

Review Article

OVERVIEW ON: RECEPTOR

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ABSTRACT

The term receptor has been used operationally to denote any cellular macromolecule to which a drug binds to initiate its effects. Among the most important drug receptors are cellular proteins whose normal function is to act as receptors for endogeneous regulatory ligands- particularly hormones, growth factors, neurotransmitters and autacoids. The function of such physiological receptors consists of binding the appropriate ligand and, in response, propogating its regulatory signal in the target cell.

Key words: Receptor, Ligand, Protein

INTRODUCTION

Identification of the two functions of a receptor, ligand binding and messege propagation, led to speculation on the existence of functional domains within the receptor: a ligand binding domain and an effector domain. The evolution of different receptors for diverse ligands that act by similar biochemical mechanisms, on the one hand and of multiple receptors for a single ligand that act by unreleted mechanisms, on the other, supports this concept.

The regulatory actions of a receptor may be exerted directly on its cellular target(s), effector protein(s), or may be conveyed to cellular targets by intermediary cellular molecules, transducers. The receptor, its cellular target, and any intermediary molecules are reffered to as a receptor-effector system or signal transduction pathway. Even the effector protein may not be the ultimate cellular component affected but may synthesize or release another signaling molecule, usually a small metabolite or iron, known as a second messenger.

An important property of physiological receptors, which also makes them excellent targets for drugs, is that they act catalytically and hence are biochemical signal amplifiers. For example, when a singal ligand molecule binds to a receptor that is an ion channel and opens it, many ions flow through the channel. Similarly, a single steroid hormone molecule binds to its receptor and initiates the transcription of many copies of specific mRNAs, which in turn can give rise to multiple copies of a single protein.^[1]

TYPES OF RECEPTORS :

Receptors elicit many different types of cellular effect. Some of them are very rapid, such as those involved in synaptic transmission, operating within milliseconds, whereas other receptor mediated effects, such as those produced by thyroid hormone or various steroid hormones, occur over hours or days. There are also many examples of intermediate timescales; catecholamines, for example, usually act in a matter of seconds, whereas many peptides take rather longer to produce their effects. On the basis of these and molecular structures, receptors can be classified in four main types.^[2]

Type 1: Ligand gated ion channels

Type 2: G-protein coupled receptors

Type 3: Kinase-linked receptors

Type 4: Nuclear receptors

LIGAND GATED ION CHANNELS:

The ligand gated ion channels are also known as ionotropic receptors. These are membrane proteins with a similar structure to other ion channels but incorporating a ligand-binding (receptor) site, usually in the Extracellular domain. Typically, these are the receptors on which fast neurotransmitters act. Examples include

- i) nicotinic acetylcholine receptors (nAChR)
- ii) serotonin receptors (5HT₃)
- iii) glycine receptors and
- iv) γ -amino butyric acid receptors

Molecular structure:

The ligand-gated ion channels have structural features in common with other ion channels. The nicotinic acetylcholine receptor (figure 1), the first to be cloned, has been studied in great detail.

It is assembled from four different types of subunit, termed α , β , γ , δ , each of molecular mass 40-58 kDa. The four subunits show marked sequence homology, and analysis of the hydrophobicity profile, which determines which sections of the chain are likely to form membrane-spanning α -helices, suggests that they are inserted into the membrane as shown in figure 2

The oligomeric structure possesses two acetylcholine-binding sites, each lying at the interface between one of the two α -subunits and its neighbour. Both must bind acetylcholine molecules in order for the receptor to be activated. This receptor is sufficiently large to be seen in electron micrographs, and figure 2 shows its structure, based mainly on a high-resolution electron diffraction study. Each subunit spans the membrane four times, so the channel comprises no less than 20 membrane-spanning helices surrounding a central pore.

