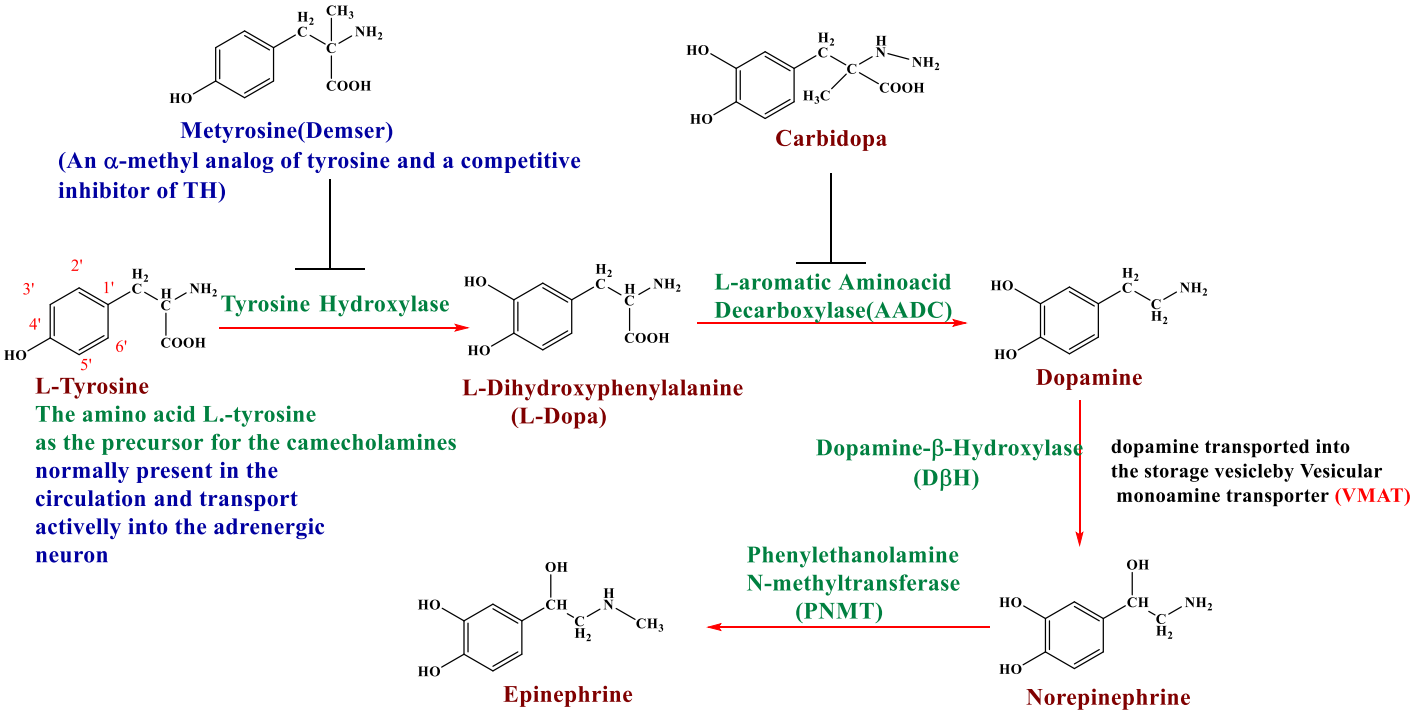
***Biosynthesis*:-**

The biosynthesis of the catecholamines dopamine, NE and E involves a sequence of enzymatic reaction.



Biosynthesis of the catecholamines dopamine, norepinephrine and epinephrine

The first step **in CA biosynthesis is the 3'-hydroxylation of the amino acid L-tyrosine to form L-dihydroxyphenylalanine by Tyrosine hydroxylase (TH, tyrosin-3-monooxygenase). TH is stereospecific and requires molecular O_2, Fe^{+2} and a tetrahydropteridine as a cofactor. TH is the rate limiting step in the biosynthesis of NE (rate limiting in CA biosynthesis) because:-**

1- **TH is the first enzyme in a biosynthesis pathway.**
2- **The inhibitors of TH markedly reduce endogenous NE and DA in the brain and NE in the heart, spleen, and other sympathetically innervated tissues.**

This enzyme plays a key role in the regulation CA biosynthesis and is therefore the logical biological target of some drug.

