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.

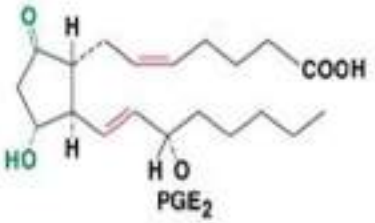
5- There are various eicosanoid molecules and of receptors causing different effects in the organism.

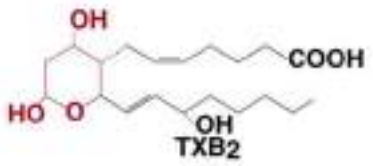
6- Participate in intercellular signaling and intracellular signal cascades.

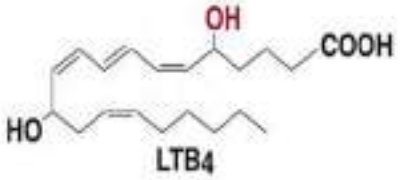
Types of eicosanoids:

- 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
  - Reproductive processes
    Control & tissue growth

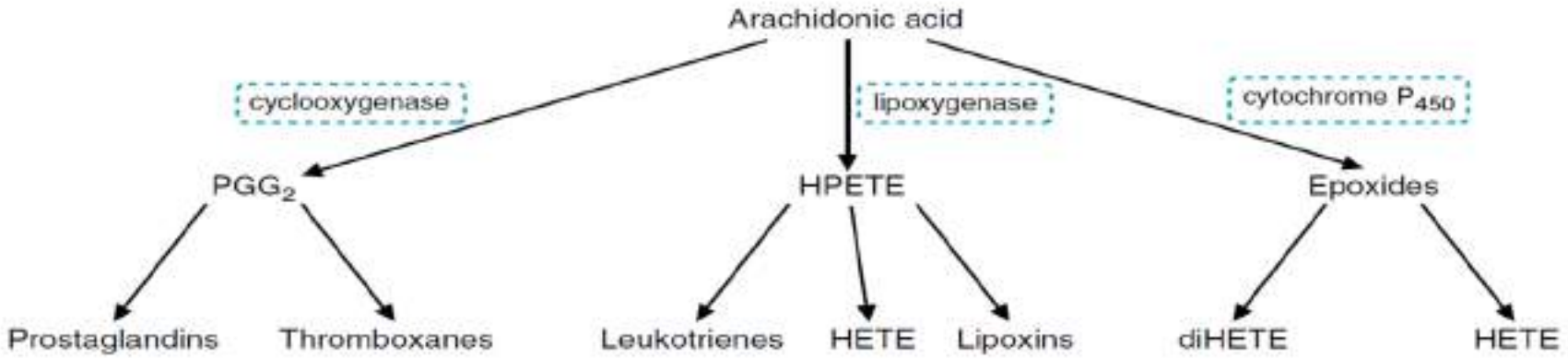






- Prostaglandins all have a cyclopentane ring, while, thromboxanes have instead a 6-member ring.

Synthesis of eicosanoids: Overview



Main eicosanoid production sites

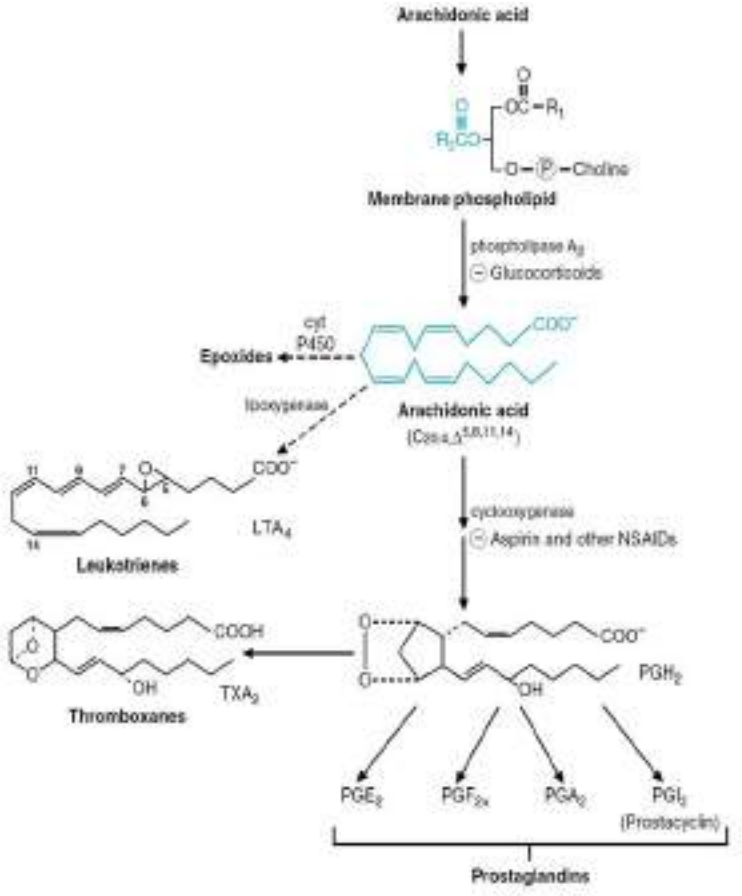
- Endothelial cells - Leukocytes - Platelets - Kidneys
- Unlike e.g. histamin, eicosanoids are not synthesized in advance and stored in granules
- In case of an emergent need, they are rapidly produced from a released arachidonate
- Eicosanoid biosynthesis takes place in every cell type except red blood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

