AS 2549

M. Pharm. I SEMESTER

EXAMINATION, 2013

BASIC AND MOLECULAR PHARMACOLOGY

SECTION- A

i) GABA B receptor are G protein coupled receptor that work by inhibiting adenylyl cyclase, inhibition of Ca^{++} channels and activation of K^{+} channels. eg baclofen (agonist) used for the treatment of spasticity and related motor disorders.

ii) Super sensitivity refers to the phenomenon of an enhanced physiological, behavioral or biochemical response eg. dopamine

iii) Guanethedine, gunaphesine, Bethanidine

iv) Time the drug takes for the blood plasma concentration of a substance to reduce to half its steady-state concentration.

v) Pharmacogenetics is the study of how people's genetic makeup affects their responses to drugs both in terms of therapeutic effect as well as adverse effects.

vi) The noradrenaline is metabolized by monoamine oxidase (MAO) and catechol ortho methyl transferase (COMT).

vii) Apparent volume of distribution describes the relationship between concentration and the amount of drug in the body.

viii) Adrenaline and dihydroergotamine, noradrenaline and chlorpromazine.

ix) Location of $\alpha_{,1}$Blood vessel; β_1 Heart; β_2 Blood vessels and β_3 adipose tissues.

x) Digoxin, neostigmine, gentamicin, atenolol, furosemide, oxytetracycline.

xi) Adrenaline is released during emergency condition that makes the body ready for "fight or flight" response, therefore called as emergency hormone.

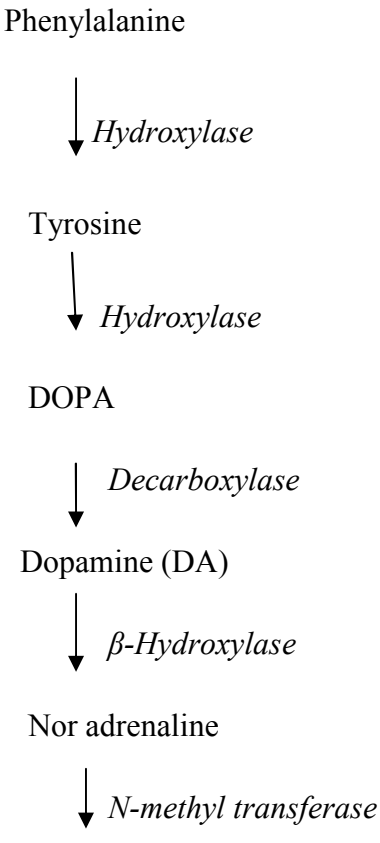
xii) Drugs that produce only agonistic action eg morphine and codeine.

SECTION- B

Q.2 Adrenergic transmission is restricted to the sympathetic division of the Autonomic Nervous System. There are 3 endogenously related catecholamines. Noradrenaline (NA); which acts as transmitter at post ganglionic sympathetic sites. Adrenaline: is secreted by adrenal medulla and have a transmitter role in the brain and Dopamine which is a major neurotransmitter in basal ganglia , limbic system , CTZ and anterior pituitary.

Synthesis of cathecholamine:

These are synthesized from the amino acid phenyalanine. Tyrosine hydroxylase is the rate limiting enzyme aand its inhibition by α methyl p tyrosine and results in depletion of catecholamines. Synthesis of NA occur in all adrenergic neurons, while theat of adrenaline occur only in the adrenal medulla.



Storage of Noradrenaline (NA) and Adrenaline:

NA is stored in the synaptic vesicles within the adrenergic terminals. The vesicular membrane actively takes up doamine from the cytoplasm and the final step of synthesis of NA takes place inside the vesicles which contain dopamine β hydoxylase. NA is then stored as a complex with ATP.

Release of Noradrenaline (NA) and Adrenaline:The nerve impulse coupled release of adrenaline takes place by exocytosis and all the vesicular contents are poured out.The release is modulated by presynaptic receptors, of which the α_2 inhibitory control is dominant. Indirectly acting sympathomometics amines also induce release of adrenaline, but they do so by displacing NA from the nerve ending binding sites.