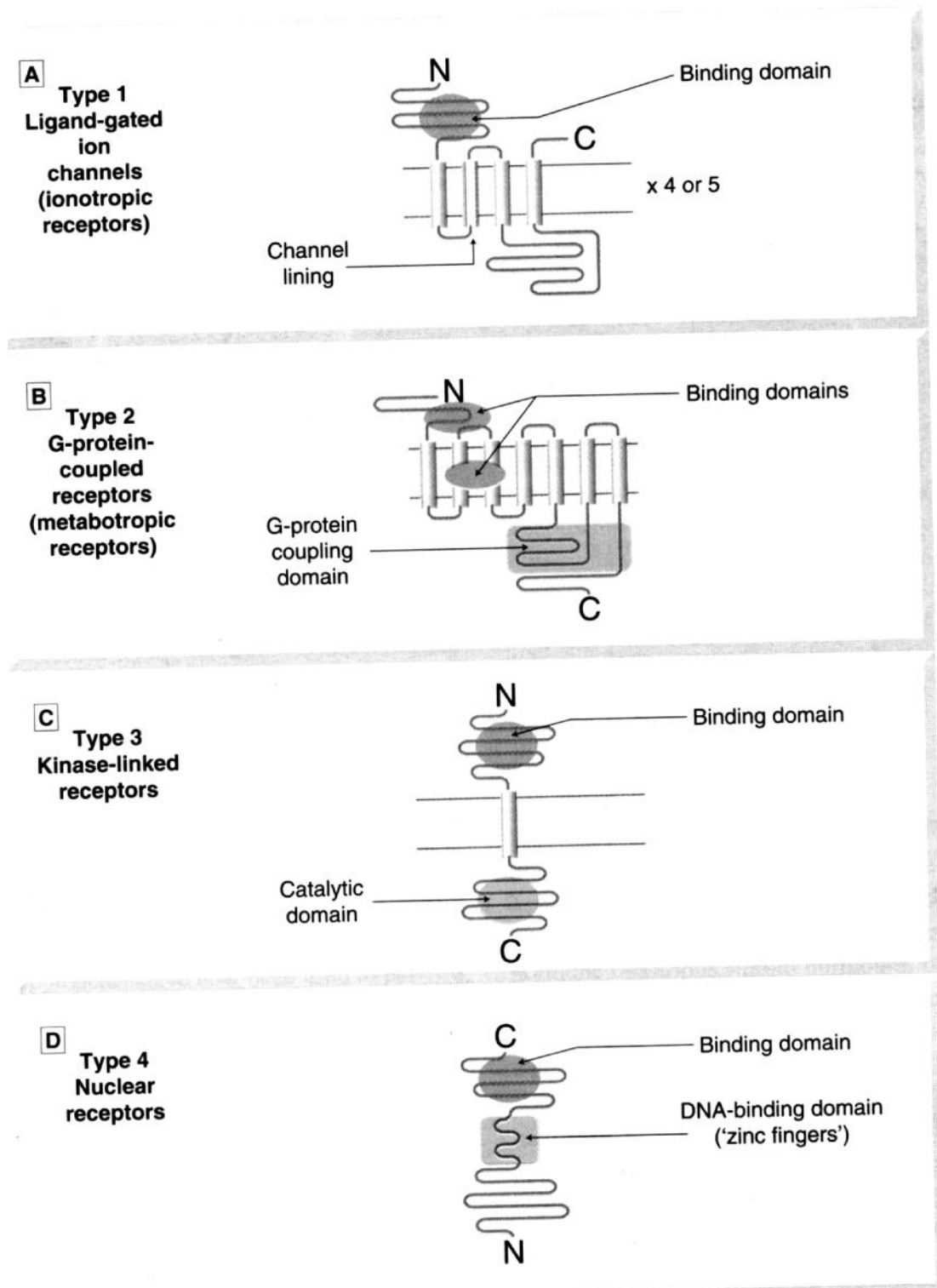


Figure 1 General structure of four receptor families

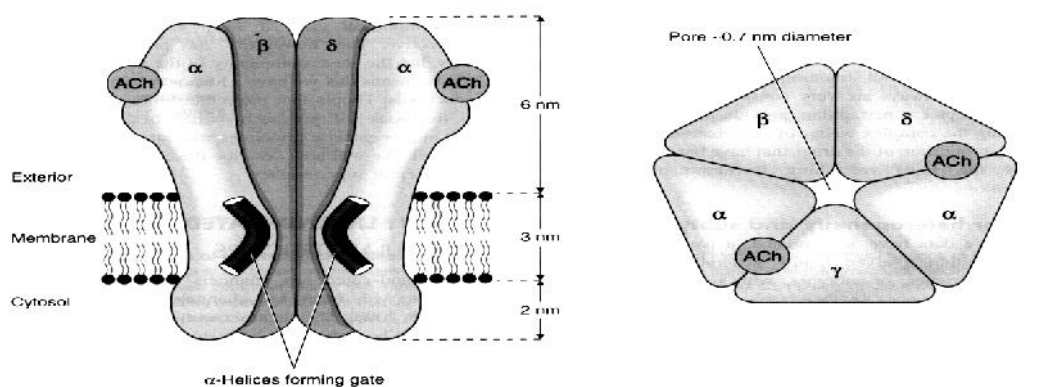


Figure 2. Structure of the nicotinic acetylcholine receptor (a typical-ligand gated ion channel) in side-view (left) and plan-view (right)

Signal transduction mechanism :

Receptors of this type control the fastest synaptic events in the nervous system, in which a neurotransmitter acts on the post synaptic membrane of a nerve or muscle cell and transiently increases its permeability to particular ions. Most excitatory neurotransmitters such as acetylcholine at the neuromuscular junction or glutamate in the central nervous system cause an increase in Na^+ and K^+ permeability. This results in a net inward current carried mainly by Na^+ , which depolarizes the cell and increases the probability that it will generate an action potential. The action of the transmitter reaches a peak in a fraction of a millisecond and usually decays within a few milliseconds. The sheer speed of this response implies that the coupling between the receptor and the ionic channel is a direct one, and the molecular structure of the receptor-channel complex agrees with this.