The second step **in CA biosynthesis is the decarboxylation of L-dopa to give dopamine. The enzyme involved is L-aromatic amino acid decarboxylase enzyme. In addition to being found in catecholaminergic neurons, L-aromatic amino acid decarboxylase is found in high concentrations in many other tissues, including the liver and kidneys. It does not remove the carboxyl groups only from L-dopa; it acts on all naturally occurring L-amino acids, such as L-tyrosine, L-phenylalanine, L-histidine, and L-tryptophan, in addition to L-dopa and L-5-hydroxytryptophan.**

The third step **in CA biosynthesis is the side chain β-hydroxylation of DA to give NE by the dopamine β- hydroxylase.**

Dopamine formed in the cytoplasm of the neuron is actively transported into storage vesicles by the vesicular monoamine transporter (VMAT) and is then hydroxylated stereo specifically at the β-carbon to NE inside the vesicle by dopamine β-monooxygenase (dopamine β-hydroxylase DBH).

The NE formed is stored in the vesicles until depolarization of the neuron initiates the process of vesicle fusion with the plasma membrane and extrusion of NE into the synaptic cleft.

The last step **in CA biosynthesis is the N-methylation of NE to give E in the adrenal medulla. This reaction is catalysed by the enzyme phenylethanolamine-N-methyltransferase (PNMT).**

PNMT is a cytosolic enzyme and the methyl donor s-adenosyl methionine (SAM) is required for the N-methylation of NE.

The reaction occurs in the cytoplasm, and the epinephrine formed is transported into the storage granules of the chromaffin cells. Although PNMT is highly localized in the adrenal medulla, it is also present in small amounts in heart and brain tissues.

Storage, Release, Uptake, and Metabolism

Storage and Release

A large percentage of the NE is located in the synaptic vesicles (granules) in sympathetic nerve endings and chromaffin cells. Much of the NE in CNS is also located within similar vesicles. The concentration in the vesicles is maintained also by the vesicular monoamine transporter (VMAT).

The NE formed is stored in the vesicles until depolarization of the neuron initiates the process of vesicle fusion with the plasma membrane and extrusion of NE into the synaptic cleft, where it interacts with specific presynaptic and post synaptic adrenoceptors, on the

effector cell, triggering a biochemical cascade that results in a physiological response by the effector cells.

Uptake of NE

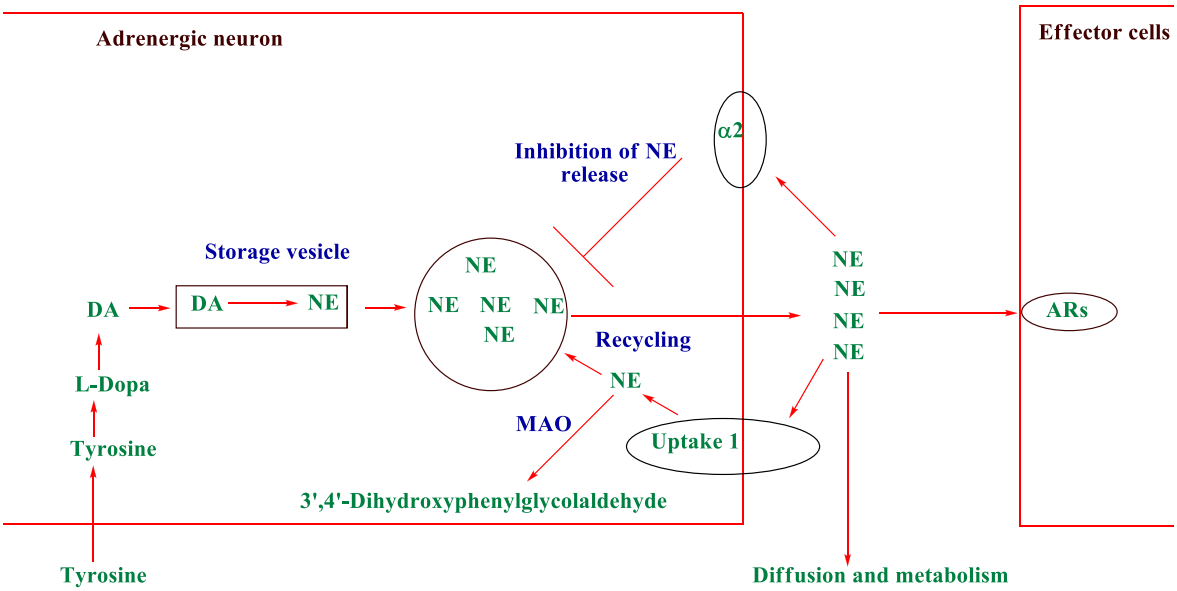
Once NE has exerted its effect at adrenergic receptors, there must be mechanisms for removing the NE from the synapse and terminating its action at the receptors. These mechanisms include:

(a) reuptake of NE into the presynaptic neuron (recycling, major mechanism) by NET and into extraneuronal tissues.
(b) Conversion of NE to an inactive metabolite.
(c) Diffusion of the NE away from the synapse.

By far, the most important of these mechanisms is recycling the NE. This process is termed uptake-1 and involves a Na^+ /C1$^-$dependent transmembrane(TM) NET that has a high affinity for NE. This uptake system also transports certain amines other than NE into the nerve terminal, and it can be blocked by such drugs as cocaine and some of the tricyclic antidepressants. Similar transporters, dopamine transporter (DAT) and serotonin transporter (SERT) are responsible for the reuptake of DA and 5-HT (serotonin), respectively into the neurons that release these transmitters.

Some of the NE that reenters the sympathetic neuron is transported from the cytoplasm into the vesicles by VMAT; then it would be released again into the synaptic cleft.

Note: In addition to the neuronal uptake of NE, there exists an extraneuronal uptake process, called uptake-2 with relatively low affinity for NE. Although its physiological significance is unknown, it may play a role in the disposition of circulating CAs, because CAs that are taken up into extraneuronal tissues are metabolized quite rapidly.



Model of the life cycle of NE

Metabolism

The second mechanism of CAs removal is metabolism. The major mammalian enzymes of importance in the CA metabolism are monoamine oxidase (MAO) and catechol-O-methyltransferase (COMT).

Both E and NE are orally inactive and have short durations of actions because of their :-

1- **High hydrophilicity.**
2- **Ionization.**
3- **Extensive first-pass metabolic deactivation by COMT and MAO.**

The first enzyme **of importance in the metabolism of CAs in the adrenergic neurons of human brain and peripheral tissues is MAO. MAOs oxidatively deaminate CAs to their corresponding aldehydes, which are rapidly oxidized to the corresponding acid by the enzyme aldehyde dehydrogenase (AD). In some circumstances, the aldehyde is reduced to the glycol by aldehyde reductase (AR). This glycol metabolite that is released into the circulation, where it undergoes methylation by the COMT that it encounters in nonneuronal tissues. The product of methylation, 3′-methoxy-4′-hydroxyphenylethylene glycol, is oxidized by alcohol dehydrogenase and AD to give 3′-methoxy-4′-hydroxymandelic acid. This metabolite commonly is referred to as vanillylmandelic acid (VMA), and is the major end product of several pathways of NE metabolism.**