Uptake of Noradrenaline (NA) and Adrenaline*:* NA released from the nerve terminal is recaptured. This occur in 2 steps:

Axonal uptake: An active pump transport NA by a Na+ coupled mechanism. This is known as Uptake 1. This is an important mechanism for terminating the actions of NA.

Vesicular uptake: Another pump is present on the vesicles that transport catecholamines from cytoplasm to vesicles. The NA constantly leaks out of the vesicles, is immediately taken back in the vesicles, which helps in maintaining the vesicular/axonal concentration of NA. This is inhibited by reserpine.

Extraneuronal uptake: This is called as Uptake 2. In this uptake is carried the pump into the postjunctional cells and tissues.It is not of any physiological importance.

Q. 3 Bioavailability: Bioavailability refers to rate and extent of the drug that reaches to systemic circulation following administration by any route. The area under the blood concentration time curve is proportional to the extent of bioavailability for a drug if its elimination is first order. For an iv route of drug administration the bioavailability is 100% and for drug administerd orally the bioavailability of the drug is always less than 100%. This is because of the two reasons first due to first pass metabolism and second due to incomplete absorption through the gut wall.

Factors influencecing bioavailability: The absolute bioavailability of a drug, when administered by an extravascular route, is usually less than one (i.e., F <100%). Various physiological factors reduce the availability of drugs prior to their entry into the systemic circulation. Whether a drug is taken with or without food will also affect absorption, other drugs taken concurrently may alter absorption and first-pass metabolism, intestinal motility alters the dissolution of the drug and may affect the degree of chemical degradation of the drug by intestinal microflora. Disease states affecting liver metabolism or gastrointestinal function will also have an effect.

Other factors may include, but are not limited to:

- Physical properties of the drug (hydrophobicity, pKa, solubility)
- The drug formulation (immediate release, excipients used, manufacturing methods, modified release – delayed release, extended release, sustained release, etc.)
- Whether the formulation is administered in a fed or fasted state
- Gastric emptying rate
- Circadian differences
- Interactions with other drugs/foods:
    - Interactions with other drugs (e.g., antacids, alcohol, nicotine)
    - Interactions with other foods (e.g., grapefruit juice, cranberry juice)
- Transporters: Substrate of efflux transporters (e.g. P-glycoprotein)
- Health of the GI tract
- Enzyme induction/inhibition by other drugs/foods:
    - Enzyme induction (increased rate of metabolism), e.g., Phenytoin induces CYP1A2, CYP2C9, CYP2C19, and CYP3A4
    - Enzyme inhibition (decreased rate of metabolism), e.g., grapefruit juice inhibits CYP3A → higher nifedipine concentrations
- Individual variation in metabolic differences
    - Age: In general, drugs are metabolized more slowly in fetal, neonatal, and geriatric populations
    - Phenotypic differences, enterohepatic circulation, diet, gender
- Disease state
    - E.g., hepatic insufficiency, poor renal function

Each of these factors may vary from patient to patient (inter-individual variation), and indeed in the same patient over time (intra-individual variation). In clinical trials, inter-individual variation is a critical measurement used to assess the bioavailability differences from patient to patient in order to ensure predictable dosing.

Compartment models: Pharmacokinetic modelling is performed by noncompartmental or compartmental methods. Compartmental methods estimate the concentration-time graph using kinetic models.In single-compartment modeling, the drug is considered to be distributed instantaneously into a unique compartment in the body. This compartment is characterized by a distribution volume. The drug input into this volume depends on the dosage regimen. The drug output from this volume is characterized by an elimination constant rate. Several dosage regimens are considered here:

1. An intravenous bolus injection: the input is equal to the dose at the time point 0 and becomes equal to 0 thereafter. The concentration at time 0, C(0), corresponds to the dose divided by the volume. Subsequently, the concentration decreases in an exponential manner.
2. Intravenous infusion: the drug input is constant and equal to the rate of infusion of the drug. Therefore, the amount of drug in the volume progressively increases until equilibrium is reached when the drug input rate equals the output rate. In other words, equilibrium is reached when the rate of elimination (which increases with the amount of drug in the volume) compensates for the rate of infusion.
3. Extravascular dose: we only consider the case when the input rate follows linear kinetics: the rate of absorption may be characterized by an absorption rate constant and is proportional to the amount of drug available for absorption. The concentration at any time point results from the drug input into the volume minus the output which both vary with time depending on the amount of drug available for absorption and for elimination.