Main steps of eicosanoid production

1- Activation of phospholipase A_2 (PLA_2)

2- Release of arachidonate from membrane phospholipids by PLA_2

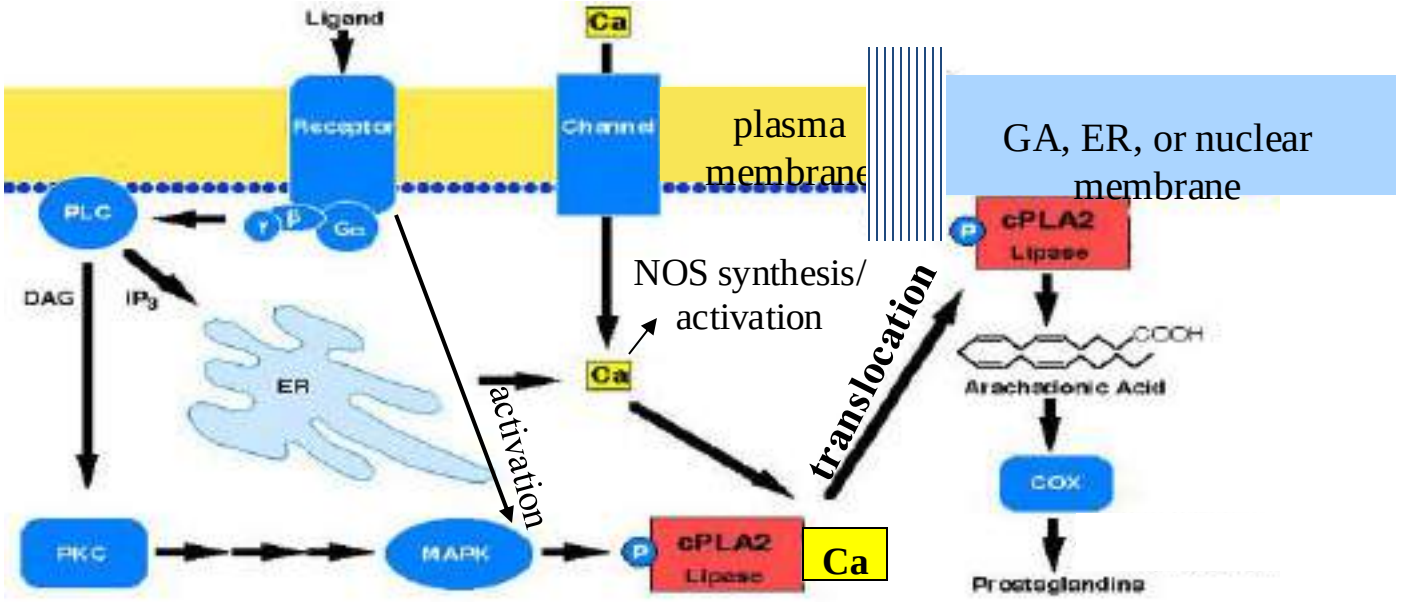
3- Eicosanoids synthesis from arachidonate: COX or LO pathway further modifications by synthases/ isomerases depending on cell type.



1- Activation of phospholipase A_2

- PLA_2 is activated by ↑ intracellular Ca^{2+} concentration.
- PLA_2 phosphorylation by MAPK and CAMKII plays a stimulating role.
- Ligand binding to a receptor → phospholipase C activation : PIP_2 → DAG + IP_3, which opens Ca^{2+} channels in ER.
- By the action of Ca^{2+} and phosphorylation, PLA_2 is translocated to the membranes of GA, ER and/or nucleus, from which arachidonate is released.

Ligand: e.g. ATP released from dying cells



- PLA_2 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 PLA_2 → ↓ arachidonate release.
- There are multiple PLA_2 enzymes, subject to activation via different signal cascades, platelet activating factor is involved in activating some PLA_2 variants.

- Success to develop drugs that can inhibit particular isoforms of PLA_2 for treating inflammatory diseases has been limited by the diversity of PLA_2 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.