G-PROTEIN COUPLED RECEPTORS :

G protein-coupled receptors (GPCRs), also known as seven transmembrane receptors, 7TM receptors, and heptahelical receptors, are a protein family of transmembrane receptors that transduce an extracellular signal (ligand binding) into an intracellular signal (G protein activation). The GPCRs are the largest protein family known, members of which are involved in all types of stimulus-response pathways, from intercellular communication to physiological senses. The diversity of functions is matched by the wide range of ligands recognized by members of the family, from photons (rhodopsin, the archetypal GPCR) to small molecules (in the case of the histamine receptors) to proteins (for example, chemokine receptors). This pervasive involvement in normal biological processes has the consequence of involving GPCRs

in many pathological conditions, which has led to GPCRs being the target of 40 to 50% of modern medicinal drugs.^[3]

Table 1 G-protein controlled receptor families :

Family	Receptors	Structural features
A. Rhodopsin family	The largest group; receptors for most amine neurotransmitters, many neuropeptides, purines, prostanoids, cannabinoids, etc.	Short extracellular (N-terminal) tail; ligand binds to transmembrane helices (amines) or to extracellular loops (peptides)
B. Secretin/glucagon receptor family	Receptor for peptide hormones, including secretin, glucagon, calcitonin	Intermediate extracellular tail, incorporating ligand-binding domain
C. Metabotropic glutamate receptor/calcium sensor family	Small group; metabotropic glutamate receptors, GABA _B receptors, calcium sensing receptors	Long extracellular tail, incorporating ligand-binding domain

Physiological roles :

GPCRs are present in a wide variety of physiological processes. Some examples include:

1. The visual sense: the opsins use a photoisomerization reaction to translate electromagnetic radiation into cellular signals. Rhodopsin, for example, uses the conversion of *11-cis*-retinal to *all-trans*-retinal for this purpose.
2. The sense of smell: receptors of the olfactory epithelium bind odorants (olfactory receptors) and pheromones (vomeronasal receptors)
3. Behavioral and mood regulation: receptors in the mammalian brain bind several different neurotransmitters, including serotonin and dopamine
4. Regulation of immune system activity and inflammation: chemokine receptors bind ligands that mediate intercellular communication between cells of the immune system; receptors such as histamine receptors bind inflammatory mediator and engage target cell types in the inflammatory response
5. Autonomic nervous system transmission: both the sympathetic and parasympathetic nervous systems are regulated by GPCR pathways. These systems are responsible for control of many automatic functions of the body such as blood pressure, heart rate and digestive processes.^[3]

Molecular structure :

GPCRs consist of a single polypeptide chain of upto 1100 residues. Their characteristic structure comprises seven transmembrane α -helices, with an extracellular N-terminal domain of varying length and an intracellular C-terminal domain. GPCRs are divided into three distinct families.

There is considerable sequence homology between the members of one family, but none between different families. They share the same heptahelical structure but differ in other respects, principally in the length of N-terminus and the location of the agonist-binding domain. Family A, related to rhodopsin, is by far the largest, comprising most monoamine and neuropeptide receptors. Family C, is the smallest, its main members being the metabotropic glutamate receptors and the calcium sensing receptors.

Site-directed mutagenesis experiments show that the long third cytoplasmic loop is the region of the molecule that couples to the G-protein, since deletion or modification of this section results in receptors that still bind ligands but cannot associate with G-proteins or produce responses. Usually, a particular receptor subtype couples selectively with a particular G-protein.

For small molecules such as noradrenaline, the ligand-binding domain appears to reside not on the extracellular N-terminal region, as with the ion channel-coupled receptors, but buried in the cleft between the α -helical segments within the membrane. Peptide ligands, such as substance P, bind more superficially to the extracellular loops. By single-cell mutagenesis experiments, it is possible to map the ligand-binding domain of these receptors, and the hope is that it may soon be possible to design synthetic ligands based on the knowledge of the structure of the receptor site—an important milestone for the pharmaceutical industry, which has relied up to now mainly on the structure of endogenous mediators (such as histamine) or plant alkaloids (such as morphine) for its chemical inspiration.^[2]

Signal transduction by GPCRs :

G-proteins consist of three subunits, α , β , and γ (figure 3). Guanine nucleotides bind to the α -subunit, which has enzymic activity, catalysing the conversion of GTP to GDP. The β - and γ -subunits remain together as a $\beta\gamma$ -complex. All three subunits are anchored to the membrane through a fatty acid chain, coupled to the G-protein through a reaction known as prenylation. In the resting state, the G-protein exists as an unattached $\alpha\beta\gamma$ -trimer, with GDP occupying the site on the α -subunit. When a GPCR is occupied by an agonist molecule, a conformational change occurs, involving the cytoplasmic domain of the receptor causing it to acquire high affinity for the $\alpha\beta\gamma$ -trimer. Association of $\alpha\beta\gamma$ -trimer with the receptor causes the bound GDP to dissociate and to be replaced with GTP (GDP/GTP exchange), which in turn causes dissociation of the G-protein trimer, releasing α -GTP and $\beta\gamma$ -subunits; these are the active forms of the G-protein, which diffuse in the membrane and can associate with various enzymes and ion channels, causing activation or inactivation as the case may be.^[2]

