Before start talking about today's lecture let's revise some points of JAKs (janus kinases)

1*.they are a family of intracellular , non-receptor tyrosine kinases*

2*. they transmit signals via JAK-STAT pathway*

3*.for Growth hormone , JAK 2 is the tyrosine kinase that's associated with its receptor, (the association of JAK2 with the receptor is not covalent , and is increased by the phosphorylation of the receptor)*

Now we will talk about other kinds of receptors in which tyrosine kinase is an integral part of them (the receptor itself is a tyrosine kinase)

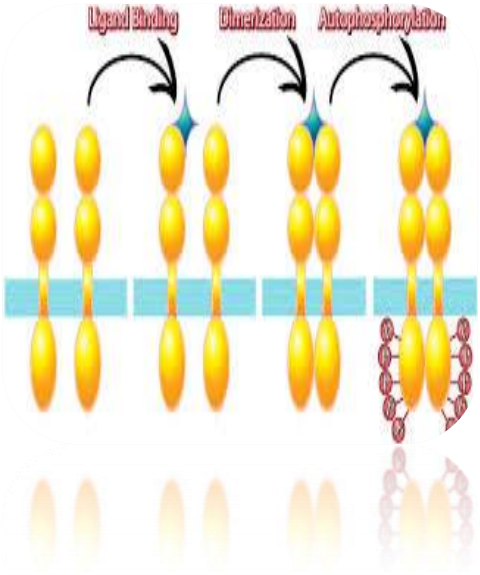
1. EGF (Epidermal growth factor)receptor :

a.**EGF**, is a hormone that is required for growth and development of skin and derma

b. it was discovered accidently

c.it,(the receptor), is monomeric (inactive)

d.EGF binding leads to dimerization of receptor then autophosphorylation , in which each monomer phosphorylates the other (cross phosphorylation),and now the receptor is activated and leads to activation of target proteins.



2.insulin receptor

a.insulin receptor composed of four subunits of (**2alpha & 2 beta**)subunits

b. each alpha subunit and beta subunit make what so called (alpha-beta pair), and the result is dimer of 2 (alpha-beta pairs)

c.these pairs are bounded together via disulfide bridge

d.thus , the receptor itself is a dimer (not monomer as EGF receptor), even when insulin is not bound

e. binding of insulin will lead to activation of integral tyrosine kinase

f. so , dimerization is important(but not sufficient) for activation of the receptor.

Do these receptors transfer information across the membrane in the same way ??

The question was answered by synthesizing a gene that encoded a chemeric of receptors (mixture of receptors), in which the extracellular part came from insulin receptor , and the membrane-spanning and cytosolic part came from EGF receptor . The striking result was that the binding of insulin induced tyrosine kinase activity , as evidenced by rapid autophosphorylation .Hence ,the insulin receptor and the EGF receptor employ a common mechanism of signal transmission across the plasma membrane.

Note that the chemeric receptor is inactive until the binding of insulin .

Epidermal growth factor signaling pathway

Phosphorylation of receptors leads to activation of subsequent proteins , these proteins act sequentially to mediate the effect of growth factor, and the proteins participate in EGF signaling are :

1. GRB2 2. SOS 3. RAS

Now ,Read the following points to understand the signaling pathway

1. first, binding of EGF hormone to its receptor leads to autophosphorylation of the receptor (as we mentioned previously),then GRB2 protein come and through its SH2 domain bind to phosphorylated tyrosine residue

2. this protein (GRB2) , also has 2 SH3 domains that bind to SOS protein, so it's activated

3.the binding of SOS allow another protein called RAS to come and bind to it,(RAS is a guanine binding protein , it binds GDP , but once binds to SOS , it exchanges GDP with GTP and become activated).

4.then , it leads to activation of specific protein kinases.

RAS PROTEIN

1. it's a monomeric protein (not a trimer as ordinary G proteins)

2.exist in 2 forms (GDP-inactive,GTP-active)

3.smaller than G protein (resembles the alpha subunit of G protein in structure & mechanism

4.is a member of small G protein family, and also it includes several groups or subfamilies.

5.has GTPase activity

6.it has a major role in growth,differentiation,cellular transport and motility.