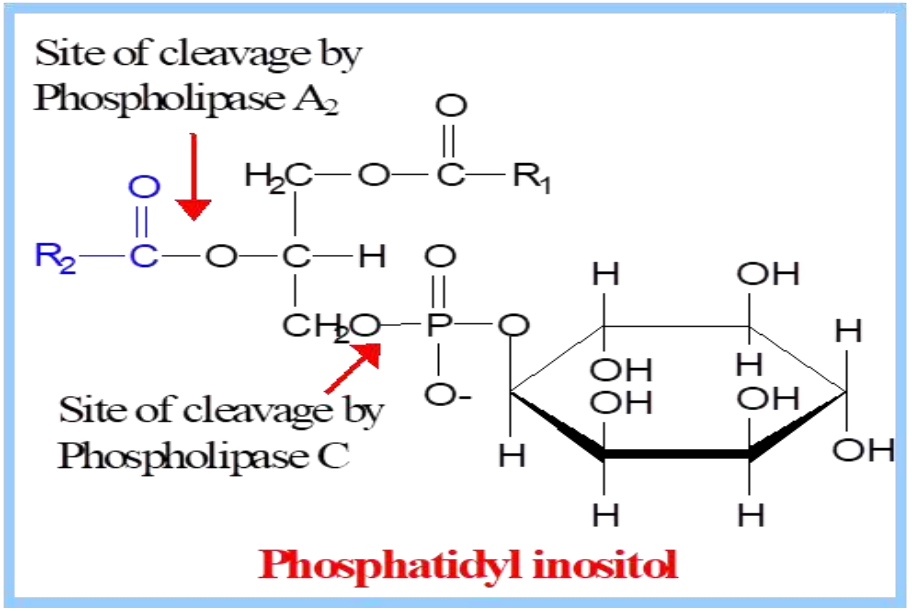
PLA_2 expression / activity stimulate:

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

PLA_2 expression / activity block:

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

2- Arachidonate mobilization for eicosanoid synthesis from membrane phospholipids, mostly by the action of PLA_2



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.

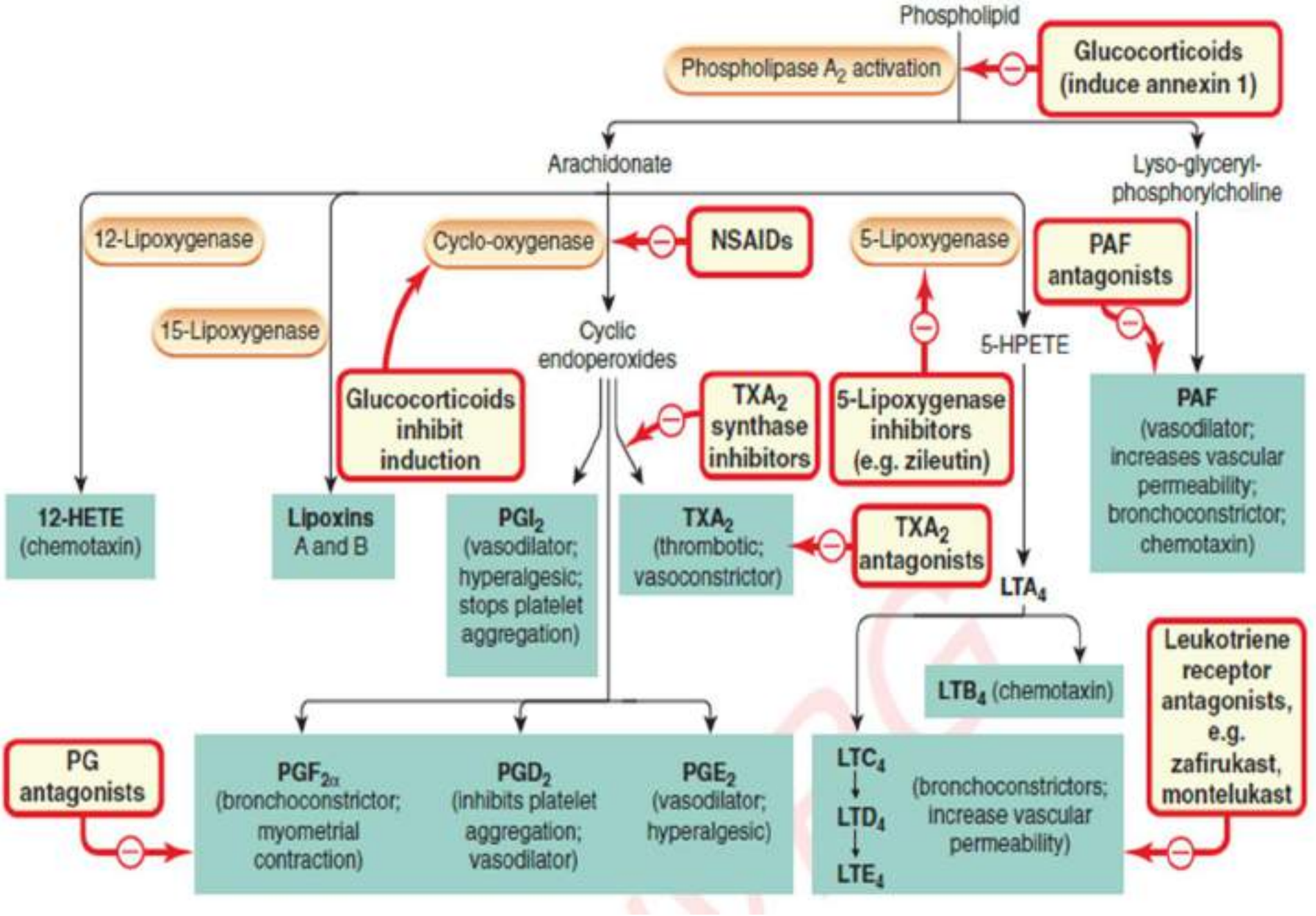
A- <u>COX pathway</u>

- PGH synthase (PGHS), catalyzes the committed step in the "cyclic pathway" that leads to the production of PGH_2, a precursor for prostaglandins, prostacyclines & 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