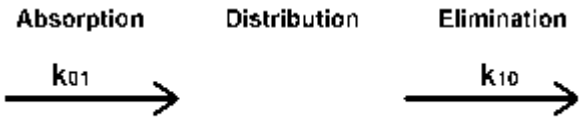
Clinical implications

This model is an easy way of representing the drug outcome in the body when the drug is rapidly distributed within the volume of distribution. Such a representation allows predictions of plasma

drug concentration profiles in different conditions and a more accurate estimation of the initial dosage regimen to be given to a patient.

Assessment

Single compartment representation



Differential equation describing this single compartmental model:

$$\frac{dA}{dt} = Input - (k10 * A)$$

Considering that:

$$CL = k10 * V$$

The following equation may apply to the model:

$$\frac{dC}{dt} = \frac{Input - (CL * C)}{V}$$

A = amount of drug

k10 = Transfer constant rate from the compartment (1) to the outside of the body (0)

V = volume of the compartment

CL = clearance

C = concentration in the volume

Q.4 Metabolism: Metabolism means chemical alteration of the drug in the body. Metabolism is also known as biotransformation. It is needed to render nonpolar compounds polar so that they are not reabsorbed in the renal tubules and excreted out. Lipohilic drugs are transformed to more polar and hence more readily excreted out. Metabolic products are often less active than the parent drug and may even be inactive.

The primary site for drug metabolism is liver and apart from that kidney, intestine, lungs and plasma. Most metabolic biotransformation occurs at some point between absorption of drug into the general circulation and its renal elimination. A few transformations occur in intestinal lumen or intestinal wall. Biotransformation of drug may lead to following things:

- Inactivation e.g. ibuprofen, chloramphenicol
- Active metabolite from an active drug e.g. morphine and losartan.
- Activation of inactive drug e.g. levodopa and enalpril

Metabolism can be classified into:

a) **Phase 1:** It may also called as nonsynthetic or functionalization reaction, in this reaction a functional group is attached or exposed and the metabolite may be inactive or active.eg oxidation,reduction, hydrolysis,cyclization. Phase 1 reactions covert the parent drug to a more polar metabolite by introducing a functional group (-OH, -NH_2, -SH)

b) **Phase 2:** It is called synthetic or conjugation reaction.eg glucuronide conjugation, acetylation, methylation and glycine conjugation.

Phase 1:

- ***Oxidation***: In the oxidation reaction either addition of oxygen or negatively charged radical or removal of positively charged radical may occur. Oxidation reaction are mostly carried out by a group of monooxygenase in the liver eg barbiturates, phenothiazines, steroids, theophylline, benzodiazapines etc
- ***Reduction***: This reaction is just opposite of the oxidation reaction. In this Cyt P-450 enzyme woking in the opposite direction. eg alcohols, aldehydes, quinines, cholralhydrate, chloramphenicol, warfarin.

- ***Hydrolysis***: In this reaction a drug molecule is broken down by addition of water molecule in presence of an enzyme. Amides and polypeptides are hydrolysed by amidases and peptidases.

Phase 2: Parent drug or their phase 1 metabolite that contain suitable chemical groups often undergo coupling or conjugation reactions.These involve any type of conjugation of the drug or its Phase 1 metabolite with an endogenous substrate, generally derived from carbohydrate or amino acid to form an ionized drug so that it may be excreted out easily. Conjugates formation involves high energy intermediates and specific transfer enzymes. Such enzymes may be located in microsomes or in the cytosol. Of these uridine 5 'diphosphate (UDP) and glucornosyl transferase are most dominant enzymes.