DEFECTS IN SIGNALING PATHWAY CAN LEAD TO CANCER AND OTHER DISEASES

*cancer is characterized by uncontrolled growth, one of the causes is that the regulatory or signaling pathways are continuously turned on without stopping and by that will progressively stimulate the growth & differentiation of cells ,leading to cancer

*certain viruses can cause cancer

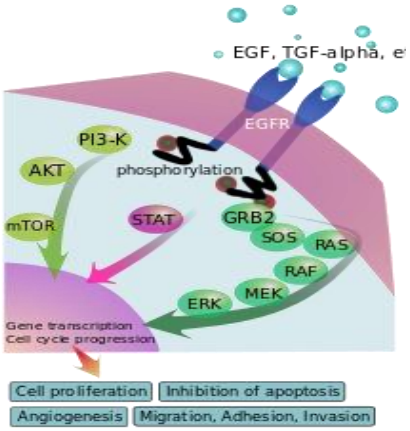
*OR can be associated with defect in signal transduction proteins ; failure of signal transduction process

1/One of the viruses that cause cancer is <u>ROUS SARCOMA VIRUS ,</u> that cause sarcoma in chickens.

How do it causes sarcoma ???

1.**Rous sarcoma virus** contains an oncogene called **v-src** ,the host form of that oncogene is called proto-oncogene **c-src.**

2. it has been shown that these tumor viruses picked up their oncogenes by incorporating normal host genes into their genomes , then



undergo some mutations that lead to changes in that gene and subsequent cancer .
a.the normal host **c-src** gene encodes a tyrosine kinase protein, its structure is composed of SH2 , SH3 domains and protein kinase domain

note that there is a minimal difference between c-src & v-src in a way that cause v –src always active (we will talk about that)

b.when SRC is inactive ,**1**. the phosphorylated tyrosine group interacts with SH2 domain , and **2**. SH3 domain interacts with poly-proline sequence on protein kinase domain, maintaining the inactive unit tightly bound
c.as we mentioned some mutations lead to conversion of proto-oncogen into oncogen that are continuously active (because the tyrosine residues are not phosphorylated , and by that no interaction will occur with SH2 domain to mediate the inactive form)
d.thus ,the presence of this oncogene leads to continuous signal transduction of growth factor and subsequent uncontrolled growth .
the oncogene is trapped in the on position in three ways:
1-SH2 displacement.
2-SH3 displacement.
3- dephosphorylation.
Protein kinase inhibitors are used in such cases of cancer.

2/Impaired GTPase activity can lead to cancer in humans
1.In mammalian cells there are 3 RAS proteins .
2. Mutations will lead to loss ability to hydrolyze GTP .
3.by that , the RAS is locked in ' ON' position and lead to continuous stimulation of growth .

Cholera & whooping cough are due to altered G-protein activity,how?

1. the cholera is a bacterial disease that causes dehydration and diarrhea, and can lead to death as well.
2. the vibrio cholera do not enter the cells.
3.the disease of cholera arises from cholera toxin that causes the toxic manifestations by the following mechanism
*first , cholera toxin is a protein composed of 2 functional subunits
a.***B subunit*** , binds to GM1 gangliosides of the intestinal epithelium
b.***A subunit*** (catalytic subunit) , which enter the cell,and catalyzes the covalent modification of G-alpha subunit protein by attachment of ADP-ribose **(derived from NAD**)to an arginine residue of G-alpha- s protein
c. this modification leads to stabilization of GTP-bound form of G-alpha s
d. the activated G protein then activates protein kinase A.
e. chloride channels will open continuously , and chloride will diffuse (along with sodium) to the lumen , and water will follow them (osmosis).
f.large amount of water will lost leading to dehydration .

EICOSANOIDS

Eicosanoids are 20 carbon signal molecules that play an important role in inflammation response and in platelet aggregation

1.composed of several classes ;(**prostaglandins, thromboxanes** and **leukotrienes**).
Prostaglandin name was originated because it was discovered for the first time in prostate secretions.

2.produced in almost all tissues

3.they act locally (not carried by circulation) , and affect neighboring cells(paracrine effect) and cells that produce them(autocrine effect).

4.they produce wide range of responses in target cells.

5.they are very potent (although they found in very low concentration).