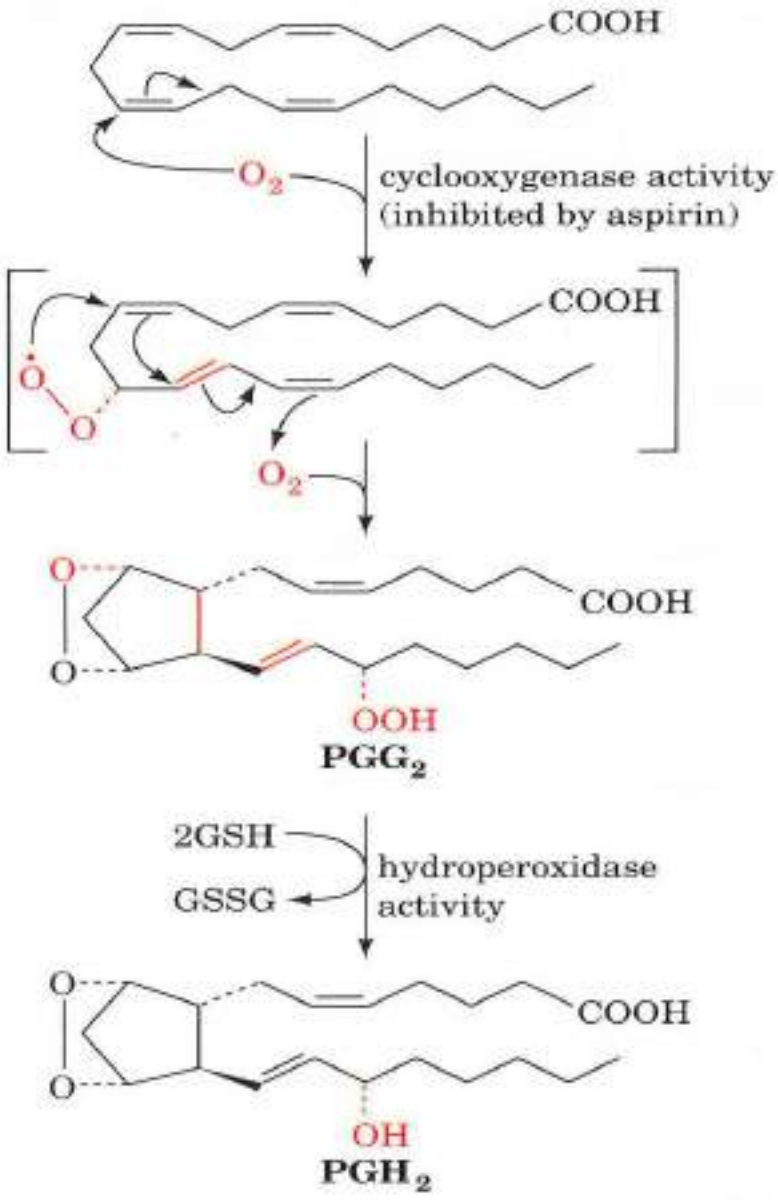
- A particular cell type produces mostly one particular prostanoid type: platelets produce almost exclusively tromboxanes (because of containing thromboxane synthase); vascular endothelial cells produce prostacyclins (because of containing prostacyclin synthase); myocardium cells produce mainly PGI_2, PGE_2, $PGF_{2\alpha}$


Phospholipid
Phospholipase A_2 activation
Glucocorticoids (induce annexin 1)
Arachidonate
Lyso-glyceryl-phosphorylcholine
12-Lipoxygenase
Cyclo-oxygenase
NSAIDs
5-Lipoxygenase
PAF antagonists
15-Lipoxygenase
Cyclic endoperoxides
5-HPETE
Glucocorticoids inhibit induction
TXA_2 synthase inhibitors
5-Lipoxygenase inhibitors (e.g. zileutin)
PAF (vasodilator; increases vascular permeability; bronchoconstrictor; chemotaxin)
12-HETE (chemotaxin)
Lipoxins A and B
PGI_2 (vasodilator; hyperalgesic; stops platelet aggregation)
TXA_2 (thrombotic; vasoconstrictor)
TXA_2 antagonists
LTA_4
LTB_4 (chemotaxin)
Leukotriene receptor antagonists, e.g. zafirukast, montelukast
PG antagonists
$PGF_{2\alpha}$ (bronchoconstrictor; myometrial contraction)
PGD_2 (inhibits platelet aggregation; vasodilator)
PGE_2 (vasodilator; hyperalgesic)
LTC_4
LTD_4
LTE_4
(bronchoconstrictors; increase vascular permeability)

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.