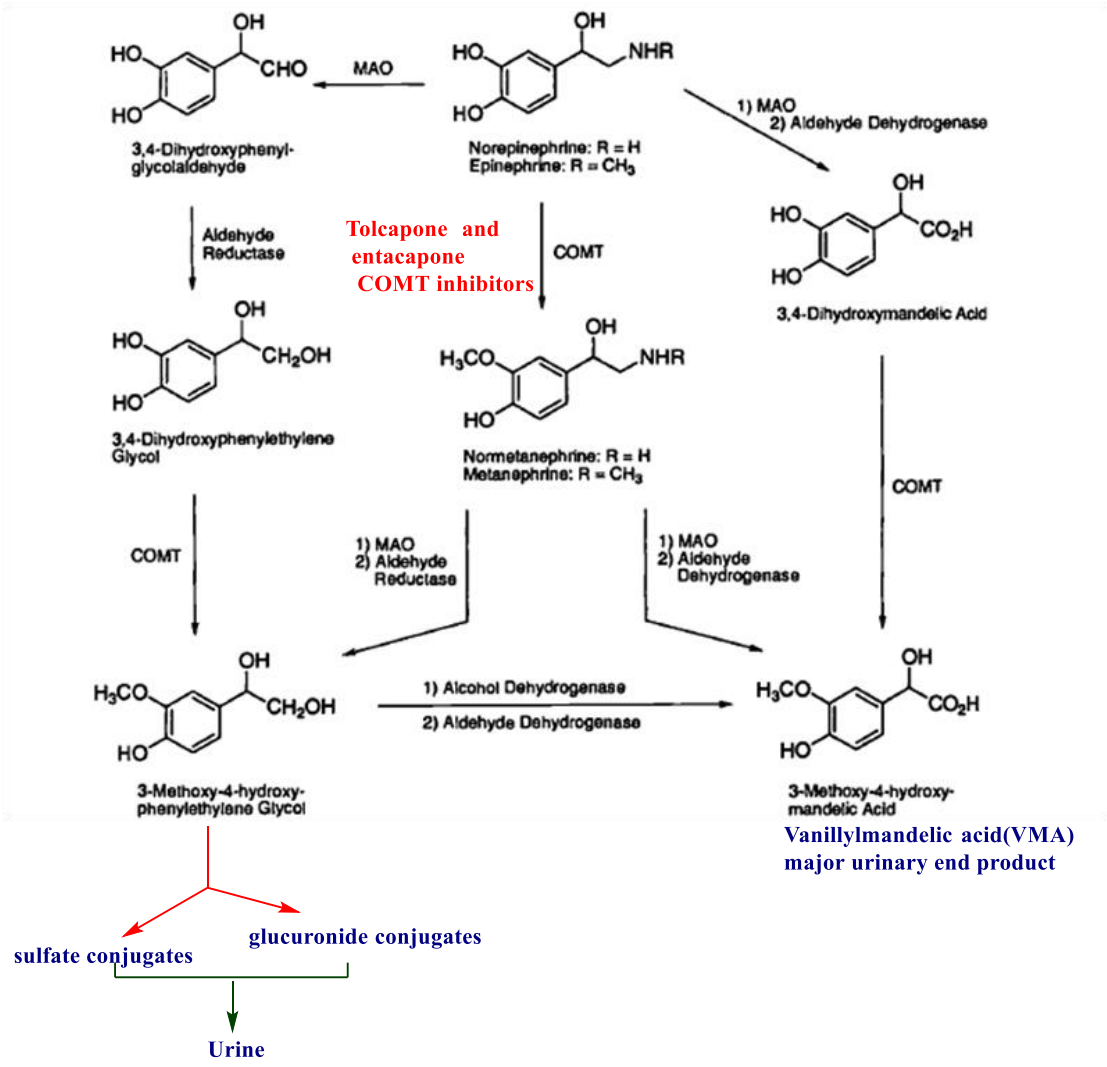
In the oxidative deamination of NE and E at extraneuronal sites such as the liver, the aldehyde formed is oxidized usually by AD to give 3,4-dihydroxymandelic acid (DOMA).

MAO inhibitors (MAOIs) prevent MAO-catalyzed deamination of NE, DA, and 5-HT following their reuptake into the nerve terminal from the synaptic cleft. As a result, higher

concentration of the NTs will be stored in the vesicles and become available for release from the presynaptic terminals on demand.