6.not stored (produced and released immediately).

7.they have short half life.

8. different classes have opposite effects .

Functions of prostaglandins and thromoxanes

The doctor will not ask about them , but you have to know that they have contrary functions.

PGI2, PGE2, PGD2 ('2' is related to number of double bonds in the structure) have the following functions

1. vasodilation

2. increase cAMP

3.decrease platelet, leukocyte aggregation

4.decrease lymphocyte migration

PGF2alpha

1. vasoconstriction

2.bronchoconstriction

3.smooth muscle contration

Thromboxanes

1. vasoconstriction

2. Induce platelet aggregation

3.increase lymphocyte proliferation

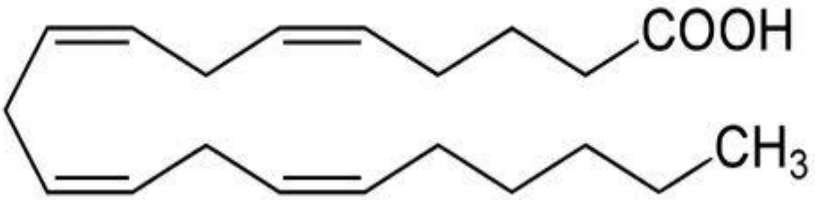
4.bronchoconstriction

Structures of eicosanoids

It's important to be able to recognize the structure of different classes of eicosanoids and differentiate between them .

1.Arachidonic acid : considered as the parent compound in the synthesis of eicosanoids , it's a 20 carbon molecule with 4 double bonds. (look at the

picture below)

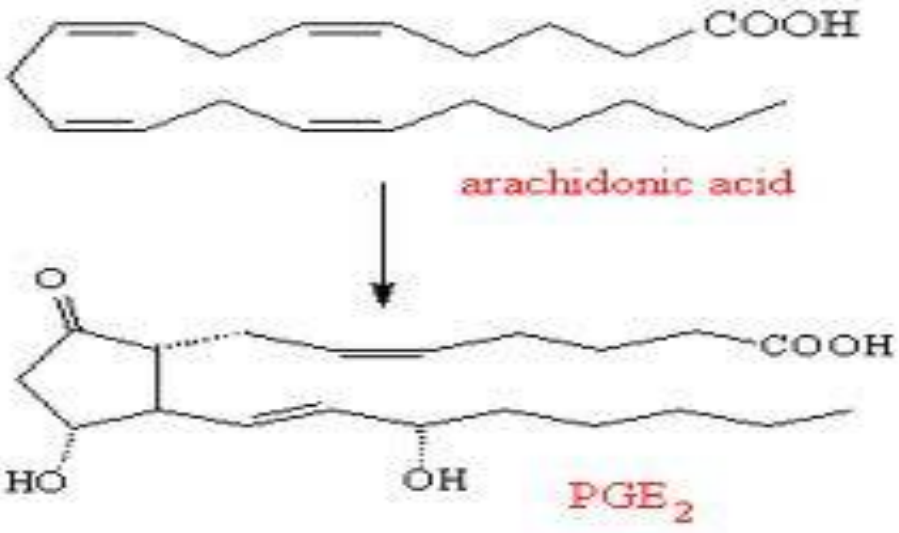


2.prostaglandins:

1. consist of five-membered ring , and 2 side chains ; one contains 7 carbons and the other contains 8 carbon

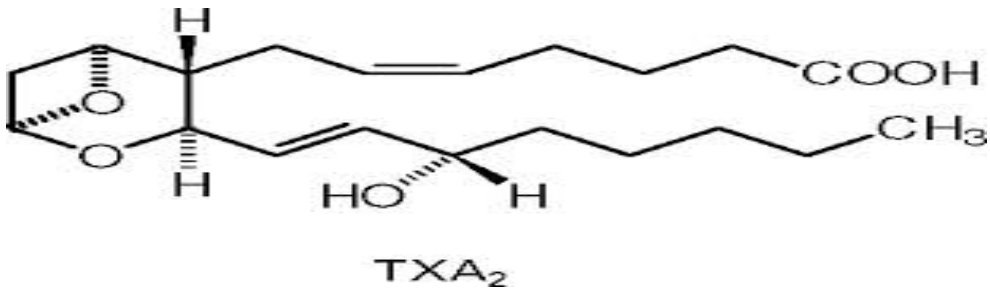
2. have several subclasses (structures are not required).