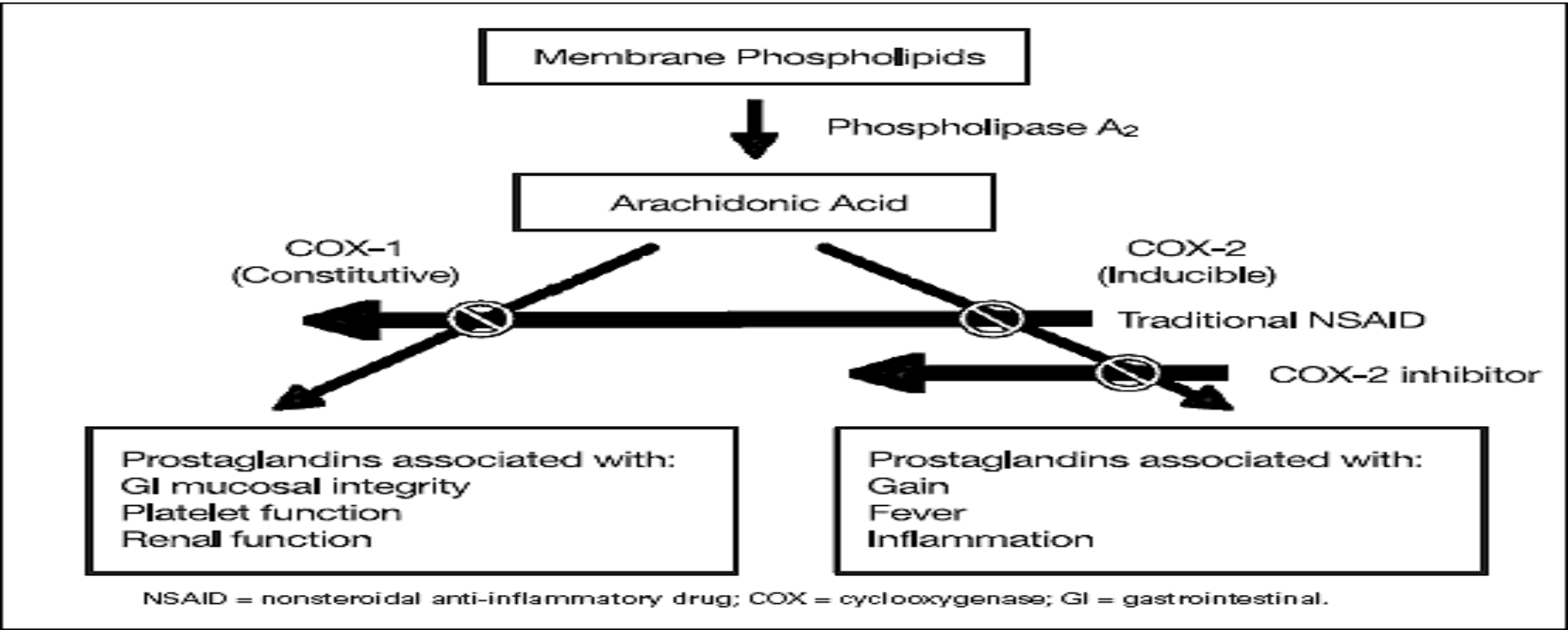
-The two isoforms of PGH_2 Synthase:

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 PGE_2 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.