The second enzyme **of importance in the metabolism of CAs is COMT that O-methylates 3′-OH group of CAs and renders them inactive. Methylation by COMT occurs almost exclusively on the meta-OH group of the catechol, regardless of whether the catechol is NE, E, or one of the metabolic products. For example, the action of COMT on NE and E gives normetanephrine and metanephrine, respectively. A converging pattern of the metabolism of NE and E in which 3′-methoxy-4′-hydroxymandelic acid (VMA) and 3′-methoxy-4′-hydroxyphenylethylene glycol are common end products thus occurs, regardless of whether the first metabolic step is oxidation by MAO or O-methylation by COMT.**

In patients with tumors of chromaffin tissue that secrete these amines (a rare cause of high blood pressure), the urinary excretion of VMA is markedly increased and is used as a diagnostic test for this condition.



Adrenergic receptors

Adrenergic receptor subtypes

There are two types of adrenergic receptors

1- **α- adrenergic receptor (α1 and α2)**
2- **β – adrenergic receptor (β1, β2, and β3)**
they all belong to the guanine nucleotide (G)- regulatory proteins (G-protein)-coupled receptors(GPCR).

ADs	Organ or tissue	Effect of activation	Physiological effect	Drugs	Therapeutic uses
α_1	Blood vessels Skin	vasoconstriction	Increase blood pressure	α_1-agonist	Shock hypotension
α_1	Mucosa membrane	vasoconstriction	Nasal decongestion	α_1-agonist	Nasal congestion
α_{1A}	Prostate smooth muscle	Contraction	Prostatic hyperplasia	α_{1A}-Antaagonist	Benign prostatic hyperplasia (BPH)
α_1	uterus	contraction			
α_2	CNS	Decrease NE release	Decrease blood pressure	α_2-agonist(clonidine)	Hypertension
				α2-antagonist	Erectile impotence
β_1	Heart muscle	Muscle contraction	Increase heart rate and force	β_1-Antagonist	Hypertension Angina, Cardiac arrhythmias
β_1	Kidney	Increase rennin secretion	Increase blood pressure		
β_2	Bronchial smooth muscle	Smooth muscle relaxant	Dilates and open airways	β_2- agonist	Asthma and COPD
β_2	uterus	Smooth muscle relaxation	Relaxes uterine muscles	β_2- agonist	Premature laber
β_3	Adipose tissue	stimulation of lipolysis			

α – adrenergic receptors

a- α1 – agonist as vasoconstriction and nasal decongestants

In blood vessels, the principal effect is vasoconstriction. Blood vessels with α1 –receptors are present in skin and during the fight- or- flight response, vasoconstriction results in the decreased blood flow to this organ. Agonist acting selectively on α1- receptors cause:-

1- **Vasoconstriction and thus can be used alongside local anesthetics in dentistry to localize and prolong the effect on the anesthetic at the site of injection.**
2- **They are also used as nasal decongestants (vasoconstriction of mucous membrane)**
3- **For raising blood pressure (vasoconstriction of blood vessels) in shock.**

b- α1 –Antagonist for treatment of hypertension.

Because α_1 – agonists are vasoconstriction and hypertension, α_1 – antagonists would be expected to be vasodilators and hypotension. Similarly, they should block α_{1A}-receptor in prostate smooth muscle and relax the muscle with implication of treating benign prostatic hyperplasia (BPH).

c- α 2- Agonists for treatment of hypertension

α2- agonists (e.g., clonidine) act at CNS sites to decrease sympathetic outflow to the periphery, resulting in decrease NE release at sympathetic nerve terminal and relaxed vascular smooth muscle.

d- β 1 – blockers for treatment of hypertension, angina, and certain cardiac arrhythmias.

Activation of the β 1- receptors in heart causes an increase in rate and force of contraction and β 1 –blockers should be expected to slow the heart rate and decrease the force of contraction.

e- β 2- agonists for treatment of asthma and premature Labor.