3. prostaglandin names include numbers such as , PGE2,PGF2 …etc,they are related to number of double bonds.



3.Thromboxanes

1. composed of six –membered ring , one of the atoms that participate in formation of the ring is **oxygen**

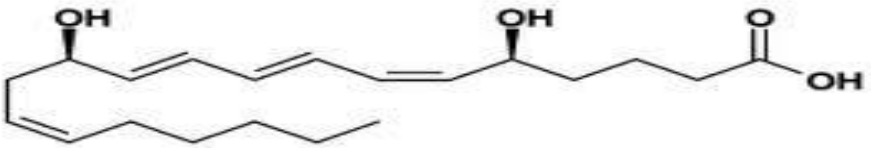


4. leukotrienes:

1 .Leuko**triene** means it contains three conjugated double bonds

2. contain no ring in their structures

3 .several subclasses , one of them **LTB4** , which contains 4 double bonds **.**



Leukotriene B4

*functional groups on the cyclopentane ring determain PG classes (PGA, PGD, PGG…etc).*not required to distinguish between PG classes though.
*PG names include numbers… what are these numbers? They are the numbers of the double bonds.
PGE2: has 2 double bonds. Also PGF2, PGI2 and TXA2.
LTA4: the total number of double bonds is four!

Overview of eicosanoid synthesis

1.As you know the dietary linoleic acid can be converted in the body to Arachidonic acid by elongation and further desaturation.
2. then Arachidonic acid is usually incorporated in the membrane phospholipids (in the inner leaflet of membrane at position 2 in glycerol phospholipids)
3. phospholipase A2 hydrolyze phospholipids at position 2 lead to release of Arachidonic acid (**This step is the rate limiting step in eicosanoid synthesis).**
4.once released different enzymes will act on it to synthesize different eicosanoids (**what makes Arachidonic acid to be converted to prostaglandin, or leukotriens or other eicosanoids ? different and specific enzymes for each class of eicosanoids)**
5. the primary products are :

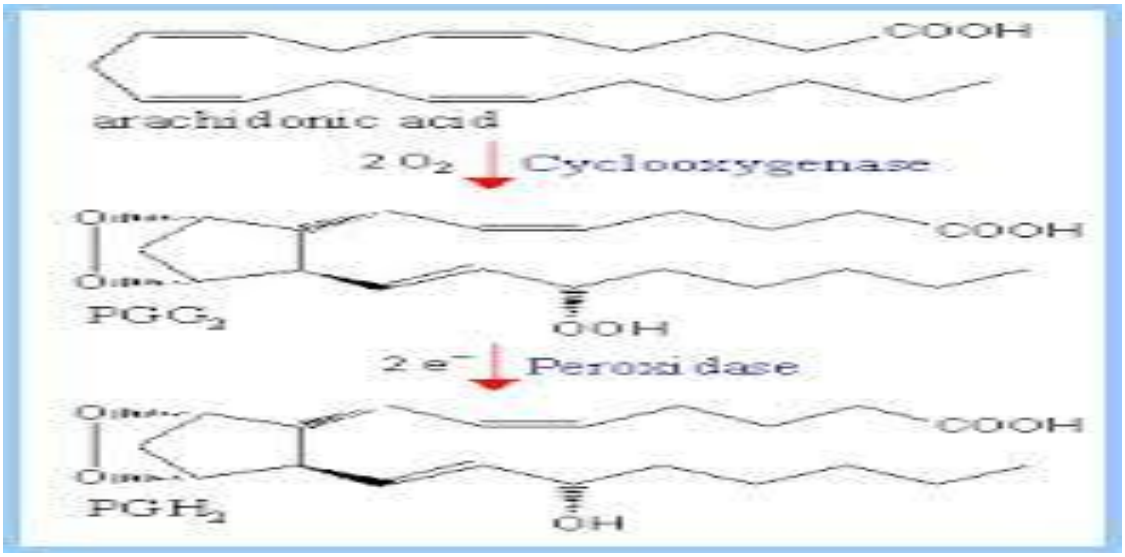
a. PGH2 (parent compound that lead to synthesis of **Thromboxanes**,prostacyclin, and **other prostaglandins**)
b. leukotrienes
c. HETE (hydroxy eicosa tetra enoic acid)

synthesis of PGH2 from Arachidonic acid

1. Arachidonic acid undergo closure of ring between carbon 8 & carbon 12, by cyclooxygenase enzyme and leads to formation of peroxide (OOH) on carbon 15 . The prostaglandin now is **PGG2**