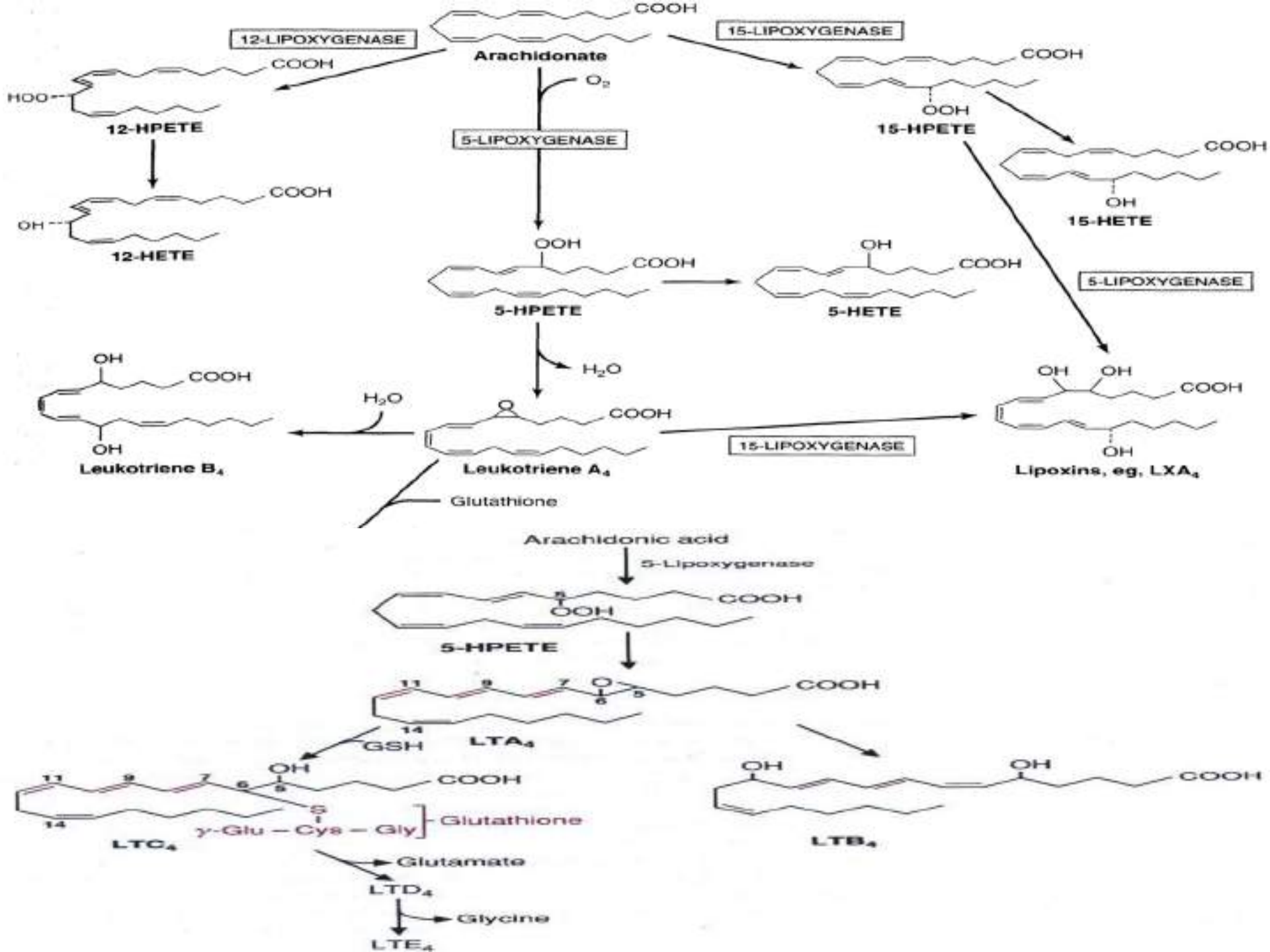


NSAID = nonsteroidal anti-inflammatory drug; COX = cyclooxygenase; GI = gastrointestinal.

	COX-1	COX-2
Tissue expression	**Constitutive** enzyme expressed in most tissues (housekeeping function).	**Inducible** in many tissues by many stimuli including growth factors (TNF-α) and cytokines (IL-1). **Constitutive** in brain, kidney and vessels.
Function	Regulates normal cellular processes , such as platelet aggregation, GIT cytoprotection, renal blood flow auto-regulation, and initiation of labor.	Inflammation, fever, pain, renal function, production of vascular PGI2
Inhibitors	Most of classical NSAIDs (diclofenac, ibuprofen, indomethacin).	Many NSAIDs drugs and also selective COX-2 inhibitors like celecoxib

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-lipooxygenase 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).

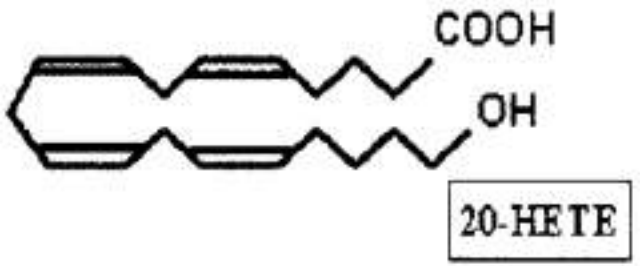

12-LIPOXYGENASE
15-LIPOXYGENASE
Arachidonate
COOH
O_2
5-LIPOXYGENASE
HOO
12-HPETE
OOH
15-HPETE
OH
12-HETE
15-HETE
5-HPETE
5-HETE
5-LIPOXYGENASE
H_2O
Leukotriene B_4
Leukotriene A_4
15-LIPOXYGENASE
Lipoxins, eg, LXA_4
Glutathione
Arachidonic acid
5-Lipoxygenase
5-HPETE
GSH
LTA_4
LTC_4
γ-Glu — Cys — Gly
Glutathione
LTB_4
Glutamate
LTD_4
Glycine
LTE_4

- **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 PLA_2 in association with the nuclear envelope has been observed during activation of leukotrienes synthesis in leukocytes.

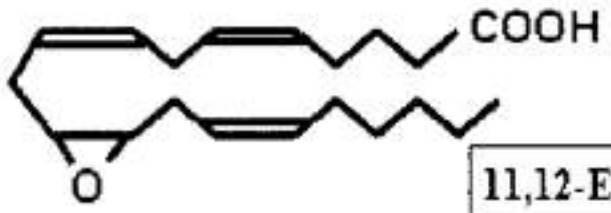
C- **<u>Synthesis of eicosanoids by enzymes CYP450</u>** (monooxygenase)

$RH + O2 + NADPH+H+ \rightarrow ROH + H2O + NADP+$

- 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.).



20-HETE



11,12-EET

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 **PPARγ**, 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 Gs or Gi proteins, leading to either ↑or ↓in cAMP formation.