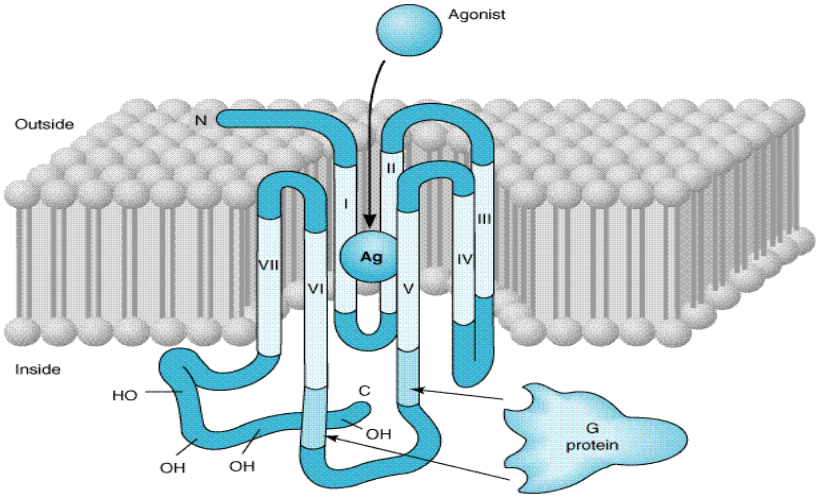
Types of conjugation reactions

- Glucuronide conjugation
- Acetylation
- Methylation
- Sulphate conjugation
- Glycine conjugation
- Glutathione conjugation

Phase 2 reactions are relatively faster than P450 catalyzed reactions thus effectively accelerating drug biotransformation. Most drugs are metabolized by many pathways. Moreover phase 1 and phase 2 reaction both are important for drug metabolism simultaneously.

Q.5 a) G Proteins & Second Messengers

This type of receptor use a transmembrane signaling system with three separate components. First, the extracellular ligand is specifically detected by a cell-surface receptor. The receptor in turn triggers the activation of a G protein located on the cytoplasmic face of the plasma membrane. The activated G protein then changes the activity of an effector element, usually an enzyme or ion channel. This element then changes the concentration of the intracellular second messenger. For cAMP, the effector enzyme is adenylyl cyclase, The corresponding Gs protein, stimulates adenylyl cyclase after being activated by hormones and neurotransmitters that act via a specific receptor



G-protein coupled receptor

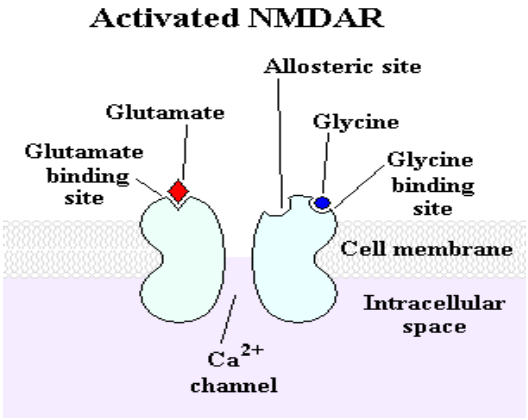
IP3/DAG Pathway:

IP3/DAG is a second messenger system involves hormonal stimulation of phosphoinositide hydrolysis. Some of the hormones, neurotransmitters, and growth factors that trigger this pathway bind to receptors linked to G proteins, while others bind to receptor tyrosine kinases. In all cases, stimulation of a membrane enzyme phospholipase C (PLC), splits a minor phospholipid component of the plasma membrane, phosphatidylinositol-4,5-bisphosphate (PIP_2) into two second messengers inositol-1,4,5-trisphosphate (IP_3) and diacylglycerol. Diacylglycerol is confined to the membrane where it activates a phospholipid- and calcium-sensitive protein kinase called protein kinase C. IP_3 is watersoluble and diffuses through the cytoplasm to trigger release of $Ca^{++}$from internal storage vesicles. Elevated cytoplasmic Ca^{++}concentration promotes the binding of Ca^{++} to the calcium-binding protein calmodulin, which regulates activities of other enzymes, including calcium-dependent protein kinases.