Cyclooxygenase complex is an enzyme complex with two catalytic activities ; cyclooxygenase activity and peroxidase activity .

2. peroxidase enzyme then convert (OOH) group into (OH) group , by the help of reduced glutathione (2 GSH). The prostaglandin now is **PGH2**.



Eicosanoids can be synthesized from other polyunsaturated fatty acid

1. they can be synthesized from any fatty acid containing 20 carbons
2.so fatty acids of 20 carbons with

a. 3 double bonds like Eicosatrienoic acid

b. 4 double bonds as Eicosatetraenoic acid (arachidonic acid)

c.or 5 double bonds(Eicosapentaneoic acid : omega-3 fatty acids)

can synthesize eicosanoids

Now , the product of these 20 carbon fatty acids can be predicted by their number of double bonds

for example:

1. Eicosatetraenoic acid (or arachidonic) contain 4 double bonds , the product will be of 2 double bonds (substract by 2) , for example **PGE2**, **PGF2alpha (4→2)**

2. Eicosatrienoic acid (omega-6) : the products are for example **PGE1 (3→1)**

3.Eicosapentaenoic acid : **PGE3 (5→3)**

Now , which one of these fatty acids (eicosa-pentenoic acid or eicosa-tetraenoic acid) is better to be taken as dietary habits for good health and lifestyle???

the answer is **eicosa-pentaenoic acid ,**because it will produce eicosanoids of 3 double bonds , such as **TxB3 ,** that inhibits platelet aggregation , while **eicosa-tetraenoic acid ,** will produce **TxB2** , that stimulates platelet aggregation.

This explains why myocardial infarction is low in some populations (in east Asia , because they depend on sea food for their diet (rich in omega-3 fatty acids , like eicosa-pentaenoic acids)

Because prostaglandins are implicated in the inflammatory response, sometimes we need to inhibit their synthesis to decrease inflammation

1.Steroidal anti-inflammatory agents : inhibit phospholipase A2 ,and thus inhibit prostaglandin synthesis.

2. NSAIDS (non-steroidal anti-inflammatory drugs) include:

a. Aspirin (acetylsalicylate) : it transfers its acetyl group to cyclooxygenase enzyme, inhibiting it.

b. Ibuprofen : it binds irreversibly to the enzyme ,inhibiting it.

Cyclooxygenase exists in two forms

1. COX 1 : Constitutive ; same concentration all the time , and is produced in cells of gastric mucosa, kidneys and platelets

2. COX 2: inducible , only produced in response to inflammatory stimuli , produced by monocytes, macrophages ,and smooth muscles

This provide us with the benefit to give selective COX-2 inhibitors for long term treatment , because if we give general inhibitor of COX it will inhibit COX-1 in addition to COX-2 , and lead to development of ulcer

Some people take Baby Aspirin (100mg) , every day , why ?

1. it's anti-platelet (inhibits platelet aggregation)

2. it inhibits the production of TxB2

3. It's taken in low dose , because all what you need is to inhibit platelet cells , which are semi-dead cells having no nucleus and no protein synthesis, once inhibiting the enzyme(irreversibly), they can't produce another enzyme molecules .

THE END

Notes from the correction team about biochemistry **sheet 3**

- **Page 3**: the enzyme become in close proximity with Interior (not anterior) leaflet.
- **Page 9**: it is Rigid molecule (Not ridge(
- **Page 12** :Cannot get it --> It's Rigor mortis
- **Page 13**:Monomeric when not bound and dimeric when bound (ya3ni when .. not WILL) receptors .. not rectors
- **Page 13** (*Last sentence*) : dimeric form is ACTIVE.
- The other mistakes are grammatical or obvious.