Prostanoid signaling

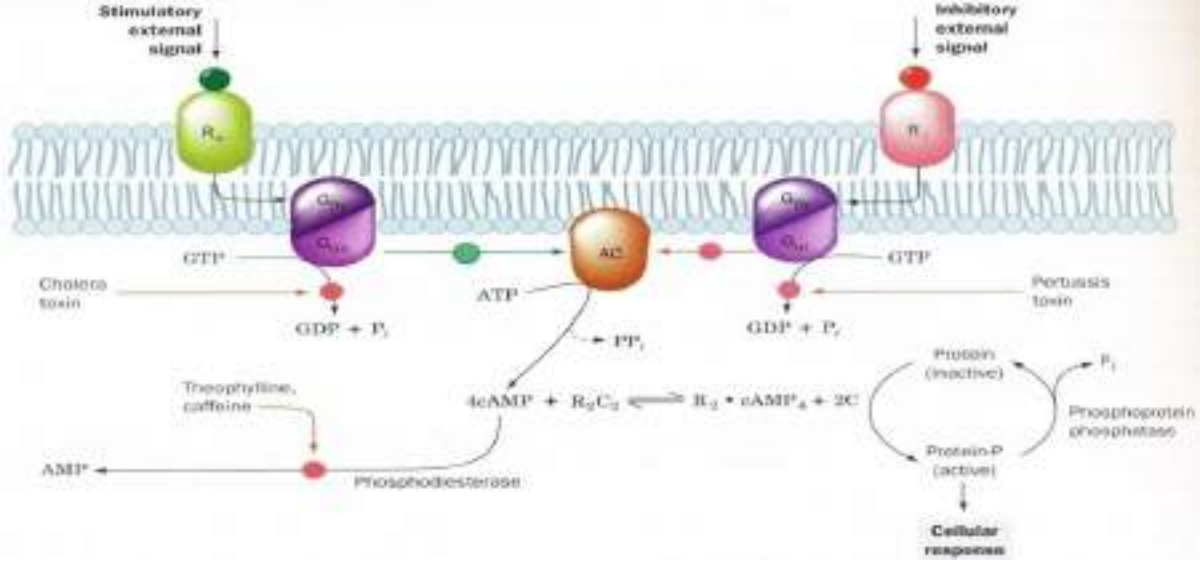
-Through G-protein-coupled receptors:

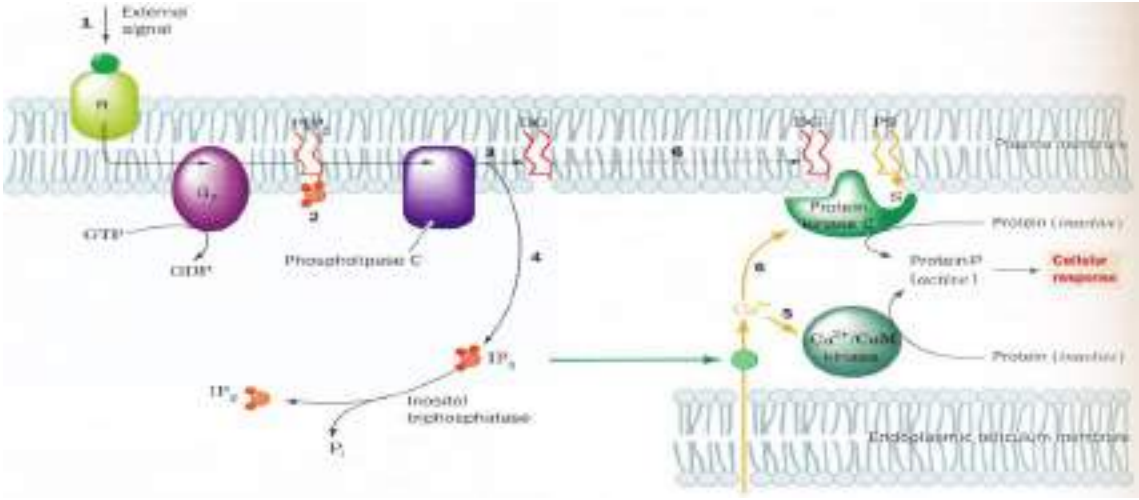
A- $G_{\alpha s}$ activate adenylate cyclase → ↑ cAMP → activates PKA

B- $G_{\alpha i}$ inhibit adenylate cyclase

C- G_q activates phospholipase C (it requires Ca^{2+}), which cleaves PIP_2 to IP3 and DAG).