With its multiple second messengers and protein kinases, the phosphoinositide signaling pathway is much more complex than the cAMP pathway. For example, different cell types may contain one or more specialized calcium- and calmodulin-dependent kinases with limited substrate specificity (eg, myosin light chain kinase) in addition to a general calcium- and calmodulin-dependent kinase that can phosphorylate a wide variety of protein substrates. Furthermore, at least nine structurally distinct types of protein kinase C have been identified. Diacylglycerol is either phosphorylated to yield phosphatidic acid, which is then converted back into phospholipids, or it is deacylated to yield arachidonic acid. IP_3 is actively removed from the cytoplasm by Ca^{2+} pumps. These and other nonreceptor elements of the calcium-

phosphoinositide signaling pathway are now becoming targets for drug development. For example, the therapeutic effects of lithium ion, an established agent for treating manic-depressive illness, may be mediated by effects on the metabolism of phosphoinositides.

b) NMDA receptor: The NMDA receptor is a specific type of ionotropic glutamate receptor. NMDA (*N*-methyl-D-aspartate) is the name of a selective agonist that binds to NMDA receptors but not to other glutamate receptors. Activation of NMDA receptors results in the opening of an ion channel that is nonselective to cations. A property of the NMDA receptor is its voltage-dependent activation, a result of ion channel block by extracellular Mg^{2+} & Zn^{2+} ions. This allows the flow of Na^{+} and small amounts of Ca^{2+} ions into the cell and K^{+} out of the cell to be voltage-dependent. Calcium flux through NMDARs is thought to be critical in synaptic plasticity, a cellular mechanism for learning and memory. The activation of NMDA receptors requires binding of glutamate or aspartate. In addition, NMDARs also require the binding of the co-agonist glycine for the efficient opening of the ion channel, which is a part of this receptor.



Glutamate and Aspartate. Glutamate and aspartate are found in very high concentrations in brain, and both amino acids have powerful excitatory effects on neurons in virtually every region of the CNS. Glutamate receptors are classed functionally either as ligand-gated ion channel ("ionotropic") receptors or as "metabotropic" (G protein-coupled) receptors. The ligand-gated ion channels are further classified according to the identity of agonists that selectively activate each receptor subtype, and are broadly divided into *N*-methyl-D-aspartate (NMDA) receptors and "non-NMDA" receptors. The non-NMDA receptors include the a-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), and kainic acid (KA) receptors. The activity of NMDA receptors is sensitive to pH and also can be modulated by a variety of endogenous modulators

including Zn^{2+}, some neurosteroids, arachidonic acid, redox reagents, and polyamines such as spermine. AMPA and kainate receptors mediate fast depolarization at most glutamatergic synapses in the brain and spinal cord. NMDA receptors also are involved in normal synaptic transmission, but activation of NMDA receptors is associated more closely with the induction of various forms of synaptic plasticity rather than with fast point-to-point signaling in the brain. AMPA or kainate receptors and NMDA receptors may be co-localized at many glutamatergic synapses. A well-characterized phenomenon involving NMDA receptors is the induction of long-term potentiation (LTP). LTP refers to a prolonged (hours to days) increase in the size of a postsynaptic response to a presynaptic stimulus of given strength. Activation of NMDA receptors is obligatory for the induction of one type of LTP that occurs in the hippocampus. NMDA receptors normally are blocked by Mg^{2+} at resting membrane potentials. Thus, activation of NMDA receptors requires not only binding of synaptically released glutamate, but simultaneous depolarization of the postsynaptic membrane. This is achieved by activation of AMPA/kainate receptors at nearby synapses by inputs from different neurons. AMPA receptors also are dynamically regulated to affect their sensitivity to the synergism with NMDA. Thus, NMDA receptors may function as coincidence detectors, being activated only when there is simultaneous firing of two or more neurons. Interestingly, NMDA receptors also can induce long-term depression (LTD; the converse of LTP) at CNS synapses. Apparently the frequency and pattern of synaptic stimulation dictates whether a synapse undergoes LTP or LTD.