Activation of β 2- receptors relaxes the smooth muscles in the bronchi, thus dilating and opening airways. Similarly, activation of β 2- receptors in the uterus relaxes the muscle, and some β 2- agonists are used to inhibit uterine contractions.

ADRENERGIC RECEPTORS

α - Adrenergic Receptors

1- **α 1-adrenergic receptors: postsynaptic receptors(excitatory receptor).**

2- **α 2-adrenergic receptors: presynaptic receptors(inhibitory receptor).**

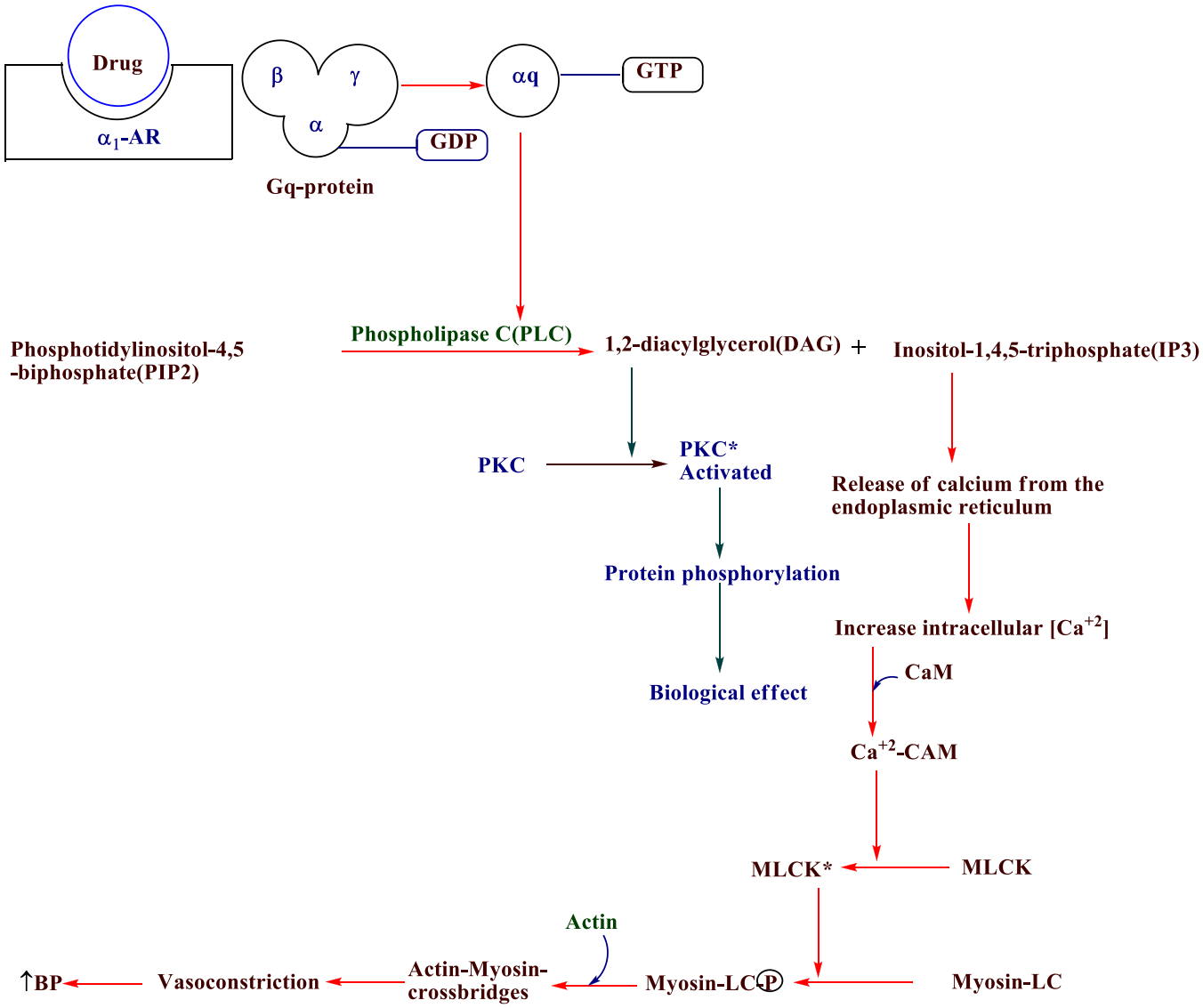
The α1 and α2 receptors each have been subdivided into at least three subtypes, which have been designated α1A , α1B, α1D and α2A, α2B and α2C respectively.