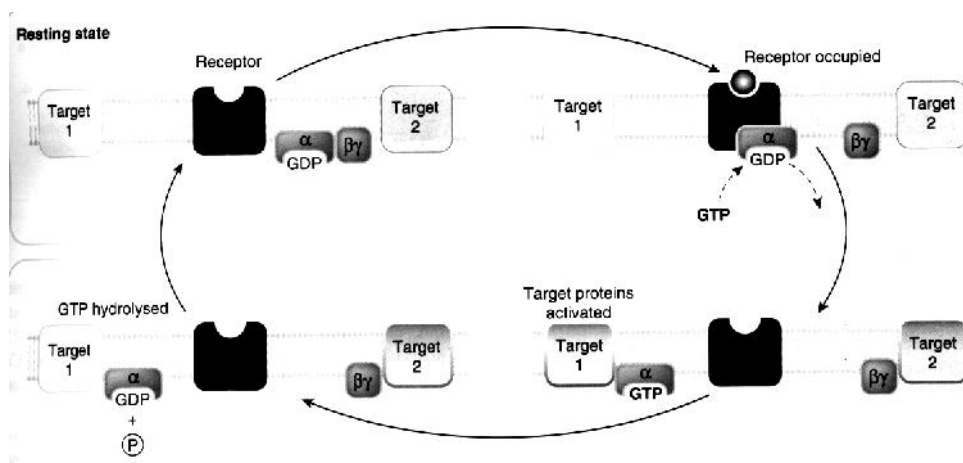


Figure 3 Signal transduction by GPCRs

The process is terminated when the hydrolysis of GTP to GDP occurs through the GTPase activity of the α -subunit. The resulting α -GDP then dissociates from the effector and reunites with the $\beta\gamma$ -complex, completing the cycle. Attachment of the α -subunit to an effector molecule actually increases its GTPase activity, the magnitude of this increase being different for different types of effector. Since GTP hydrolysis is the step that terminates the ability of α -subunit to produce its effects, regulation of its GTPase activity by the effector protein means that the activation of the effector tends to be self-limiting. The mechanism results in amplification because a single agonist-receptor complex can activate several G-protein molecules in turn, and each of these can remain associated with the effector enzyme for long enough to produce many molecules of product.

There are three main classes of G-protein (Gs, Gi and Gq) which show selectivity with respect to both the receptors and the effectors with which they couple, having specific recognition domains in their structure complementary to specific g-protein binding domains in the receptor and effector molecules. Gs and Gi produce respectively stimulation and inhibition of the enzyme adenylate cyclase, and a similar bidirectional control operates on other effectors.

The α -subunits of these G-proteins differ in structure. One functional difference, which has been useful as an experimental tool to distinguish which type of G-protein is involved in different situations, concerns the action of two bacterial toxins, cholera toxin and pertussis toxin. These toxins, which are enzymes, catalyse a conjugation reaction (ADP-ribosylation) on the α -subunit of G-proteins. Cholera toxin acts only on Gs, and it causes persistent activation. Many of the symptoms of cholera, such as an excessive secretion of fluid from the gastrointestinal epithelium, are the result of the uncontrolled activation of adenylate c yclase that occurs. Pertussis toxin acts on Gi in a similar way.^[2]

Targets for G-proteins :

The main targets for G-proteins, through which GPCRs control different aspects of cell function are :adenylate cyclase : the enzyme responsible for cAMP formation phospholipase c : the enzyme responsible for inositol phosphate and diacylglycerol formation ion channels : particularly calcium and potassium channels.

The adenylylase/cAMP system :

cAMP is a nucleotide synthesised within the cell from ATP by the action of a membrane bound enzyme, adenylylase. cAMP is produced continuously and inactivated by hydrolysis to 5'-AMP through the action of a family of enzymes known as phosphodiesterases. Many different drugs, hormones and neurotransmitters act on GPCRs and produce their effects by increasing or decreasing the catalytic activity of adenylylase, thus raising or lowering the concentration of cAMP within the cell.

cAMP regulates many aspects of cellular function including, for example, enzymes involved in energy metabolism, cell division and cell differentiation; ion transport; ion channels; and the contractile proteins in smooth muscle. These varied effects are, however, all brought about by a common mechanism, namely the activation of protein kinases by cAMP. Protein kinases regulate the function of many different cellular proteins by catalysing the phosphorylation of serine and threonine residues, using ATP as a source of phosphate groups. Phosphorylation either activates or inhibits target enzymes or ion channels. Figure 4 shows the ways in which increased cAMP production in response to β -adrenoceptor activation affects enzymes involved in glycogen and fat metabolism in liver, fat and muscle cells. The result is a coordinated response in which stored energy in the form of glycogen and fat is made available as glucose to fuel muscle contraction.

Other examples of regulation by cAMP-dependent protein kinases include the increased activity of voltage-activated calcium channels in heart muscle cells; phosphorylation of these channels increases the amount of Ca^{2+} entering the cell during the action potential and, thus, increases the force of contraction of the heart.

In smooth muscle, cAMP dependent protein kinase phosphorylates (thereby inactivating) another enzyme, myosin-light-chain kinase, which is required for contraction. This accounts for the smooth muscle relaxation produced by many drugs that increase cAMP production in smooth muscle.