Q.6 i) Myasthenia gravis: is an autoimmune neuromuscular disease leading to fluctuating muscle weakness and fatigue. Muscle weakness is caused by circulating antibodies that block acetylcholine receptors at the postsynaptic neuromuscular junction, inhibiting the excitatory effects of the neurotransmitter acetylcholine on nicotinic receptors at neuromuscular junctions.The myasthenia gravis is characterized by fatigability. Muscles become progressively weaker during periods of activity and improve after periods of rest. Muscles that control eye and eyelid movement, facial expressions, chewing, talking and swallowing are especially susceptible. The muscles that control breathing and neck and limb movements can also be affected.

Symptoms, which vary in type and severity, may include asymmetrical ptosis (a drooping of one or botheyelids), diplopia due to weakness of the muscles that control eye movements, an

unstable or waddling gait, weakness in arms, hands, fingers, legs, and neck, a change in facial expression, dysphagia, shortness of breath and dysarthria .

Treatment: Treatment is usually started with the neostigmine. 15mg orally 6hrly. The dosage requirement may fluctuate from time to time and patient to patient. Pyridostigmine is an alternative which require less frequent dosing. If muscarinic side effects are produced atropine can be added to block them. Cortocosteroids offer improvement by its immunosuppressant action eg prednisolone.

ii) Protein binding of drugs:

A drug's efficiency may be affected by the degree to which it binds to the proteins within blood plasma. The less bound a drug is, the more efficiently it can traverse cell membranes. Common blood proteins that drugs bind to are human serum albumin, lipoprotein, glycoprotein, and α, β, and γ globulins.

A drug in blood exists in two forms: bound and unbound. Depending on a specific drug's affinity for plasma protein, a proportion of the drug may become bound to plasma proteins, with the remainder being unbound. If the protein binding is reversible, then a chemical equilibrium will exist between the bound and unbound states, such that:

Protein + drug ⇌ Protein-drug complex

Protein binding can influence the drug's biological half-life in the body. The bound portion may act as a reservoir or depot from which the drug is slowly released as the unbound form. Since the unbound form is being metabolized and/or excreted from the body, the bound fraction will be released in order to maintain equilibrium.

Since albumin is alkaline, acidic and neutral drugs will primarily bind to albumin. If albumin becomes saturated, then these drugs will bind to lipoprotein. Basic drugs will bind to the acidic, alpha-1 acid glycoprotein. This is significant because various medical conditions may affect the levels of albumin, alpha-1 acid glycoprotein, and lipoproteins.

Only the unbound fraction of the drug undergoes metabolism in the liver and other tissues. As the drug dissociates from the protein more and more drug undergoes metabolism. Changes in the levels of free drug change the volume of distribution because free drug may distribute into the tissues leading to a decrease in plasma concentration profile. For the drugs which rapidly

undergo metabolism, clearance is dependent on the hepatic blood flow. For drugs which slowly undergo metabolism, changes in the unbound fraction of the drug directly change the clearance of the drug.

The fraction unbound can be altered by a number of variables, such as the concentration of drug in the body, the amount and quality of plasma protein, and other drugs that bind to plasma proteins. Higher drug concentrations would lead to a higher fraction unbound, because the plasma protein would be saturated with drug and any excess drug would be unbound. If the amount of plasma protein is decreased, there would also be a higher fraction unbound. Additionally, the quality of the plasma protein may affect how many drug-binding sites there are on the protein. The change in pharmacologic effect also could have adverse consequences.The effect of protein binding is most significant with drugs that are highly protein-bound (>95%) and have a low therapeutic index, such as warfarin. A low therapeutic index indicates that there is a high risk of toxicity when using the drug. Since warfarin is an anticoagulant with a low therapeutic index, warfarin may cause bleeding if the correct degree of pharmacologic effect is not maintained. If a patient on warfarin takes another drug that displaces warfarin from plasma protein, such as a sulfonamide antibiotic, it could result in an increased risk of bleeding.