The α_1-adrenergic **receptor is coupled to the enzyme phospholipase C via a Gq protein. When stimulated by activation of the α 1,-adrenergic receptor, phospholipase C hydrolyzes phosphatidylinositol-4.5-bisphosphate (PIP_2) to give the second messengers inositol- I ,4,5-**

triphosphate(IP_3) and 1,2-diacylglycerol (DAG). In the smooth muscle, IP_3 stimulates the release of Ca^{2+} from the sarcoplasmic reticulum, resulting in an increase in free intracellular calcium levels. Increased free intracellular calcium is correlated with smooth muscle contraction, whereas DAG activates protein kinase C (PKC), which phosphorylates proteins, and may induce slowly developing contractions of vascular smooth muscle.

Vasoconstriction is commonly initiated by the opening of voltage-gated L-type $Ca2^{+}$ channels in the sarcolemma during plasma membrane depolarization, which mediates $Ca2^{+}$ flux into the cytoplasm. Ca^{2+} entry into the cell activates calmodulin (CaM). The Ca^{2+}-CaM complex activates myosin light chain kinase (MLCK) to phosphorylate myosin light chain (myosin-LC). The phosphorylated myosin-LC interacts with actin to form actin-myosin cross-bridges, a process that initiates vascular smooth muscle cell contraction. The vascular constriction thus causes an increase in blood pressure. In contrast, relaxation is a coordinated series of steps that act to dephosphorylate and hence inactivate myosin-LC.

Activation of α2
-adrencrgic receptors leads to a reduction in the catalytic activity of adenylyte cyclase (AC), which in turn results in a lowering of intracellular levels of cyclic- 3,5-adenosine monophosphate (cAMP). The α2-adrenergic receptor—mediated inhibition of adenylyt cyclase is regulated by the G protein Gi.


Drug
α_1-AR
β
γ
α
αq
GTP
GDP
Gq-protein
Phosphotidylinositol-4,5 -biphosphate(PIP2)
Phospholipase C(PLC)
1,2-diacylglycerol(DAG) +
Inositol-1,4,5-triphosphate(IP3)
PKC
PKC* Activated
Protein phosphorylation
Biological effect
Release of calcium from the endoplasmic reticulum
Increase intracellular [Ca^{+2}]
CaM
Ca^{+2}-CAM
MLCK*
MLCK
Actin
↑BP
Vasoconstriction
Actin-Myosin-crossbridges
Myosin-LC-P
Myosin-LC

β – Adrenergic receptors

All three β -receptors are coupled to adenylyl cyclase (AC), which catalytes the conversion of ATP to cAMP. This coupling is via the guanine nucleotide protein (G- protein) Gs. In the absence of agonist, guanosine diphosphate (GDP) is bound reversibly to the Gs protein. Interaction of the agonist with the receptor causes a conformational change in the receptor, which decreases the affinity of the Gs protein for GDP and a concomitant increase in affinity for guanosine triphosphate (GTP). The αs subunit of the Gs protein complex dissociates from the receptor—G protein tertiary complex and then binds to and activates AD.

The intracellular function of the second-messenger cAMP is activation of protein kinases, which phosphorylate specific proteins, thereby altering their function. The action of cAMP is terminated by a class of enzymes known as phosphodiesterases (PDE) that catalyzes the hydrolysis of cAMP to AMP.