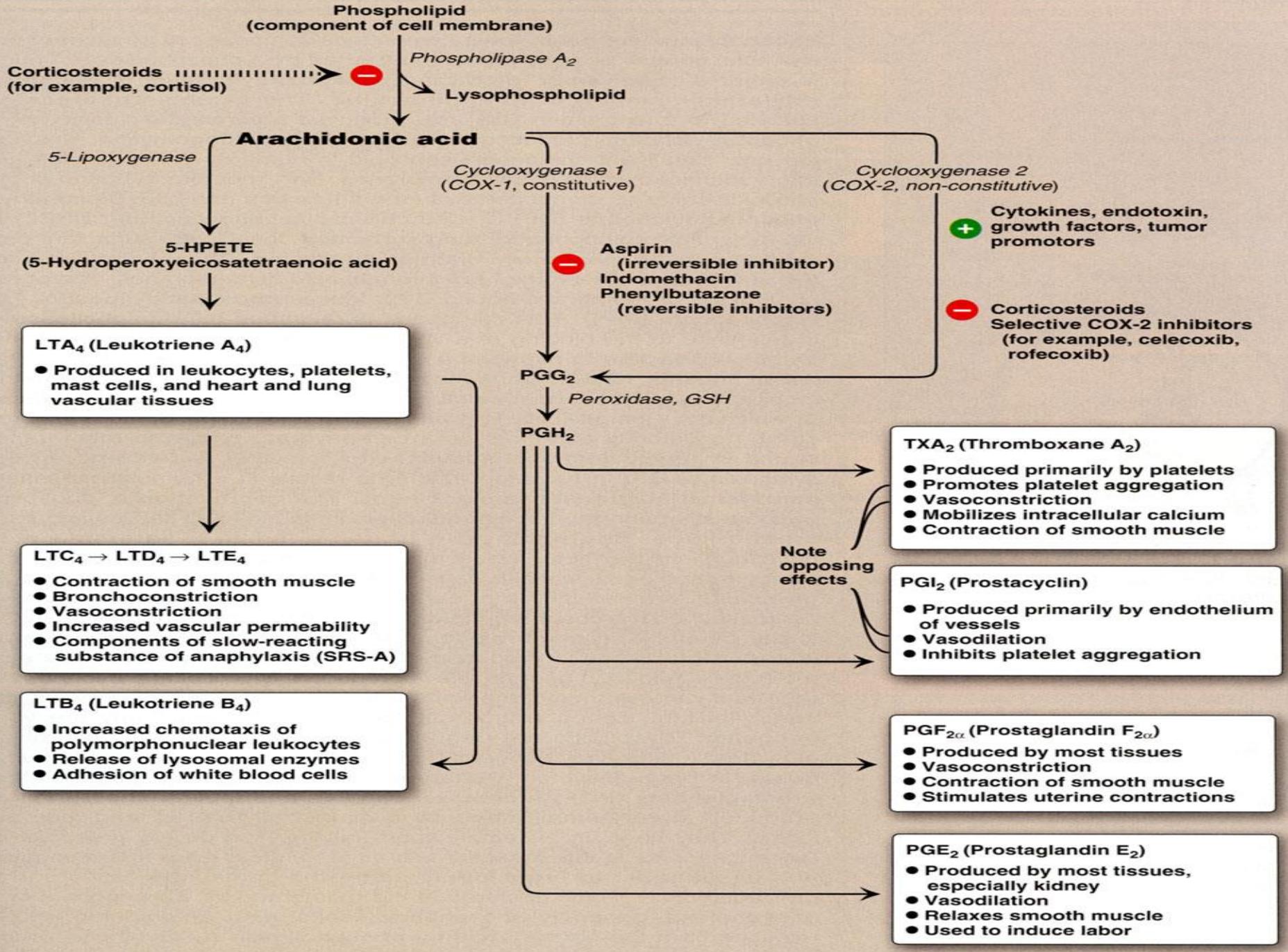
C- G_q activates phospholipase C (it requires Ca^{2+}), which cleaves PIP_2 to IP_3 and DAG).

- DAG and Ca^{2+} activate PKC,
- IP_3 opens Ca^{2+} channels in ER.



Slow reacting substance of anaphylaxis (SRS-A)

- It is a mixture of LTC₄, LTD₄ and LTE₄
- More potent than histamine, constrictor of bronchial airway musculature
- Increase vascular permeability
- Attraction and activation of leucocytes.





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