Q.7 a) Pharmacological actions of acetyl choline:

Cardiovascular system:

Heart: Acetyl choline hyperpolarize the SA node and decrease the rate of impulse generation which leads to bradycardia. It also decrease the force of contraction. The M2 receptors are maily present on the heart. It also decreases the ventricular contractility.

Blood vessels: All blood vessels are dilated. It leads to fall in blood pressure and flushing. Stimulation of cholinergic nerves to the penis cause erection by releasing NO and dilating cavernosal vessels.

Respiratory System: The acetyl choline contract the smooth muscle of the bronchial tree. In addition, the glands of the tracheobronchial mucosa are stimulated to secrete. This combination of effects can occasionally cause symptoms, especially in individuals with asthma.

Gastrointestinal Tract: It increases the secretory and motor activity of the gut. The salivary and gastric glands are strongly stimulated; the pancreas and small intestinal glands. Peristaltic activity is increased throughout the gut, and most sphincters are relaxed. Stimulation of

contraction in this organ system involves depolarization of the smooth muscle cell membrane and increased calcium influx.

Genitourinary Tract: It stimulate the detrusor muscle and relax the trigone and sphincter muscles of the bladder, thus promoting voiding. The human uterus is not notably sensitive to muscarinic agonists.

Miscellaneous Secretory Glands: Acetyl choline stimulate secretion by thermoregulatory sweat, lacrimal and

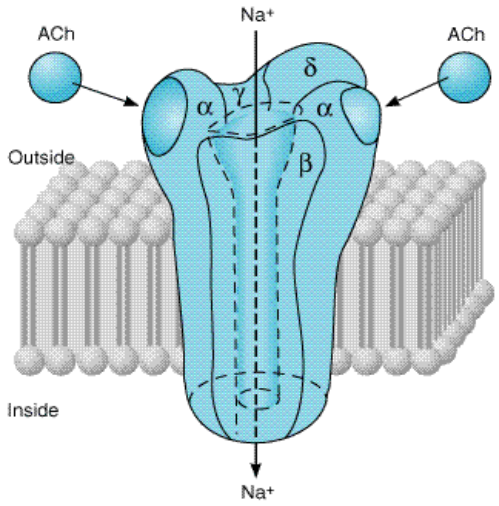
Nasopharyngeal.

CNS: Acetyl choline injected iv does not enter BBB therefore no effect is seen. But if injected directly in the brain produce a complex pattern of stimulation followed by depression.

b)Inotropic receptor:

Many of the most useful drugs in clinical medicine act by mimicking or blocking the actions of endogenous ligands that regulate the flow of ions through plasma membrane channels. The natural ligands include acetylcholine, serotonin, -aminobutyric acid (GABA), and the excitatory amino acids (eg, glycine, aspartate, and glutamate). All of these agents are synaptic transmitters. Each of their receptors transmits its signal across the plasma membrane by increasing transmembrane conductance of the relevant ion and thereby altering the electrical potential across the membrane. For example, acetylcholine causes the opening of the ion channel in the nicotinicacetylcholine receptor (AChR), which allows Na^+ to flow down its concentration gradient into cells, producing a localized excitatory postsynaptic potential a depolarization. The AChR is one of the best-characterized of all cell-surface receptors for hormones or neurotransmitters. One form of this receptor is a pentamer made up of five polypeptide subunits viz two α, one β, one γ and one δ. When acetylcholine binds to sites on the subunits, a conformational change occurs that results in the transient opening of a central aqueous channel through which sodium ions penetrate from the extracellular fluid into the cell. The time elapsed between the binding of the agonist to a ligand-gated channel and the cellular response can often be measured in milliseconds. The rapidity of this signaling mechanism is crucially important for moment-to-moment transfer of information across synapses. Ligand-gated ion channels can be regulated by multiple mechanisms, including phosphorylation and internalization. In the central

nervous system, these mechanisms contribute to synaptic plasticity involved in learning and memory.



Inotropic receptor