As mentioned above, receptors linked to G_i rather than G_s inhibit adenylylase and thus reduce cAMP formation. Examples include certain types of muscarinic acetylcholine

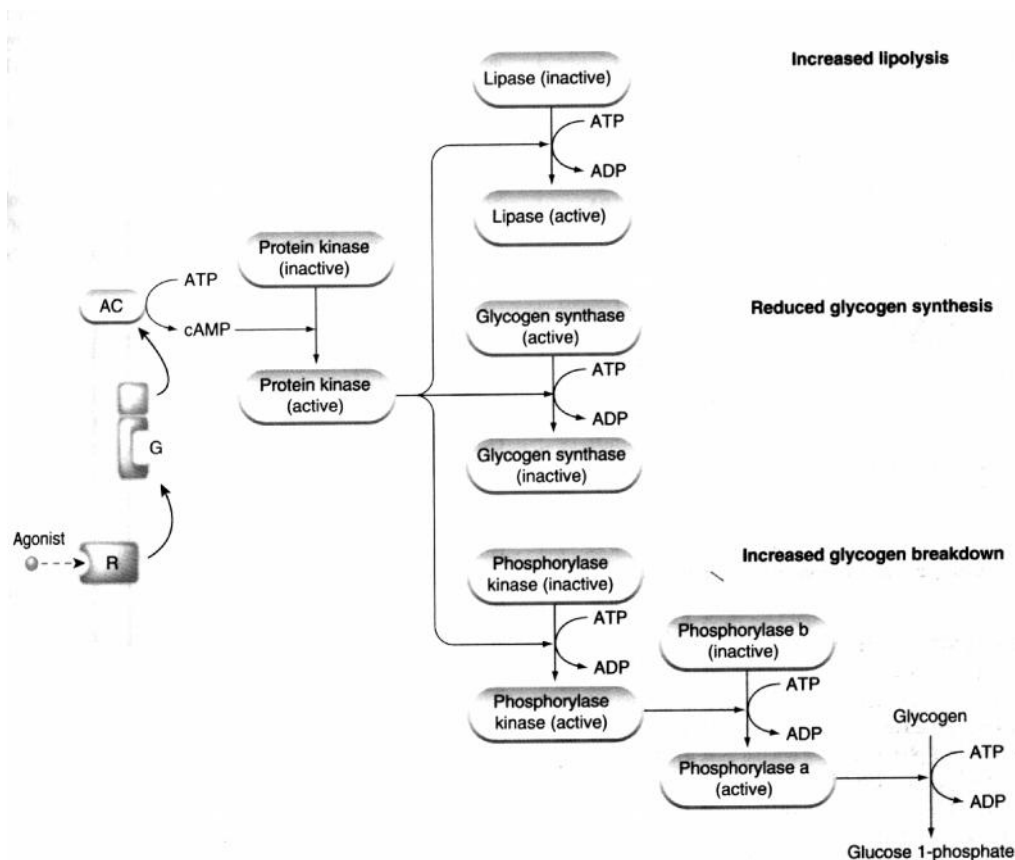


Figure.4 Regulation of energy metabolism by cAMP

As mentioned above, receptors linked to G_i rather than G_s inhibit adenylate cyclase and thus reduce cAMP formation. Examples include certain types of muscarinic acetylcholine receptor (e.g. the M_2 -receptor of cardiac muscle), α_2 -adrenoceptors in smooth muscle and opioid receptors.

cAMP is hydrolysed within cells by phosphodiesterases, a family of enzymes that is inhibited by drugs such as methylxanthines (e.g. theophylline, caffeine) and sildenafil. Various tissue specific isoforms of phosphodiesterase exist, and selective inhibitors of this enzyme have applications in cardiovascular and respiratory diseases.^[2]

The phospholipase C/inositol phosphate system :

The phosphoinositide system, an important intracellular second messenger system, was first discovered by Hokin and Hokin in the 1950s, whose interests centered on the mechanism of salt secretion by the nasal glands of seabirds. They found that secretion was accompanied by increased turnover of a minor class of membrane phospholipids known as phosphoinositides (PI). Subsequently, Berridge found that many hormones which produce an increase in free intracellular Ca^{2+} concentration also increase PI turnover. Subsequently, it was found that one

particular member of the PI family, namely phosphatidylinositol 4,5-bisphosphate (PIP_2), which has additional phosphate groups attached to the inositol ring, plays a key role. PIP_2 is the substrate for a membrane-bound enzyme, PLC, which splits it into diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP_3 ; figure 5), both of which function as second messengers. After cleavage of PIP_2 the status quo is restored, as shown in figure 4.5.2, DAG being phosphorylated to form phosphatidic acid, while the IP_3 is dephosphorylated and then recoupled with phosphatidic acid to form PIP_2 once again