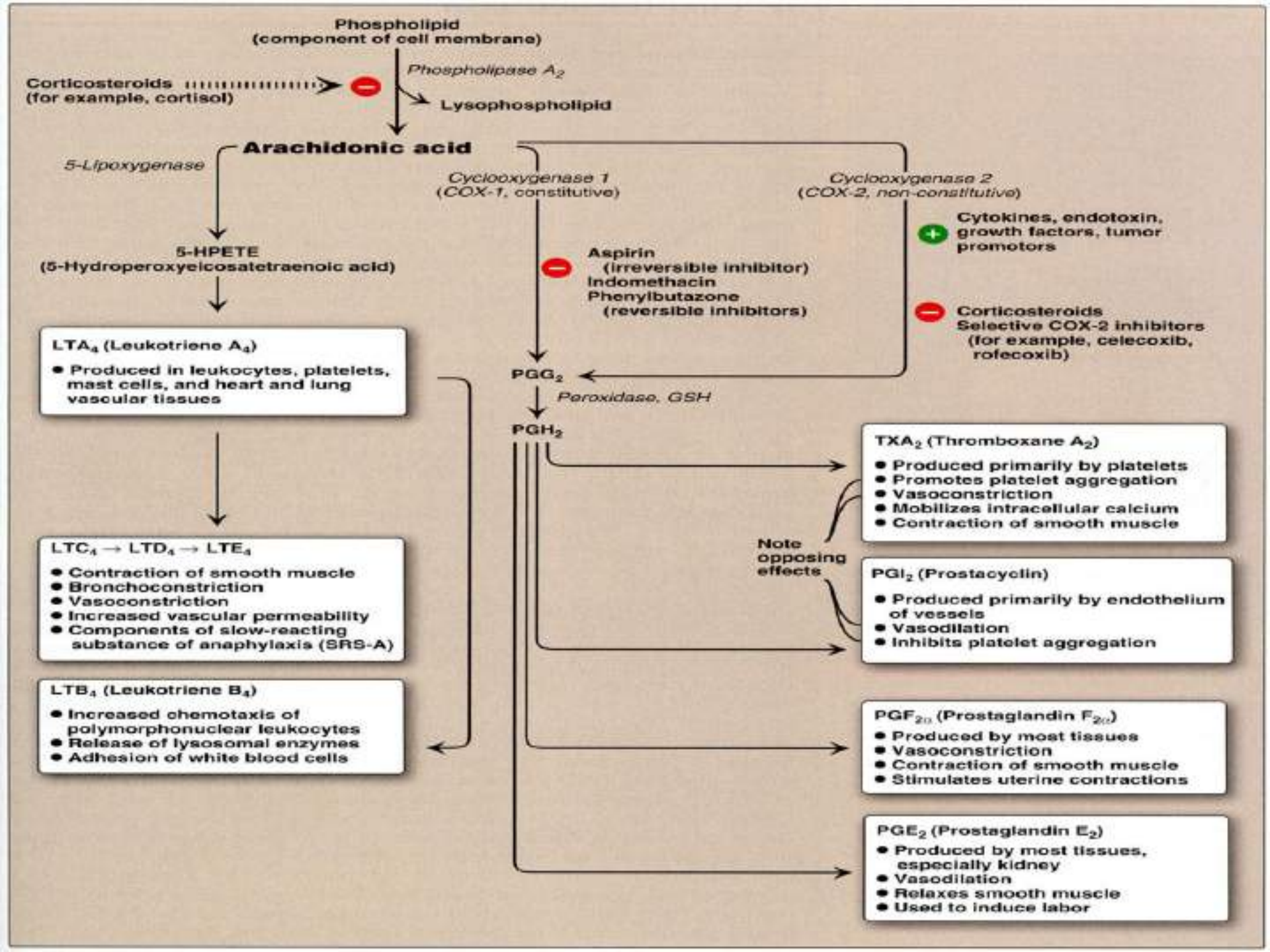
- DAG and Ca^{2+} activate PKC,
- IP_3 opens Ca^{2+} channels in ER.





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.


Phospholipid
(component of cell membrane)
Corticosteroids
(for example, cortisol)
Phospholipase A2
Lysophospholipid
Arachidonic acid
5-Lipoxygenase
Cyclooxygenase 1
(COX-1, constitutive)
Cyclooxygenase 2
(COX-2, non-constitutive)
Cytokines, endotoxin, growth factors, tumor promotors
5-HPETE
(5-Hydroperoxyeicosatetraenoic acid)
Aspirin
(irreversible inhibitor)
Indomethacin
Phenylbutazone
(reversible inhibitors)
Corticosteroids
Selective COX-2 inhibitors
(for example, celecoxib, rofecoxib)
LTA4 (Leukotriene A4)
Produced in leukocytes, platelets, mast cells, and heart and lung vascular tissues
PGG2
Peroxidase, GSH
PGH2
TXA2 (Thromboxane A2)
Produced primarily by platelets
Promotes platelet aggregation
Vasoconstriction
Mobilizes intracellular calcium
Contraction of smooth muscle
Note opposing effects
LTC4 → LTD4 → LTE4
Contraction of smooth muscle
Bronchoconstriction
Vasoconstriction
Increased vascular permeability
Components of slow-reacting substance of anaphylaxis (SRS-A)
PGI2 (Prostacyclin)
Produced primarily by endothelium of vessels
Vasodilation
Inhibits platelet aggregation
LTB4 (Leukotriene B4)
Increased chemotaxis of polymorphonuclear leukocytes
Release of lysosomal enzymes
Adhesion of white blood cells
PGF2α (Prostaglandin F2α)
Produced by most tissues
Vasoconstriction
Contraction of smooth muscle
Stimulates uterine contractions
PGE2 (Prostaglandin E2)
Produced by most tissues, especially kidney
Vasodilation
Relaxes smooth muscle
Used to induce labor

G
O
O
D
L
U
C
K