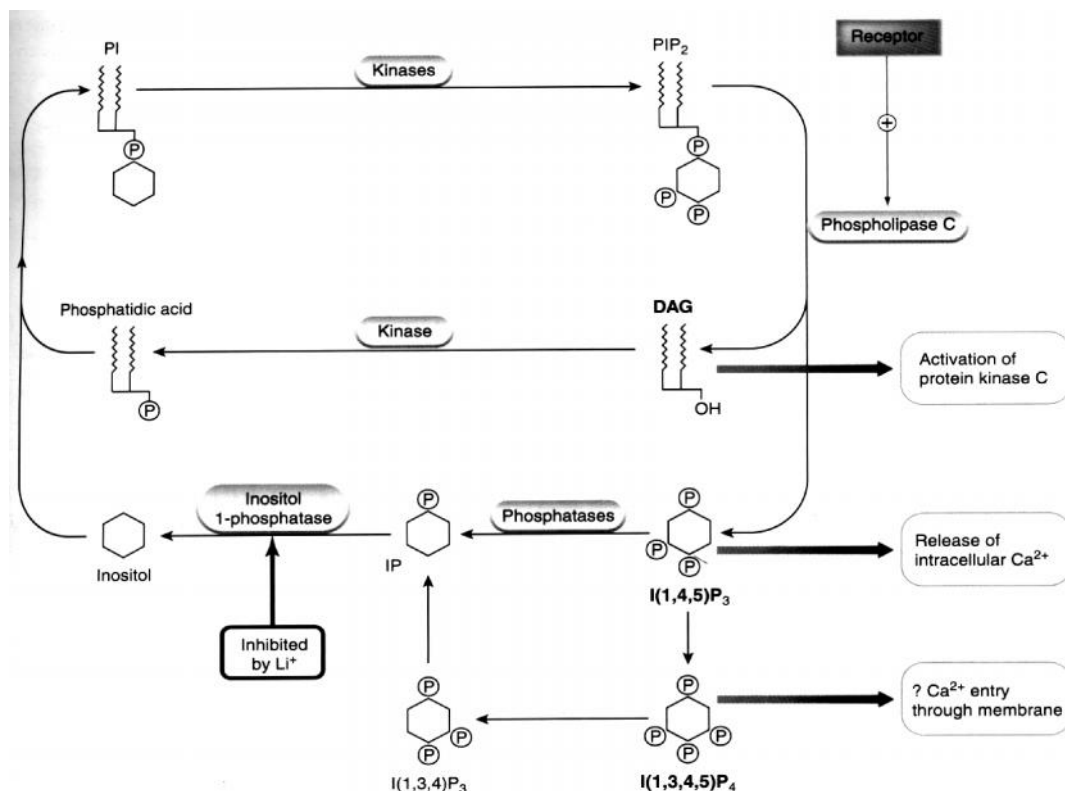


Figure 5 The phosphatidylinositol cycle

Inositol phosphates and intracellular calcium :

IP_3 is water-soluble mediator that is released into the cytosol and acts on a specific receptor-the IP_3 receptor- which is a ligand-gated calcium channel present on the membrane of the endoplasmic reticulum. The main role of IP_3 is to control the release of Ca^{2+} from intracellular stores. IP_3 is converted inside the cell to the 1,3,4,5-tetraphosphate (IP_4) by a specific kinase. The exact role of IP_4 remains unclear, but there is evidence that it too is involved in Ca^{2+} signalling.

Diacylglycerol and protein kinase C :

DAG is produced as well as IP_3 whenever receptor induced PI hydrolysis occurs. The main effect of DAG is to activate a membrane-bound protein kinase C (PKC), which catalysis the phosphorylation of a variety of intracellular proteins. DAG, unlike the Ips, is highly lipophilic

and remains within the membrane. It binds to a specific site on the PKC molecule, which migrates from the cytosol to the cell membrane in the presence of DAG, thereby becoming activated. There are at least 12 different PKC subtypes, which have distinct cellular distributions and phosphorylate different proteins. Most are activated by DAG and raised intracellular Ca^{2+} levels, both of which are produced by activation of GPCRs.

Ion channels as targets for G-proteins :

GPCRs can control ion channel function directly by mechanisms that do not involve second messengers such as cAMP or Ips. This was first shown for cardiac muscle. In cardiac muscle, for example, muscarinic acetylcholine receptors are known to enhance K^+ permeability (thus hyperpolarizing the cells and inhibiting electrical activity). Similar mechanisms are believed to operate in neurons, where opiate analgesics reduce excitability by opening potassium channels. These actions are produced by direct interaction between the G-protein subunit and the channel, without the involvement of second messengers. As shown in figure 6 either the free β -subunit, or the $\beta\gamma$ -subunit complex of the G-protein may be the mediator that controls the channel.

Desensitisation of G-protein coupled receptors :

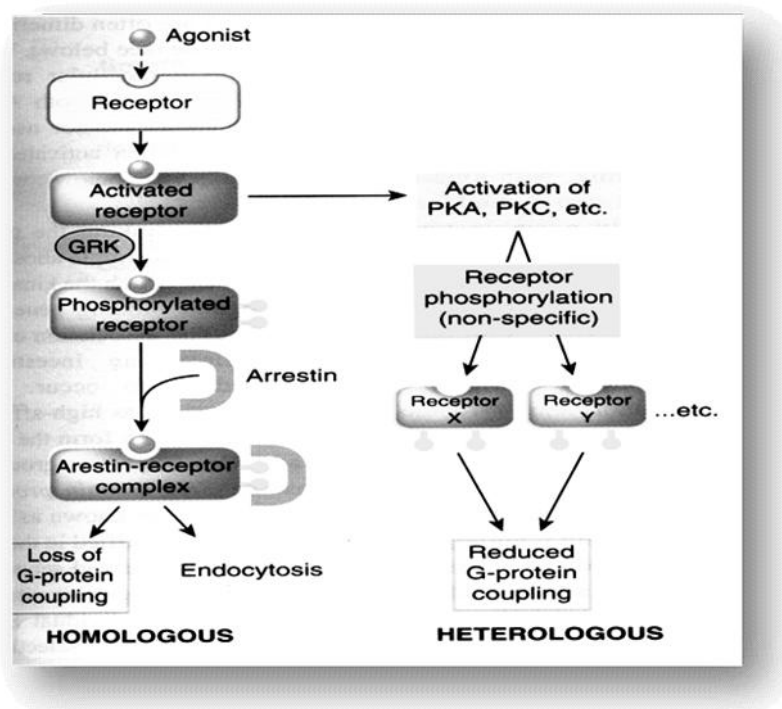


Figure 6. Desensitisation of G-protein coupled receptors

Homologous (agonist-specific) desensitization involves phosphorylation of the activated receptor by a specific kinase (GRK). The phosphorylated receptor then binds to arrestin, causing the

receptors to lose its ability to associate with a G-protein and undergoes endocytosis, which removes the receptor from the membrane. Heterologous (cross-) desensitization occurs as a result of phosphorylation of one type of receptor through activation of kinases by another. (figure 6)^[2]

KINASE-LINKED RECEPTORS :

The kinase-linked receptors are quite different in structure and function from either the ligand-gated channels of GPCRs. They mediate the actions of a wide variety of protein mediators, including growth factors and cytokines, and hormones such as insulin and leptin. Reflecting their inherent enzymic properties, growth factor receptors are often referred to as receptor tyrosine kinases.

Molecular structure :

The basic structure of these receptors is shown in figure 3.1.1C. They comprise very large extracellular (ligand-binding) and intracellular (effector) domains, with about 400-700 residues in each. In the case of insulin receptor, the extracellular domain consists of a separate polypeptide, which is linked to the rest of the molecule by disulfide bonds. In contrast, the growth factor receptors consist of a single long chain of over 1000 residues. Cytokine receptors are generally similar but are often dimeric. In all cases, the receptors trigger a kinase cascade. With growth factors and insulin receptors, the intracellular region possesses tyrosine kinase activity and incorporates both ATP- and substrate binding sites. Cytokine receptors do not usually have intrinsic kinase activity but associate, when activated by ligand binding, with kinases known as Jaks (see below), which are the first step in the kinase cascade.

Signal transduction mechanism :

First step of signal transduction involves dimerisation of pairs of receptors. The association of the two intracellular kinase domains allows autophosphorylation of tyrosine residues to occur (figure 7). The autophosphorylated tyrosine residues then serve as high affinity binding sites for other intracellular proteins, which form the next stage in the signal transduction cascade. One important group of such adaptor proteins is known as the SH2-domain proteins (because they contain a highly conserved domain known as SH2 standing for 'src homology', since it was first identified in the src oncogene product). These possess a highly conserved sequence of about 100 amino acid residues forming a recognition site for the phosphotyrosine residues of the receptor. Individual SH2-domain proteins, bind selectively to particular receptors, so the pattern of events triggered by particular growth factors is highly specific.

Two well-defined signal transduction pathways are summarised in figure 7. The Ras/Raf pathway (figure 7) mediates the effect of many growth factors and mitogens. Ras, which is a proto-oncogene product, functions like a G-protein and

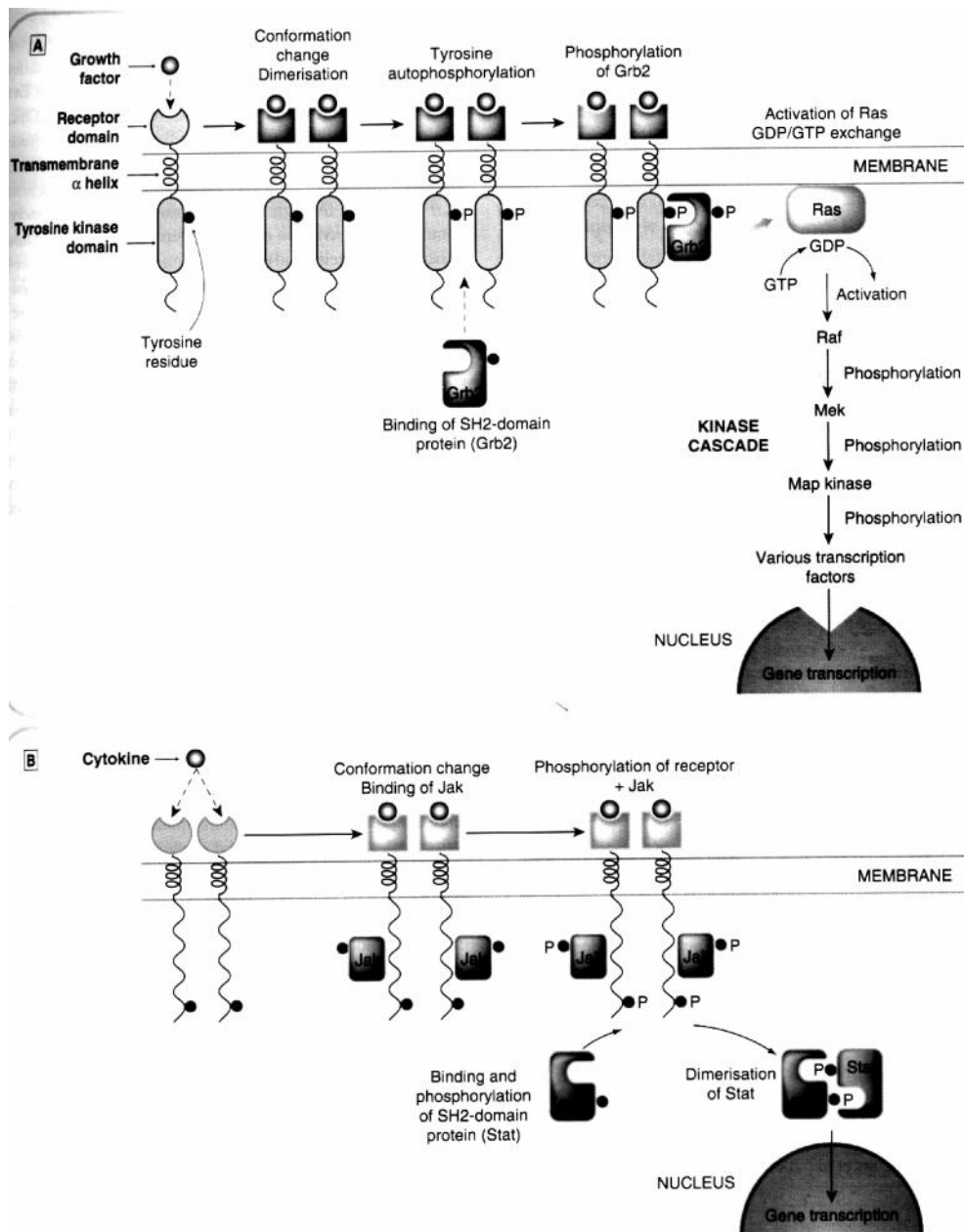


Figure 7 Transduction mechanism of kinase-linked receptors

conveys the signal from the SH2-domain protein Grb, which is phosphorylated by the receptor tyrosine kinase. Activation of Ras, in turn activates Raf, which is the first of a sequence of serine/threonine kinases, each of which phosphorylates, and activates, the next in line. The last of these, MAP (mitogen-activated protein) kinase, phosphorylates one or more transcription factors that initiate gene expression, resulting in a variety of cellular responses, including cell division.

A second pathway, the Jak/Stat pathway (figure 7), is involved in responses to many cytokines. Dimerisation of these receptors occurs when the cytokine binds, and this attracts a cytosolic tyrosine kinase unit (Jak) to associate with, and phosphorylate the receptor dimer. Jaks belong to a family of proteins, different members having specificity for different cytokine receptors. Among the targets for phosphorylation by Jak are a family of transcription factors (Stats). These are Sh2-domain proteins that bind to the phosphotyrosine groups on the receptor-Jak complex, and are themselves phosphorylated. Thus activated, Stat migrates to the nucleus and activates gene expression.

NUCLEAR RECEPTORS :

Nuclear receptors are a class of intracellular receptors. They are located inside the cell rather than on its outer cell membrane. The ligands that bind to nuclear receptors are often lipophilic hormones like steroid hormones. Practically all intracellular receptors are transcription factors. When activated they migrate to the cell nucleus and bind to DNA and stimulate or suppress gene transcription^[4]

Molecular structure :

They possess a highly conserved region of about 60 residues in the middle of the molecules that constitutes the DNA-binding domain of the receptor. It contains two loops of about 15 residues each (zinc fingers), knotted together by a cluster of four cysteine residues surrounding a zinc atom; these structures occur in many proteins that regulate DNA transcription, and the fingers are believed to wrap around the DNA helix. The hormone-binding domain lies downstream of this central region, while upstream lies a variable region that is responsible for controlling gene transcription.

Types of nuclear receptors :

- Steroid receptor
 - Sex hormone receptors (sex hormones)
 - Estrogen receptor
 - Androgen receptor
 - Progesterone receptor
 - Vitamin D receptor (vitamin D)
 - Glucocorticoid receptor (glucocorticoids)
 - Mineralocorticoid receptor (mineralocorticoids)
- Thyroid hormone receptor
- Retinoid receptor (vitamin A and related compounds);
- Peroxisome proliferator-activated receptors (PPARs)

Signal transduction pathway :

Nuclear (or cytoplasmic) receptors are soluble proteins localized within the cytoplasm or the nucleoplasm. The hormone has to pass through the plasma membrane, usually by passive diffusion, to reach the receptor and initiate the signal cascade. The nuclear receptors are ligand-

activated transcription activators; on binding with the ligand (the hormone), they will pass through the nuclear membrane into the nucleus and enable the production of a certain gene and, thus, the production of a protein.^[5]

On binding a steroid molecule, the receptor changes its conformation, which facilitates the formation of receptor dimers. These dimers bind to specific sequences of the nuclear DNA, known as ‘hormone-responsive elements’, which lie about 200 base pairs upstream from the genes that are regulated. An increase in RNA polymerase activity and the production of specific mRNA occur within a few minutes of adding the steroid, though the physiological response may take hours or days to develop. The different steroid hormones are able to induce or repress specific genes, and thus initiate completely different patterns of protein synthesis and produce different physiological effects. For example, glucocorticoids inhibit transcription of the gene for cyclooxygenase-2 (COX-2), which may account for their anti-inflammatory properties, whereas mineralocorticoids stimulate the production of various transport proteins that are involved in renal tubular function.

Other ligands that act on nuclear receptors of this family include thyroid hormones, vitamin D and retinoic acid. Retinoic acid is an important regulator of embryonic development, and gradients of this substance arising during development play a key role in controlling the development of limbs and organs.^[2]

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